

On two-phase flow models for cell motility



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Abstract

The ability of cells to move through their environment and spread on surfaces is fundamental to a host of biological processes; including wound healing, growth and immune surveillance. Controlling cell motion has wide-ranging potential for medical applications; including prevention of cancer metastasis and improved colonisation of clinical implants. The relevance of the topic coupled with the naturally arising interplay of biomechanical and biochemical mechanisms that control cell motility make it an exciting problem for mathematical modellers.

Two-phase flow models have been widely used in the literature to model cell motility; however, little is known about the mathematical properties of this framework. The majority of this thesis is dedicated to improving our understanding of the two-phase flow framework. We first present the simplest biologically plausible two-phase model for a cell crawling on a flat surface. Stability analyses and a numerical study reveal a number of features relevant to modelling cell motility. That these features are present in such a stripped-down two-phase flow model is notable. We then proceed to investigate how these features are altered in a series of generalisations to the minimal model. We consider the effect of membrane-regulated polymerization of the cell's actin network, the effect of describing the network as viscoelastic, and the effect of explicitly modelling myosin, which drives contraction of the actin network.

Validation of hydrodynamical models for cell crawling and spreading requires data on cell shape. The latter part of the thesis develops an image processing routine for extracting the three-dimensional shape of cells settling on a flat surface from confocal microscopy data. Models for cell and droplet settling available in the literature are reviewed and we demonstrate how these could be compared to our cell data. Finally, we summarise the key results and highlight directions for future work.

Statement of originality

Chapter 1 is a discussion, based on the literature of what is currently known about how cells crawl and spread, and mathematical modelling of cell motility. This material is substantially updated, extended and re-organised from that found in the introduction to my MSc thesis, [62].

Chapter 2 is based closely on [92]. I am the first author on this paper, my supervisors Dr. J. Oliver, Dr. J. Whiteley and Dr. S. Waters and our collaborator Prof. J. King are co-authors. The following sections §2.1.7, §2.2.3, §2.4 and the comparison between our constitutive law for network swelling and contraction and that used in [97] in §2.1.2 are wholly my own work. The remainder of this chapter is joint work with my supervisors and the code used in §2.4 was written by Dr. J. Whiteley.

All of the work contained in Chapter 3 is new and my own work. I modified Dr. J. Whiteley's code for the minimal model to obtain the codes used for the numerical simulations in this chapter.

All of the work contained in Chapter 4 is new and my own work, apart from the numerical code used in this chapter which was written by Dr. J. Whiteley. I ran all the simulations. The content of this chapter has been submitted for publication.

Chapter 5 is wholly my own work and new for this thesis with the exception of §5.3.1 where we present the results of stability analyses previously carried out in [105] and by me in [62]. I wrote the code used for the numerical simulations in this chapter based on Dr. J. Whiteley's codes from the previous chapters.

The image processing techniques used in Chapter 6 are available in the literature. The current initial image segmentation technique has grown out of an earlier MATLAB code written by Dr. J. Ratcliffe (University of Nottingham), but I have improved it significantly. The active contours routine is a development of a code

written by Dr. J. Lee (King's College London). Where existing functions beyond those available in MATLAB have been used, this has been clearly indicated. It is well known that droplets where surface tension dominates form constant mean curvature surfaces and the models reviewed in §6.4 are available in the literature. We are not aware of the Hocking and Rivers model previously being used as a model for cell spreading. We derive a power law for cell spreading in the long time limit from the Hocking and Rivers model, which is in agreement with that obtained from total wetting theory in [42]. The comparison of the models to our cell data in §6.5 is new.

Appendix A follows the derivation presented in [102] and is included for reference. Throughout the thesis wherever results or methods from the literature have been used, they have been identified and attributed.

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Chapter 1

Introduction

The ability of cells to crawl through their surroundings is crucial to many biological processes, including wound healing, immunity and embryonic development [20]. Questions about how cells crawl are not posed solely for their intellectual challenge; there are medical applications including improved design for biomaterial implants and prevention of metastasis in cancer treatment. The interplay of mechanical forces and biochemical control mechanisms encapsulated in cell motility has caught the attention of many experimentalists and mathematical modellers alike. Yet despite considerable efforts, how cells achieve motility is still only partially understood and consensus remains elusive [114]. This thesis will investigate what progress can be made in understanding cell motility by mathematically modelling crawling and spreading cells within a hydrodynamical framework. First, however, we present an introduction to what is known biologically about how cells crawl and spread.

1.1 Biological description of cell motility

Experimental observations of cell crawling have revealed considerable variety in the ways in which vertebrate cells crawl. However, common principles can be observed. Cells form protrusions, extensions at their front which adhere to the substrate, the surface the cell crawls over. The bulk of the cell is pulled forwards and adhesions at the rear of the cell weaken and detach [20, 114]. These stages can occur sequentially or simultaneously and the mode of crawling seems to be related to cell size. Once moving, smaller cells tend to employ all the stages simultaneously, whereas larger cells often use a sequential combination of the stages. Cells of medium size can typically exhibit both types of motion [122].

The protrusions cells make come in various shapes, from finger-like pseudopodia, to “bulbous” protrusions termed lobopodia. Fibroblasts extend both lamellipodia and filopodia. Lamellipodia are thin sheet-like protrusions. Filopodia are very small “hairlike” protrusions that seem to be used either to sense the cell’s environment or to achieve movement [20]. It has been observed experimentally that cells can switch between different modes of motility in three dimensional environments, exhibiting either elongated or rounded morphologies, see [134] and references therein.

1.1.1 Cellular constituents

It is widely accepted that a cell’s actin network is the main generator of protrusive force and a key component of a cell’s motile machinery. In fact actin is a highly conserved protein and to date has been found in every cell whose crawling has been studied, with the exception of the nematode sperm [20]. Actin monomers polymerize to form filaments that make up part of the cytoskeleton, which gives the cell its shape. Microtubules and intermediate filaments also form part of the cytoskeleton [113]. Microtubules are composed of polymerized filaments of α - and β -tubulin and undergo rapid turnover. Intermediate filaments are more stable. Myosin II (referred to as myosin henceforth) is a small motor protein important for cell motility. Myosin “walks” along actin filaments and generates stress in the actin network [20].

The cytoskeleton exists in a cytoplasm crowded with organelles including the cell nucleus and mitochondria which produce energy used by the cell in the form of adenosine triphosphate (ATP). The cell nucleus is responsible for controlling many processes within the cell, but experiments presented in [148] demonstrated that fragments of keratocyte cells without a nucleus crawl in much the same way as whole keratocyte cells so the nucleus is not thought to be very important in controlling cell motility. The actin network is, however, highly regulated by many auxiliary proteins as discussed in the next section. Finally, the cell membrane is crucial for cell crawling: not only does it ensure the cell’s integrity, it is the site across which the cell interacts with other cells and the environment it crawls through [113].

1.1.2 Control and co-ordination of actin

Actin monomers polymerize into filaments. The filament ends are chemically distinct, denoted barbed and pointed, and have different polymerization rates. It has been shown

in vitro that at intermediate monomer concentrations (between $0.2\mu\text{M}$ and $2\mu\text{M}$), actin filaments polymerize at their barbed ends and depolymerize at their pointed ends. An equilibrium can be attained where the filament remains at a constant length, but a labelled monomer added to the barbed end moves backwards along the filament, a phenomenon termed treadmilling [20].

Within cells many actin filaments make up a highly regulated and dynamic actin network. The accepted tale of this regulation near to the leading edge, the ‘dendritic nucleation’ model, is beautifully described and drawn in [125], whose explanation we now summarise. A diagram from [125] is reproduced in Figure 1.1. ATP plays a key role in actin treadmilling: it binds to actin monomers to form ATP-actin, which is readily incorporated into actin filaments at the barbed end. ATP-actin in the network is hydrolysed to ADP-actin, which is vulnerable to attack from ADF/cofilin proteins, which depolymerize the network. By this process the filament is increasingly degraded towards its pointed end. The protein profilin catalyses the conversion of ADP-actin monomers to ATP-actin monomers and promotes the addition of ATP-actin to the barbed ends of filaments. Thymosin- β 4 is a protein that binds to ATP-actin monomers less favourably than profilin does and blocks polymerization to provide a second storage buffer for ATP-actin monomers. Together, thymosin- β 4 and profilin supply monomers for network growth. The model further proposes that receptors in the cell membrane respond to extracellular signals by initiating a cascade that produces active Rho-family GTPases and PIP2, which activate WASp/Scar proteins, which activate Arp2/3 near the cell membrane, thus providing a direction for the growth of the actin network. Arp2/3 complexes attach to the existing actin network and nucleate the growth of new filaments which branch from the existing network at an angle of approximately 70° , hence the term “dendritic”. The polymerizing filaments push the membrane forwards. Finally the model proposes that there is an optimal number of growing filaments, which balances the ability of more filaments to generate additional force against their faster depletion of the actin monomer supply, and that this optimality is obtained by capping proteins attached to the barbed ends of some actin filaments to halt polymerization.

Whilst established and largely accepted, the dendritic nucleation model described above is not without opposition. Most notably the work of Small and Urban *et al.*, [141, 145], challenged the accepted role of Arp2/3, after finding no branches in the lamellipodial actin network of migrating cells. The ensuing debate can be followed in [75, 85, 142] and a re-

The diagram originally presented here cannot be made freely available via ORA because of copyright. The diagram can be found in Figure 3 of [125].

Figure 1.1: A schematic diagram of the dendritic nucleation hypothesis taken from [125].

assessment of the original data which does find branching is detailed in [157]. The emerging consensus, as far as it can be drawn, is that dendritic nucleation is still a valid framework for understanding the growth of actin networks, but Arp2/3 branching is probably not as common as previously thought [158]. Network rigidity in the newly proposed, less-branched network is maintained by cross-linking proteins that fasten actin filaments which pass near to each other. A further modification to the theory is the inclusion of proteins, such as formins, that bind to the cell membrane and promote polymerization when they cap the barbed ends of actin filaments [158]. Recent experiments have contributed a number of interesting results. Wu *et al.* [155] find that modified cells without Arp2/3 complexes are capable of chemotaxis, but unable to respond to gradients of extracellular matrix (ECM) and Wiggan *et al.* [152] report that ADF/Cofilin proteins inhibit myosin binding to the actin network. Intriguingly, Risca *et al.* [128] find that Arp2/3 binds preferentially to the convex side of curved actin filaments *in vitro*, a mechanism for the force on the network to

influence the direction of branching. More detailed reviews of the chemical control of actin networks may be found in *e.g.* [127, 129]; the former emphasizes the differences observed between different modes of protrusion.

1.1.3 Distribution and dynamics of actin

Much of the available data pertains to fish epidermal keratocytes, which are popular with experimental scientists, and hence theoreticians, for the simple way in which they crawl. When keratocytes crawl, a large lamellipodium “fans” out ahead of the cell body [20]. Typical dimensions for such a lamellipodium are a length of $10\mu\text{m}$ and a thickness of $0.1\mu\text{m}$ [115]. Their polarisation and hence their direction of movement is “persistent,” they move quickly with the speed of a “few tens of micrometer per minute” and the cell’s shape exhibits “remarkable conservation” [143].

Verkhovsky *et al.* work with fragments of keratocyte cytoplasm in [148]. They find stationary, near symmetric, disc shaped fragments with a ring of high actin density just inside the fragment membrane, see Figure 1.2(a). Mechanical stimulus triggers polarization of the fragment and transition to a stable locomoting configuration, which has a crescent moon shape, a high actin density at the front of the fragment and arcs of bundled actin fibres at the rear, as illustrated in Figure 1.2(b).

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The image originally presented here cannot be made freely available via ORA due to copyright. The image can be found in Figure 2(a) of [148].

(a) Stationary fragment

(b) Locomoting fragment

Figure 1.2: Fluorescence microscopy images of stationary and crawling fragments of keratocyte cells. Actin is stained cyan, myosin is stained red, the scale bar in (b) is $2\mu\text{m}$. Pictures taken from [148]. The yellow lines are drawn on and will be referred to in subsequent chapters.

Whilst numerous visualisation techniques are used to investigate cell motility, one which

provided a considerable step forward is fluorescent speckle microscopy (FSM). A small number of fluorescently labelled actin monomers are microinjected into a cell, which enables polymerization, depolymerization and movement of monomers in the network to be tracked in live, motile cells [149].

In whole cells there exist distinct regions within the cytoplasm. The cortex is an actin rich layer just inside the cell membrane which helps to maintain the cell's shape [20]. At the front of a locomoting cell is the lamellipodium, characterised by high actin density and a highly branched network in which filaments predominantly have their barbed ends pointed forwards [143]. There is evidence emerging that this “criss-crossed” network is associated with rapid protrusion and more slowly protruding networks have a different configuration with filaments predominantly orthogonal to the leading edge [151]. FSM has provided evidence for strong polymerization of the network at the leading edge and transport of the actin network backwards away from the leading edge, termed retrograde flow [136, 146, 147]. This work also identifies a thin band of strong depolymerization a small distance behind the leading edge, where the lamellipodium meets the lamellum. The lamellum is characterised by lower network density and a more bundled network, which also undergoes retrograde flow [103]. At the rear of the lamellum, where the protrusion joins the main body of the cell, arced bundles of actin align predominantly parallel to the cell's leading edge [143]. This band of actin network is comparable in density to the leading edge, and the density exceeds that in the lamellum and the cell body [143]. FSM data suggests the network is pulled into this region, moving parallel to the leading edge, and that there is considerable depolymerization of the network. This region is called the convergence zone [103, 147]. In the cell body there is anterograde flow, but at a velocity smaller than the cell velocity [136], so in a frame that moves with the cell this would be seen as retrograde flow.

The description above suggests a steady, translating configuration for the actin network in a locomoting cell. Whilst that is broadly true it neglects some interesting dynamical features of actin networks. Many cells crawl with periodic protrusion and retraction cycles of their leading edge. Burnette *et al.* studied these fluctuations in [24] and found that during protrusion lamellipodial actin turns over rapidly and is contained in the lamellipodium. In contrast during retraction it is swept back into the lamellum, gathering into arcs which stack into bundles in the lamellum. These retraction events have been linked to the formation of “ruffles”, actin-filled folds of the cell membrane which travel backwards over the lamellum

and disappear at the transition to the cell body [17, 65, 77]. Possible mechanisms for waves in the actin network that travel along the cell edge are discussed in [3], who suggest such waves enable motility when polymerization resources are scarce. Returning to consider spatial regulation on the scale of the whole cell we note the recent work, [38], which casts doubt on the traditional view that polarization starts by forming protrusions at the cell front. They suggest instead that in the absence of chemoattractants the rear of the cell forms first.

1.1.4 Distribution and dynamics of myosin

We now turn our attention to myosin. A myosin molecule can be characterised as a tail with two heads, which can bind to and use ATP to move along actin filaments, towards their barbed end. When the tails of multiple myosin molecules aggregate they can cause actin filaments to slide relative to each other, generating contractile stress in the actin network [20]. A series of *in vitro* experiments in [126] beautifully demonstrate that the arrangement and alignment of fibres in the actin network is crucial to the function of myosin. They added myosin to randomly oriented filaments and observed rapid contraction followed by depolymerization of the filaments. When the experiments were conducted with parallel filaments no contraction was seen, whereas antiparallel filaments contracted to form antiparallel bundles. The authors suggest network structures similar to the randomly oriented filaments exist in regions of the highly branched, lamellipodial network and that parallel and antiparallel arrangements exist in filopodia and in the convergence zone respectively. There is also FSM evidence for myosin moving towards the barbed ends of filaments in travelling cells [136].

Figure 1.2 displays typical distributions of myosin in a keratocyte fragment. When the fragment is stationary myosin is distributed symmetrically, but once the fragment is crawling, the symmetry is broken and myosin is found at high concentrations at the rear of the fragment [148]. In a whole cell, myosin accumulates in small patches in the lamellipodia and attains high concentrations at the convergence zone, indicating its role in the network contraction and depolymerization associated with that region [143]. The main cell body has a much lower concentration of myosin. Distributions of both actin and myosin in a moving keratocyte can be seen in Figure 1.3, taken from [143].

The role of myosin in cell motility has been further elucidated by experiments that promoted myosin activity and found increased retrograde flow and depolymerization of the

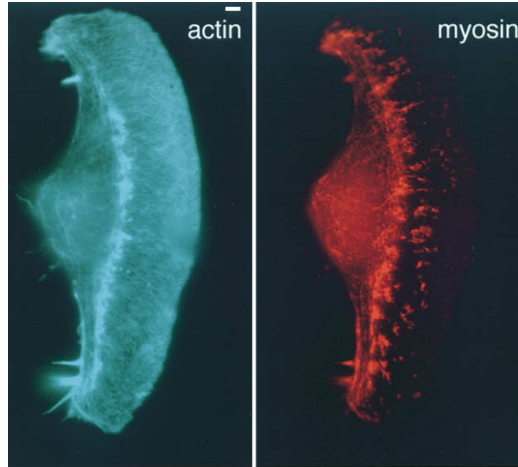


Figure 1.3: Fluorescence microscopy images of a keratocyte moving from left to right. The image on the left is a staining of the actin network. The image on the right is a staining of the myosin. The scale bar is $2\mu\text{m}$ and the image is taken from [143]. Reproduced under a ‘Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported license’ (<http://creativecommons.org/licenses/by-nc-sa/3.0/>), the figure was first published by Rockefeller University Press in *The Journal of Cell Biology*, vol. 139, no. 2, copyright belongs to the authors T. M. Svitkina, A. B. Verkhovsky, K. M. McQuade, and G. G. Borisy, 1997.

network in the lamellum with little change in the lamellipodia [147]. Inhibiting myosin results in reduced cell velocity, a disorganised network and often separation of lamellipodia from the cell body [136]. One effect of myosin is to allow the actin network to flow like a fluid, as demonstrated experimentally in [84]. The recent review [103], concerning the biomechanical properties of actomyosin networks, highlights the complex behaviours they can exhibit on different timescales and the competing factors that result in an active material which is short-time (seconds) elastic and long-time (tens of seconds to minutes) viscous.

1.1.5 Cell adhesion and forces

Understanding how cells adhere to the substrates they crawl over is crucial to understanding both cell crawling and cell spreading. A cell in suspension must form adhesions to the substrate, whilst deforming to increase the area of its ‘footprint’ in order to settle and spread on a flat surface. For a cell to crawl it must be able to form adhesions at its front and break them at its rear. The basic principle is to form an attachment between the ECM and the actin network, via integrins (a class of transmembrane protein) with the help of other auxiliary proteins [114]. A more detailed description of adhesion complexes can be found in the recent review [69] on which the following summary is based. It is estimated

that over 200 different molecules play some role in forming adhesion complexes. There are four main types of cell adhesion complex. The smallest ($< 0.25\mu\text{m}$) and most transient ($< 1\text{min}$) of these is the nascent adhesion which forms in the presence of polymerizing actin in lamellipodia. Some of these mature, aided by myosin and Rac, into focal complexes at the lamellipodia–lamellum interface. Focal complexes are around $1\mu\text{m}$ in size and have a typical life time of several minutes. Rho encourages their maturation into focal adhesions which are elongated in shape, a few microns in length and situated at the ends of actin bundles that form stress fibres. They have a typical half–life of 20 minutes. The fourth type of adhesion, termed a fibrillar adhesion, is the largest, the most stable and is associated with large bundles of actin filaments.

Force has also been indicated as having a role in adhesion maturation [69]. This is just one example of mechanotransduction, *i.e.* cells responding to applied force, possible mechanisms for which are discussed in [86]. Adhesion complexes are believed to breakdown with time, so that as the cell moves forwards over the adhesions it has set down, those at the rear of the cell will weaken allowing the cell to detach [114]. There is also increasing evidence that adhesion complexes can act in either “slipping” or “gripping” modes, with gripping adhesions localised to the cell front and slipping adhesions found in the flanks of rapidly locomoting keratocytes [60, 89]. Crawling cells have a preferred adhesiveness of substrate at which they crawl fastest; they crawl more slowly on surfaces that are too sticky or too slippery [123].

The forces that cells exert, via their adhesions, on the surface they crawl over is investigated by observing wrinkles and markers in a thin elastic substrate in [25]. They find that the lamellipodia exert a force on the substrate opposing the direction of cell motion. The flanks of the cell exert a large inwards and slightly forwards force on the substrate. The rear of the cell exerts a force on the substrate in the direction of cell motion. A similar experiment in [101] calculates traction forces from the displacement of small beads in an elastic substrate. They again find large, lateral forces directed inwards in the flanks and rear of the cell. However, they do not observe rearward directed force under lamellipodia, but instead find a very small force in the direction of motion at the leading edge of the cell. A study by Herant *et al.* [74] calculated the force a crawling neutrophil can generate by making the cells crawl in a pipette whilst subjected to a counter–pressure. They found a roughly linear relationship between crawling speed and counter–pressure; unopposed the cells crawl

at approximately $0.33\mu\text{ms}^{-1}$, but approximately $1.8 \times 10^4 \text{ dyn cm}^{-2}$ of counter-pressure halts them.

1.2 Mathematical modelling of cell crawling

There have been numerous approaches to the mathematical modelling of cell motility. We aim here to provide an overview of the key themes in the literature and refer those seeking a more detailed discussion of the field to recent reviews [114, 156]. First we discuss the models which focus on the structure and dynamics of actin filaments at the leading edge of a cell. These include the seminal paper by Mogilner and Oster [117] which proposes the “elastic Brownian ratchet” model. They model an actin filament growing towards the cell membrane as a polymer polymerizing under the effect of a load. Bending of the polymer and small fluctuations in the position of the load vary the size of the gap between the load and the filament. If this gap becomes large enough, an extra monomer can polymerize onto the filament causing it to elongate. The load is prevented from returning to its original range of positions due to resistance to bending in the filament. In this way the load is pushed forwards by the polymerizing filament. Mogilner and Oster derive an equation for the velocity of a load, pushed by a single actin filament. They find an optimal angle of 48° between the filament and the load which maximises the velocity. This prediction is in reasonable agreement with that observed in lamellipodia [117].

1.2.1 Filament based models

The work of Mogilner and Oster has been extended by a number of authors who consider a stochastic, computational simulation of an actin network growing against a load. For example, [81, 82, 137] seek to quantify the effect of branching, capping and anti-capping proteins in the dendritic nucleation hypothesis. Similar stochastic simulations in [150] reveal two distinct patterns in the branched network that emerge from the dynamics depending on the simulation parameters. Each pattern has a characteristic angle between the actin filaments and the direction of motion. The interactions between actin filaments are considered in [66]. Tensegrity models are another class of model that describe the actin network as a collection of rod-like structures. They model not only actin filaments, but also microtubules, intermediate filaments and actin bundles as “cables” under tension and “struts” under compression,

which are connected to each other and the ECM. To date these models have been more useful in describing the properties of stationary cells than motile cells, see Chapter 6 of [113] and references therein. Drawbacks to modelling approaches such as these, which track the position of individual actin filaments, include the high computational costs of introducing additional modelling components and of running multiple stochastic simulations in order to obtain trends.

1.2.2 Reaction–diffusion models

We proceed to consider modelling approaches that still focus on capturing the organisation of the actin network, but do so via continuous variables obeying reaction, advection and diffusion equations. Edelstein–Keshet’s group have made a significant contribution to this field with both one- and two-dimensional, numerical simulations [40, 41, 107, 108, 115]. They consider a density of barbed actin filaments and investigate how the capping and branching of these filaments is controlled by the local concentrations of auxiliary proteins. The model presented in [115] predicts an optimal numbers of polymerizing barbed ends: too few and the force on the membrane is weak and the cell speed small, too many and the actin monomers are used up rapidly, stalling polymerization. Travelling wave solutions for spontaneous and side branching nucleation of actin filaments, in which the cell’s velocity is a function of Arp2/3 activation and capping rate, are presented in [41]. The two-dimensional models, [107, 108], split the domain into a hexagonal lattice and use a Cellular Potts based model, wherein each hexagon is labelled part of the cell or not part of the cell and these labels are updated at each timestep according to an energy minimisation. A similar approach with an energy minimisation, depending on actin alignment, over a piecewise linear cell membrane has simulated motile cells avoiding obstacles in [119]. Grimm *et al.* [68] use a similar methodology to model an actin network with densities of left and right filaments which are orientated at $\pm 35^\circ$ to the direction of motion. They relate the membrane velocity to the density of barbed actin filaments at the leading edge and investigate the effects of different capping rates. Lower rates were found to promote “persistent, steady locomotion” [68].

Another reaction–diffusion–equation–driven approach to modelling cell motility solves equations on the cell surface as opposed to in the bulk. Typically, an excitable, coupled system with an activator and inhibitor is considered. Localised regions of high concentration

of the activator species develop on the curve that represents the cell membrane of a two-dimensional, model cell. The membrane is then evolved according to rules that make it protrude at regions of high activator concentration [70, 118]. This modelling approach has captured tip-splitting of advancing pseudopods [70], an experimentally observed feature. Typically generic chemical species are considered and specific, experimentally identified, signalling molecules are not modelled. A notable exception to this is the work of Gamba *et al.* [63] which considers the stochastic reaction and diffusion of identified cell molecules on the surface of a sphere. Their model displays symmetry breaking in the distributions of these chemicals.

One hurdle to simulating reaction-diffusion equations in motile cells is the computational cost of explicitly tracking the moving cell boundary. Phase-field formulations address this issue with a continuous variable equal to one inside the cell and zero outside, or *vice versa*. The cell membrane is identified as the narrow region in which this phase variable changes rapidly. For example, [139] present a two-dimensional model of the concentration of cross-linked actin filaments, that cause membrane protrusion, and bundled actin filaments, which cause membrane retraction. The cell position is updated, via the phase-field variable, according to the forces due to the actin network, surface tension and membrane stiffness and a pressure which enforces a constant cell area. A similar phase-field approach [132] models degradation of the ECM ahead of the cell protrusion, although forces acting on the cell membrane are not considered. The phase-field formulation has been used for computation in different modelling frameworks as we shall see in §1.2.4.

Reaction-diffusion based models of cell motility are in essence about the spatial localisation of specific substances, which break the cell's symmetry, cause polarization and drive motion. This symmetry breaking can be thought of as pattern formation. The classical reaction-diffusion, pattern-forming system of Turing illustrates that parameter sizes, and in particular domain sizes, are very important in determining the patterns that develop. This may be concerning for modelling cell motility, since the same biochemical systems (so presumably the same rate constants and diffusion coefficients) are observed generating remarkably similar behaviours in cells from a considerable range of sizes. More importantly, whilst reaction-diffusion systems can well describe the biochemical aspects of cell motility, they fail to address the biomechanical aspects.

1.2.3 Mechanical component models

We proceed to consider models of cell motility that use more mechanical descriptions of the cell. The cell is treated as six connected, viscoelastic, Voigt elements coupled to a model for integrin dynamics in [49]. Asymmetry is imposed by making adhesion stronger at the predefined front of the cell than at the rear, and the model captures the biphasic dependence of the cell speed on adhesion strength. A two-dimensional model that describes the cell as a collection of viscoelastically connected nodes is presented in [50]. Again the cell front is predetermined and nodes have different elastic and contractile properties based on their position in the cell. Other modelling approaches that focus on the adhesion dynamics include [23], which describes the cell as a two-dimensional elastic plate connected to the substrate by elastic spring adhesion complexes, and [130], which describes a cell as a two-dimensional, fluid-filled, elastic membrane that rolls as adhesions detach at the cell rear. These models are all characterised by the interaction of discrete elements and whilst that may be a realistic representation of adhesion complexes, it may be somewhat less satisfactory to carve up the cell cytoskeleton into arbitrary components.

1.2.4 Continuum mechanics models

Next we consider approaches that model the cell cytoskeleton as a single continuous material. Such models may describe the cell as a viscous fluid [6, 138], or a viscoelastic material [67, 79, 100, 131, 133, 135], typically with additional terms to model the active nature of the cytoskeleton. A phase-field formulation is used for two-dimensional simulations in [138], which models adhesion via discrete adhesion sites that can either grip or slip. The model demonstrates the experimentally observed bell-shaped dependence on adhesion strength. Gracheva *et al.* [67] demonstrate the same qualitative dependence on adhesion strength in their one-dimensional, viscoelastic, model of a crawling cell. In [100], another one-dimensional model, the cytoplasm's elastic modulus, viscous coefficient and adhesion to the substrate depend linearly on the actin concentration. The model exhibits a travelling-wave solution with qualitatively plausible distributions of actin and myosin, but much of the shape of the distribution is prescribed by the boundary conditions. Similar constitutive laws are used in [135] to model a cell crawling over a substrate with an adhesion gradient, although the authors impose a linear distribution of actin which increases towards the *a priori* defined front of the travelling strip. Another interesting continuum model for a crawling cell can

be found in [131]. The authors deal only with the cell’s lamellipod and impose a spatially varying adhesion strength in the cell *a priori*, before solving the viscoelastic equations for the actin network’s velocity in a two-dimensional, realistically shaped domain. A three-dimensional, ellipsoid-shaped cell is considered in [133], although quantities vary only with axial length, so the model is, in effect, one-dimensional. The authors investigate the ability of cells to switch between different modes of locomotion. Finally we draw the readers attention to a different continuum based approach in [79], which models cell motility as a combination of surface growth and bulk deformation processes.

An alternative continuum approach to those discussed above is based on polar gels, see for example [27, 28, 51, 52, 53, 54, 57, 87, 88, 96, 160]. Proponents of this methodology argue that actin filaments have a preferred direction for polymerization, which carries through to directionality of the actin network that is not adequately captured by an isotropic continuous medium. The cytoplasm is modelled as a fluid with an associated vector field indicating the local filament orientation. This modelling draws on techniques from modelling liquid crystals and has led to a number of interesting results. The filamentous fluid can self-organize into spirals, asters or waves and the model yields solutions that replicate polarized travelling cells observed experimentally, see in particular [57, 160]. (Note that [160] uses a phase-field formulation.) A simplification to this modelling approach is presented in [27], which assumes that the network direction is in the radial direction in a circular domain. Polar gels have also been coupled to the two-phase flow framework, which we discuss next [28, 87].

1.2.4.1 Two-phase flow models

A very promising framework for the continuum modelling of cell motility is the two-phase, poroviscous, reactive flow (TPRF) formulation, established by [46], further developed in [4, 5, 44, 45, 74, 93, 97, 122, 159] and discussed in [35]. The framework allows for the incorporation of both biophysical and biochemical mechanisms, both in the cell bulk and on the cell membrane, and naturally handles the free boundary inherent in cell motility. The two-phase mixture consists of a ‘network’ phase and a ‘solution’ phase. The network phase consists of the actin-myosin network, which generates actively the forces responsible for motion. The solution phase lumps together the aqueous solvent and actin monomers, which flow passively through the porous network. ‘Reactive’ refers to the polymerization and depolymerization of the network. Advantages of the TPRF framework can be seen by

comparison to some of the alternatives. The one-phase formulations discussed above treat only the actin network and have nowhere for monomers of depolymerized actin to go. As a result, they cannot describe the effects of relative motion between the network and the solution and do not capture swelling of the network [35]. A flow with more than two phases poses a considerably tougher challenge for analysis, which is why we advocate the TPRF framework as sufficiently detailed to be of interest for modellers of cell motility, whilst simple enough for its study to be informative. A more general introduction to two-phase flow can be found in [56].

Early successes that point to the strength of this approach have included: the representation of fountain streaming in amoeboid cells [44]; the prediction of ruffling dynamics in the actin network in a one-dimensional model [5]; and the onset of polarization for a two-dimensional cell fragment [97]. Both [5] and [97] impose different boundary conditions on different parts of the lamellipodium to describe its interaction with the substrate or cell body. The model results show a peak in actin density where the lamellipodium meets the cell body, at the rear of their domain. Cell adhesion receives a fairly sophisticated treatment in [97]. The integrins are modelled in four stages: free; bound to the substrate; bound to the actin network; and bound to both the substrate and the actin network. Numerical simulations are presented for a three-dimensional model in [72] and Herant and Dembo have developed a three-dimensional code, called Cytopede, to solve three-dimensional, two-phase flow cell models [71]. Both [93] and [122] derive three-dimensional models and exploit the cell's geometry to consider thin-film reductions of these models. In [93] a transfer of actin from solution to network at the leading edge is considered.

Further weight behind the argument that the cell cytoplasm is properly represented by a two-phase material is provided in [110] and [111]. Both model the network as an elastic, porous, solid through which the passive cytosol fluid flows. In [111] rapid indentation experiments validate the poroelastic assumption on a number of cell types in a variety of conditions. The cell rheology is measured only over timescales which are much shorter than typical crawling timescales, so their results are not incompatible with our two-fluid model. Having discussed models for cell crawling we proceed to consider models of cell spreading.

1.3 Mathematical models of cell spreading

A number of biological observations have highlighted the hydrodynamical nature of cell spreading. Fardin *et al.* compare the spreading of a single cell to the driven spreading of a droplet during spin coating [59]. They also collect observations of three stages of cell spreading. First the cell makes contact with the surface, but maintains a constant contact radius, then it spreads rapidly with the contact radius scaling like $t^{1/2}$ (where t is time), before entering a slower spreading regime in which the contact radius scales like t^n , with different experiments estimating n to be between $1/10$ and $1/4$. They propose a lubrication type model for cell spreading.

We note that a cellular aggregate is observed to spread like a droplet in [55]. The cellular aggregate presents a slightly more complicated scenario in which both the material properties of the individual cells and the strength of adhesion between cells contributes to the observed phenomena. For strongly adhering cells, a cell monolayer forms a precursor ahead of the spreading drop. For more weakly adhering cells, individuals escape the aggregate and move on the substrate.

Returning to single cells, the work of Frisch and Thoumine, [61], compares experimental data of fibroblasts in suspension, settling and then spreading on a flat surface to predictions from the theory of partially wetting droplets. A similar investigation is carried out for osteosarcoma cells in [29]. An alternative approach, which models a spreading cell as a deformable, adhesive shell can be found in [39]. Models of cell spreading are discussed further in Chapter 6.

1.4 Thesis outline

Two-phase flow models are widely used in the literature and are likely to become more widely used as experimentalists discover more quantitative information about the material properties of cells. However, these models have quickly become complicated and typically their investigation relies solely on numerical simulation. The first aim of this thesis is to improve our understanding of such two-phase flow models. To do this, we begin in Chapter 2 by formulating and analysing the simplest, biologically plausible, one-dimensional, two-phase flow model of a single cell crawling on a flat substrate. Our goal is to understand the mathematical properties of the two-phase flow equations, particularly the features it has that are relevant to cell crawling.

The next three chapters explore different developments of the minimal model. In Chapter 3 we consider the effect of alternative boundary conditions, modelling membrane-regulated actin polymerization. The effect of the rheology of the actin network is considered in Chapter 4. In Chapter 5 we couple equations describing the transport and binding dynamics of myosin to the minimal model. After identifying several physically relevant parameter sub-limits, based on myosin binding, crawling and diffusion rates, we use stability analysis to select two models as clean examples of the different types of myosin behaviour and investigate these numerically.

The topic of Chapter 6 is cell spreading. We develop an image processing routine to extract the three-dimensional shape of spreading cells from confocal microscope images. Models for cell spreading available in the literature are discussed and we explain how the image processing could be used to validate these models against appropriate cell spreading data. Finally, in Chapter 7, we summarise the key results of the thesis and identify directions for future work.

Chapter 2

A minimal model for cell motility

In this chapter¹ we investigate a simple two-phase flow model of a crawling cell. The motivation for this modelling approach is the desire to understand how the two-phase flow framework behaves in its most transparent form, before clouding the analysis with increased complexity. We seek to understand which physical phenomena arise from a mathematical description of the actin network and aqueous cytosol as two, inter-penetrating, reactive fluids (where one fluid is allowed to flow up its volume fraction gradient), before we consider additional biophysical effects to more accurately describe cell motility. This approach allows us to begin to address the fundamental scientific question about the extent to which cell crawling can be captured by hydrodynamical processes, and when more biological considerations are required.

We investigate the idealised situation of a one-dimensional strip of cytoplasm, free to crawl on a flat surface. We do not impose a front and back on the strip, so that we are able to investigate how the direction of motion depends on the initial conditions. This is a significant step forwards in comparison to many modelling approaches that impose *a priori* a front and back for the cell. The model formulation is set out in §2.1. In §2.2 a stability analysis suggests that the system has the potential for a rich variety of behaviour. In §2.3 an analysis appropriate in the small-growth-rate limit enables us to say more about the initial direction of motion of the crawling strip. Finally, in §2.4, numerical simulations demonstrate that for a given set of parameters there exist multiple, qualitatively distinct, travelling-wave solutions. Within each family of solutions the steady crawling speed has

¹The majority of this chapter was first published as ‘Multiple travelling-wave solutions in a minimal model for cell motility’ by L.S. Kimpton, J.P. Whiteley, S.L. Waters, J.R. King and J.M. Oliver in *Math. Med. Biol.*, 30(3), p241-272, 2013 by Oxford University Press.

the experimentally observed ‘bell-shaped’ dependence on the strength of network–substrate adhesion [123].

2.1 Model formulation

2.1.1 Mass and momentum conservation

The two-phase flow framework is discussed in §1.2.4.1 and a brief explanation of the form of the two-phase flow equations is provided in Appendix A. We follow the framework used in [5] and consider a one-dimensional model in which x measures the distance along a strip of cytoplasm in the direction of motion as indicated by the yellow lines on the cell fragments in Figure 1.2. The variables $\theta(x, t)$ and $1 - \theta(x, t)$ denote respectively the volume fractions of network and solution at time t , there being no voids. Assuming each phase to be incompressible and, for simplicity, of the same density, the conservation of mass equations are given by

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad \frac{\partial}{\partial t}(1 - \theta) + \frac{\partial}{\partial x}((1 - \theta)v) = J, \quad (2.1a, b)$$

where $u(x, t)$ and $v(x, t)$ are the network and solution velocities, respectively, and J is the net rate at which mass is transferred from the network phase into the solution phase by microscopic polymerization ($J < 0$) and depolymerization ($J > 0$) processes.

Experiments to determine the rheology of actin solutions have found nonlinear viscoelastic behaviours that depend on network cross-linking, filament length and experimental procedure [22, 91]. However, the timescale of motion for a crawling cell is typically much longer than the elastic relaxation timescale, which is of the order of one second in a pure actin–myosin network at physiological actin concentrations [84]. In this chapter we neglect elastic behaviour and model the actin network as a viscous fluid. The force balances on the network and solution phases are given by

$$-\theta \frac{\partial p}{\partial x} - \frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x} \left(\mu \frac{\partial u}{\partial x} \right) = H(u - v) + \beta u, \quad -(1 - \theta) \frac{\partial p}{\partial x} = H(v - u), \quad (2.2a, b)$$

where $p(x, t)$ is the hydrodynamic pressure, Ψ is the swelling/contractile pressure in the network, μ is the (effective) network viscosity, H is the network–solution drag coefficient and β is the adhesion or network–substrate drag coefficient. We note that Ψ , μ , H and β will be prescribed as functions of θ .

We have modelled the network phase as isotropic for simplicity. The balance between the forces associated with hydrodynamic pressure, swelling and contraction of the network,

network–solution drag and cell–substrate adhesion is described in (2.2a). The flow of network is driven down gradients of p and Ψ and this flow is resisted by the effects of viscosity and drag between the network and the solution or the substrate. Noting that Ψ depends on θ , so that $\partial\Psi/\partial x = \Psi'(\theta)\partial\theta/\partial x$, we see that for $\Psi'(\theta) > 0$ this term acts to drive the transport of network down its volume fraction gradient and hence transport of the solution up the network volume fraction gradient, thus modelling swelling of the network. For $\Psi'(\theta) < 0$ this term acts to drive the transport of network up its volume fraction gradient, modelling contraction of the network. The solution phase is modelled as inviscid on the macro–scale, which is consistent with the network viscosity being at least three orders of magnitude larger than that of water [45]. The force balance (2.2b) describes Darcy flow of the aqueous solution through the porous network.

Summing (2.2a,b) we obtain the total force balance on the mixture

$$\frac{\partial\Sigma}{\partial x} = \beta u, \quad (2.3)$$

where the total-mixture stress is given by

$$\Sigma = -p - \Psi + \mu \frac{\partial u}{\partial x}. \quad (2.4)$$

We note that (2.1)–(2.3) is Galilean invariant if and only if $\beta \equiv 0$. In the absence of adhesion to the substrate, the strip is free to crawl at any speed.

2.1.2 Constitutive laws

In order to solve the governing equations (2.1)–(2.2) for the dependent variables θ , u , v and p we must prescribe constitutive laws for J , Ψ , μ , H and β . Our aim in formulating these constitutive laws is not to accurately capture every interaction between actin filaments, the detailed biochemistry of the dendritic nucleation model, the maturation of individual adhesion complexes or any of the other microscale features of cell motility described in §1.1. Instead, we seek the simplest possible constitutive laws that encapsulate the principle effects of the microscale interactions at the appropriate macroscale level of our continuum model. In keeping with our ‘simplest problem first’ approach, we restrict J , Ψ , μ , H and β to depend only on the network volume fraction θ . Wherever possible we shall keep our analysis general and assume only a θ dependence for our constitutive laws. Where it is necessary to prescribe explicit expressions for the constitutive laws, for instance in §2.4, we shall use those given below.

For the rate of mass transfer J we assume first-order kinetics, with

$$J(\theta) = \frac{\theta - \theta_E}{T}, \quad (2.5)$$

where θ_E is the equilibrium network volume fraction and T is the timescale for relaxation, to this equilibrium value. This is the same form of polymerization/depolymerization rate as used in [122].

Attempts to describe the behaviour of polymers in solution by considering the thermodynamics and formulating a free energy for the polymer exist in the literature. See for example Flory Huggins theory discussed in [36]. However, within cells the picture is far more complicated and the details of actin filament interactions in the presence of myosin and ATP are unknown. We follow a more constitutive approach with the hope of formulating a more tractable model. We assume that myosin is distributed throughout the cell proportionally to the volume fraction of the actin network, so that at intermediate volume fractions there is sufficient myosin for the network to contract. At low network volume fractions we assume network contraction is inhibited by the low levels of myosin. At high network volume fractions we assume network contraction is inhibited by high levels of filament cross-linking and entanglement. Experimental evidence that network contraction stalls at high network volume fractions (for a reduced system) can be found in [13].

We summarise the key features of the constitutive law used in [4, 5, 97] as being an increasing function of the network volume fraction for small and large volume fractions, decreasing for intermediate volume fractions and tending to infinity as the network volume fraction tends to unity. We choose the simplest law that satisfies these criteria, namely

$$\Psi'(\theta) = \Psi_0 \frac{(\theta - \theta_L)(\theta - \theta_R)}{(1 - \theta)}, \quad (2.6)$$

where Ψ_0 , θ_L and θ_R are positive constants with $\theta_L < \theta_R$. We are able to specify straightforwardly the boundaries to the swelling regime in which $\Psi'(\theta) > 0$ and Ψ drives the transport of network down its gradient ($\theta < \theta_L$ or $\theta > \theta_R$), and the contractile regime in which $\Psi'(\theta) < 0$ and Ψ drives the transport of network up its gradient ($\theta_L < \theta < \theta_R$). The denominator $(1 - \theta)$ ensures that dense packing of the network, *i.e.* θ approaching unity, is counteracted by a large swelling pressure. Integrating $\Psi'(\theta)$ with respect to θ we obtain the swelling/contractile pressure in the form

$$\Psi(\theta) = -\Psi_0 \left((1 - \theta_L)(1 - \theta_R) \ln(1 - \theta) + (1 - \theta_L - \theta_R)\theta + \theta^2/2 \right). \quad (2.7)$$

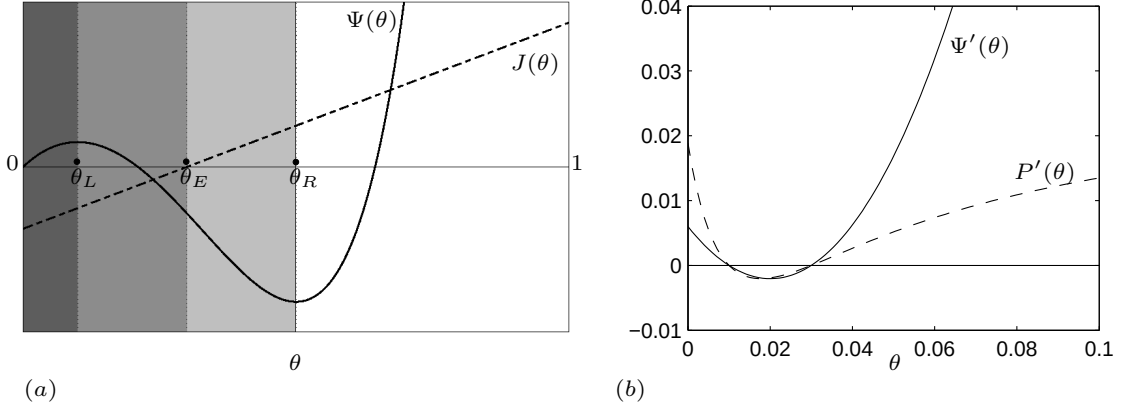


Figure 2.1: Schematic of the swelling/contractile pressure $\Psi(\theta)$ and of the mass transfer rate $J(\theta)$ are shown in (a) for the constitutive laws (2.5) and (2.7). The shading indicates network volume fractions at which the network: swells and depolymerizes (white); contracts and depolymerizes (light grey); contracts and polymerizes (mid grey); swells and polymerizes (dark grey). Plots of $\Psi'(\theta)$ and $P'(\theta)$ are compared in (b) for $\theta_L = 0.01$, $\theta_R = 0.03$, $\Psi_0 = 20$, $L_1 = 0.019$, $L_2 = 4.0$ and $\theta_{\text{sat}} = 0.036$ to two significant figures.

We shall select parameter values so that the polymerization/depolymerization kinetics drive the network volume fraction towards the contractile regime, explicitly we pick $\theta_L < \theta_E < \theta_R$. Thus, we identify four swelling/contractile, polymerizing/depolymerizing regimes which are shaded in Figure 2.1(a). It is possible to identify combinations of our coefficients with the constants in the Flory–Huggins functional (see, *e.g.* [36].) We also compare our constitutive law to

$$P(\theta) = -L_1 \ln(1 - \theta) - \frac{L_2}{2} \frac{\theta^2}{(1 + \theta/\theta_{\text{sat}})^2}, \quad (2.8)$$

which is used in [97], where L_1 is identified as the strength of the swelling pressure and L_2 is the contractile stress. To find parameter choices so that we can compare this constitutive law to ours we factor L_1 out of (2.8) and see that this sets the scale of $P'(\theta)$. The two remaining parameters L_2/L_1 and θ_{sat} control the position of the roots of $P'(\theta)$. After selecting values for θ_L , θ_R and Ψ_0 we numerically minimise $P'(\theta_L)^2 + P'(\theta_R)^2$ over the parameters L_2/L_1 , and θ_{sat} . Finally we determine L_1 by setting the minimum of $\Psi'(\theta)$ equal to the minimum of $P'(\theta)$. In Figure 2.1(b) we plot our swelling/contractile pressure against (2.8) and show they are qualitatively similar.

For the effective network viscosity, network–solution and network–substrate drag coefficients, we adopt the simplest physically plausible constitutive laws, namely

$$\mu(\theta) = \mu_0 \theta, \quad H(\theta) = H_0 \theta (1 - \theta), \quad \beta(\theta) = \beta_0 \theta, \quad (2.9a-c)$$

where μ_0 , H_0 and β_0 are positive constants; μ_0 and H_0 are the network viscosity and hydraulic resistance, respectively.

2.1.3 Boundary and initial conditions

In order to model a travelling cell we shall consider our governing equations on a moving domain $a(t) < x < b(t)$. This represents a strip of cell cytoplasm, aligned with the direction of cell motion. Crucially we do not impose a direction of motion, so that both the position of the strip and which end of the strip is the front must be determined as part of the solution. In this chapter we work with the simplest possible, biologically reasonable assumption that there is no flow of network or solution through the ends of the travelling strip and no polymerization or depolymerization of the actin network on the boundaries, an assumption that will be revisited in Chapter 3. The boundary conditions are given by

$$\text{at } x = a(t), \quad \theta(u - \dot{a}) = 0, \quad (1 - \theta)(v - \dot{a}) = 0, \quad \Sigma = 0, \quad (2.10a-c)$$

$$\text{at } x = b(t), \quad \theta(u - \dot{b}) = 0, \quad (1 - \theta)(v - \dot{b}) = 0, \quad \Sigma = 0, \quad (2.11a-c)$$

where here and hereafter, a dot $\dot{}$ on a variable denotes a derivative with respect to t . The zero stress boundary condition models the simplest situation in which the crawling strip feels no stress from the medium it moves through. Since the boundaries $x = a(t)$ and $x = b(t)$ move with the network velocity, characteristics to the hyperbolic equation (2.1a) trace the edge of the domain, meaning that no boundary conditions are required for θ . The system (2.1)–(2.2) and (2.10)–(2.11) is closed by the initial conditions

$$a(0) = 0, \quad b(0) = L; \quad \text{for } 0 < x < L, \quad \theta(x, 0) = \theta^*(x); \quad (2.12a-c)$$

where L is the initial length of the strip and θ^* the initial network volume fraction.

2.1.4 Reduced system

Here we present manipulations that simplify our system of governing equations. Summing (2.1a,b) and integrating with respect to x yields the volume-averaged-mixture velocity

$$V(t) = \theta u + (1 - \theta)v, \quad (2.13)$$

which depends only on time. Using (2.13) to eliminate v from the Darcy-flow law (2.2b) implies that the hydrodynamic pressure gradient is given by

$$\frac{\partial p}{\partial x} = \alpha(u - V), \quad (2.14)$$

where

$$\alpha(\theta) = H(\theta)/(1 - \theta)^2, \quad (2.15)$$

which we shall work with from now on. Using (2.13)–(2.14) to eliminate v and $\partial p/\partial x$, we find that (2.1)–(2.2) may be reduced to

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad -\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x} \left(\mu \frac{\partial u}{\partial x} \right) = \alpha(u - V) + \beta u, \quad (2.16a,b)$$

for θ and u . We note that $V(t)$, $a(t)$ and $b(t)$ are further unknowns: the manner in which they are specified is described below. We can consider (2.16a) a hyperbolic equation for θ if u is known. If θ and V are known we can consider (2.16b) a second-order ordinary differential equation for u .

For the moving-strip problem, the reduced system (2.16) is to be solved on $a(t) < x < b(t)$, with (2.10a,b), (2.11a,b) and (2.13) implying that

$$V(t) = \dot{a}(t) = \dot{b}(t). \quad (2.17)$$

It follows that length of the cell is fixed by the initial conditions (2.12a,b) to be of length L , *i.e.* $b(t) - a(t) \equiv L$, and that the mixture velocity is equal to that of the strip. By (2.17), the location of the leftmost edge of the strip is given by

$$a(t) = \int_0^t V(\tau) \, d\tau. \quad (2.18)$$

The boundary conditions for the reduced system (2.16) with nonzero θ become

$$u(a(t), t) = V(t), \quad u(b(t), t) = V(t), \quad (2.19a,b)$$

and it remains to determine V . Integrating the mixture-force balance (2.3) from $x = a(t)$ to $x = b(t)$ and using the fact that the mixture stress is equal to zero at the ends of the strip by (2.10c) and (2.11c), we find that the global force balance is given by

$$\int_{a(t)}^{b(t)} \beta(\theta(x, t)) u(x, t) \, dx = 0, \quad (2.20)$$

which provides the solvability condition for $V(t)$, the stress within the cell being given by

$$\Sigma(x, t) = \int_{a(t)}^x \beta(\theta(x', t)) u(x', t) \, dx'. \quad (2.21)$$

2.1.5 Nondimensionalization

Scaling distance, time and the dependent variables by setting

$$x = Lx^\dagger, \quad t = Tt^\dagger, \quad u = \frac{L}{T}u^\dagger, \quad V = \frac{L}{T}V^\dagger, \quad a = La^\dagger, \quad b = Lb^\dagger, \quad (2.22)$$

the reduced system (2.16) is unchanged only if we scale the constitutive laws by setting

$$J = \frac{1}{T}J^\dagger, \quad \Psi = \frac{\mu_0}{T}\Psi^\dagger, \quad \mu = \mu_0\mu^\dagger, \quad \alpha = \frac{\mu_0}{L^2}\alpha^\dagger, \quad \beta = \frac{\mu_0}{L^2}\beta^\dagger. \quad (2.23)$$

The corresponding scalings for v and p are L/T and μ_0/T , respectively, and the daggers represent dimensionless quantities.

2.1.6 Travelling frame

Since the length of the strip is constant and the network velocity is equal to that of the strip at its ends, it is convenient to consider a frame moving with the strip by setting

$$x^\dagger = \int_0^{t^\dagger} V^\dagger(\tau) d\tau + \hat{x}, \quad \theta^\dagger(x^\dagger, t^\dagger) = \hat{\theta}(\hat{x}, t^\dagger), \quad u^\dagger(x^\dagger, t^\dagger) = V^\dagger(t^\dagger) + \hat{u}(\hat{x}, t^\dagger). \quad (2.24a-c)$$

Making the transformation (2.24) for the nondimensional versions of (2.16)–(2.20) gives (dropping the hats and daggers) the governing equations we shall work with from now on, namely

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad -\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x} \left(\mu \frac{\partial u}{\partial x} \right) = \alpha u + \beta(u + V), \quad (2.25a,b)$$

for $t > 0$, $0 < x < 1$, subject to the boundary and initial conditions

$$\text{for } t > 0, \quad u(0, t) = 0, \quad u(1, t) = 0; \quad \text{for } 0 < x < 1, \quad \theta(x, 0) = \theta^*(x). \quad (2.26a-c)$$

We also require the solvability condition

$$\langle \beta(u + V) \rangle = 0, \quad (2.27)$$

for $t \geq 0$ where, here and hereafter, $\langle \cdot \rangle = \int_0^1 \cdot dx$ is the average of a quantity over the strip.

Rearranging (2.27) we see that

$$V = -\frac{\langle \beta u \rangle}{\langle \beta \rangle}, \quad (2.28)$$

so this model will display treadmilling on the scale of the strip, in the sense that the cell moves forwards when on average the network flows backwards. The equation for the cell

position in the travelling frame is still given by (2.18) for $t \geq 0$. The dimensionless versions of the constitutive laws (2.5)–(2.9) are given by

$$J(\theta) = \theta - \theta_E, \quad \Psi(\theta) = -\Psi^* \left((1 - \theta_L)(1 - \theta_R) \ln(1 - \theta) + (1 - \theta_L - \theta_R)\theta + \theta^2/2 \right), \quad (2.29a,b)$$

together with

$$\mu(\theta) = \theta, \quad \alpha(\theta) = \alpha^* \theta / (1 - \theta), \quad \beta(\theta) = \beta^* \theta. \quad (2.30a-c)$$

The three dimensionless parameters in addition to θ_L , θ_E and θ_R are given by

$$\Psi^* = \frac{\Psi_0 T}{\mu_0}, \quad \alpha^* = \frac{H_0 L^2}{\mu_0}, \quad \beta^* = \frac{\beta_0 L^2}{\mu_0}, \quad (2.31a-c)$$

which quantify the typical ratios of swelling/contractile pressure, network–solution drag and network–substrate drag to network viscous forces, respectively.

2.1.7 Dimensionless parameters

The parameter estimates in Table 2.1 for L , T , μ_0 , H_0 , Ψ_0 and θ_E were obtained from experimental results, while the estimate for β_0 is based on values used in a previous mathematical model in [5], and we consider a suitable range for β_0 to be $0.01 - 1 \text{ kPa min}/\mu\text{m}^2$. We will use $\theta_E = 0.02$ in our numerical simulations and pick θ_L and θ_R to be the same order and such that $\theta_L < \theta_E < \theta_R$. If we fix the estimates in which we have most confidence at $L = 20 \mu\text{m}$ and $T = 1 \text{ min}$, and combine the remaining ranges of the dimensional parameters, we obtain estimates for our nondimensional parameters as $\Psi^* = 0 - 5 \times 10^6$, $\alpha^* = 4 \times 10^{-5} - 4 \times 10^3$ and $\beta^* = 2 - 2 \times 10^7$. This is a considerable region of parameter space to consider and our numerical investigations in §2.4 will encompass only a small fraction of it. However, our nondimensionalization provides the useful information that improved estimates of network viscosity would significantly reduce uncertainty in the parameter values.

2.1.8 System classification

Our governing equations (2.25)–(2.27) are nonlinear and nonlocal in the sense that V depends on an integral of θ and u via (2.27). Further information can be obtained from a potential hodograph transformation, *cf.* [93], which will allow us to eliminate u as we shall now describe.

Parameter	Values	Units	Reference
Cell length (L)	20 – 100	μm	HOBs [122]
	10 – 20	μm	FKs [143]
Network–turnover time (T)	0.2 – 10	min	[45]
Network viscosity (μ_0)	$2 \times 10^{-4} - 2$	kPa min	[45]
Network–solution drag (H_0)	$2 \times 10^{-4} - 2$	Pa min μm^{-2}	[45]
Network–substrate drag (β_0)	0.26 – 0.4	kPa min μm^{-2}	[5]
Swelling/contractile pressure (Ψ_0)	0 – 10^2	kPa	[45]
Lower contractile threshold (θ_L)	0.01	-	Assumed
Equilibrium volume fraction (θ_E)	$10^{-4} - 10^{-2}$	-	[45]
	$10^{-3} - 10^{-1}$	-	HOBs [122]
Upper contractile threshold (θ_R)	0.03	-	Assumed

Table 2.1: Parameter estimates based on experimental data or models. Human osteoblasts have been abbreviated to HOBs and fish keratocytes have been abbreviated to FKs.

2.1.8.1 Potential hodograph transformation

Following [93], we define the integrated mass variable ρ for the network phase by setting

$$\rho = \int_0^{\chi(\rho,t)} \theta(\xi, t) d\xi, \quad \chi(0, t) = 0, \quad (2.32a, b)$$

and make the change of variables from x to ρ by writing

$$\theta(\chi(\rho, t), t) = \Theta(\rho, t), \quad u(\chi(\rho, t), t) = U(\rho, t). \quad (2.33a, b)$$

Since θ is continuous and strictly positive, $\chi(\rho, t)$ is differentiable and (2.32a) gives

$$1 = \theta(\chi(\rho, t), t) \frac{\partial \chi}{\partial \rho}(\rho, t), \quad 0 = \int_0^{\chi(\rho,t)} \frac{\partial \theta}{\partial t}(\xi, t) d\xi + \theta(\chi(\rho, t), t) \frac{\partial \chi}{\partial t}(\rho, t). \quad (2.34a, b)$$

Substituting (2.25a) into (2.34b) and integrating gives

$$U = \frac{\partial \chi}{\partial t} + I, \quad (2.35)$$

and hence, by (2.34a),

$$\frac{\partial U}{\partial \rho} = \frac{\partial \phi}{\partial t} + \frac{\partial I}{\partial \rho}, \quad (2.36)$$

where, after using the boundary condition $U(0, t) = 0$,

$$\phi(\rho, t) = \frac{1}{\Theta(\rho, t)}, \quad I(\rho, t) = -\phi(\rho, t) \int_0^\rho \phi(\eta, t) J(1/\phi(\eta, t)) d\eta. \quad (2.37a, b)$$

We note that $I(\rho, t)$ depends on the integral of a dependent variable and is nonlocal. We also note that $\chi(\rho, t)$ is given in terms of $\phi(\rho, t)$ by

$$\chi(\rho, t) = \int_0^\rho \phi(\eta, t) d\eta. \quad (2.38)$$

Transforming (2.25b) and using (2.36) we obtain U in terms of ϕ , I and V (for $\alpha + \beta \neq 0$)

$$U = A(\phi) \frac{\partial \phi}{\partial \rho} + B(\phi) \frac{\partial}{\partial \rho} \left(C(\phi) \left(\frac{\partial \phi}{\partial t} + \frac{\partial I}{\partial \rho} \right) \right) - V(t) D(\phi), \quad (2.39)$$

where

$$A(\phi) = \frac{\Psi'(1/\phi)}{\phi^3(\alpha(1/\phi) + \beta(1/\phi))}, \quad B(\phi) = \frac{1}{\phi(\alpha(1/\phi) + \beta(1/\phi))}, \quad (2.40a, b)$$

$$C(\phi) = \frac{\mu(1/\phi)}{\phi}, \quad D(\phi) = \frac{\beta(1/\phi)}{\alpha(1/\phi) + \beta(1/\phi)}. \quad (2.41a, b)$$

The final step is to eliminate U by substituting (2.39) back into (2.36), which shows that $\phi(\rho, t)$ satisfies the equation

$$\frac{\partial \phi}{\partial t} = \frac{\partial}{\partial \rho} \left\{ A \frac{\partial \phi}{\partial \rho} + B \frac{\partial}{\partial \rho} \left(C \left(\frac{\partial \phi}{\partial t} + \frac{\partial I}{\partial \rho} \right) \right) - VD - I \right\}. \quad (2.42)$$

We see that the potential hodograph transformation has enabled us to reformulate our problem as a single third-order, nonlocal, integro-partial-differential equation for $\phi(\rho, t)$ with one extra variable V which depends only on time.

We may use (2.39) to write the boundary and solvability conditions in terms of ϕ and V , so that the problem can be formulated for these variables on a moving domain $0 < \rho < R(t)$, where, by (2.38), $R(t)$ is determined by the condition

$$1 = \chi(R(t), t) = \int_0^{R(t)} \phi(\eta, t) d\eta. \quad (2.43)$$

The initial condition (2.26b) becomes $\phi(\rho, 0) = \phi^*(\rho)$ for $0 < \rho < R(0)$, with $\phi^*(\rho) = 1/\theta^*(\chi(\rho, 0))$.

2.1.8.2 Remarks

The potential hodograph transformation shows clearly that both the velocity of the crawling strip V , and network polymerization and depolymerization cause the system to be nonlocal, via an integral over the travelling strip. This formulation also allows us to identify competing physical effects in our model. Contraction of the actin network occurs for $\Psi'(\theta) < 0$, which corresponds to $A(\phi) < 0$, which we recognise as a backwards diffusion term in (2.42). Therefore, network contraction has a destabilising effect which is regularised by the viscosity since $B(\phi)C(\phi) > 0$. If we eliminate the nonlocal terms, by considering for example a stationary strip without network polymerization and depolymerization, we obtain

$$\frac{\partial \phi}{\partial t} = \frac{\partial}{\partial \rho} \left\{ A \frac{\partial \phi}{\partial \rho} + B \frac{\partial}{\partial \rho} \left(C \frac{\partial \phi}{\partial t} \right) \right\}, \quad (2.44)$$

which is pseudo-parabolic (*cf.* [93]). It can be thought of as a regularized nonlinear diffusion equation [120], which shares some similarities with a limit case of the viscous Cahn-Hilliard equation [30]. These types of equations have been studied previously in the context of phase separation processes. We also note that when $J = 0$, ρ is a Lagrangian co-ordinate by (2.35).

Despite the insight it provides, there are clearly considerable difficulties with this formulation. The independent variable ρ is far from intuitive and the transformed problem must be solved on the moving domain $0 < \rho < R(t)$ for nonzero J . This could be resolved by instead defining ρ as the integral of a strictly positive dependent variable which is conserved globally, but we shall not pursue such issues here. Instead we return to the Eulerian formulation and consider a linear stability analysis.

2.2 Linear stability analysis

We now consider the linear stability analysis of the reduced system in order to understand how a steadily crawling strip responds to small perturbations in the initial network volume fraction about the equilibrium value θ_E . We seek insight into the biologically relevant questions of how the actin network polarizes and how this determines the direction of cell motion. We begin with the most general case, considering (2.25) on an unbounded domain, free from choices about boundary conditions.

2.2.1 Unbounded domain

On an infinite strip $-\infty < x < \infty$, a uniform steady-state solution to (2.25) is given by

$$\theta = \theta_E, \quad u = u_E, \quad V = V_E, \quad (2.45a-c)$$

for any positive constant V_E , provided

$$J(\theta_E) = 0, \quad u_E = -\frac{\beta(\theta_E)V_E}{\alpha(\theta_E) + \beta(\theta_E)}; \quad (2.46a,b)$$

hereafter we will use a subscript E on a constitutive law to indicate that it is evaluated at the equilibrium network volume fraction θ_E , *i.e.* $\alpha_E = \alpha(\theta_E)$ and so on. Note the constraint placed on J is satisfied by (2.5). In the usual way we seek a solution to (2.25) by linearizing about (2.45) as follows

$$\theta \sim \theta_E + \varepsilon\theta_1 + \dots, \quad u \sim u_E + \varepsilon u_1 + \dots, \quad V \sim V_E + \varepsilon V_1 + \dots, \quad (2.47a-c)$$

where $0 < \varepsilon \ll 1$ is a small parameter associated with small prescribed variations in the initial network volume fraction. We obtain at $O(\varepsilon)$ the linearized system

$$\frac{\partial \theta_1}{\partial t} + u_E \frac{\partial \theta_1}{\partial x} + \theta_E \frac{\partial u_1}{\partial x} = -J'_E \theta_1, \quad (2.48)$$

$$-\Psi'_E \frac{\partial \theta_1}{\partial x} + \mu_E \frac{\partial^2 u_1}{\partial x^2} = \alpha_E u_1 + \beta_E (u_1 + V_1) + (\alpha'_E u_E + \beta'_E (u_E + V_E)) \theta_1, \quad (2.49)$$

where a prime $'$ on a constitutive law indicates its derivative with respect to θ , so that $\alpha'_E = d\alpha/d\theta(\theta_E)$ and so on. Differentiating (2.49) once with respect to x , then eliminating u_1 using (2.48) shows that small disturbances in the network volume fraction are governed by the linear partial differential equation

$$\left(\frac{\partial}{\partial t} + u_E \frac{\partial}{\partial x} + J'_E \right) \left(\theta_1 - \frac{\mu_E}{(\alpha_E + \beta_E)} \frac{\partial^2 \theta_1}{\partial x^2} \right) + \gamma_E u_E \frac{\partial \theta_1}{\partial x} = \frac{\theta_E \Psi'_E}{(\alpha_E + \beta_E)} \frac{\partial^2 \theta_1}{\partial x^2}, \quad (2.50)$$

for θ_1 , where

$$\gamma_E = -\frac{\theta_E}{(\alpha_E + \beta_E)} \left(\alpha'_E + \beta'_E \left(1 + \frac{V_E}{u_E} \right) \right) = -\frac{\theta_E \beta_E}{(\alpha_E + \beta_E)} \left(\frac{\alpha}{\beta} \right)'_E. \quad (2.51)$$

We note from the advection terms that the network is transported with velocity $(1 + \gamma_E)u_E$ where γ_E combines the linearized effects of the network-solution drag and the cell adhesion. Seeking a solution to (2.50) of the form $\theta_1(x, t) = \text{Re}[\hat{\theta}_1 \exp(ikx + \Omega(k)t)]$, where $\hat{\theta}_1$ is a complex constant, leads immediately to the dispersion relation (*cf.* [46])

$$\Omega(k) = -J'_E - \frac{\theta_E \Psi'_E k^2}{\alpha_E + \beta_E + \mu_E k^2} - ik u_E \left(1 + \frac{(\alpha_E + \beta_E) \gamma_E}{\alpha_E + \beta_E + \mu_E k^2} \right), \quad (2.52)$$

for $\Omega(k)$. A small, spatially periodic perturbation of wavenumber k (where k is taken to be non-negative without loss of generality) has the growth rate $\text{Re}[\Omega(k)]$. The corresponding perturbation in the network velocity is given by

$$u_1 = \frac{\hat{\theta}_1}{\theta_E} \left(\frac{(\alpha_E + \beta_E) \gamma_E u_E - \theta_E \Psi'_E ik}{\alpha_E + \beta_E + \mu_E k^2} \right) \exp(ikx + \Omega(k)t). \quad (2.53)$$

Thus, in general, small perturbations in θ and u have different amplitudes and are not in phase.

Figure 2.2 depicts the growth rate as a function of wavenumber k for a number of different parameter regimes. We first make three observations: $\text{Re}(\Omega(0)) = -J'_E$; the growth rate is monotonic in k ; and $\text{Re}(\Omega(k)) \rightarrow \Omega_\infty$ as $k \rightarrow \infty$, where

$$\Omega_\infty := - \left(J'_E + \frac{\theta_E \Psi'_E}{\mu_E} \right). \quad (2.54)$$

The growth rate curves can be characterised by J'_E and Ω_∞ . If there is no polymerization or depolymerization of the network then $J'_E = 0$ and the growth rate is zero for $k = 0$. For $J'_E = 0$: if $\Omega_\infty > 0$, then every wavenumber $k > 0$ is linearly unstable; if $\Omega_\infty < 0$, then every wavenumber $k > 0$ is linearly stable; and if $\Omega_\infty = 0$, then every wavenumber $k > 0$ is neutrally stable. If $J'_E > 0$, as for the constitutive law (2.29a), then we are in one of the three regimes depicted in Figure 2.2(b). All wavenumbers are linearly stable if $\Omega_\infty < 0$. If $\Omega_\infty = 0$, then all finite wavenumbers are linearly stable and the neutral stability point lies at $k = \infty$. If $\Omega_\infty > 0$, then there exists a critical wavenumber

$$k = k_c := \left(\frac{(\alpha_E + \beta_E)J'_E}{\mu_E \Omega_\infty} \right)^{1/2}, \quad (2.55)$$

which is the point of neutral stability and perturbations are linearly stable (unstable) for $k < k_c$ ($k > k_c$).

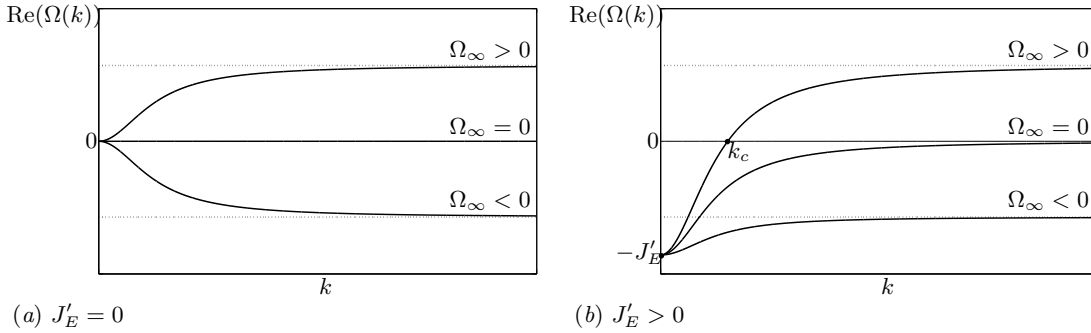


Figure 2.2: Plots of the typical growth rates given by (2.52) in the cases (a) $J'_E = 0$ and (b) $J'_E > 0$, where Ω_∞ and k_c are given by (2.54) and (2.55); see text for details.

For $J'_E > 0$ we see that the polymerization kinetics stabilise small wavenumber perturbations. We also see the destabilising effect of network contraction via (2.54). As the network contraction gets stronger, the magnitude of $\Psi'_E < 0$ increases. So for sufficiently strong contractile forces $\Psi'_E < -\mu_E J'_E / \theta_E$, which implies Ω_∞ is positive and large wavenumbers are unstable.

The phase speed is given by

$$c(k) := -\frac{\text{Im}(\Omega(k))}{k} = u_E \left(1 + \frac{(\alpha_E + \beta_E)\gamma_E}{\alpha_E + \beta_E + \mu_E k^2} \right), \quad (2.56)$$

so there is a convected instability, perturbations travel downstream or upstream for $\gamma_E > 0$ or $\gamma_E < 0$ respectively. This behaviour may be relevant to the travelling actin ruffles discussed in §1.1.3. We note that the constitutive laws (2.30d,e) imply $\gamma_E < 0$. The linear

stability analysis predicts that perturbations with larger wavenumbers travel more slowly. Finally, we note that the swelling/contractile pressure does not feature in the phase speed, because it is associated with diffusive, not advective, transport.

2.2.2 Effect of boundary conditions

We now consider the effect of imposing the boundary conditions $u(0, t) = u(1, t) = 0$ and see that after substituting (2.47) into (2.25) we obtain, at $O(\epsilon)$, (2.48) and (2.49) with $u_E = V_E = 0$. Thus the speed at which the strip travels is now of the same order as the initial perturbation in network volume fraction about θ_E . We seek solutions of the form $\theta_1 = \hat{\theta}_1(x)e^{\omega t}$, $u_1 = \hat{u}_1(x)e^{\omega t}$, $V_1 = \hat{V}_1e^{\omega t}$, which after dropping the hats and eliminating θ_1 gives

$$\left(\frac{\theta_E \Psi'_E}{J'_E + \omega} + \mu_E \right) \frac{d^2 u_1}{dx^2} - (\alpha_E + \beta_E) u_1 = \beta_E V_1, \quad (2.57)$$

for $J'_E + \omega \neq 0$. We note that for $\theta_E \neq 0$ and $\Psi'_E \neq 0$, $J'_E + \omega = 0$ only at $k = 0$. For $V_1 = 0$, (2.57) becomes a linear, homogeneous, second-order ordinary differential equation, leading to solutions of the form

$$u_1 = A \sin(n\pi x) e^{\omega_n t}, \quad \theta_1 = -\frac{An\pi\theta_E}{J'_E + \omega_n} \cos(n\pi x) e^{\omega_n t}, \quad (2.58a, b)$$

for n an integer, where the growth rate is in agreement with (2.52), so that $\omega_n = \Omega(n\pi)$ with $u_E = 0$; A is a constant and the linearized form of (2.27), namely

$$\langle \beta_E (u_1 + V_1) \rangle = 0, \quad (2.59)$$

implies that n is even. Thus, small perturbations to the network volume fraction about θ_E that are symmetric about $x = 1/2$ do not cause the strip to move. For $V_1 \neq 0$, the solutions of (2.57) are given by

$$u_1 = \frac{\hat{V}_1 \beta_E}{\alpha_E + \beta_E} \left(\frac{\cos(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)} - 1 \right) e^{\omega_{(\lambda_n)} t}, \quad (2.60)$$

$$\theta_1 = \frac{\hat{V}_1 \beta_E \theta_E \lambda_n}{(\alpha_E + \beta_E)(J'_E + \omega_{(\lambda_n)})} \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)} e^{\omega_{(\lambda_n)} t}, \quad (2.61)$$

where n is an integer again the growth rates are in agreement with (2.52) and $\omega_{(\lambda_n)} = \Omega(\lambda_n)$ for $u_E = 0$. It remains to determine λ_n by substituting u_1 from (2.60) into the linearized version of (2.27). We find that λ_n is the root of

$$\frac{\alpha_E}{\beta_E} + \frac{2}{\lambda_n} \tan\left(\frac{\lambda_n}{2}\right) = 0, \quad (2.62)$$

such that $(2n - 1)\pi < \lambda_n < 2\pi n$. We note that the boundary conditions do not alter the growth rate curves depicted in Figure 2.2; they simply identify a discrete set of points on these curves. The growth rate curve plateaus and remains in the unstable regime for large wavenumbers, when $\Omega_\infty > 0$, so the solution leaving the linear regime depends on how the coefficients of the short wavelength modes in the initial perturbation depend on the wavenumber. The fact that small perturbations to the network about its equilibrium volume fraction only cause the strip to move if they break the symmetry of the network is an interesting insight into the polarization of the network in crawling cells.

2.2.3 Solution of the linearized initial value problem

It is possible to show by direct calculation, using (2.62), that the θ_1 modes in (2.58b) and (2.61) are orthogonal. Working with these modes we seek to understand how the strip travels in response to a general small perturbation of the network about the equilibrium volume fraction. We seek a solution of the form

$$\theta_1(x, t) = \frac{a_0(t)}{2} + \sum_{n \text{ even}} a_n(t) \cos(n\pi x) + \sum_{n=1}^{\infty} b_n(t) \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad (2.63)$$

where the λ_n are given by (2.62) and following (2.47a) we expand the initial condition $\theta^* \sim \theta_E + \varepsilon\theta_1^* + \dots$ so that

$$a_n(0) = 2 \int_0^1 \theta_1^*(\xi) \cos(n\pi\xi) d\xi, \quad b_n(0) = \frac{2\lambda_n \cos(\lambda_n/2)}{\lambda_n - \sin(\lambda_n)} \int_0^1 \theta_1^*(\xi) \sin(\lambda_n(\xi - 1/2)) d\xi. \quad (2.64a, b)$$

Substituting (2.63) into (2.50) with $u_E = 0$ and using orthogonality to equate the coefficients of like terms, we find that

$$\dot{a}_0 + J'_E a_0 = 0, \quad (2.65)$$

for n even

$$(\alpha_E + \beta_E + \mu_E n^2 \pi^2) \dot{a}_n + J'_E (\alpha_E + \beta_E + \mu_E n^2 \pi^2) a_n = -\theta_E \Psi'_E n^2 \pi^2 a_n, \quad (2.66)$$

and for n an integer

$$(\alpha_E + \beta_E + \mu_E \lambda_n^2 \pi^2) \dot{b}_n + J'_E (\alpha_E + \beta_E + \mu_E \lambda_n^2 \pi^2) b_n = -\theta_E \Psi'_E \lambda_n^2 \pi^2 b_n. \quad (2.67)$$

After some manipulation we see that

$$\text{for } n \text{ even, } a_n(t) = a_n(0)e^{\omega_n t}; \quad \text{for integer } n, \quad b_n(t) = b_n(0)e^{\omega_{(\lambda_n)} t}, \quad (2.68a, b)$$

where, in agreement with (2.52), $\omega_n = \Omega(n\pi)$ and $\omega_{(\lambda_n)} = \Omega(\lambda_n)$, both with $u_E = 0$. Now we substitute (2.63) into (2.48) with $u_E = 0$, integrate and apply the boundary conditions that $u_1 = 0$ at $x = 0, 1$, to find

$$u_1 = \sum_{n=1}^{\infty} \frac{J'_E + \omega_{(\lambda_n)}}{\lambda_n \theta_E} b_n(t) \left(\frac{\cos(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)} - 1 \right) - \sum_{n \text{ even}} \frac{J'_E + \omega_n}{n\pi \theta_E} a_n(t) \sin(n\pi x). \quad (2.69)$$

We substitute this expression for u_1 into (2.49) along with (2.63) to obtain, after some manipulation, the linear prediction for the crawling speed of the strip in terms of the initial conditions and the model parameters in the form

$$V_1 = -\frac{\Psi'_E(\alpha_E + \beta_E)}{\beta_E} \sum_{n=1}^{\infty} \frac{\lambda_n b_n(0) e^{\omega_{(\lambda_n)} t}}{\alpha_E + \beta_E + \mu_E \lambda_n^2}. \quad (2.70)$$

This expression is verified against our numerics in §2.4. We again see that only the perturbations to the network that are asymmetric about $x = 1/2$ contribute, via b_n , to the speed of the crawling strip. Having found an expression for the moving-strip speed in terms of a small perturbation to the network equilibrium volume fraction θ_E , one might anticipate we could make some general statement about the direction in which a stationary strip of cytoplasm starts to crawl when disturbed. However, the plateauing growth rate curve for large wavenumbers leads to an expression for V_1 that depends on how the large wavenumber modes are excited. All the large wavenumber modes grow at a comparable rate, so the initial amplitude of each mode is important in determining which mode dominates as the solution exits the linear regime. The implication for modelling cell crawling is that the perturbation response displayed by this deterministic model is rich and depends on both the modes that are excited, their relative amplitudes and the symmetry of the perturbation.

2.2.4 An alternative solution of the linearized initial value problem

It is also possible to write the initial condition θ_1^* as a Fourier cosine series, which is advantageous because completeness of the Fourier modes is well known. The resulting calculation for V_1 is somewhat more involved, due to the coupling of the Fourier modes that are asymmetric about $x = 1/2$. In the usual way we seek a series solution that satisfies the boundary conditions for u_1 as follows

$$\theta_1(x, t) = \frac{a_0(t)}{2} + \sum_{n=1}^{\infty} a_n(t) \cos(n\pi x), \quad u_1(x, t) = \sum_{n=1}^{\infty} b_n(t) \sin(n\pi x), \quad (2.71a, b)$$

where the initial condition for θ_1 gives

$$\text{for } n \in \{0, 1, \dots\}, \quad a_n(0) = 2 \int_0^1 \theta_1^*(\xi) \cos(n\pi \xi) d\xi, \quad (2.72)$$

so the a_n and b_n are different to those in the previous section. Substituting (2.71) into the linearized equations (2.48) and (2.49), with $u_E = V_E = 0$, and equating coefficients of $\sin(n\pi x)$ and $\cos(n\pi x)$ gives

$$\dot{a}_0(t) + J'_E a_0(t) = 0, \quad (2.73)$$

and for $n \in \{1, 2, \dots\}$

$$\dot{a}_n(t) + J'_E a_n(t) = -\theta_E n \pi b_n(t), \quad b_n = \frac{\Psi'_E n \pi a_n(t) - \beta_E c_n(t)}{\alpha_E + \beta_E + \mu_E n^2 \pi^2}, \quad (2.74a,b)$$

where the $c_n(t)$ are the Fourier coefficients in the series

$$V_1(t) = \sum_{n=1}^{\infty} c_n(t) \sin(n\pi x), \quad (2.75)$$

and are related to $V_1(t)$ by

$$\text{for } n \in \{1, 2, \dots\}, \quad c_n(t) = \frac{2(1 - (-1)^n)}{n\pi} V_1(t). \quad (2.76)$$

It follows from these expressions and (2.74) that

$$\dot{a}_n(t) - \omega_n a_n(t) = \begin{cases} 0 & \text{for } n \in \{0, 2, 4, \dots\}, \\ \left(\frac{4\beta_E \theta_E}{\mu_E}\right) \frac{V_1(t)}{\nu^2 + n^2 \pi^2} & \text{for } n \in \{1, 3, 5, \dots\}, \end{cases} \quad (2.77a,b)$$

where the growth rates $\omega_n = \text{Re}(\Omega(n\pi))$, with $\Omega(k)$ given by (2.52). For algebraic convenience, we rewrite ω_n and define the parameter ν as

$$\omega_n = -J'_E - \left(\frac{\theta_E \Psi'_E}{\mu_E}\right) \frac{n^2 \pi^2}{\nu^2 + n^2 \pi^2}, \quad \nu = \left(\frac{\alpha_E + \beta_E}{\mu_E}\right)^{1/2}. \quad (2.78a,b)$$

The solvability condition (2.59) implies that

$$\sum_{n \text{ odd}} \frac{b_n(t) + c_n(t)}{n\pi} = 0. \quad (2.79)$$

Substituting (2.76) and (2.74b) into (2.79), we find that $V_1(t)$ is given by

$$V_1(t) = 2 \left(\frac{\alpha_E + \beta_E}{\alpha_E + \beta_E \frac{2}{\nu} \tanh \frac{\nu}{2}} \right) \left(-\frac{\Psi'_E}{\mu_E} \right) \sum_{n \text{ odd}} \frac{a_n(t)}{\nu^2 + n^2 \pi^2}. \quad (2.80)$$

Thus, we see the separation of the even n modes and the odd n modes. The even n modes are symmetric about $x = 1/2$ and decouple. The odd n modes are asymmetric about $x = 1/2$ and are governed by the following infinite set of ordinary differential equations:

$$\text{for } n \in \{1, 3, 5, \dots\}, \quad \dot{a}_n(t) - \omega_n a_n(t) = \frac{A}{\nu^2 + n^2 \pi^2} \sum_{m \text{ odd}} \frac{a_m(t)}{\nu^2 + m^2 \pi^2}, \quad (2.81)$$

where the parameter A is given by

$$A = 8 \left(\frac{\alpha_E + \beta_E}{\alpha_E + \beta_E \frac{2}{\nu} \tanh \frac{\nu}{2}} \right) \left(-\frac{\Psi'_E}{\mu_E} \right) \left(\frac{\beta_E \theta_E}{\mu_E} \right). \quad (2.82)$$

Using an integrating factor in (2.77b) we obtain an integral expression for $a_n(t)$, which we substitute into (2.80) to obtain

$$V_1(t) = \sum_{n \text{ odd}} \frac{A}{\nu^2 + n^2 \pi^2} \left(\frac{\mu_E}{4\beta_E \theta_E} a_n(0) e^{\omega_n t} + \frac{1}{\nu^2 + n^2 \pi^2} \int_0^t V_1(s) e^{\omega_n(t-s)} ds \right). \quad (2.83)$$

Defining by

$$\bar{V}_1(\rho) = \int_0^\infty e^{-\rho t} V_1(t) dt$$

the Laplace transform of $V_1(t)$, we obtain from (2.83) the expression

$$\bar{V}_1(\rho) = \sum_{n \text{ odd}} \frac{A}{(\nu^2 + n^2 \pi^2)(\rho - \omega_n)} \left(\frac{\mu_E a_n(0)}{4\beta_E \theta_E} + \frac{\bar{V}_1(\rho)}{\nu^2 + n^2 \pi^2} \right). \quad (2.84)$$

Summing the series involving $\bar{V}(\rho)$ on the right-hand side of (2.84) and rearranging shows that

$$\bar{V}_1(\rho) = -\frac{2\Psi'_E}{\beta_E} \sum_{n \text{ odd}} \frac{\nu^2 a_n(0)}{(\nu^2 + n^2 \pi^2) f(\rho)(\rho - \omega_n)}, \quad (2.85)$$

where

$$f(\rho) = \frac{\alpha_E}{\beta_E} + \frac{2}{\nu} \left(\frac{\rho - \Omega_\infty}{\rho - \omega_0} \right)^{1/2} \tanh \frac{\nu}{2} \left(\frac{\rho - \omega_0}{\rho - \Omega_\infty} \right)^{1/2}. \quad (2.86)$$

The expressions (2.84) and (2.85) are consistent provided we use the branch of $((\rho - \omega_0)/(\rho - \Omega_\infty))^{1/2}$ that is real and positive on the real axis away from the branch cut $(\omega_0, \Omega_\infty)$. The function $1/f(\rho)$ is analytic except at the branch points $\rho = \omega_0, \Omega_\infty$ and at the simple poles

$$\rho = \omega_{(\lambda_k)} = \Omega_\infty + \left(\frac{\theta_E \Psi'_E}{\mu_E} \right) \frac{\nu^2}{\nu^2 + \lambda_k^2}, \quad (2.87)$$

for $k = 1, 2, 3, \dots$, where λ_k is defined as the k th positive root of (2.62) and $\omega_{(\lambda_k)} = \Omega(\lambda_k)$ as in (2.52) with $u_E = 0$. Note that Ω_∞ is an accumulation point of the family of poles $\omega_{(\lambda_k)}$. However, the sum of contributions from these poles is convergent, so a careful choice of contour (see Figure 2.3) enables us to invert (2.85) to obtain

$$V_1(t) = -\frac{2\Psi'_E}{\beta_E} \sum_{n \text{ odd}} \frac{\nu^2 a_n(0)}{\nu^2 + n^2 \pi^2} \left\{ \frac{e^{\omega_n t}}{f(\omega_n)} + \sum_{k=1}^{\infty} \frac{e^{\omega_{(\lambda_k)} t}}{(\omega_{(\lambda_k)} - \omega_n) f'(\omega_{(\lambda_k)})} \right\}. \quad (2.88)$$

It is also possible to show that the Laplace transforms of (2.88) and (2.70) agree. We first calculate the Fourier cosine series coefficients of (2.63) and substitute for the $a_n(0)$ in (2.84), then manipulate the expression to find $\bar{V}$ is the Laplace transform of (2.70), so we claim that the modes used to construct (2.63) are complete.

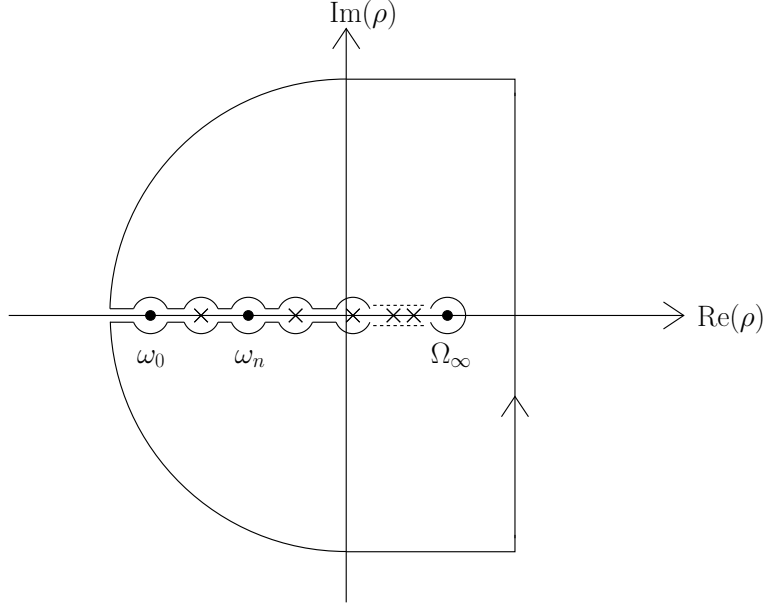


Figure 2.3: Schematic diagram of the contour used to invert (2.85), poles at ω_0 , ω_n and Ω_∞ are labelled, crosses depict the first few $\omega_{(\lambda_k)}$ poles.

2.3 Small growth rate limit

We now consider a parameter limit in which we can say a little more about the early response of the model to a small perturbation to the stationary steady state, namely the parameter regime in which the maximum growth rate is small and positive *i.e.* $0 < \Omega_\infty \ll 1$. Note this is different to a traditional weakly nonlinear analysis, because we are unable to isolate and excite only the fastest growing mode. We tackle this regime by parametrizing Ψ with Ω_∞ . Now, defining $\varepsilon = \Omega_\infty$ for convenience, (2.54) holds provided

$$\frac{\partial \Psi}{\partial \theta}(\theta_E; \varepsilon) = -\frac{\mu_E}{\theta_E}(J'_E + \varepsilon). \quad (2.89)$$

Note that for the constitutive laws (2.29) and (2.30), (2.89) implies $\Psi^* \sim 1/((\theta_E - \theta_L)(\theta_R - \theta_E))$ as $\varepsilon \rightarrow 0$, which gives $\Psi^* \approx 10^4$ for $\theta_L = 0.01$, $\theta_E = 0.02$ and $\theta_R = 0.03$, the θ values used in our numerical simulations in §2.4. Returning to our general expression, we fix $k_c = O(1)$, so assuming $J'_E = O(1)$, (2.55) shows we must rescale

$$\alpha = \varepsilon \hat{\alpha}, \quad \beta = \varepsilon \hat{\beta}, \quad (2.90a, b)$$

so we consider a small network-solution drag and small adhesion limit. Hereafter, we drop the hats. Clearly this very weak adhesion limit does not belong to the range of values for

β^* discussed in §2.1.7, this analysis could be applicable to an experiment with cells placed on a less adhesive surface than those typically encountered *in vivo*.

2.3.1 Small time problem $t = O(1)$

We begin with the early evolution on the timescale $t = O(1)$ by substituting (2.90) into the governing equations (2.25) and dropping the hats to obtain

$$\theta_t + (\theta u)_x = -J, \quad -\Psi_x(\theta; \varepsilon) + (\mu u_x)_x = \varepsilon(\alpha u + \beta(u + V)). \quad (2.91a, b)$$

We Taylor expand the swelling/contractile pressure, Ψ , and use asymptotic series expansions $\theta \sim \theta_E + \varepsilon\theta_1 + \varepsilon^2\theta_2 + \dots$, $u \sim \varepsilon u_1 + \varepsilon^2 u_2 + \dots$ and $V \sim \varepsilon V_1 + \varepsilon^2 V_2 + \dots$, to obtain at $O(\varepsilon)$

$$\frac{\partial \theta_1}{\partial t} + \theta_E \frac{\partial u_1}{\partial x} = -J'_E \theta_1, \quad \frac{\mu_E J'_E}{\theta_E} \frac{\partial \theta_1}{\partial x} + \mu_E \frac{\partial^2 u_1}{\partial x^2} = 0. \quad (2.92a, b)$$

Integrating the first equation once and the second twice with respect to x (using $u_1 = 0$ at $x = 0, 1$), then manipulating and integrating with respect to t reveals that

$$\theta_1(x, t) - \langle \theta_1 \rangle(t) = \theta_1^*(x) - \langle \theta_1^* \rangle, \quad \langle \theta_1 \rangle(t) = \langle \theta_1^* \rangle e^{-J'_E t}. \quad (2.93a, b)$$

Hence we see our choice of nondimensional timescale (2.22) returned as the timescale over which relaxation of the network, via polymerization and depolymerization, occurs. There is no leading order change in shape of the network profile. Moreover, the network velocity is independent of t , as is the moving-strip velocity:

$$u_1(x, t) = -\frac{J'_E}{\theta_E} \left(\int_0^x \theta_1^*(\xi) d\xi - x \langle \theta_1^* \rangle \right), \quad V_1(t) = \mathcal{V}(\theta_1^*) = \frac{J'_E}{\theta_E} \left(\langle \theta_1^* \rangle - \frac{1}{2} \langle \theta_1^* \rangle \right), \quad (2.94a, b)$$

where, here and hereafter

$$\langle \langle \theta_1^* \rangle \rangle := \int_0^1 \int_0^x \theta_1^*(\xi) d\xi dx. \quad (2.95)$$

In this parameter regime the initial speed of the moving strip is constant and depends only on how θ^* varies along the strip from the mean of θ^* . Loosely speaking, if one side of the strip has a higher than mean network volume fraction and the other side of the strip has a lower than mean volume fraction, then the side with the higher volume fraction becomes the rear of the strip.

2.3.2 Large time problem $t = O(1/\varepsilon)$

By our choice of parameter regime the growth rate is of $O(\varepsilon)$, so perturbations to the network evolve slowly. Here we investigate them on the long timescale $t = \tau/\varepsilon$, with $\tau = O(1)$, for which the governing equations (2.25) become

$$\varepsilon\theta_\tau + (\theta u)_x = -J, \quad -\Psi_x(\theta; \varepsilon) + (\mu u_x)_x = \varepsilon(\alpha u + \beta(u + V)). \quad (2.96a, b)$$

We expand $\theta \sim \theta_E + \varepsilon\hat{\theta}_1 + \varepsilon^2\hat{\theta}_2 + \dots$, $u \sim \varepsilon\hat{u}_1 + \varepsilon^2\hat{u}_2 + \dots$ and $V \sim \varepsilon\hat{V}_1 + \varepsilon^2\hat{V}_2 + \dots$, as in the previous section and obtain at $O(\varepsilon)$, after rearranging

$$\hat{\theta}_1 = -\frac{\theta_E}{J'_E} \frac{\partial \hat{u}_1}{\partial x}, \quad (2.97)$$

as well as the derivative of this equation with respect to x . In this form (2.97) reflects how the flow of the network changes the local network volume fraction. The derivative of (2.97) with respect to x represents a balance between swelling/contractile forces and viscous forces. There is still insufficient information to close the problem, so we seek a solvability condition by considering (2.96) at $O(\varepsilon^2)$. After some manipulation, we obtain the expressions

$$-\left(J'_E\hat{\theta}_2 + \theta_E \frac{\partial \hat{u}_2}{\partial x}\right) = \frac{\partial \hat{\theta}_1}{\partial \tau} + \frac{\partial}{\partial x}(\hat{\theta}_1\hat{u}_1) + \frac{1}{2}J''_E\hat{\theta}_1^2, \quad (2.98)$$

$$\begin{aligned} -\mu_E \frac{\partial}{\partial x} \left(J'_E\hat{\theta}_2 + \theta_E \frac{\partial \hat{u}_2}{\partial x} \right) &= \frac{\partial}{\partial x} \left(\mu_E\hat{\theta}_1 - \frac{1}{2}\theta_E\Psi''_E\hat{\theta}_1^2 + \theta_E\mu'_E\hat{\theta}_1 \frac{\partial \hat{u}_1}{\partial x} \right) \\ &\quad - \theta_E(\alpha_E\hat{u}_1 + \beta_E(\hat{u}_1 + \hat{V}_1)), \end{aligned} \quad (2.99)$$

where $\Psi''_E = \Psi_{\hat{\theta}\hat{\theta}}(\theta_E; 0)$. Differentiating (2.98) with respect to x and substituting it into (2.99) we obtain the solvability condition

$$\begin{aligned} \mu_E \frac{\partial}{\partial x} \left(\frac{\partial \hat{\theta}_1}{\partial \tau} + \frac{\partial}{\partial x}(\hat{\theta}_1\hat{u}_1) + \frac{1}{2}J''_E\hat{\theta}_1^2 \right) &= \\ \frac{\partial}{\partial x} \left(\mu_E\hat{\theta}_1 - \frac{1}{2}\theta_E\Psi''_E\hat{\theta}_1^2 + \theta_E\mu'_E\hat{\theta}_1 \frac{\partial \hat{u}_1}{\partial x} \right) &- \theta_E(\alpha_E\hat{u}_1 + \beta_E(\hat{u}_1 + \hat{V}_1)) \end{aligned} \quad (2.100)$$

for $\hat{\theta}_1$ and $\hat{u}_1$. Using (2.97) to eliminate $\hat{\theta}_1$ from (2.100), we obtain

$$\frac{\partial}{\partial x} \left\{ \frac{\partial}{\partial x} \left(\frac{\partial \hat{u}_1}{\partial \tau} + \hat{u}_1 \frac{\partial \hat{u}_1}{\partial x} - \hat{u}_1 \right) - a_E \left(\frac{\partial \hat{u}_1}{\partial x} \right)^2 \right\} = b_E(\alpha_E\hat{u}_1 + \beta_E(\hat{u}_1 + \hat{V}_1)) \quad (2.101)$$

for $\hat{u}_1(x, \tau)$, where

$$a_E = \frac{\theta_E}{2\mu_E J'_E} (\theta_E \Psi''_E + \mu_E J''_E + 2\mu'_E J'_E), \quad b_E = \frac{J'_E}{\mu_E}. \quad (2.102a, b)$$

In summary, we have two partial differential equations (2.97) and (2.100) and the condition $\langle \hat{u}_1 + \hat{V}_1 \rangle = 0$ from (2.27) for the three unknowns $\hat{u}_1$, $\hat{\theta}_1$ and $\hat{V}_1$. This system must be solved subject to an initial condition for $\hat{\theta}_1$ and boundary conditions $\hat{u}_1 = 0$ at $x = 0$ and $x = 1$. We obtain the initial conditions by matching to the early time solution:

$$\lim_{\tau \rightarrow 0} \hat{\theta}_1(x, \tau) = \lim_{t \rightarrow \infty} \theta_1(x, t) = \theta_1^*(x) - \langle \theta_1^* \rangle. \quad (2.103)$$

Equation (2.97) reveals that we require $\langle \hat{\theta}_1 \rangle = 0$ in order to satisfy the boundary conditions on $\hat{u}_1$. The early time calculations show how arbitrary initial network profiles evolve to provide a consistent initial condition for the long-time problem, with zero mean. A further check on the matching between the two timescales is obtained by considering $\hat{V}_1$. We see from the integral constraint that

$$\hat{V}_1(\tau) = -\langle \hat{u}_1(x, \tau) \rangle, \quad (2.104)$$

so substituting in (2.97) we find that

$$\hat{V}_1(\tau) = \frac{J'_E}{\theta_E} \langle \langle \hat{\theta}_1(\xi, \tau) \rangle \rangle, \quad (2.105)$$

which is consistent with (2.94b) as $\tau \rightarrow 0$. When the large time problem is formulated in terms of $\hat{u}_1$, as in (2.101), then the initial condition for $\hat{u}_1$ is given by (2.94a).

The large time analysis reveals that the moving-strip speed is no longer constant and depends on the network velocity. Unfortunately, the nonlinear, pseudo-parabolic equation (2.101) controlling $\hat{u}_1$ does not represent a significant simplification of the full problem, so making predictions about the motion of the travelling strip and the evolution of the network, without simulating numerically, would be involved. We also note that if we set $a_E = b_E = 0$, (2.101) reduces to the forced, inviscid Burgers' equation. This small growth rate analysis is verified against numerical solutions in the appropriate parameter regime in §2.4.1. This analysis holds in the slightly contrived situation where the viscosity, contractile strength and polymerization rates are such that the maximum growth rate Ω_∞ is small and positive and the substrate permits only weak adhesion, although it may be possible to design experiments for weakly contracting cells on suitable substrates. This model predicts that such an experiment would see the mean network volume fraction decay to zero on the order of minutes in dimensional units.

2.4 Numerical simulations

2.4.1 Description and validation of the scheme

We obtain numerical solutions to (2.25)–(2.27) using a fully coupled finite element scheme (see, for example, [58]), with the resulting discretized system of nonlinear algebraic equations solved using the PETSc libraries [7, 8, 9]. The scheme uses a piecewise linear basis for the spatial approximations and on each time step the solution at the previous time step is used as an initial guess for the Newton iteration used to solve the nonlinear system. The scheme is fully implicit in time. We prescribe initial conditions for the network distribution satisfying $\langle \theta^* \rangle = \theta_E$ and solve the unsteady problem on the interval $[0, 1]$ for the network profile, network velocity and strip speed.

In order to ensure that the code converges with respect to the grid size we take the same initial condition, namely a small perturbation to the uniform steady state, and run the code for a series of different uniform discretizations in both space and time. First, to check spatial convergence, we select a time step of 5×10^{-3} and a sequence of six nested grids ranging from 500 to 16000 elements, where each successive grid is obtained by doubling the number of elements in the previous grid. We find that the maximum absolute difference in V over time decreases as the number of elements increases, as illustrated in Figure 2.4(a). To check the temporal convergence we compute the same difference in V over a sequence of grids with 4000 elements and a time step that halves each time starting with 8×10^{-2} and finishing with 5×10^{-3} . Demonstration of temporal convergence is shown in Figure 2.4(b).

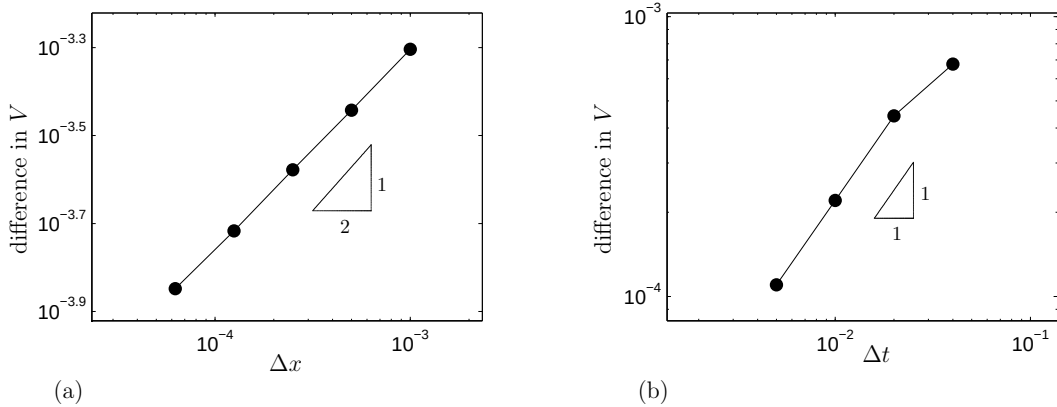


Figure 2.4: Graphs showing how differences between successive simulations decrease on increasingly fine grids. The parameters are $\Psi^* = 14,700$, $\alpha^* = 0.00495$ and $\beta^* = 0.495$ to three significant figures.

We validate the code by comparison to the small growth rate analysis. The numerical solution agrees well with (2.97) for appropriate parameter values as shown in Figure 2.5(a). We also compare the prediction for the travelling speed $\hat{V}_1$ as a function of the network velocity $\hat{u}_1$ in (2.104) to the full numerical solution in Figure 2.5(b). We also validate the code by comparing it to the predictions made in our linear stability analysis (see Figure 2.6).

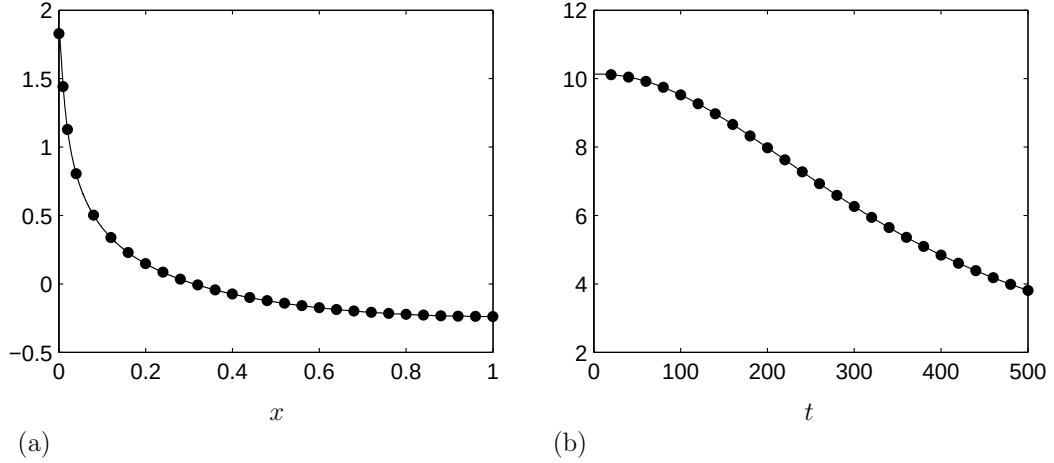


Figure 2.5: Figure (a) displays $\hat{\theta}_1$ (continuous) and the right hand side of (2.97) (dots), both at time $t = 500$. Figure (b) displays V/ϵ (continuous) from the numerical solution to the full problem and $\hat{V}_1$ (dots) calculated from the numerical solution for u according to (2.104). The dotted curves are sparsely sampled to aid visualization. The initial conditions are $\theta^* = \theta_E + 10^{-4} \cos(\pi x)$ and the parameter values are $\Psi^* = 9,800$, $\alpha^* = 10^{-5}$ and $\beta^* = 8.98 \times 10^{-5}$ to three significant figures, which correspond to $k_c = 1$ and $\Omega_\infty = \epsilon = 10^{-4}$.

2.4.2 Results

Having validated the code we consider the solution to the initial value problem (2.25)–(2.27), with parameter values $\Psi^* = 15,000$, $\alpha^* = 0.4$ and $\beta^* = 3$. The initial conditions are a small perturbation to the uniform steady state, such that $\theta^*(x) = \theta_E + \epsilon \cos(\pi x)$ with $\epsilon = 10^{-4}$. Figure 2.6 shows how the speed of the travelling strip increases from near stationary and oscillates, before settling to a constant speed. We also plot the linear stability prediction, (2.70), which holds for small times (*i.e.* until the perturbation to the steady state grows and exceeds the linear regime). The initial conditions have an elevated network volume fraction at $x = 0$ and the network is in the contractile regime ($\theta_L < \theta < \theta_R$) so the network flows up the concentration gradient towards $x = 0$. The strip starts to crawl in the direction opposite to that in which the network is flowing, so the model displays the biologically

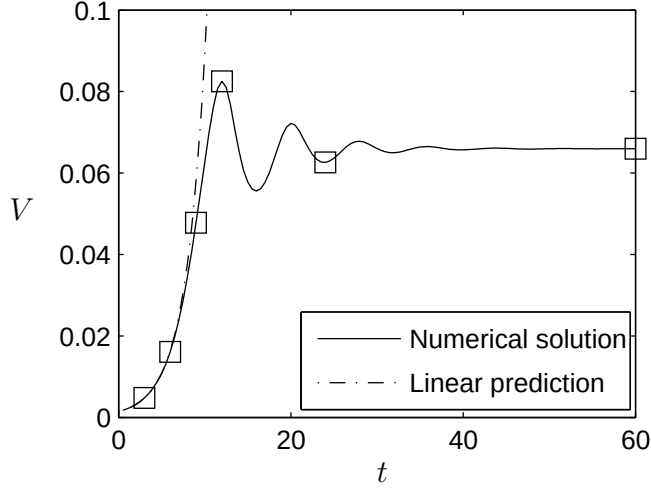


Figure 2.6: Speed of the travelling strip and comparison to linear stability predictions. The parameters used are $\Psi^* = 15,000$, $\alpha^* = 0.4$ and $\beta^* = 3$ and the initial condition is a perturbation to the equilibrium volume fraction: see text. Network volume fraction and velocity are plotted for the boxed times in Figure 2.7.

observed retrograde flow on the scale of the strip. The peak in network volume fraction is thus located at the rear of the travelling strip. The network dips at the front of the travelling strip. We plot snapshots of the network volume fraction and velocity in Figure 2.7, at the times indicated by the boxes in Figure 2.6. The nondimensional force exerted on the substrate by the crawling strip is given by

$$F = \beta(\theta)(u + V), \quad (2.106)$$

and plotted in Figure 2.7 for the times indicated in Figure 2.6. The force is largest at the rear of the strip and has the same direction as the motion of the strip at both the front and rear of the strip. Once the travelling strip in effect attains a constant speed the network volume fraction and velocity are also constant in time and our unsteady code has found a steady travelling-wave solution as the large-time attractor. Noting our choice of $L = 20\mu\text{m}$ and $T = 1\text{min}$ to nondimensionalize the model in §2.1.5 we see that a travelling strip velocity of 0.07 corresponds to a cell locomoting at $1.4\mu\text{mmin}^{-1}$. This would be a slow fish keratocyte cell [143], but is a reasonable speed for human osteoblast cell [122]. To have a reasonable cell speed, without having to tune parameter values, is quite encouraging. In the steady travelling-wave solution the retrograde flow reaches a speed of $2\mu\text{mmin}^{-1}$, which is comfortably within the range of values reported in the literature. Retrograde flow speed is reported to be of order $1\mu\text{mmin}^{-1}$ in [146], with values up to $4.2\mu\text{mmin}^{-1}$ found in [24].

We shall proceed to consider when such travelling waves can be found and how they depend on the model parameters.

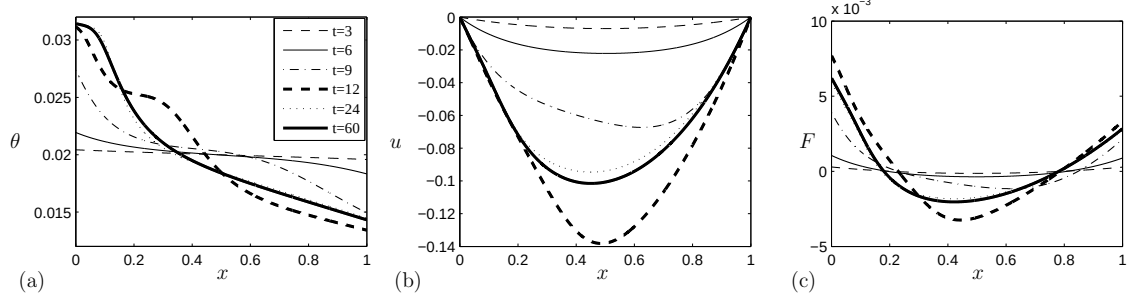
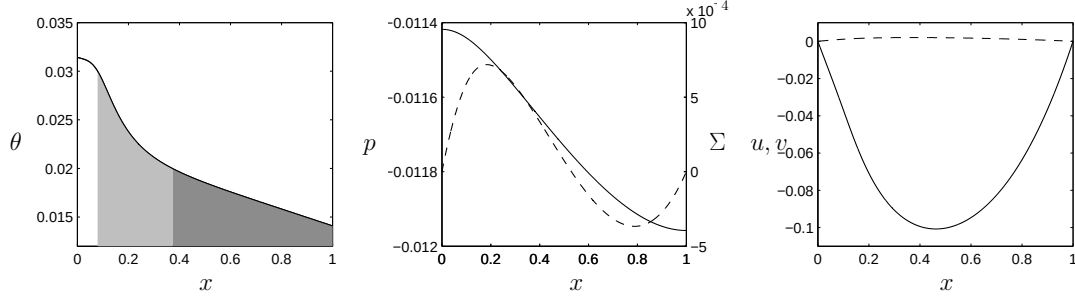


Figure 2.7: Snapshots of the network volume fraction (a), velocity (b) and force exerted on the substrate (c) at the times indicated by the boxes in Figure 2.6. The parameters used are $\Psi^* = 15000$, $\alpha^* = 0.4$ and $\beta^* = 3$ and the force is calculated using (2.106).

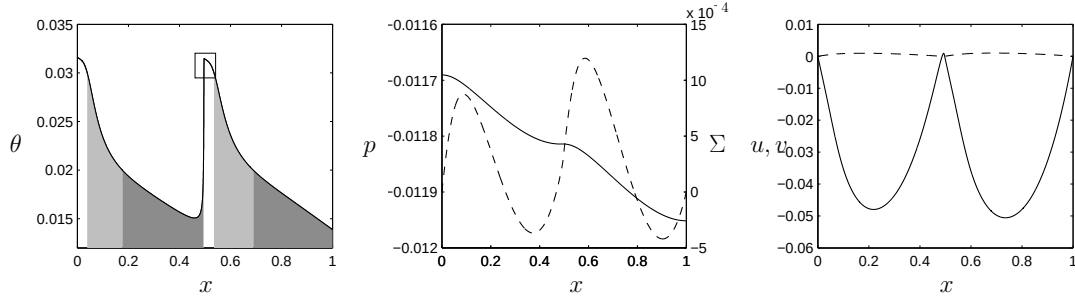
2.4.3 Effect of adhesion strength

Our numerical experiments have indicated that the travelling strip attains a steady travelling wave for a wide range of parameters, although there are a number of qualitatively distinct forms the travelling wave can take. The top three rows of Figure 2.8 show travelling-wave solutions with one, two and three peaks in the network volume fraction. These peaks are regions where substantial flow of the network up its concentration gradient has formed a large gradient in network volume fraction. The peak is prevented from growing further once the volume fraction exceeds the contractile regime ($\theta > \theta_R$) and network swelling resists contraction. The bottom row of Figure 2.8 contains a slightly different travelling-wave solution which has a peak at both ends of the travelling strip. The first column displays the network volume fraction, colour coded to identify swelling or contracting and polymerizing or depolymerizing regions of the strip. The central column shows variation in hydrodynamic pressure and mixture stress across the strip. We note that for all four travelling-wave solutions the pressure is largest at the rear of the strip and decreases towards the front. The mixture stress oscillates, with a local maximum just in front of each of the network volume fraction peaks. The final column shows the network and solution velocities, in the frame that travels with the strip. The network flows backwards displaying retrograde flow, whilst the solution flows forwards much more slowly. At each of the interior peaks in network volume fraction and at the front of the split peak solution, there is a small region where the network flows forwards.

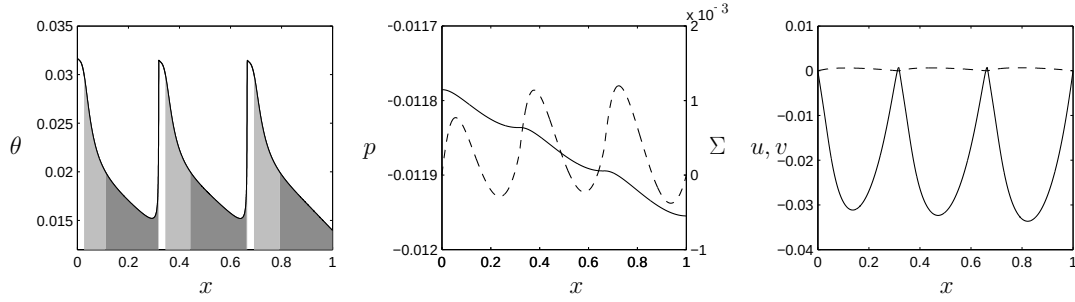
One peak $\beta^* = 4$, $V = 0.0661$



Two peaks $\beta^* = 21$, $V = 0.0320$



Three peaks $\beta^* = 44$, $V = 0.0208$



Split peak $\beta^* = 9$, $V = 0.0581$

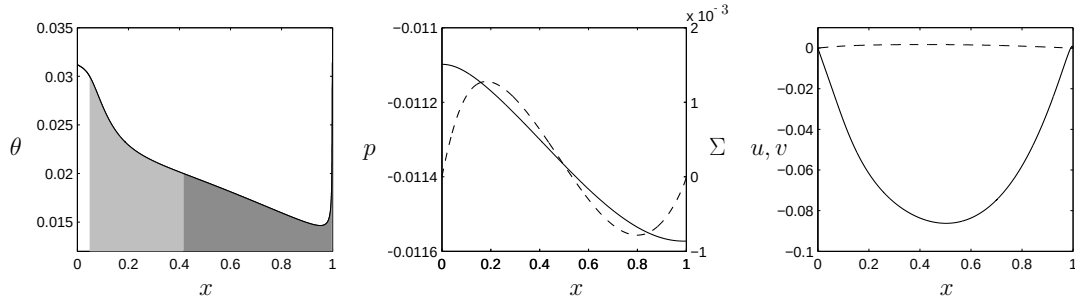


Figure 2.8: Steady state plots for a range of different β^* values with $\Psi^* = 15,000$, $\alpha^* = 0.4$. The speed V is stated to three significant figures. For all these simulations $V > 0$, so $x = 0$ is the rear of the travelling strip. The first column displays the network volume fraction (shading: white is swelling and depolymerizing; light grey is contracting and depolymerizing; mid grey is contracting and polymerizing: see (2.5), (2.6) and Figure 2.1.) The middle column displays the hydrodynamic pressure p (continuous, left axis) and mixture stress Σ (dashed, right axis). The final column shows the network velocity u (continuous) and the solution velocity v (dashed), both in a frame that travels with the strip. The box on the peak in network volume fraction for $\beta^* = 21$ indicates the zoomed region (not to scale) displayed in Figure 2.10.

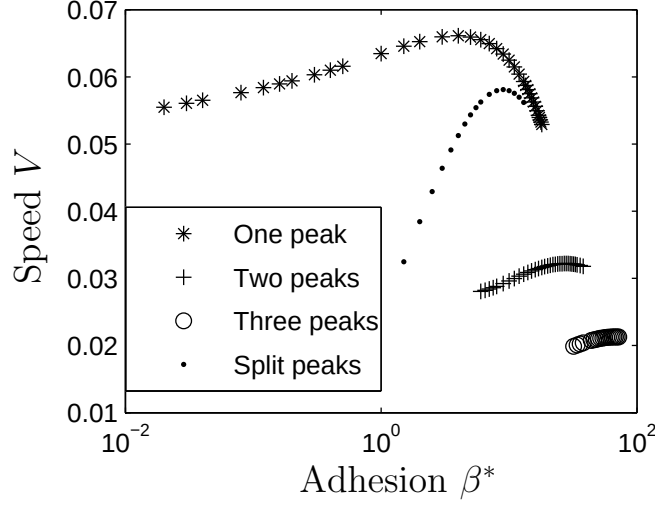


Figure 2.9: Travelling speed of steady states as a function of adhesion strength, typical solutions for each family of points can be seen in Figure 2.8. The parameters used are $\Psi^* = 15,000$ and $\alpha^* = 0.4$.

Some of the numerical simulations generated travelling-wave solutions with a negative crawling speed V . We note that for any solution the transformation $(\theta(x), u(x), V) \rightarrow (\theta(1-x), -u(1-x), -V)$ yields another solution. Since, in this section, we are concerned with travelling-wave solutions and not transient behaviour, any numerical solution obtained with negative V has been transformed to the corresponding positive V solution before plotting it in either Figure 2.8 or Figure 2.9.

The effect of adhesion strength β^* on the speed of the travelling strip V is shown in Figure 2.9 for fixed contractile strength $\Psi^* = 15,000$ and network-solution drag $\alpha^* = 0.4$. To reduce the time taken for a simulation to attain a steady state, we have employed continuation. The steady state network profile obtained in Figure 2.7 was used as the first point on the one peak curve and the initial condition for the problems with a slightly smaller and larger β^* . The first solutions in each of the other qualitatively distinct families were obtained when the continuation failed, *i.e.* the final network profile from a simulation that had not settled to a steady state was used as the initial condition to the problem with a different β^* . The solutions plotted in Figure 2.9 were all calculated on grids with five thousand and ten thousand elements and the largest relative error in steady strip speed was 7.1×10^{-3} .

We notice first that the qualitatively distinct families of solutions overlap in parameter

space, so for a given set of parameters different initial conditions can yield different non-trivial steady travelling solutions. We also note the bell-shaped dependence of speed on adhesion strength displayed within each family. As the number of peaks increases, the speed drops and the bell-shaped curve flattens for the one, two and three peak families. Within the family of split peaks, as we increase cell-substrate adhesion, β^* , the actin peak at the front narrows substantially. For smaller, decreasing β^* values the speed of the split peak solution decreases rapidly as the profile becomes more symmetric. This may be interpreted as cells without a strongly defined polarity travelling more slowly.

2.4.4 Remarks

Finding numerical solutions to (2.25)–(2.27) has proved more computationally expensive than one might anticipate for a one-dimensional problem, not least because of sharp peaks in network volume fraction that dance in the transient solutions, before settling to a steady state. Preliminary numerical investigations found some steady travelling-wave solutions with more than three peaks in the network profile for different parameter values and initial conditions of a small perturbation to the equilibrium network volume fraction. We are confident that the results of numerical simulations displayed here have sufficient resolution to capture the actin peaks as shown in Figure 2.10. Moving actin peaks have previously been seen in other models within this framework, see *e.g.* [5, 46], and may be relevant to the ruffling phenomena observed in cells. Many of the transient solutions displayed oscillations in V around a constant. We took great care to understand whether these oscillations were a numerical artefact (amplitude reduced by spatial refinement), a slowly decaying transient (amplitude decreases with time) or a true oscillation (amplitude persists when the simulation is run for a longer time and is unaffected by spatial and temporal refinement.) Only when the relative amplitude of the oscillation is less than 9.1×10^{-4} for at least the final twenty nondimensional time units of the simulation have we accepted the solution as settled to a steady state.

Since all our solutions are obtained with an unsteady code, we reason that they must be stable to sufficiently small perturbations. Our intuitive understanding, based on running simulations, is that for more extreme β^* values within a given family smaller steps in the continuation parameter β^* are required in order for the simulation to settle to a steady state in a reasonable amount of time. In some of the overlap regions, too large a step in β^* causes the code to settle on a steady state solution belonging to a different family. For

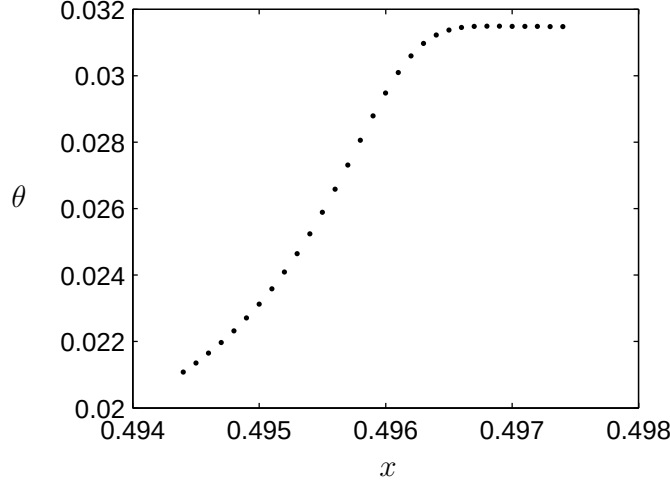


Figure 2.10: A zoomed in view of the peak in network volume fraction near the centre of the domain, for $\beta^* = 21$ in Figure 2.8. The dots show the discretization used, which was 10,000 elements. The other parameters used were $\Psi^* = 15,000$, $\alpha^* = 0.4$.

some of the split peak travelling-wave solutions, a steady state, with V positive, generated on the coarser grid (5000 elements) and used as initial conditions on the finer grid (10,000 elements) results in a simulation which changes direction and settles to the corresponding steady state with V negative. These simulations were included as converged in Figure 2.9 as discussed in §2.4.3.

2.5 Chapter summary

We have presented stability analyses and numerical simulations for a stripped-down, two-phase, poroviscous, reactive flow model applicable to cell crawling. The linear stability analysis about a uniform stationary state predicts an initial crawling direction that depends on both which modes are excited and the relative amplitude of these modes in the initial perturbation. It also provides a mechanism by which an asymmetric perturbation is required to cause the strip to travel, suggesting this framework could be useful in explaining the polarization of crawling cells. This analysis was done without employing specific constitutive laws and the positive plateauing growth rate for large wavenumbers is obtained as long as $\Psi'_E < 0$, so we would expect similar results for different constitutive laws in this class. Numerical investigations into the effect of adhesion strength on cell speed found families of travelling-wave solutions, with each family displaying the bell-shaped dependence of speed on adhesion strength that has been experimentally observed, (see *e.g.* [123]). To our

knowledge this has not previously been demonstrated in the two-phase flow framework for such a simple model, although it has been found in viscoelastic models for crawling cells [49, 67, 138] and in a much more complicated two-phase flow model [4].

The network velocities associated with our travelling states also display the experimentally observed phenomenon of retrograde flow of the actin network. Whilst we have not explicitly modelled the transport of actin monomers, the flow of solution carries them forwards, making them available for network polymerization. Across the one, two and three peak solutions the actin volume fraction dips at the front of the cell. By construction, the network undergoes net polymerization wherever the network volume fraction is below the equilibrium value θ_E . Therefore whilst these solutions display strong polymerization at the cell front in agreement with experiments, they cannot also capture the high actin densities observed at the front of travelling cells. This will be addressed in Chapter 3 by allowing a mass transfer between the network and solution phases to occur on the boundary. The actin distribution in the split peak family of solutions is very interesting: this solution could be biologically relevant to modelling cell motility, specifically the lamellipodium, which has a high actin density both at the leading edge and the transition to the cell body [143] as discussed in Chapter 1. That the actin profile declines so sharply behind the front peak allows polymerization to take place close to the front of the travelling strip.

Due to the increased computational cost of resolving more peaks we limited our study of the effect of adhesion strength to three peak solutions. However, it is probable that families of travelling-wave solutions with more than three peaks exist. We believe that the plateauing growth rate is responsible for many of the exciting features of this model, and in the subsequent chapters we investigate how this growth rate is modified by altering the network rheology, Chapter 4, and including myosin in the model explicitly, Chapter 5. First however, we consider the effect of membrane associated molecules on polymerization and depolymerization in Chapter 3.

Chapter 3

Modelling membrane-regulated actin polymerization

Whilst the minimal model of Chapter 2 is a valuable step to understanding the two-phase flow framework and its potential for describing cell motility, it lacks many features observed in crawling cells. Most notably the low network volume fractions at the front of the travelling strip are not compatible with experimental observations. The minimal model also makes no attempt to capture differences in the behaviour of the actin network near to cell membranes. In this chapter we begin to address these concerns.

As discussed in §1.1.2 the actin network is highly regulated by auxiliary proteins and there is considerable evidence that signalling cascades initiated by receptors in the membrane at the leading edge cause increased network polymerization nearby. Possible mechanisms include the competing effects of activated Arp2/3 and capping molecules creating a narrow band behind the leading edge where branching is promoted, and formin mediated polymerization at the leading edge. Therefore, it is pertinent to investigate the effect of polymerization and depolymerization of the actin network on the cell boundaries in our mathematical model. These questions are also mathematically intriguing due to the way in which modifying the boundary conditions alters the path of the characteristics of the continuity equation (which traced the edge of the solution domain in the minimal model) and changes the mathematical character of the problem.

Models that modify polymerization of the network in the vicinity of the cell membrane in the two-phase flow framework include [72, 73, 122]. They all allow the equilibrium volume fraction to be a function of a chemical messenger, which is emitted from the cell boundaries and diffuses through the cell with a constant sink term, so that network polymerization is

promoted near to the leading edge. Polymerization on the boundary is considered in [93] and we shall use a similar form of boundary condition in the second part of this chapter.

In keeping with our earlier philosophy that we should seek to first understand the simplest interesting problem, we continue to work with the one-dimensional, two-phase, poro-viscous, reactive flow framework used in Chapter 2 and consider the effects of imposing polymerization and depolymerization at the boundaries. In this chapter we consider two distinct modifications to the minimal model.

The first, in §3.1, models constant polymerization of the network at one end of the strip, $x = b(t)$. We present the model derivation in §3.1.1 including a discussion of the estimation of parameter values in §3.1.1.4. We then proceed to a small polymerization analysis of a nonlinear steady state of the model in §3.1.3 and demonstrate that such a state exists only when the homogeneous steady state of the minimal model is stable to small perturbations. Finally we investigate the model numerically in §3.1.4 and demonstrate the effect of varying polymerization and adhesion parameters on steady, travelling-wave solutions. We also draw the readers attention to some interesting numerical results discussed in §3.1.4.5.

The second model, in §3.2, prescribes polymerization and depolymerization at opposite ends of the travelling strip and allows the rates of polymerization and depolymerization to depend on the speed of the travelling strip. First the model is formulated in §3.2.1, then we present some numerical simulations in §3.2.2. Specifically we investigate the effect of (i) the ratio of timescales that govern establishing a cell front and network turnover, (ii) the rate of the polymerization imposed on the boundaries, and (iii) the strength of adhesion to the substrate. Finally the results of this chapter are summarised in §3.3.

3.1 Polymerization at the right-hand-side

3.1.1 Model formulation

We use the governing equations for the minimal model stated in §2.1.1 as a starting point and modify the boundary conditions as follows.

3.1.1.1 Boundary conditions

We consider first the effect of prescribing network polymerization at $x = b(t)$ in our minimal model for cell motility. The boundary conditions for the minimal model stated in (2.10) and

(2.11) are

$$\text{at } x = a(t), \quad \theta(u - \dot{a}) = 0, \quad (1 - \theta)(v - \dot{a}) = 0, \quad \Sigma = 0, \quad (3.1a-c)$$

$$\text{at } x = b(t), \quad \theta(u - \dot{b}) = 0, \quad (1 - \theta)(v - \dot{b}) = 0, \quad \Sigma = 0, \quad (3.2a-c)$$

We retain the no stress conditions (3.1c) and (3.2c) and the no-flux conditions (3.1a,b) on u and v at $x = a(t)$. Instead of the no-flux boundary conditions (3.2a,b) at $x = b(t)$, we impose

$$\text{at } x = b(t), \quad \theta(u - \dot{b}) = -K_0, \quad (1 - \theta)(v - \dot{b}) = K_0, \quad (3.3a,b)$$

where $K_0 > 0$ represents polymerization of the network on the boundary and $K_0 < 0$ represents depolymerization. The condition can also be understood as a flux of network into the strip of cytoplasm and a corresponding flux of solution out of the strip, for $K_0 > 0$. Summing (3.3a,b), we obtain

$$\theta u + (1 - \theta)v = \dot{b} \quad (3.4)$$

at $x = b(t)$, where the left-hand side is the mixture velocity V (defined in (2.13)) that depends only on time, so we see there is no flux of mixture in or out of the strip of cytoplasm and mass is conserved. Reducing the system as in §2.1.4, the boundary condition on the network velocity at $x = b(t)$ becomes

$$\theta(u - V) = -K_0. \quad (3.5)$$

For now we shall take K_0 to be a prescribed positive parameter; however, in general it could be a function of x , u , V and θ , *etc.* Equation (2.16a) is hyperbolic in θ given u , so $K_0 > 0$ implies that the characteristics at $x = b(t)$ flow into the domain. We therefore require an additional boundary condition for θ at $x = b(t)$, and we impose

$$\theta = \theta_F, \quad (3.6)$$

where θ_F is a positive constant. This models the biologically plausible situation in which the volume fraction of the highly regulated actin network is kept at some desirable value at the leading edge of the cell. References [4] and [5] tackle the problem of in-flow conditions for the hyperbolic equation describing continuity of the network in a two-phase flow framework in a slightly different way. Their models allow the network either to stick to or be ‘disrupted’ from the leading edge of the cell. When the network is disrupted the network velocity is less than the velocity of the leading edge and the network volume fraction imposed at the leading edge is zero.

3.1.1.2 Nondimensionalization

Employing the same scalings in (3.5) as in §2.1.5 and setting $K_0 = LK^\dagger/T$ we obtain

$$\theta \left(u^\dagger - V^\dagger \right) = -K^\dagger, \quad (3.7)$$

at $x^\dagger = 1 + a^\dagger$ where the daggers represent dimensionless quantities.

3.1.1.3 Travelling frame

We make the change of variables given by (2.24) into a frame that moves with the travelling strip, then drop hats and daggers. The resulting model is summarized as follows:

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad -\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x} \left(\mu \frac{\partial u}{\partial x} \right) = \alpha u + \beta(u + V), \quad (3.8a,b)$$

for $t > 0$, $0 < x < 1$, subject to the boundary conditions

$$\text{for } t > 0, \quad u(0, t) = 0, \quad u(1, t) = -\frac{K}{\theta_F}, \quad \theta(1, t) = \theta_F \quad (3.9a-c)$$

and the initial condition

$$\text{for } 0 < x < 1, \quad \theta(x, 0) = \theta^*(x), \quad (3.10)$$

where in order for the boundary and initial conditions to be compatible we must set $\theta^*(1) = \theta_F$. The solvability condition is

$$\langle \beta(u + V) \rangle = 0. \quad (3.11)$$

The constitutive laws are as given in (2.29) and (2.30) and restated here for convenience

$$J(\theta) = \theta - \theta_E, \quad \Psi(\theta) = -\Psi^* \left((1 - \theta_L)(1 - \theta_R) \ln(1 - \theta) + (1 - \theta_L - \theta_R)\theta + \theta^2/2 \right), \quad (3.12a,b)$$

$$\mu(\theta) = \theta, \quad \alpha(\theta) = \alpha^*\theta/(1 - \theta), \quad \beta(\theta) = \beta^*\theta. \quad (3.13a-c)$$

In addition to the six dimensionless parameters θ_L , θ_E , θ_R , Ψ^* , α^* and β^* from the minimal model in Chapter 2, there are two other dimensionless parameters θ_F and K .

3.1.1.4 Dimensionless parameters

A discussion of parameter values for the minimal model can be found in §2.1.7, so we restrict our attention here to estimating the size of K and θ_F . First, we assume that θ_F will be of the same order as the network volume fractions θ_L , θ_E and θ_R . An estimate of K can be made from the retrograde flow speeds observed experimentally in epithelial cells

[24]: examples of a large retrograde flow speed and large cell velocity are $4.2 \mu\text{m min}^{-1}$ and $0.3 \mu\text{mmin}^{-1}$, respectively. We sum these and multiply by a large network volume fraction estimate of $\theta = 0.04$ to obtain an upper estimate, $K_0 = 0 - 0.18 \mu\text{m min}^{-1}$. The corresponding nondimensional interval is $K = 0 - 0.009$, if we use $T = 1\text{min}$ and $L = 20\mu\text{m}$. The possible range for K is clearly larger if we take into account a range of values for T and L and the fact that other cells, for instance fish keratocytes crawl more quickly [24].

3.1.2 Steady state actin profile

Since much of the motivation for this chapter lies in the biologically unrealistic actin profiles that were obtained by the minimal model in Chapter 2, we pause to consider what can be said immediately about the steady actin profile. At steady state (3.8a) becomes

$$\frac{d}{dx}(\theta u) = -J = \theta_E - \theta, \quad (3.14)$$

for J given by (2.29a). Integrating, applying the boundary conditions (3.9) and rearranging implies

$$\int_0^1 \theta dx = \theta_E + K. \quad (3.15)$$

Therefore, as a rough guide, if we assume that localized polarization at $x = 1$ will make $x = 1$ the front of the travelling strip and if we want the network volume fraction to have a local maximum towards the front, then θ_F should not be chosen to be smaller than $\theta_E + K$.

3.1.3 Asymptotic analysis in the small polymerization limit

We aim to understand how the minimal model of Chapter 2 is altered by imposing a small flux of network into the right-hand-side of the travelling strip. We might reasonably expect that the small polymerization limit, obtained by allowing $K \rightarrow 0$, returns the minimal model studied in Chapter 2. However, closer inspection reveals that $K \rightarrow 0$ is in some sense a singular limit of our model, because when $K = 0$ we lose the ability to impose a boundary condition for θ at $x = 1$. Note that if we set $\theta_F = \theta_E$, then $u = V = 0$ and $\theta = \theta_E$ is a solution to (3.8)–(3.11). We seek a steady state solution to (3.8)–(3.11) in the limit $|\theta_E - \theta_F| \ll 1$ and $0 < K \ll 1$. Below we show the steady state solution has a boundary layer at $x = 1$.

We start by rescaling $K = \varepsilon \hat{K}$ for $0 < \varepsilon \ll 1$ and seeking a steady solution by considering the governing equations (3.8) and (3.11) at steady state, subject to the boundary conditions

$u(0) = 0$, $u(1) = -\varepsilon K/(\theta_E + \varepsilon\theta_G)$ and $\theta(1) = \theta_E + \varepsilon\theta_G$, after dropping the hat on K . We expand the boundary condition on u at $x = 1$ in terms of ε as follows:

$$u(1) = -\frac{\varepsilon K}{\theta_E} \left(1 - \frac{\varepsilon\theta_G}{\theta_E} + O(\varepsilon^2) \right) \quad \text{as } \varepsilon \rightarrow 0. \quad (3.16)$$

We substitute expansions of the form

$$u \sim \varepsilon \tilde{u}_1 + \varepsilon^2 \tilde{u}_2 + \dots, \quad V \sim \varepsilon \tilde{V}_1 + \varepsilon^2 \tilde{V}_2 + \dots, \quad \theta \sim \theta_E + \varepsilon \tilde{\theta}_1 + \varepsilon^2 \tilde{\theta}_2 + \dots \quad (3.17a-c)$$

into the steady governing equations to obtain at $O(\varepsilon)$ the expressions

$$\theta_E \tilde{u}'_1 = -\tilde{\theta}_1 J'_E, \quad -\Psi'_E \tilde{\theta}'_1 + \mu_E \tilde{u}''_1 = (\alpha_E + \beta_E) \tilde{u}_1 + \beta_E \tilde{V}_1, \quad \tilde{V}_1 = -\langle \tilde{u}_1 \rangle, \quad (3.18a-c)$$

subject to the boundary conditions $\tilde{u}_1(0) = 0$, $\tilde{u}_1(1) = -K/\theta_E$ and $\tilde{\theta}_1(1) = \theta_G$, although we shall not be able to satisfy all three boundary conditions with this second-order system. As before a subscript E on a function denotes that the function is evaluated at $\theta = \theta_E$, dashes on $\tilde{u}$ and $\tilde{\theta}$ denote differentiation with respect to x and dashes on J and Ψ denote differentiation with respect to θ .

Eliminating $\tilde{\theta}_1$ between (3.18a) and (3.18b) we obtain the second-order, inhomogeneous, ordinary differential equation

$$\tilde{u}''_1 + \lambda^2 \tilde{u}_1 = \frac{\beta_E J'_E \tilde{V}_1}{\theta_E \Psi'_E + \mu_E J'_E}, \quad (3.19)$$

where

$$\lambda^2 = -\frac{(\alpha_E + \beta_E) J'_E}{\theta_E \Psi'_E + \mu_E J'_E}; \quad (3.20)$$

note that λ may or may not be real depending on the parameter values, so henceforth the real parts of $\tilde{u}_1$ and $\tilde{\theta}_1$ are understood. Solving (3.19) yields

$$\tilde{u}_1 = A \cos(\lambda x) + B \sin(\lambda x) + \frac{\beta_E J'_E \tilde{V}_1}{\lambda^2 (\theta_E \Psi'_E + \mu_E J'_E)}. \quad (3.21)$$

for arbitrary constants A and B . The perturbed crawling speed $\tilde{V}_1$ is yet to be determined by (3.18c). Counting degrees of freedom remaining in the problem, we note that $\tilde{\theta}_1$ is given in terms of $\tilde{u}_1$ by (3.18a), so we have three boundary conditions to determine the two constants A and B . Since expanding about $u = 0$ means that we lose the $d\theta/dx$ term in (3.8a) it seems natural to anticipate a boundary layer at $x = 1$, in order to satisfy the boundary condition for $\tilde{\theta}_1$ there. We impose the boundary condition $\tilde{u}_1(0) = 0$ which implies that

$$\tilde{u}_1 = B \sin(\lambda x) + \frac{\beta_E \tilde{V}_1}{\alpha_E + \beta_E} [\cos(\lambda x) - 1], \quad (3.22)$$

and hence by (3.18a),

$$\tilde{\theta}_1 = \frac{\beta_E \theta_E \tilde{V}_1 \lambda}{J'_E(\alpha_E + \beta_E)} \sin(\lambda x) - \frac{B \theta_E \lambda}{J'_E} \cos(\lambda x), \quad (3.23)$$

which are our outer solutions.

In order to promote the $d\theta/dx$ in (3.8a) we consider a boundary layer with $O(\varepsilon)$ width by rescaling $x = 1 + \varepsilon X$ for $X \in (-\infty, 0]$. We replace the tildes with hats to signify that we are now working with inner variables. At $O(1)$, (3.18a) becomes

$$\theta_E \frac{d\hat{u}_1}{dX} = 0, \quad (3.24)$$

while at $O(\varepsilon)$,

$$\theta_E \frac{d\hat{u}_2}{dX} + \hat{u}_1 \frac{d\hat{\theta}_1}{dX} + \hat{\theta}_1 \frac{d\hat{u}_1}{dX} = -J'_E \hat{\theta}_1. \quad (3.25)$$

Similarly (3.18b) becomes

$$\mu_E \frac{d^2 \hat{u}_1}{dX^2} = 0 \quad (3.26)$$

at $O(1/\varepsilon)$, and

$$-\Psi'_E \frac{d\hat{\theta}_1}{dX} + \mu_E \frac{d^2 \hat{u}_2}{dX^2} + \mu'_E \frac{d}{dX} \left(\hat{\theta}_1 \frac{d\hat{u}_1}{dX} \right) = 0 \quad (3.27)$$

at $O(1)$. Now, (3.24) and (3.26) imply that $\hat{u}_1$ is constant in the boundary layer, so the boundary condition $\tilde{u}_1(1) = -K/\theta_E$ implies

$$\hat{u}_1 = -\frac{K}{\theta_E}. \quad (3.28)$$

Substituting (3.28) into (3.25) and (3.27) gives

$$\theta_E \frac{d\hat{u}_2}{dX} - \frac{K}{\theta_E} \frac{d\hat{\theta}_1}{dX} = -J'_E \hat{\theta}_1, \quad -\Psi'_E \frac{d\hat{\theta}_1}{dX} + \mu_E \frac{d^2 \hat{u}_2}{dX^2} = 0. \quad (3.29a,b)$$

We integrate (3.29b) once with respect to X and introduce an arbitrary constant C , then eliminate $\hat{u}_2$ using (3.29a) to find

$$\frac{d\hat{\theta}_1}{dX} = \frac{\theta_E^2 C}{K \mu_E} + \frac{\theta_E}{K \mu_E} (\mu_E J'_E + \theta_E \Psi'_E) \hat{\theta}_1, \quad (3.30)$$

which has the solution

$$\hat{\theta}_1 = -\frac{\theta_E C}{\mu_E J'_E + \theta_E \Psi'_E} + D e^{qX}, \quad (3.31)$$

where D is an arbitrary constant and

$$q = \frac{\theta_E}{K \mu_E} (\mu_E J'_E + \theta_E \Psi'_E). \quad (3.32)$$

Applying the boundary condition $\hat{\theta}_1(0) = \theta_G$, we obtain

$$\hat{\theta}_1 = -\frac{\theta_E C}{\mu_E J'_E + \theta_E \Psi'_E} + \left(\frac{\theta_E C}{\mu_E J'_E + \theta_E \Psi'_E} + \theta_G \right) e^{qX}. \quad (3.33)$$

To summarize we have found $O(\varepsilon)$ expressions for the inner and outer solutions of u and θ up to the unknown constants B , C and $\tilde{V}_1$, which will be determined by matching and imposing the integral constraint (3.11). First we match for u to obtain the boundary condition

$$\tilde{u}_1(1) = -\frac{K}{\theta_E}, \quad (3.34)$$

so by (3.22) we obtain the first expression relating B , C and $\tilde{V}_1$, namely

$$B \sin(\lambda) + \frac{\beta_E \tilde{V}_1}{\alpha_E + \beta_E} (\cos(\lambda) - 1) = -\frac{K}{\theta_E}. \quad (3.35)$$

Now matching for θ works if and only if $q > 0$, due to the exponential in (3.33), in which case $\tilde{\theta}_1(1) = \hat{\theta}_1(-\infty)$ and (3.23) and (3.33) give the second expression

$$-\frac{B\theta_E \lambda \cos(\lambda)}{J'_E} + \frac{\beta_E \theta_E \tilde{V}_1 \lambda \sin(\lambda)}{J'_E(\alpha_E + \beta_E)} = -\frac{\theta_E C}{\mu_E J'_E + \theta_E \Psi'_E}. \quad (3.36)$$

We postpone a discussion on the implications of the sign of q to §3.1.3.1. Finally we consider the integral constraint (3.11), which we write in the form

$$0 = \int_0^1 \beta(u + V) dx = \int_0^{1-\delta} \beta(\tilde{u} + \tilde{V}) dx + \varepsilon \int_{-\delta/\varepsilon}^0 \beta(\hat{u} + \tilde{V}) dX, \quad (3.37)$$

where choosing δ such that $\varepsilon \ll \delta \ll 1$ ensures the boundary layer is contained in the second integral, although this integral makes no contribution at leading order because $\hat{u}$ is of order unity in the boundary layer. At leading order we obtain

$$\tilde{V}_1 = -\int_0^1 \tilde{u}_1 dx, \quad (3.38)$$

giving the third expression

$$\tilde{V}_1 \left(\frac{\alpha_E \lambda + \beta_E \sin(\lambda)}{\lambda(\alpha_E + \beta_E)} \right) = B \left(\frac{\cos(\lambda) - 1}{\lambda} \right). \quad (3.39)$$

We now have three equations (3.35), (3.36) and (3.39) for three unknowns B , C and $\tilde{V}_1$, and we can eliminate each of the constants in turn to give

$$\tilde{V}_1 = \frac{K(1 - \cos(\lambda))(\alpha_E + \beta_E)}{\theta_E[\alpha_E \lambda \sin(\lambda) + 2\beta_E(1 - \cos(\lambda))]}, \quad (3.40)$$

$$B = -\frac{K(\alpha_E \lambda + \beta_E \sin(\lambda))}{\theta_E[\alpha_E \lambda \sin(\lambda) + 2\beta_E(1 - \cos(\lambda))]}, \quad (3.41)$$

$$C = -\frac{K\lambda(\mu_E J'_E + \theta_E \Psi'_E)(\alpha_E \lambda \cos(\lambda) + \beta_E \sin(\lambda))}{J'_E \theta_E [\alpha_E \lambda \sin(\lambda) + 2\beta_E(1 - \cos(\lambda))]} \quad (3.42)$$

Finally, we note that up to $O(\varepsilon)$ the expression for $\tilde{u}_1$ holds throughout the domain and the composite expansion for θ , upto $O(\varepsilon)$ is given by

$$\tilde{\theta}_1 = \frac{\beta_E \theta_E \tilde{V}_1 \lambda}{J'_E(\alpha_E + \beta_E)} \sin(\lambda x) - \frac{B \theta_E \lambda}{J'_E} \cos(\lambda x) + \left(\frac{\theta_E C}{\mu_E J'_E + \theta_E \Psi'_E} + \theta_G \right) e^{q(x-1)/\varepsilon}. \quad (3.43)$$

This analysis is verified by comparing with numerical simulations for appropriate parameter values in §3.1.4.1.

3.1.3.1 Remarks

The matching condition (3.36), can only be obtained for $q > 0$; if the sign on q is reversed then the inner solution grows exponentially as it exits the boundary layer and cannot be matched to the outer solution. This switch in behaviour as q passes through zero prompts further investigation. Note that

$$q = -\frac{\theta_E \Omega_\infty}{K}, \quad (3.44)$$

where Ω_∞ is defined in (2.54) and characterises the stability of the spatially homogeneous, steady state in the minimal model of the previous chapter. When $\Omega_\infty < 0$, $q > 0$, the steady state in the minimal model is stable, and there exists a steady solution to the small polymerization model which is close to the steady state in the minimal model. However, when $\Omega_\infty > 0$, the steady state in the minimal model is unstable to some small perturbations and we have been unable to find a steady state with small polymerization on the boundary close to the one in the minimal model.

It would be interesting to formally investigate the stability of the steady state found above for $q > 0$. However, since perturbing the composite solution (3.43) is likely to be sufficiently intractable as to require numerical investigation we proceed directly to solving the nonlinear initial value problem.

3.1.4 Numerical simulations

3.1.4.1 Description and validation of the numerical scheme

We employ a modified version of the code used in §2.4 to solve (3.8)–(3.13). The only alterations to the code were to implement the new boundary conditions at $x = 1$. When the code is used to obtain steady, travelling-wave solutions via continuation in the parameter

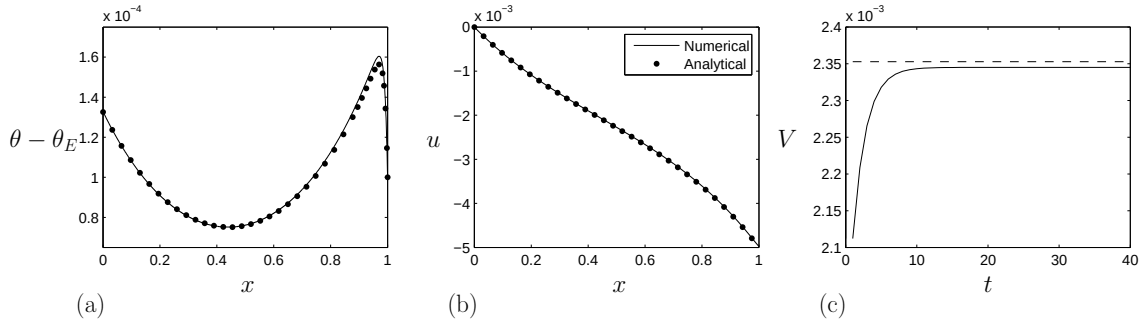


Figure 3.1: Comparison of the steady state network volume fraction θ in (a) and network velocity u in (b), obtained by numerical simulation (continuous) and the steady state found analytically in (3.22) and (3.43) (dots). The travelling-strip velocity V is shown in (c), the dashed line is the speed predicted in (3.40). Parameters used are $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\alpha^* = 0.4$, $\beta^* = 3$, $\Psi^* = 5000$, $\theta_G = 1$, $K = 1$ and $\varepsilon = 10^{-4}$ which correspond to $q > 0$. The initial condition is $\theta^*(x) = \theta_E - \varepsilon \theta_G \cos(\pi x)$.

θ_F , we allow the boundary condition on $\theta(1, t)$ to relax linearly to the new value for θ_F .

The code implements

$$\theta(1, t) = (1 - t)\theta_{F,\text{old}} + t\theta_F \quad (3.45)$$

for $t \in [0, 1]$ and $\theta(1, t) = \theta_F$ for $t > 1$, where $\theta_{F,\text{old}}$ is the previous value for the continuation parameter θ_F , which was used in the previous simulation.

We verified that the code converges with respect to the grid refinement in both time and space by running the same calculation on a sequence of nested grids, as in §2.4. To check spatial convergence the number of elements in the grid was doubled from 125 to 2000 and temporal convergence was checked for time steps doubling from 2.5×10^{-3} to 4×10^{-2} . The convergence is second-order with respect to the size of the spatial element and first-order with respect to the time step (details not shown). The numerical scheme is also validated against the boundary layer analysis for small polymerization in §3.1.3. This simulation evolves to a steady travelling-wave solution which agrees well with the analytical prediction, as shown in Figure 3.1. The numerical solution and asymptotic prediction were compared for three different values of ε . The error between them shrinks by a factor of four (to one significant figure) as ε is halved, which validates the form of the asymptotic expansion in (3.17).

3.1.4.2 Results

Confident in the validity of our numerical solutions, we proceed to investigate the effect of the polymerization boundary condition at $x = 1$. We use the same parameter values as

those used to produce Figures 2.6 and 2.7, along with the additional parameters $\theta_F = 0.03$ and $K = 0.01$. We solve the initial value problem (3.8)–(3.11) subject to the initial conditions $\theta(x, 0) = \theta^*(x) = \theta_E + (\theta_E - \theta_F) \cos(\pi x)$ for $0 < x < 1$, which can be thought of as a perturbation to the equilibrium volume fraction that satisfies the boundary conditions, although $\theta_E - \theta_F$ need not be small. Figure 3.2 shows that the speed of the travelling strip initially increases and then plateaus to a constant value by $t = 20$. The speed attained corresponds to approximately $3.2 \mu\text{mmin}^{-1}$ in dimensional units, which is biologically plausible. Between $t = 10$ and $t = 20$ the relative maximum absolute deviation in V from the mean value of V (in that interval) is less than 10^{-5} . We observe that the strip attains a steady, travelling-wave solution. The network volume fraction, network velocity and force per unit

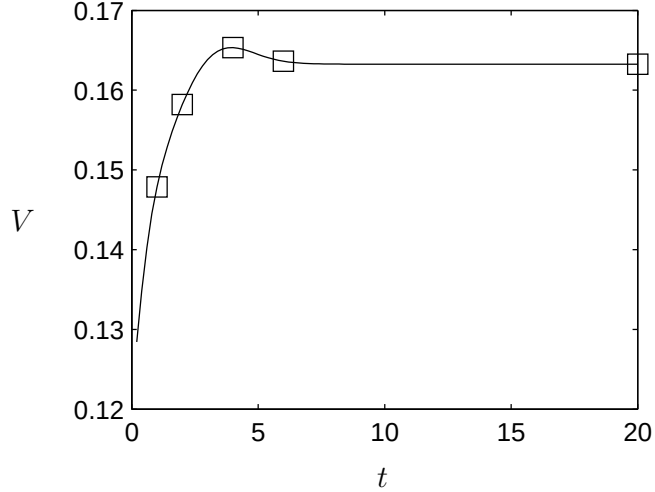


Figure 3.2: Speed of the travelling strip as a function of time for a solution of the initial value problem. Snapshots of the network volume fraction, velocity and force that the strip imposes on the substrate are plotted for the boxed times in Figure 3.3. Parameters used are $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$, $\beta^* = 3$, $\theta_F = 0.03$ and $K = 0.01$.

distance in the x -direction imposed on the substrate by the moving strip and calculated according to

$$F = \beta(\theta)(u + V), \quad (3.46)$$

are plotted at time $t = 1, 2, 4, 6, 20$ in Figure 3.3. The network volume fraction is initially highest at $x = 1$ and flowing in the negative x direction at $x = 1$ due to the boundary conditions. Also at earlier times the network volume fraction is in the contractile regime towards $x = 0$, so the network flows in the negative x direction and the strip moves in the positive x direction, displaying retrograde flow and establishing $x = 1$ as the leading

edge. The final steady network profile sits near to the swelling/contractile boundary and has mean 0.03 in agreement with (3.15). The force that the strip imposes on the substrate evolves to a steady state which is approximately linear, with forces directed towards the centre of the strip and the magnitude of the force being largest at the edges. These profiles are substantially different to those obtained from the minimal model displayed in Figure 2.7. In particular the velocity profile is not ‘U’ shaped, because there is flow through the end of the strip at $x = 1$ and the network volume fraction has no substantial peak at the rear of the strip.

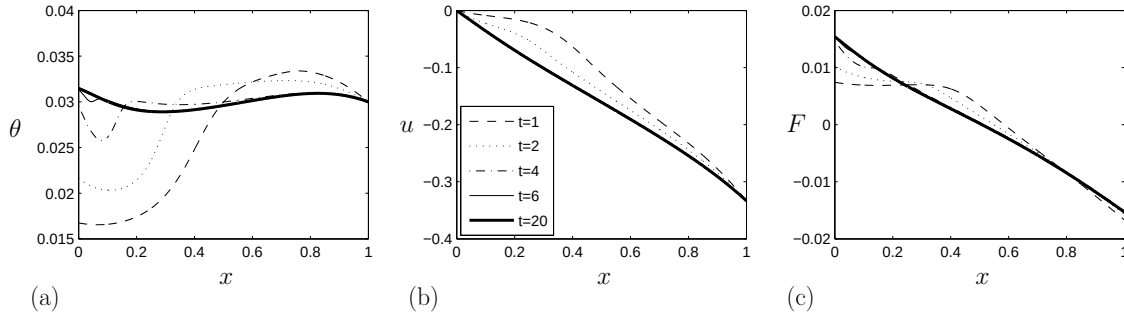


Figure 3.3: Snapshots of the network volume fraction (a), velocity (b), and force on the substrate generated by the travelling strip (c) are plotted at times $t = 1, 2, 4, 6, 20$. Parts of the later time profiles are obscured by the $t = 20$ profiles. The speed of the strip at each of these times is identified by a box in Figure 3.2. See caption to Figure 3.2 for parameter values.

3.1.4.3 Effect of polymerization parameters θ_F and K

Having obtained the steady travelling-wave solution displayed in Figure 3.3, we proceed to investigate what effect the polymerization rate K and the network volume fraction θ_F imposed at $x = 1$ have on the travelling-wave profiles. These profiles were obtained by continuation: either K or θ_F was fixed whilst the other one was varied. All other parameters were fixed as in Figure 3.3, with $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$ and $\beta^* = 3$. All the solutions in this section are calculated on grids with 1000, 2000 or 4000 spatial elements and then recalculated on a grid with twice as many spatial elements. The largest relative error in travelling speed between grids for the solutions displayed in this section is less than 1.4×10^{-4} .

As in the minimal model without polymerization at the boundary, we obtain qualitatively distinct families of travelling-wave solutions that co-exist in parameter space. These families are distinguished by their network profiles: one set are smooth across the whole

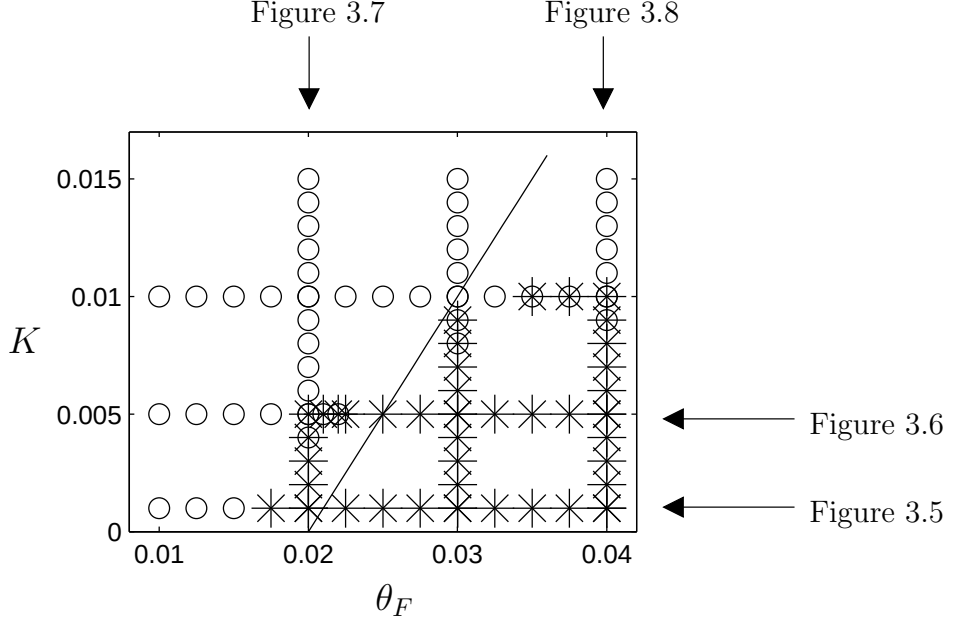


Figure 3.4: Travelling-wave solutions that are smooth ($\circ$) and have a corner ($*$) exist at the θ_F and K values indicated. For other parameter values used, see text. Line is given by $K = \theta_F - \theta_E$.

strip, whereas the other set have a region where the actin volume fraction changes sharply over a very small distance. We term this a ‘corner’, although great care has been taken to ensure that our simulations have sufficient spatial resolution to smoothly resolve these features. First in Figure 3.4, we show where in (θ_F, K) parameter space steady travelling-wave solutions have been found. All travelling-wave solutions discussed in this subsection have $V > 0$, so the imposed polymerization of the network at $x = 1$ occurs at the front of the travelling strip. The line in Figure 3.4 is given by $\theta_F = \theta_E + K$, *cf.* (3.15). Solutions below this line have θ_F greater than the mean network volume fraction. We anticipate that these solutions will be more biologically relevant, with high actin volume fractions at the front of the travelling strip. We now describe the qualitatively distinct classes of travelling-wave solutions we have found, which are displayed in Figures 3.5–3.8.

Figure 3.5 displays the steady, travelling-wave profiles for network volume fraction θ and velocity u , force on the substrate F and total mixture stress Σ attained for $K = 0.001$ and a range of θ_F values. We see in (a) that for smaller values of θ_F ($\theta_F = 0.01, \theta_F = 0.015$) the network profile is smooth, while increasing θ_F leads to a peak in the network volume fraction in the interior of the strip with a ‘corner’ on the rear side of the peak. As θ_F is increased further, this peak travels forwards. For large θ_F values there is a band of high

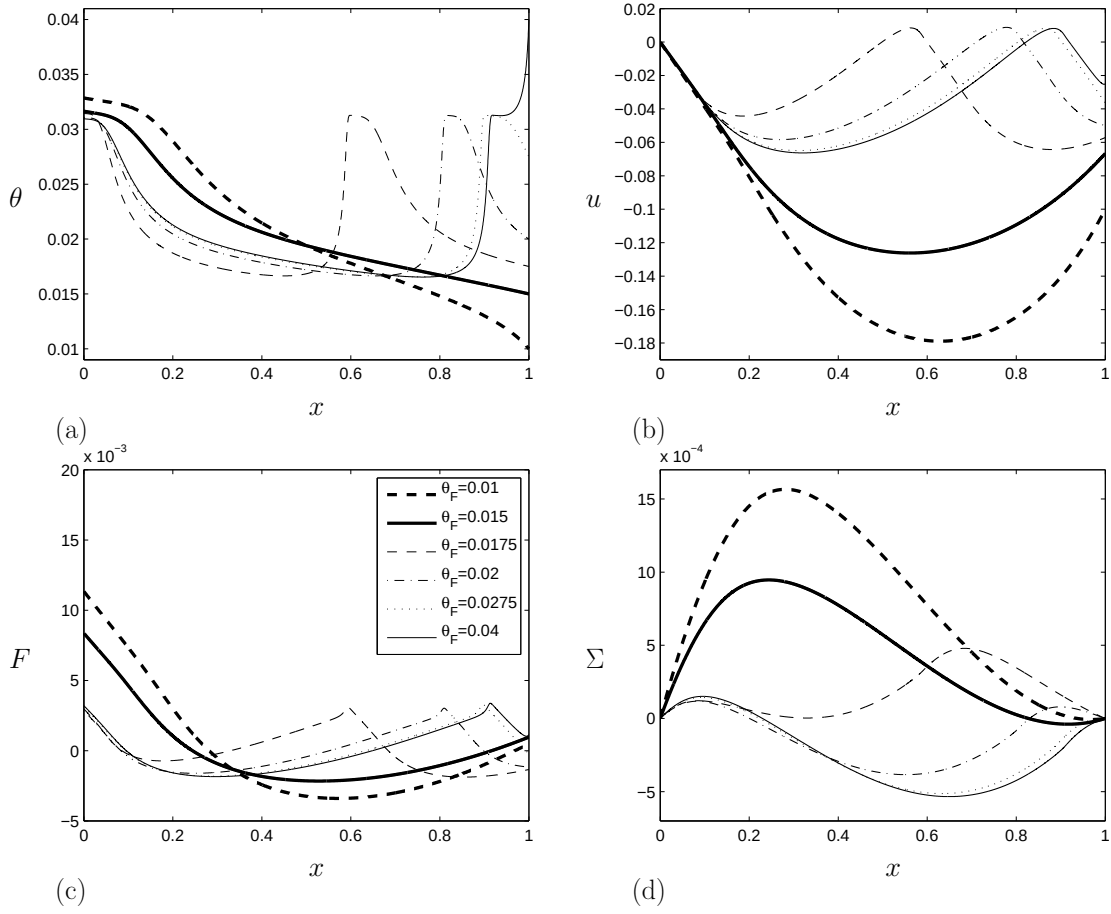


Figure 3.5: Effect of θ_F on steady state: (a) network volume fraction; (b) network velocity; (c) force on substrate and (d) total mixture stress for $K = 0.001$. Other parameters remain fixed, see text.

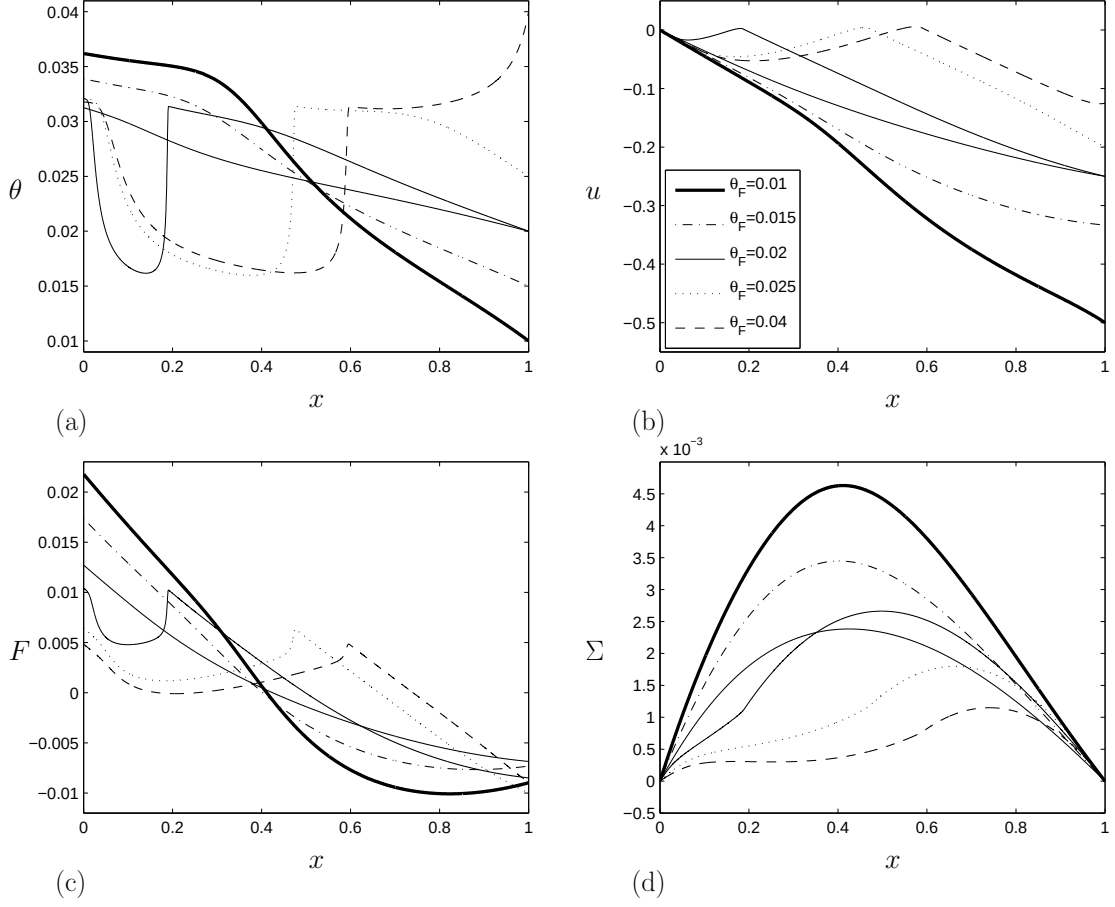


Figure 3.6: Effect of θ_F on steady state: (a) network volume fraction; (b) network velocity; (c) force on substrate and (d) total mixture stress for $K = 0.005$. Other parameters remain fixed, see text.

network volume fraction near the front of the strip. All the profiles in (b) depict retrograde flow of the network in most of the strip. For larger θ_F values there is a small region of the strip, associated with the ‘corner’ in the network profile, where the network stalls or has a small forwards flow. This region moves forwards as θ_F increases, but does not reach the front of the strip. From (c), for all θ_F values the force exerted on the substrate is large and in the direction of motion at the rear of the travelling strip. The force exerted by the smooth profiles is opposite to the direction of motion in the middle of the strip and in the direction of motion at the front. The forces exerted by the profiles with a corner have a local maximum in the interior of the strip. We also note that varying θ_F changes the shape of the total mixture stress, see (d).

We proceed to consider the effect of θ_F on steady travelling-wave solutions for a different

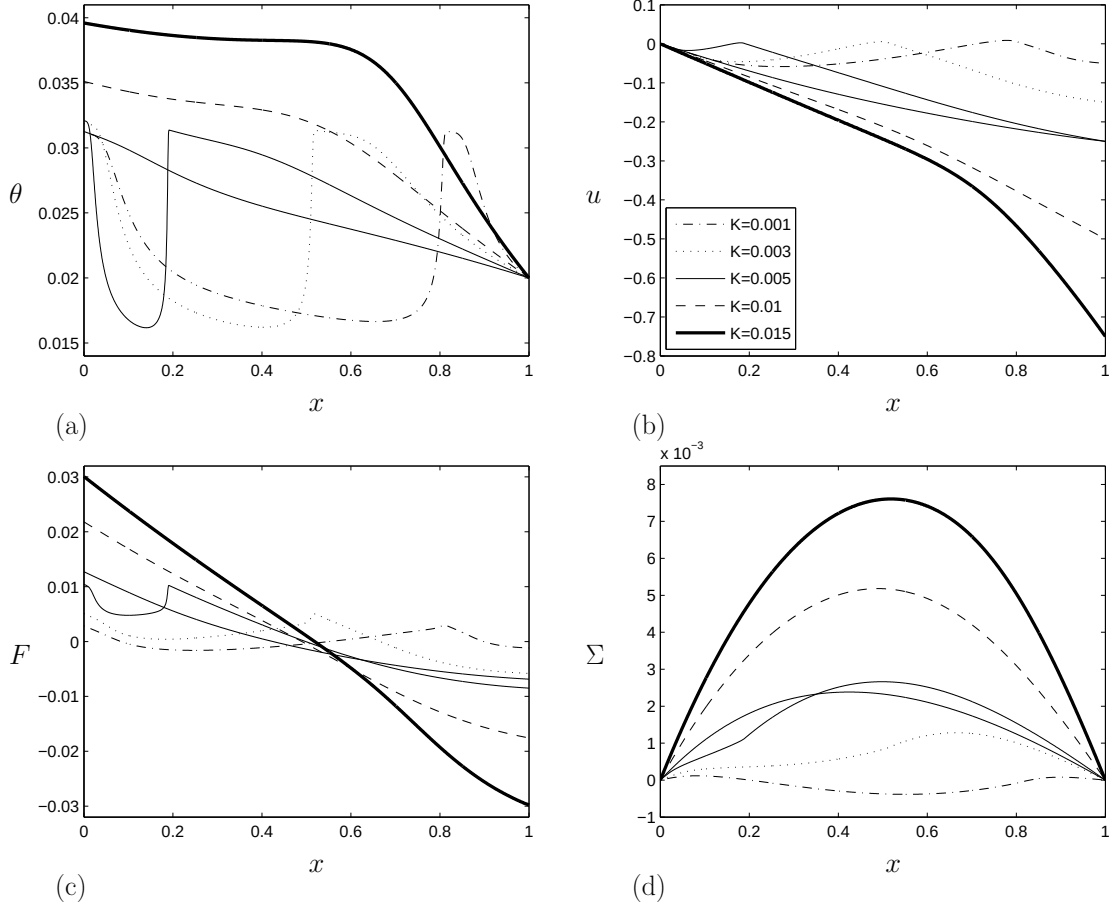


Figure 3.7: Effect of K on steady state: (a) network volume fraction; (b) network velocity; (c) force on substrate and (d) total mixture stress for $\theta_F = 0.02$. Other parameters fixed, see text.

polymerization rate, $K = 0.005$, shown in Figure 3.6. Again we see in (a) that the network profiles transition from smooth profiles to those that have a ‘corner’ with increasing θ_F . Note that we plot two profiles for $\theta_F = 0.02$, showing explicitly that the two families of solutions overlap in parameter space. The velocity profiles in (b) display retrograde flow. The velocities associated with network profiles that have ‘corners’ have a small region where the network flows forwards. The force exerted on the substrate, displayed in (c), is in the direction of motion at the rear of the cell and opposing the direction of motion at the front of the cell for all but the largest values of θ_F . The maximum magnitude of the total mixture stress decreases as θ_F increases as shown in (d). The effect of θ_F on the solution variables shown in Figure 3.6 for $K = 0.005$ is different, but not dissimilar to that seen for $K = 0.001$ in Figure 3.5.

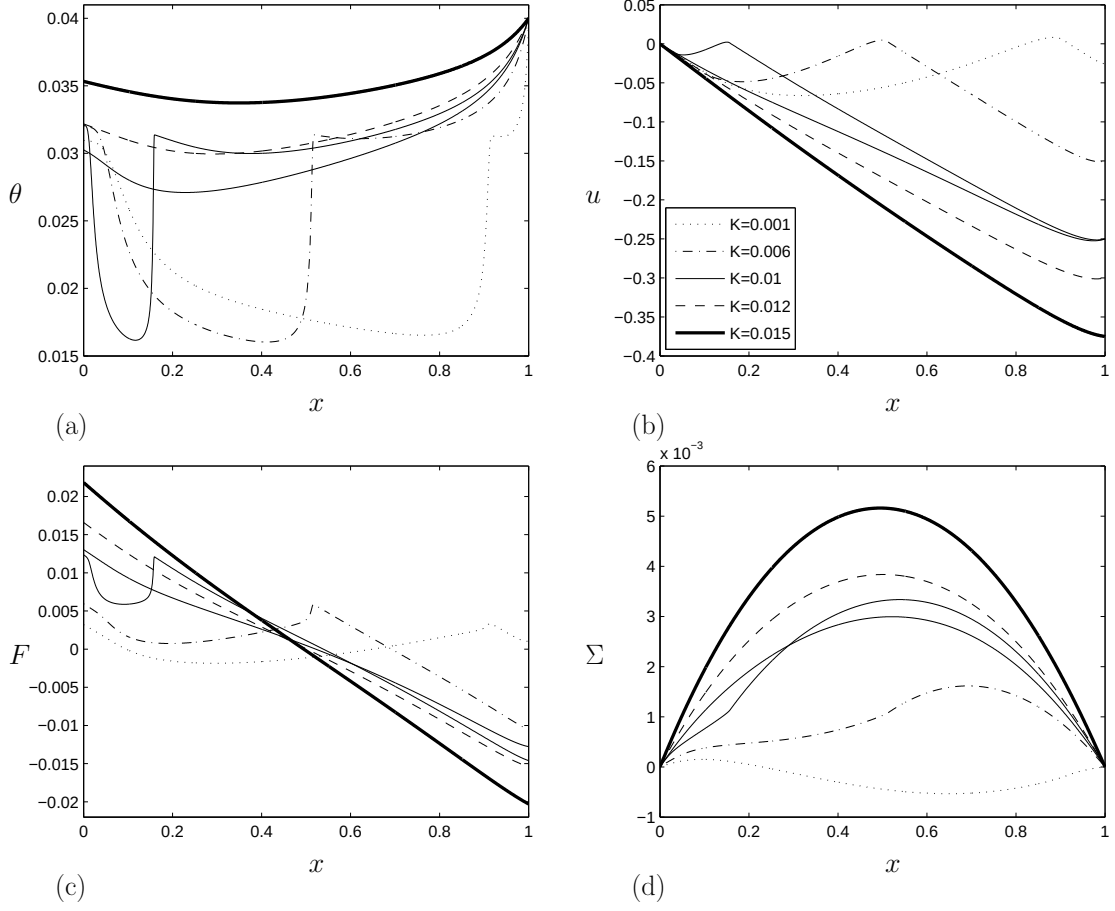


Figure 3.8: Effect of K on steady state: (a) network volume fraction; (b) network velocity; (c) force on substrate and (d) total mixture stress for $\theta_F = 0.04$. Other parameters remain fixed, see text.

Shifting our focus, we investigate the effect of varying polymerization rate K on the steady travelling-wave solutions for $\theta_F = 0.02$ in Figure 3.7. As expected we see in (a) that the total volume fraction of network in the travelling strip increases as K increases. The profiles have a ‘corner’ for small K values and become smooth as K increases, with both smooth and corner profiles plotted for $K = 0.005$. The velocity profiles depicted in (b) display retrograde flow in the majority of the travelling strip; the profiles associated with network profiles that have a ‘corner’ have a small region where the network flows forwards. Increasing K in (c) has a similar effect on the force exerted on the substrate as decreasing θ_F for $K = 0.005$ in Figure 3.6. The internal stress increases as K increases, shown in (d), and becomes negative in the centre of the strip for the smallest value of K displayed, $K = 0.001$.

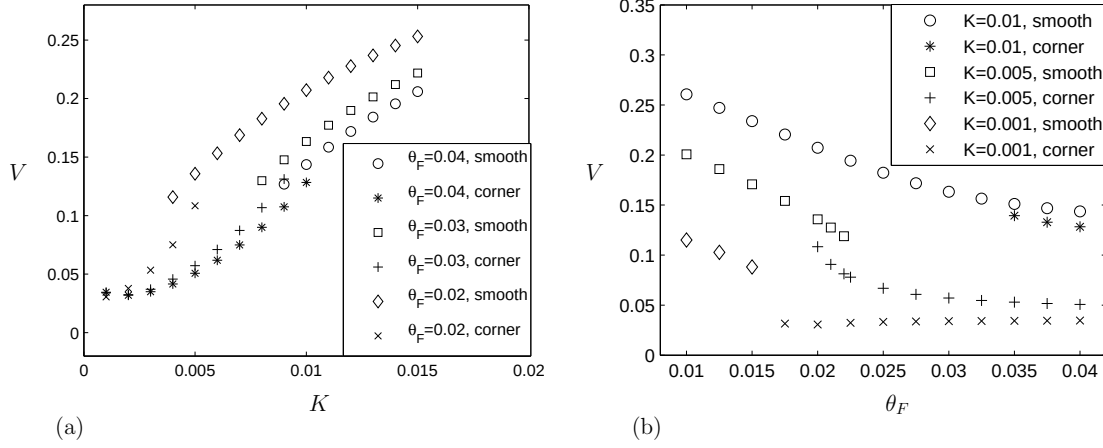


Figure 3.9: The effect of polymerization speed K (shown in (a)) and boundary network volume fraction θ_F (shown in (b)) on the speed of the travelling strip. The hollow polygons ($\circ$, $\square$, $\diamond$) represent solutions with a smooth network profile and the marks ($*$, $+$, $\times$) represent solutions with a ‘corner’ in the network profile.

Finally in Figure 3.8, we consider the effect of K on solution profiles for $\theta_F = 0.04$. For large K values the network volume fraction decreases smoothly behind the front, before increasing slightly at the rear of the strip. We show both a ‘corner’ and a smooth profile for $K = 0.01$. For smaller K the profile develops a ‘bucket-like’ feature, a region of low network volume fraction near the rear of the travelling strip, which extends forwards as K decreases. The trends in network velocity (b), force exerted on the substrate (c) and total mixture stress (d) are similar to those seen in Figure 3.7 for $\theta_F = 0.02$.

In order to further understand the different solution profiles obtained for different K and θ_F , we consider the associated velocity of the travelling strip. In Figure 3.9(a) we plot V as a function of K for three different values of θ_F and distinguish the speeds of smooth and ‘corner’ profiles. The overall trend, for most values of K , is that travelling speed increases with K and that when travelling-wave solutions belonging to both families exist for a given K the smooth profile travels faster. For very small K , when $\theta_F = 0.03$ or $\theta_F = 0.04$, there is a slight increase in travelling velocity as K decreases. It is unsurprising that larger K values, which correspond to large negative velocities for the network flowing into the front of the travelling strip, cause greater retrograde flow and hence faster locomotion of the travelling strip. However, the dependence of V on K is not simply monotonic, suggesting that for small polymerization rates other factors may be more important in determining the travelling-strip speed.

In Figure 3.9(b) we plot the steady travelling-strip speed V as a function of θ_F for three different values of K . The general trend is that V decreases as θ_F increases and as in (a), smooth solutions travel faster than their ‘corner’ counterparts where comparison is possible. The reduction in travelling speed as θ_F increases could be due to the associated increase in adhesion strength at the front of the strip. Also for fixed K an increase in θ_F means less retrograde flow of the network at $x = 1$ which could also contribute to the slower travelling speeds. Interestingly, the travelling speeds of the profiles containing a ‘corner’ for $K = 0.001$ do not fit this trend; their velocity is near constant. This again suggests that for small K some other feature of the solution is more important for determining the speed of the travelling-wave solution.

3.1.4.4 Effect of adhesion strength

Having studied the effect of the two parameters K and θ_F that control the polymerization at $x = 1$, we turn our attention to understanding the effect of β^* , our nondimensional measure of adhesion strength. Primarily we would like to know if our polymerizing boundary model retains the bell-shaped dependence of crawling speed on adhesion strength exhibited by the minimal model. We pick three points in (θ_F, K) parameter space, namely $(\theta_F, K) = (0.04, 0.01)$, $(\theta_F, K) = (0.02, 0.01)$ and $(\theta_F, K) = (0.04, 0.001)$ and obtain steady travelling-wave solutions from the unsteady code by continuation in β^* . All the solutions in this section have been obtained on a grid with 1000, 2000, 4000 or 8000 spatial elements and then repeated on a grid with twice the number of spatial elements to check convergence. The largest, relative error in steady, travelling velocity between grids for the solutions displayed in this section is less than 2×10^{-4} .

The first set of simulations we present is for $(\theta_F, K) = (0.04, 0.01)$. From Figure 3.4 we note that both ‘corner’ and smooth solutions exist for these parameters. We also note from Figure 3.8 that both solutions display the biologically realistic behaviour of a large actin volume fraction at the front of the travelling strip. Figure 3.10 displays the dependence of crawling speed on adhesion strength within each of the two families of solutions. The associated network profiles are shown in Figure 3.11; the smooth profiles are shown in (a) and profiles with a ‘corner’ are shown in (b). The steady crawling velocity of the smooth network profiles is maximal for intermediate values of β^* and decreases for very large β^* values, but plateaus for very weak adhesion. The network profile is near linear in the bulk with an increase at the front for small β^* ; as β^* increases a smooth peak in the

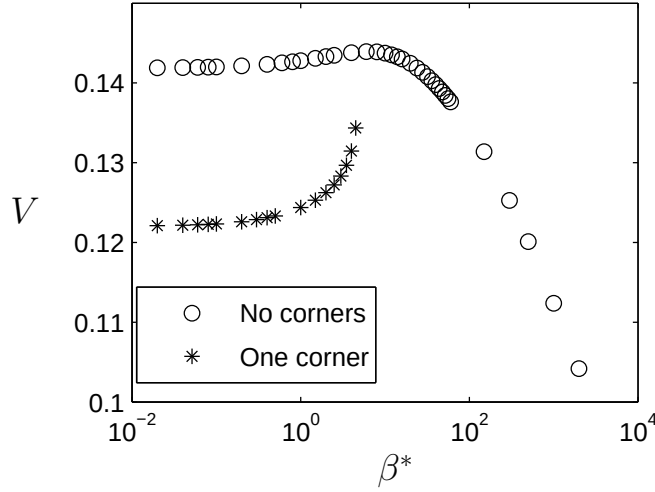


Figure 3.10: Effect of adhesion strength β^* on steady, crawling speed V . For $K = 0.01$, $\theta_F = 0.04$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$ and $\alpha^* = 0.4$.

network volume fraction grows just behind the front of the cell and the network volume fraction is elevated at the rear of the cell. The speed of the ‘corner’ profiles increases monotonically with β^* whilst the ‘bucket-like’ dip in the network volume fraction at the rear of the travelling strip gets narrower.

Next we consider the effect of β^* on crawling speed for $(\theta_F, K) = (0.02, 0.01)$. All the travelling-wave solutions obtained were smooth and the travelling speed plateaus for small β^* and decreases for large β^* as shown in Figure 3.12(a). The corresponding network profiles are shown in Figure 3.12(b) and show similar trends to the smooth profiles obtained previously for $(\theta_F, K) = (0.04, 0.01)$. The solutions for $(\theta_F, K) = (0.02, 0.01)$ appeared to be of less biological interest in §3.1.4.3, because the network volume fraction is low at the front of the travelling strip. However we see that for large adhesion strength the network volume fraction does peak just behind the front of the travelling strip.

Finally we turn our attention to $(\theta_F, K) = (0.04, 0.001)$, identifiable from Figure 3.8 as biologically interesting because the network volume fraction is high at the front of the travelling strip. Figure 3.4 suggests there is one family of solutions with a corner for these parameter values. As before we searched for steady solutions by continuation in β^* . We found no smooth steady profiles. However, the simulations did uncover three families of travelling-wave solutions, with one, two or three ‘corners’ in their actin profiles. The crawling speed of these solutions is displayed in Figure 3.13 along with the network volume fraction profiles associated with each family in Figure 3.14. Both the one ‘corner’ and

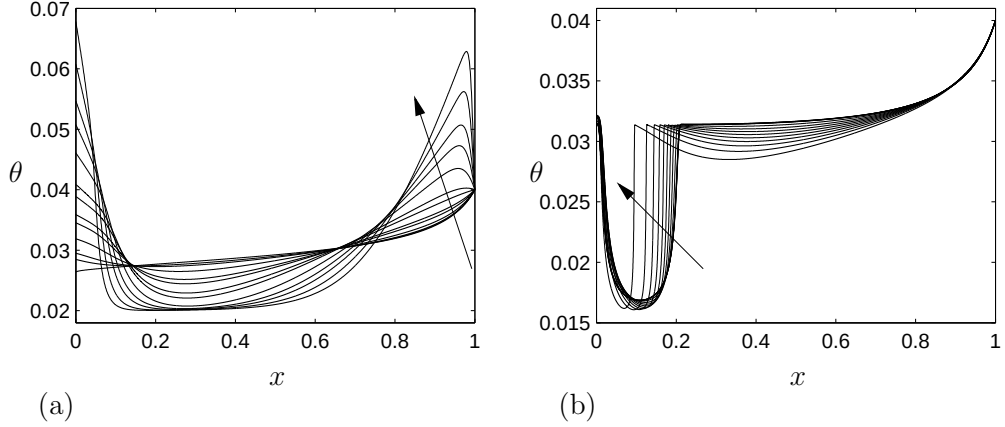


Figure 3.11: Network profiles for the steady travelling-wave solutions from Figure 3.10 without a ‘corner’ (a) and with a ‘corner’ (b). Parameters used were $K = 0.01$, $\theta_F = 0.04$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$ and $\alpha^* = 0.4$, with β^* ranging from 0.02 to 2000, arrows indicate increasing β^* . (β^* values used in (a) were 0.02, 1, 2, 6, 14, 20, 40, 60, 150, 300, 500, 1000 and 2000. β^* values used in (b) were 0.02, 0.04, 0.06, 0.08, 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5.

two ‘corner’ families of solutions display a ‘bell-shaped’ dependence of velocity on adhesion strength, reminiscent of that seen in the minimal model. The multiple corner solutions have regions of high actin volume fraction in the interior of the strip, which shift forwards and steepen as β^* increases. All these solutions have a band of high network volume fraction at the front of the travelling strip, with a profile that is locally insensitive to changes in β^* .

3.1.4.5 Some surprises

Further numerical investigation of the parameter space revealed some other interesting solutions. Figure 3.15 shows the results of the simulation for $K = 0.01$, $\theta_F = 0.04$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 40$ and $\beta^* = 3$ with the initial condition $\theta(x, 0) = \theta^*(x) = 3\theta_F/4 - \theta_F \cos(\pi x)/4$ for $0 < x < 1$. The network volume fraction is shown in Figure 3.15(a) at $t = 250$. Notice that the volume fraction peaks just behind the front of the strip, then falls almost linearly to near the middle of the strip. Behind this the volume fraction drops sharply and we observe two interior peaks in the volume fraction, which finally rises at the rear of the strip. The network velocity, shown in Figure 3.15(b) undergoes retrograde flow almost everywhere in the strip. Small regions of forward flowing network are identifiable with the actin peaks. The strip’s velocity is plotted in Figure 3.15(c), it settles to a small amplitude oscillation after about $t = 130$. Further simulations (not shown) reveal that the amplitude of this oscillation decays slowly with

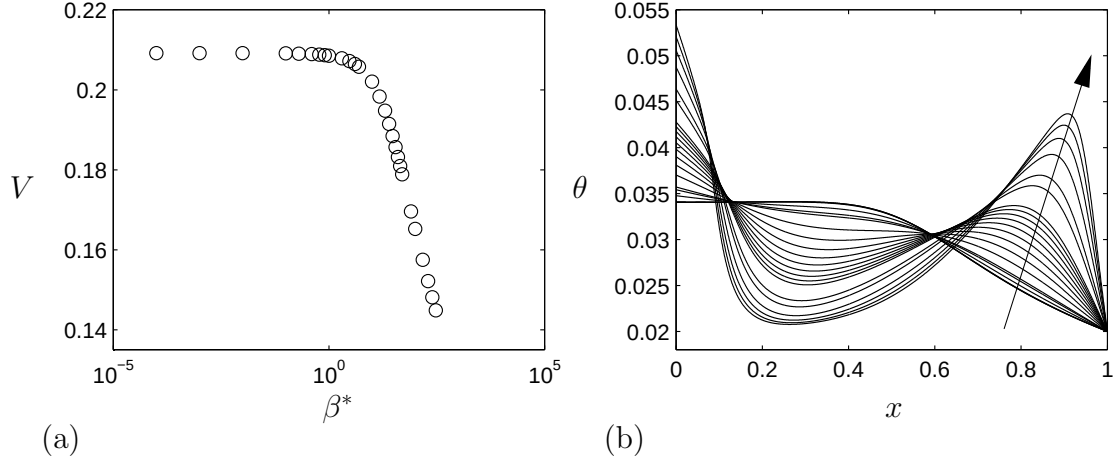


Figure 3.12: Effect of adhesion strength on (a) crawling speed and (b) actin volume fraction, for the parameters $K = 0.01$, $\theta_F = 0.02$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$ and $\alpha^* = 0.4$. Adhesion strength β^* ranges from 0.001 to 300 and arrow in (b) indicates increasing β^* . β^* values used in (b) were 0.001, 0.1, 0.2, 2, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 80, 100, 150, 200, 250 and 300.

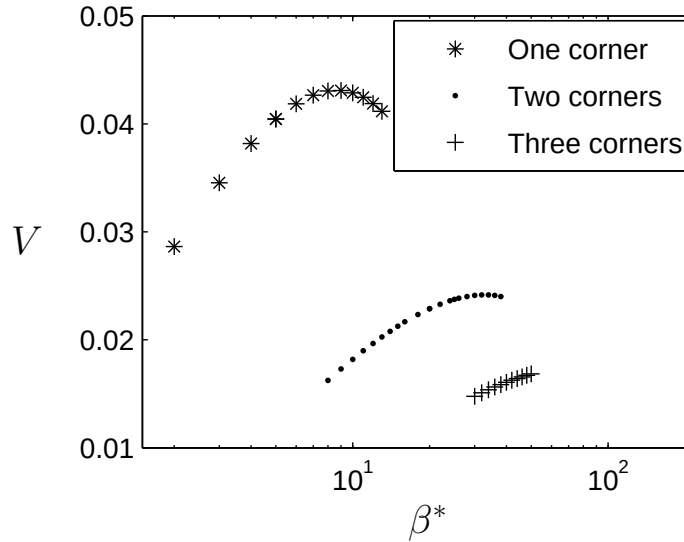


Figure 3.13: Effect of adhesion strength on crawling speed for the parameters $K = 0.001$, $\theta_F = 0.04$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$ and $\alpha^* = 0.4$.

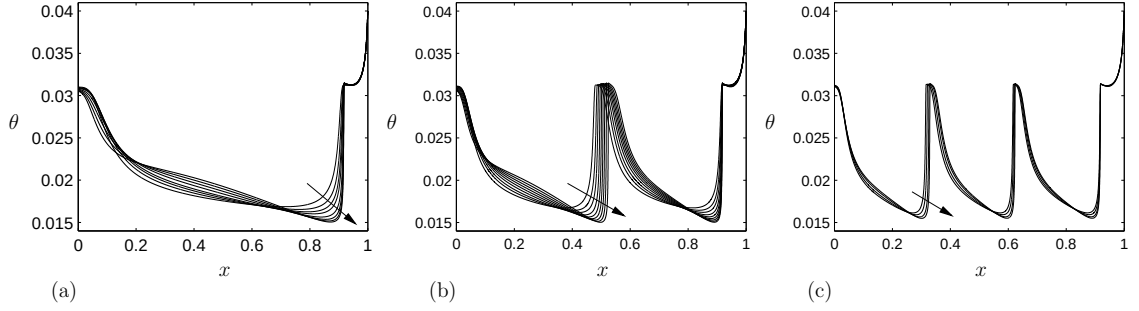


Figure 3.14: Network profiles for the simulations in Figure 3.13 with (a) one corner, (b) two corners and (c) three corners. Parameters are $K = 0.001$, $\theta_F = 0.04$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$ and $\alpha^* = 0.4$. Adhesion strength β^* ranges from 2 to 50 and arrows indicate increasing β^* . The β^* values used in (a) are 2, 3, 4, 5, 7, 9, 11 and 13. The β^* values used in (b) are 8, 11, 14, 18, 22, 26, 30, 34 and 38. The β^* values used in (c) are 30, 36, 44 and 50.

time. The network volume fraction is plotted for late times in Figure 3.15(d). The curve at the bottom of the graph is the network volume fraction $\theta(x, 200)$, the next curve up is $\theta(x, 205) + 5 \times 10^{-3}$, and we continue plotting $\theta(x, t) + (t - 200) \times 10^{-3}$ at intervals of $t = 5$ for $t \in [200, 400]$. This spreads out the θ profiles and allows us to observe how the profile changes with time. We see small oscillations in the positions of the two interior peaks. There is more movement on the interior peak at the back, while the smooth region of actin volume fraction at the front of the travelling strip appears to be constant in time. The amount of oscillation on the rear peak decreases as time progresses along with the oscillations in the velocity of the travelling strip. The most striking feature of this solution is the formation of two different regions in the actin network that behave differently. Near the front of the travelling-strip swelling dominates the network dynamics, whereas in the rear half of the strip the majority of the network is in the contractile regime. These simulations were run on three grids, the first has 4000 spatial elements and a timestep 1.25×10^{-3} , the second has 4000 spatial elements and a timestep of 2.5×10^{-3} , the third has 8000 spatial elements and a timestep of 1.25×10^{-3} . The largest relative error in V is less than 6×10^{-4} . The largest relative error in the volume fraction, θ , at $t = 250$ is less than 2.4×10^{-2} and at $t = 500$ is less than 1.7×10^{-2} .

Another interesting observation is the existence of steady travelling-wave solutions with negative crawling speed V . Polymerization is imposed on the boundary at $x = 1$, so these are crawling strips with polymerization at the rear. The volume fraction and velocity profiles for such a solution are presented in Figure 3.16. The network volume fraction is high at the

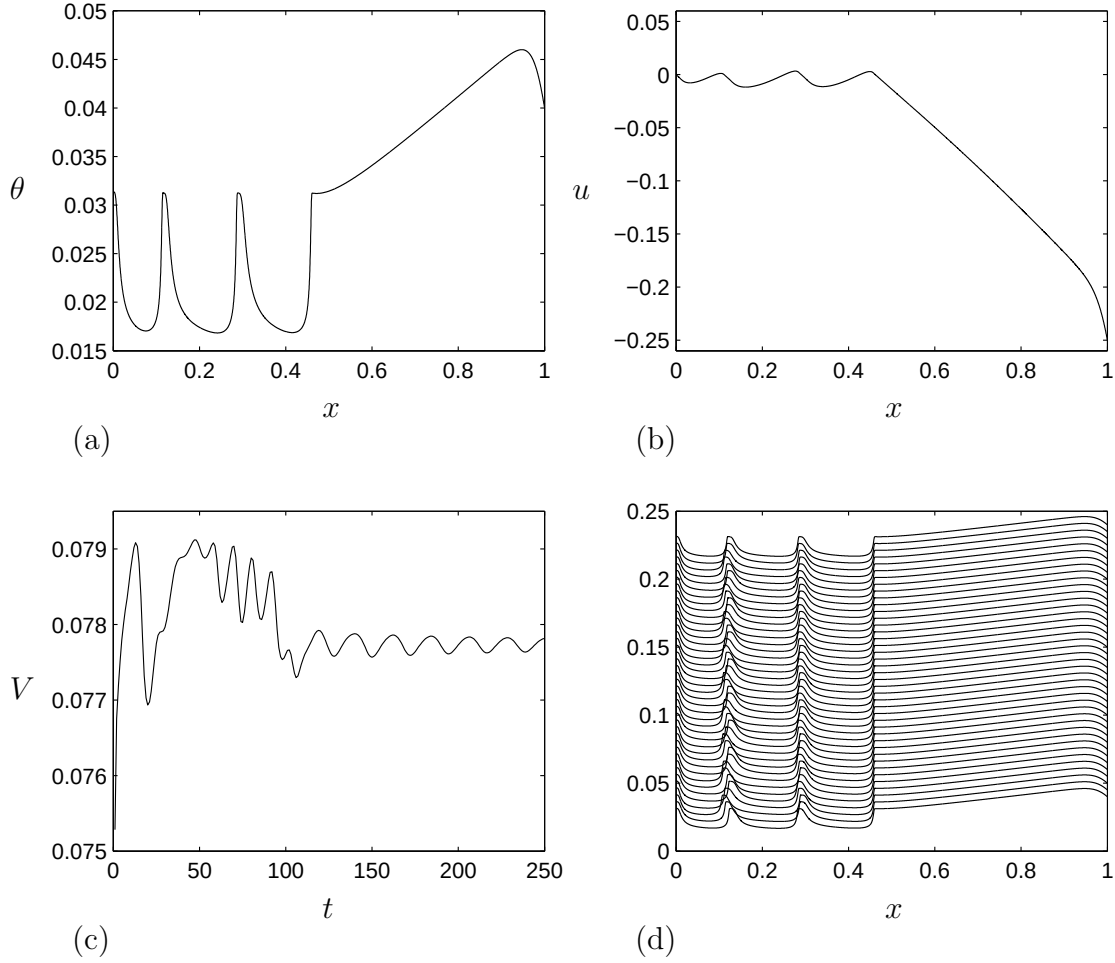


Figure 3.15: Simulation results for an initial value problem that develops multiple actin peaks. Network volume fraction and velocity profiles at $t = 250$ are shown in (a) and (b) respectively. The velocity of the travelling-strip is displayed in (c) and the evolution of the network profile is shown in (d), see text for details.

front and rear of the crawling strip; the peak at the front is much narrower than that at the back. The network undergoes retrograde flow in all but a small region at the front and back of the travelling strip where the network flows forwards. The rate of polymerization K in this simulation is fairly small and similar ‘backwards-crawling’ solutions have not been found for large values of K . These results were computed on grids with 2500 and 5000 spatial elements, and the relative error between the steady crawling speeds attained on these grids was 2.5×10^{-5} to two significant figures.

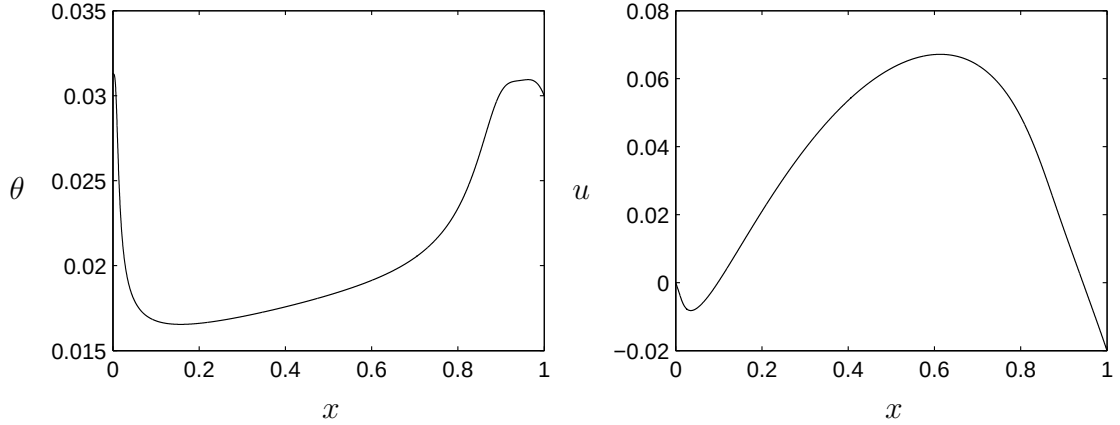


Figure 3.16: Steady travelling-wave profiles of (a) network volume fraction and (b) network velocity, for $K = 0.0006$, $\theta_F = 0.03$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$ and $\beta^* = 3$. These profiles translate with velocity $V = -0.0342$ to three significant figures.

3.2 Velocity dependent polymerization

3.2.1 Model formulation

3.2.1.1 Boundary conditions

Allowing network polymerization on the boundary of the travelling strip has yielded some interesting, new actin profiles. It is however somewhat unsatisfactory to choose which end of the strip polarization occurs at and thus strongly influence the direction of motion. We turn our attention now to an alternative model that does not impose a front and back on the travelling strip. We will use the model to investigate the biologically plausible situation, in which polymerization and depolymerization of the actin network are promoted by biochemical signals at the front and rear of the travelling strip, respectively. Instead of seeking to simulate these biochemical signals, we model the polymerization and depolymerization as functions of the cell velocity. We consider the minimal model presented in §2.1.4 and following [93] we replace (2.19) with

$$\theta(u - V) = -K_V \tanh\left(\frac{V}{V_0}\right) \quad (3.47)$$

at $x = a(t)$ and $x = b(t)$, where K_V and V_0 are positive parameter values. The form of the boundary condition is motivated by assuming that faster travelling cells have a more established front and back with faster rates of polymerization and depolymerization. We note that we also need to impose a boundary condition for θ whenever the characteristics flow into the domain. When $V > 0$ we need a boundary condition for θ at $x = b(t)$ and when

$V < 0$ we need a condition for θ at $x = a(t)$, which requires turning on and off boundary conditions if V changes sign. In order to avoid introducing discontinuities in the network volume fraction we solve

$$\frac{d\theta_c}{dt} = \frac{\theta_F - \theta_c}{\tau}, \quad (3.48)$$

subject to the initial conditions $\theta_c(0) = \theta(x, t_S)$ where $x = a(t)$ if $V < 0$ and $x = b(t)$ if $V > 0$ and t_S is the time at which the travelling strip most recently changed direction, so that $V(t_S) = 0$. For $V < 0$ ($V > 0$) we then obtain the boundary condition $\theta(a(t), t) = \theta_c(t - t_S)$ ($\theta(b(t), t) = \theta_c(t - t_S)$). Both τ and θ_F are positive constants. This models a situation in which τ is the timescale for the cell machinery associated with polymerization to organise itself once a boundary becomes the front of the cell and θ_F is the volume fraction of network maintained by biochemical signalling once those signals are established.

3.2.1.2 Nondimensionalization

Using the same scalings in (3.47) and (3.48) as in §2.1.5 and setting $K_V = LK^*/T$, $V_0 = LV^*/T$ we obtain

$$\theta(u^\dagger - V^\dagger) = -K^* \tanh\left(\frac{V^\dagger}{V^*}\right), \quad (3.49)$$

at $x^\dagger = a^\dagger, 1 + a^\dagger$ and

$$\frac{d\theta_c}{dt^\dagger} = \frac{(\theta_F - \theta_c)T}{\tau}, \quad (3.50)$$

with $\theta = \theta_c$ at $x^\dagger = a^\dagger$ if $V^\dagger < 0$ or $\theta = \theta_c$ at $x = 1 + a^\dagger$ if $V > 0$. Daggers denote dimensionless variables and stars denote dimensionless parameters.

3.2.1.3 Travelling frame

Here we summarize the governing equations obtained after moving into a frame that travels with the strip as detailed in (2.24). Solving (3.50) and dropping hats and daggers, we find

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad -\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x}\left(\mu \frac{\partial u}{\partial x}\right) = \alpha u + \beta(u + V), \quad (3.51a,b)$$

for $t > 0$, $0 < x < 1$, subject to the boundary conditions

$$\theta(0, t)u(0, t) = \theta(1, t)u(1, t) = -K^* \tanh\left(\frac{V(t)}{V^*}\right) \quad \text{for } t > 0, \quad (3.52)$$

with, for $V > 0$

$$\theta(1, t) = \theta_F + (\theta(1, t_S) - \theta_F)e^{(t_S - t)/t_R} \quad \text{for } t > 0, \quad (3.53)$$

and, for $V < 0$

$$\theta(0, t) = \theta_F + (\theta(0, t_S) - \theta_F)e^{(t_S - t)/t_R} \quad \text{for } t > 0, \quad (3.54)$$

where $t_R = \tau/T$ is the ratio of the timescale for establishing a front to the timescale for network turnover. The initial condition is

$$\text{for } 0 < x < 1, \quad \theta(x, 0) = \theta^*(x), \quad (3.55)$$

and the solvability condition is

$$\langle \beta(u + V) \rangle = 0. \quad (3.56)$$

The constitutive laws are as stated in (2.29) and (2.30) and restated here for completeness

$$J(\theta) = \theta - \theta_E, \quad \Psi(\theta) = -\Psi^* \left((1 - \theta_L)(1 - \theta_R) \ln(1 - \theta) + (1 - \theta_L - \theta_R)\theta + \theta^2/2 \right), \quad (3.57a, b)$$

$$\mu(\theta) = \theta, \quad \alpha(\theta) = \alpha^* \theta / (1 - \theta), \quad \beta(\theta) = \beta^* \theta. \quad (3.58a-c)$$

In addition to the six dimensionless parameters $\theta_L, \theta_E, \theta_R, \Psi^*, \alpha^*$ and β^* from the minimal model, this model also has parameters θ_F, K^*, V^* and t_R .

3.2.2 Numerical simulations

3.2.2.1 Description and validation of the numerical scheme

We compute numerical solutions to the system of equations (3.51)–(3.58) with the code used in §2.4 adapted to deal with the new boundary conditions. The boundary conditions for u are implemented explicitly, using the value of V at the previous time step. Changes in crawling direction are detected by considering the sign of the product of V at the current time step and V at the previous time step. When the sign is negative t_S is updated. In order to validate the code we run it on a series of nested grids to ensure it converges with respect to the grid size. To check spatial convergence the number of elements was repeatedly doubled from 250 to 8000 and to check temporal convergence the time step was repeatedly doubled from 2.5×10^{-5} to 8×10^{-4} . Graphs displaying the rate of convergence can be found in Appendix B.

3.2.2.2 Results

An example solution for the initial value problem (3.51)–(3.58) is shown in Figures 3.17–3.19. From Figure 3.17 we see that the strip velocity is initially negative, then the strip changes direction at $t = 12.6$. Figure 3.18 shows the evolution of the network volume

fraction and velocity, while snapshots to display the shape of the profiles at a sample of times are shown in Figure 3.19. We see that the network volume fraction is initially low at the front of the travelling strip ($x = 0$). Retrograde flow transports network towards $x = 1$ and the boundary conditions increase θ at $x = 0$. The thin white diagonal streak in Figure 3.18 (a) and the snapshots at $t = 5$ and $t = 8$ in Figure 3.19 show a peak of network volume fraction travelling backwards through the cell where it coalesces with the peak that has formed at $x = 1$. This event coincides with the cell changing direction. After $t = 15$

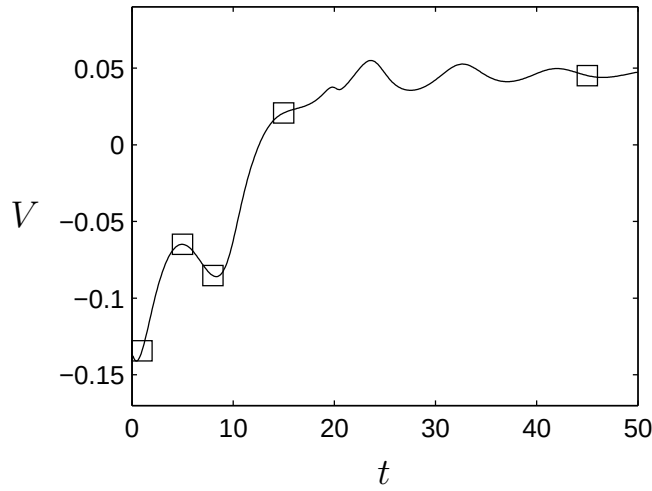


Figure 3.17: Crawling speed of the strip for the initial value problem with initial condition $\theta^*(x) = 3\theta_F/4 - \theta_F \cos(\pi x)/4$ and parameters, $K^* = 0.01$, $V^* = 1$, $\theta_F = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$, $\beta^* = 3$, $t_R = 50$, $\theta_L = 0.01$, $\theta_E = 0.02$ and $\theta_R = 0.03$. This solution was obtained on a grid with 4000 elements in space and a time step 2×10^{-4} . The relative error in V between this solution and that obtained with 2000 space elements is less than 8×10^{-5} . The relative error in V when the time step is changed to 1×10^{-4} is less than 0.02. The boxes indicate times for which the network volume fraction, velocity and force are displayed in Figure 3.19.

the peak in network volume fraction at the front of the strip ($x = 1$) is maintained by polymerization on the boundary and peaks that form in the cell interior undergo retrograde flow and are transported to $x = 0$. By $t = 45$ the network has attained a profile which resembles some of the steady states attained in the previous model, with peaks in the network volume fraction at both the front and rear of the travelling strip. Figure 3.19(b) shows that the network undergoes retrograde flow in most of the strip, with a small region of forward flowing network located just behind the actin peak at the front of the strip for later times. The plots of the force exerted on the substrate in Figure 3.19(c) all display a

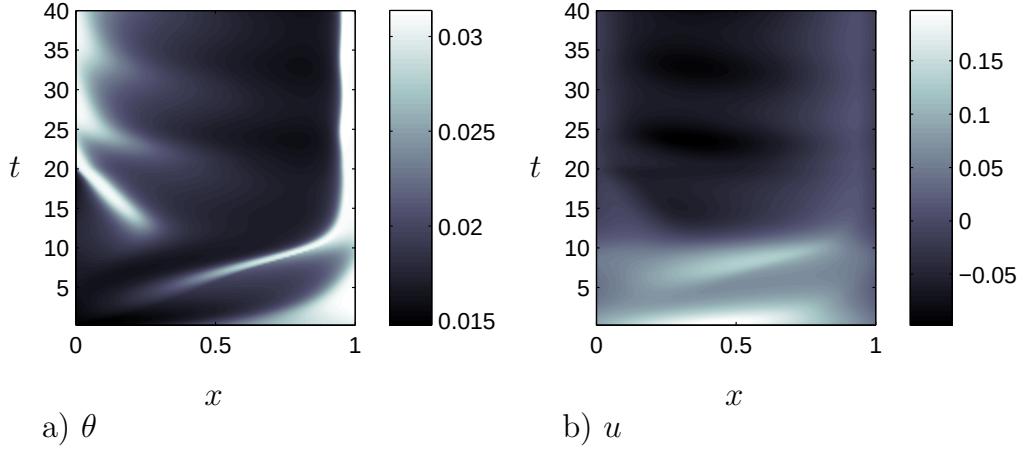


Figure 3.18: Graphs showing the variation in space and time of solutions to the initial value problem in Figure 3.17 for (a) the network volume fraction and (b) the network velocity.

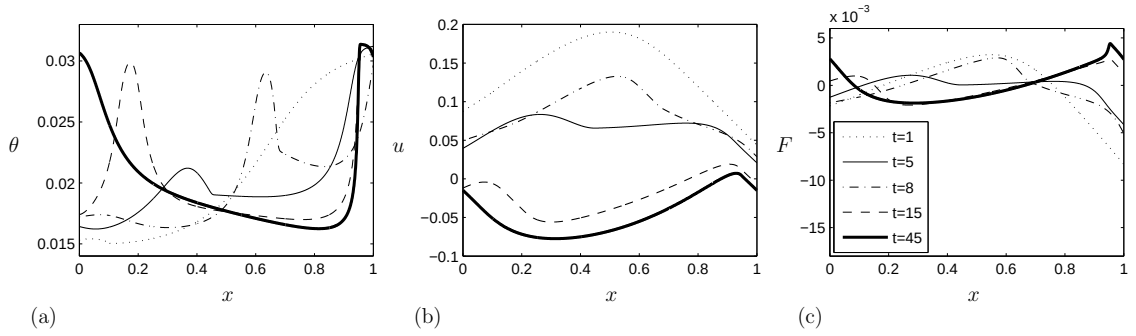


Figure 3.19: Snapshots of (a) the network volume fraction, (b) the network velocity and (c) the force exerted by the cell for the initial value problem shown in Figure 3.17 at the boxed times.

force in the direction of motion exerted at the rear of the cell and large force in the direction of motion associated with established peaks at the front of the travelling strip.

3.2.2.3 Effect of polarization timescale

In order to understand the effect of the boundary conditions in this model we investigate first the effect of varying the polarization timescale, t_R . The crawling velocities shown for $t_R = 25$ and $t_R = 50$ in Figure 3.20(a) are comparable and appear to settle to the same value. The network volume fractions attained at $t = 150$ for each value of t_R and shown in (c) also look the same. Closer inspection in (b) reveals that at late times the velocity for $t_R = 25$ exhibits decreasing oscillations around a constant value, whereas the velocity for $t_R = 50$ appears to be slowly increasing, with small, superimposed oscillations. Figure 3.20(d) reveals that the network volume fraction on the boundary $x = 1$ at $t = 150$ is closer

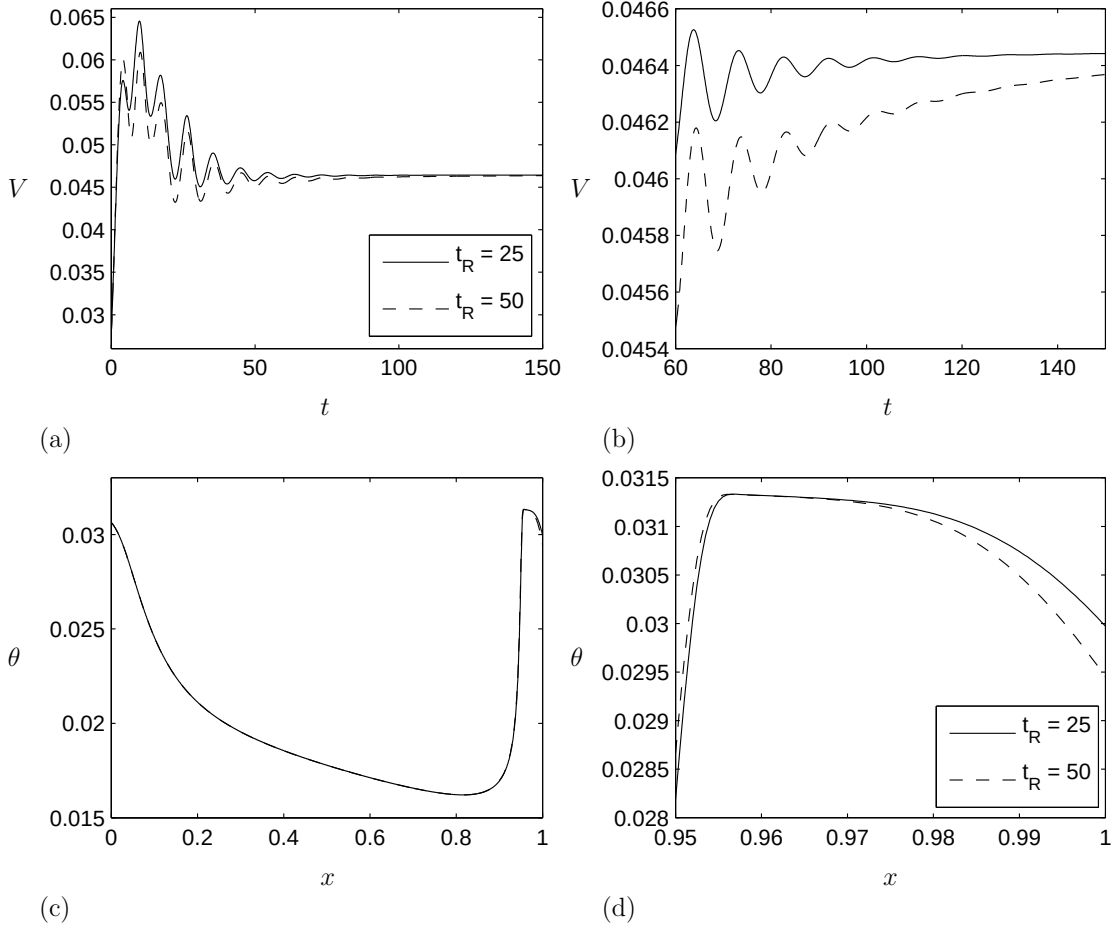


Figure 3.20: (a) Comparison of crawling strip velocity and (c) network volume fraction at $t = 150$, for $t_R = 25$ and $t_R = 50$. Zoomed in versions of (a) and (c) are shown in (b) and (d) respectively. Other parameters used were $K^* = 0.01$, $V^* = 1$, $\theta_F = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$, $\beta^* = 3$, $\theta_L = 0.01$, $\theta_E = 0.02$ and $\theta_R = 0.03$ along with initial condition $\theta^* = \theta_E + 0.001 \cos(\pi x)$.

to θ_F for $t_R = 25$ than for $t_R = 50$. Recalling (3.53) and (3.54), the boundary conditions for θ , we see that relaxation of θ to θ_F at the front of a travelling strip is slower for larger values of t_R . The slowly increasing $\theta(1, t)$ for $t_R = 50$ causes the slower decay to steady state. At sufficiently late times the difference in θ_F and the imposed boundary condition becomes negligible if the crawling direction of the strip persists.

Steady states of this model can only be approached asymptotically as $t \rightarrow \infty$. In practice, since we solve this problem numerically, we shall deem that a simulation has reached a steady state when the relative variation in V from the average value of V over the final, say, thirty time units is sufficiently small. Once such a steady solution is obtained t_R has no further influence on the θ , u and V . Running the simulation for longer we obtain

a steady travelling-wave solution that crawls with velocity $V = 0.0464$ to three significant figure for $t_R = 50$ and the relative difference in V from the average travelling velocity for the final thirty time units is less than 10^{-6} .

The limit in which $t_R \rightarrow 0$ is perhaps of little biological interest, since it describes a somewhat implausible situation in which the actin network can organise itself and establish a front on a much faster timescale than the network turnover timescale. However, it is of considerable mathematical interest, because in the limit we would be introducing jumps in the network volume fraction at the ends of the travelling strip. Analysis of a simpler case can be found in the literature in [10], which considers a single fluid flowing through fissured and porous rock within a two-phase flow formulation. Their pressure is governed by a linear pseudo-parabolic equation of the form

$$\frac{\partial p}{\partial t} = \frac{\partial^2 p}{\partial x^2} + \frac{\partial^3 p}{\partial x^2 \partial t}. \quad (3.59)$$

They consider a jump in pressure created by conflicting boundary and initial conditions, which remains at the domain boundary (as opposed to propagating into the domain). They demonstrate that the size of the jump decays exponentially with time.

3.2.2.4 Effect of polymerization parameter K^*

We proceed to consider K^* , the flux parameter, another parameter in the boundary conditions that distinguish this model from the minimal model considered in Chapter 2 and the constant boundary polymerization model considered in §3.1. Figure 3.21 shows the velocity attained by steady travelling-wave solutions that were obtained with our unsteady code by continuation in K^* . We see that the crawling velocity of the strip increases with K^* . All of these computations were performed on grids with 2000 and 4000 spatial elements. The relative difference between the velocity and the mean velocity over the last thirty time units is less than 1.7×10^{-6} , the relative error in the network volume fraction on the two nested grids is less than 1.9×10^{-4} , while the relative error in V on the two nested grids is less than 5×10^{-5} .

Figure 3.22(a) shows how the network profile varies for different K^* . For all K^* shown the network volume fraction peaks at the front and rear of the travelling strip. As K^* increases the peak at the front fattens, while the peak at the rear decreases. The network velocities displayed in Figure 3.22(b) appear to be (but are not) generated by a periodic solution that is shifted backwards as K^* increases; there is larger retrograde flow at the

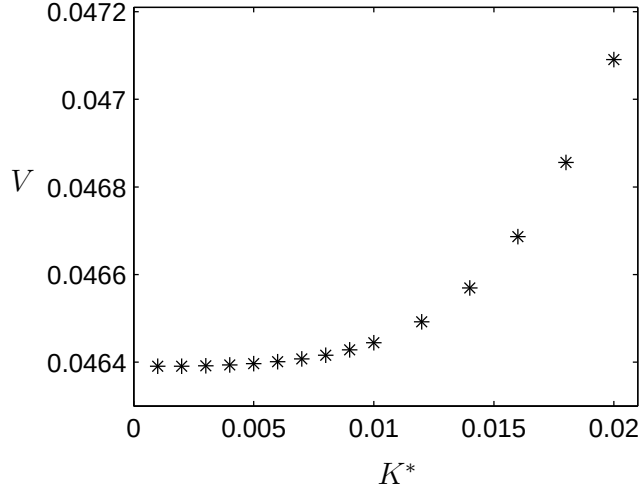


Figure 3.21: Velocity of steady, travelling-wave solutions as a function of polymerization speed K^* for $V^* = 1$, $\theta_F = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$, $\beta^* = 3$, $t_R = 50$, $\theta_L = 0.01$, $\theta_E = 0.02$ and $\theta_R = 0.03$.

boundaries for larger K^* . Retrograde flow occurs almost everywhere in the strip with a small region of forward flowing network just behind the peak in network volume fraction. The force exerted by the travelling strip, shown in Figure 3.22(c), is in the direction of motion at either end of the strip and reversed in the interior. The magnitude of the force at the rear is reduced as K^* increases. Figure 3.22(d) shows how the total internal mixture stress obtained at steady state varies with K^* .

3.2.2.5 Effect of adhesion strength

To find out if this model displays the bell-shaped dependence of crawling speed on adhesion strength seen in the minimal model of Chapter 2, we seek steady travelling-wave solutions numerically by continuation in β^* . As in the minimal model we find families of travelling-wave solutions that co-exist in parameter space. The network profiles are reminiscent of the one, two and three peak solutions obtained in Chapter 2, but all also have a peak at the front of the travelling strip. However, to be consistent with the rest of this chapter we count ‘corners’ as opposed to peaks. The one ‘corner’ family of solutions has a high network volume fraction at both the front and rear of the travelling strip, with a ‘corner’ at the back of the front peak. The two and three ‘corner’ families of solutions have one and two interior peaks respectively in their network profiles. It is clear from Figure 3.23 that the family of solutions with only one ‘corner’ in its actin network has the desired bell-shaped dependence

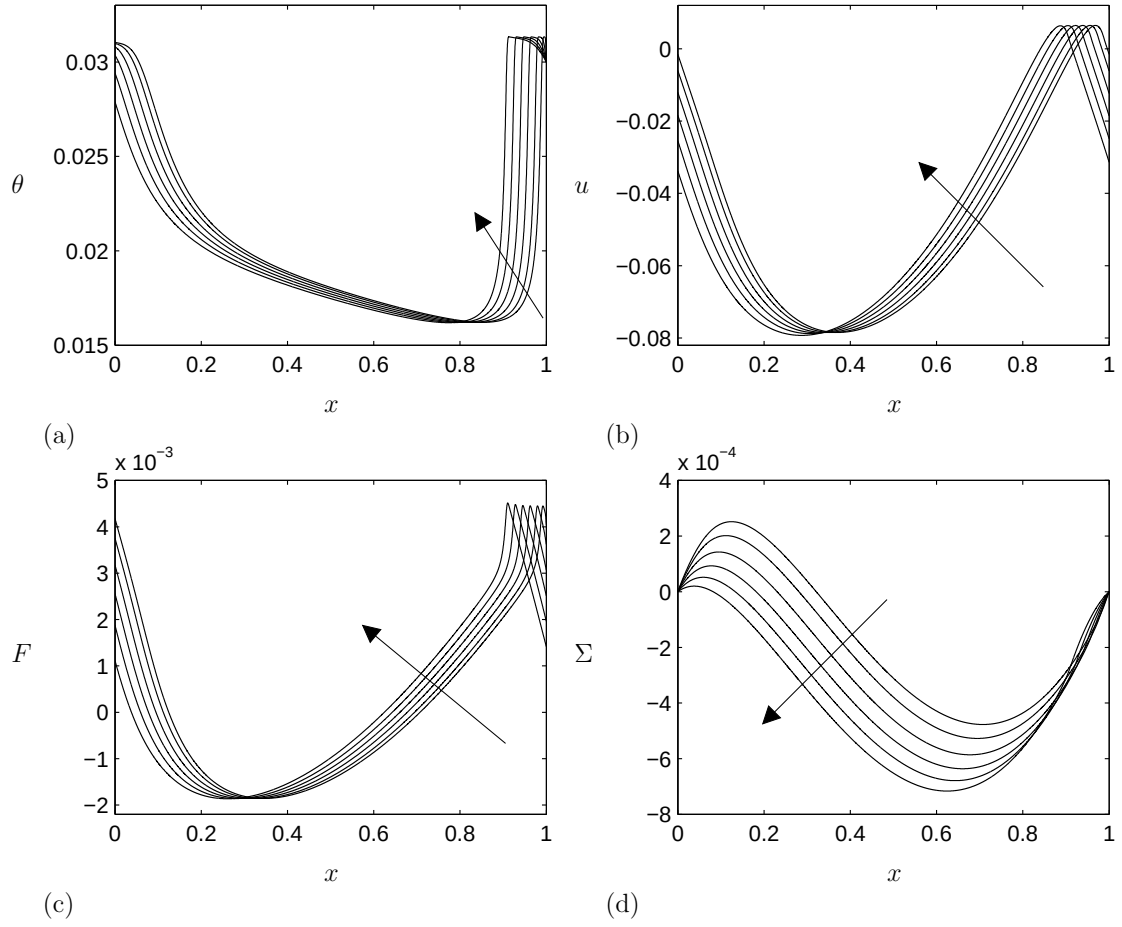


Figure 3.22: (a) network volume fraction, (b) network velocity, (c) force exerted by cell and (d) mixture stress profiles for some of the solutions in Figure 3.21. The K^* values used are 0.001, 0.004, 0.008, 0.012, 0.016 and 0.02, and arrows identify increasing K^*

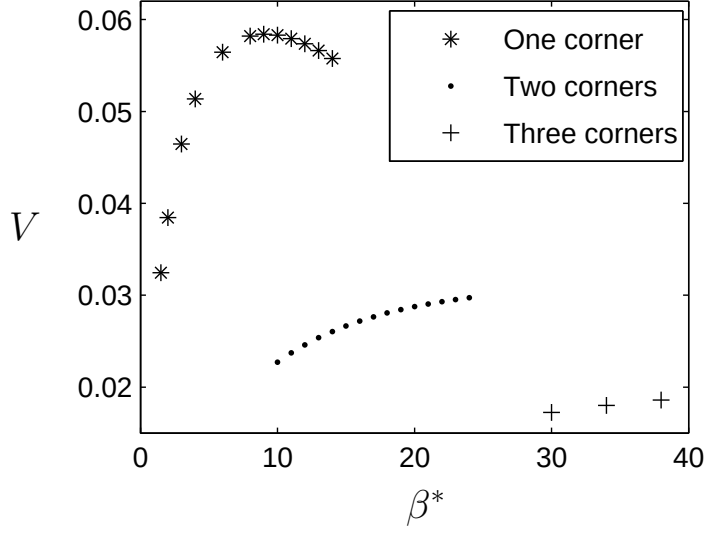


Figure 3.23: Velocity of the travelling-wave solutions as a function of adhesion strength β^* , with the parameters $K^* = 0.01$, $V^* = 1$, $\theta_F = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$, $t_R = 50$, $\theta_L = 0.01$, $\theta_E = 0.02$ and $\theta_R = 0.03$. Each solution was computed on two grids, one with twice as many spatial elements as the other, for a number of different grids ranging from 2000 to 16000 spatial elements. The largest relative error in network volume fraction calculated on consecutively nested grids was less than 4.1×10^{-3} . The network profiles associated with each family of travelling-wave solutions are shown in Figure 3.24.

on adhesion. Examples of the profiles associated with each family are shown in Figure 3.24, showing that for each family as β^* increases the peaks in the actin network steepen.

3.3 Chapter summary

In this chapter we have presented two alternative adaptations of the minimal model from Chapter 2. In each case boundary conditions have been used to model polymerization or depolymerization occurring in a small region next to the cell membrane. In the first model, a constant polymerization speed for the actin network was imposed on the boundary at $x = b(t)$. The second model prescribes velocity dependent polymerization (depolymerization) at the front (back) of the travelling strip. Some analytical progress was made for the first model: we found a steady state with a boundary layer structure at $x = 1$ for small polymerization. This state only exists for parameters that make the spatially uniform, stationary, steady state in the minimal model stable. This exercise highlighted the delicate nature of the problem and the need for compatible boundary conditions, and provided a valuable check for the numerical simulations. Both models were investigated numerically

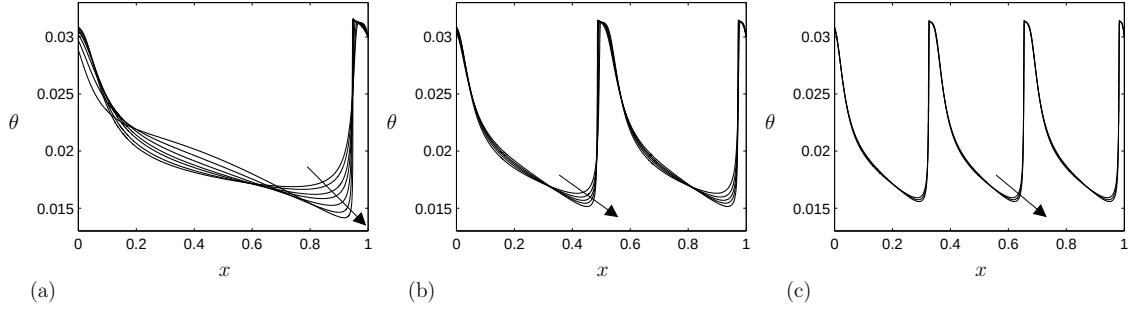


Figure 3.24: Network volume fraction profiles corresponding to some of the crawling speeds displayed in Figure 3.23 for profiles with: (a) one corner, the β^* values displayed are 1.5, 2, 3, 4, 6, 10 and 14; (b) two corners, the β^* values displayed are 10, 13, 16, 20 and 24 and (c) three corners, the β^* values displayed are 30, 34 and 38. Arrows indicate increasing β^* .

with a modified version of the code used for the minimal model.

Resolving numerically the rapidly changing regions of actin volume fraction in both models is computationally expensive, particularly due to the movement of peaks in the actin network in the transient solutions. The numerical solution displayed in Figure 3.17, on a grid with 4000 spatial elements and a timestep of 2×10^{-4} took approximately 17 hours to compute on a desktop machine with an AMD Athlon 64 X2 processor with speed 2.7GHz and 1.84GB of RAM. Sufficient temporal resolution places a stringent restriction on the time step, particularly in the velocity dependent polymerization model. It seems likely this is largely due to the explicit implementation of the boundary condition in this model. An implicit implementation was attempted, but each time step required many Newton iterations to deal with the added nonlinearity and was found to be slower than the explicit implementation of the boundary conditions. Investigating steady, travelling-wave solutions reduces the need for such a high degree of temporal resolution and is also biologically interesting. With finite time and resources, the travelling-wave solutions displayed in this chapter are by no means an exhaustive collection, though we believe we have captured the majority of the biologically plausible cases.

For the constant polymerization model in §3.1, the general trends observed include that it is possible to generate biologically relevant actin network profiles that peak at the front and rear of the travelling strip when we impose a sufficiently large network volume fraction at the right-hand side of the strip. The solutions with a narrow region of higher than equilibrium actin volume fraction are very similar to cells described in §1.1.3, which have a narrow band of depolymerization where the lamellipodium meets the lamellum. The

network mainly undergoes retrograde flow except in a small region located just behind ‘corners’ in the actin network, where the network stalls or flows forwards. Similarities can be drawn between this and experimentally observed convergence zones in the actin network, also discussed in §1.1.3.

The force profiles vary from almost linear with a negative gradient to profiles that represent force in the direction of motion at the front and rear of the travelling strip, with a reversed direction in the interior. These two force patterns are reminiscent of those observed experimentally in different cells and discussed in §1.1.5. Local peaks in the force exerted on the substrate are associated with ‘corners’ in the actin network. The overall trends observed are that the crawling velocity of the strip increases with the flux of network into the strip, K , and decreases with the network volume fraction, θ_F , imposed at the boundary. The numerical investigation into the effect of adhesion strength indicates that, as in the minimal model, a ‘bell-shaped’ dependence of crawling speed on adhesion strength can be observed in qualitatively distinct families of travelling-wave solutions that co-exist in parameter space. This behaviour is strongly modulated by the polymerization parameters θ_F and K .

Similar general trends to those observed in the constant polymerization model are seen in the velocity dependent model of §3.2. The speed of the travelling strip increases with K^* and we recover much of the same ‘bell-shaped’ dependence of crawling speed on adhesion strength as seen in the minimal model. The main modification to the minimal model network profiles is that the network volume fraction peaks at the front of the travelling strip when we allow velocity dependent polymerization on the boundaries. The network velocity profiles found exhibit retrograde flow except in a small region behind the ‘corner’ in the actin network. All the steady travelling-wave force profiles display a force exerted on the substrate in the direction of motion at the front and rear of the cell and associated with interior peaks in the network volume fraction. In between these regions the force exerted on the substrate is opposite to the direction of motion. We are not currently aware of any experimental evidence measuring force exerted on the substrate under a crawling cell with sufficient resolution to capture peaks in forces associated with regions of high actin volume fraction. It would be interesting to see if similar force profiles are associated with, say, fibrillar adhesions.

The main advantage of the velocity dependent polymerization model is that the direction

of motion is not imposed, *a priori*, so we are able to simulate solutions where the travelling-strip changes direction. We can also investigate a cell responding to a small perturbation to the equilibrium volume fraction without needing to also restrict $\theta_F - \theta_E$ to be small: see for example the simulations in §3.2.2.3. The model determines its own direction providing insight into how cells polarize and select a direction in which to travel.

The solution to the constant polymerization model displayed in Figure 3.15 is an actin network that is organised into two apparently distinct regimes in the front and back parts of the travelling strip. This is particularly interesting because regions of different actin behaviour have been observed biologically, most notably actin ruffles are typically confined to the lamellum and there is a higher volume fraction of actin in the lamellum and lamellipodium than in the cell body as discussed in §1.1.3. We could even draw similarities between the numerical solution and the actin staining in Figure 1.2(a), which depicts a high actin density at the front of the fragment, small localised spots of high actin density in the region of lower actin density behind the front, and a very narrow band of high actin density at the rear of the fragment. The temptation in observing different behaviours in different regions of a crawling cell is to assume that different biology or physics governs the different regions and to write down different models for the perceived separate compartments and some boundary condition between the compartments. See for example [5], where the authors use a two-phase flow framework to describe just the cell lamellipodium and impose different conditions on different boundaries to model the interaction between: the lamellipodium and the substrate; the lamellipodium and the surrounding media; and the lamellipodium and the cell body. Here we see it is possible for the two-phase, poroviscous, reactive flow framework to generate these types of feature spontaneously. It is also interesting that even imposing polymerization at the right-hand side of the travelling strip is not enough to force this to be the front of the travelling strip as illustrated in Figure 3.16. This may have implications for the modelling of retracting filaments at the front of protruding cells.

Both of the models presented in this chapter highlight the versatility of the two-phase, poroviscous, reactive, flow framework for modelling cell motility. A more sophisticated model of cell polarization or membrane dynamics, including a description of the auxiliary proteins that regulate the actin network, could be used to provide polymerization speeds and network volume fractions for boundary conditions. A more mathematically motivated

avenue of further investigation would be to consider the effect of instantaneously imposing a switch in the network volume fraction when the crawling strip changes direction and a boundary condition for the network volume fraction is ‘switched on’ in the velocity dependent polymerization model. For now, however, we proceed to consider the effect of a viscoelastic actin network in Chapter 4.

Chapter 4

Investigating the effect of network rheology

As detailed in the literature review in §1.2 a range of different continuum descriptions of cells and their components have previously been employed. In the minimal model of Chapter 2 we claim that, on timescales relevant for crawling, the actin network can be reasonably described as a viscous fluid. In this chapter we revisit this assumption by considering the effect of a viscoelastic model for the actin network.

We first formulate our one-dimensional, two-phase, reactive, poroviscoelastic flow model in §4.1. In §4.1.7 we consider a potential hodograph transform of the viscoelastic model. Results of a stability analysis are presented in §4.2. We show in §4.3 that in the limit in which perturbations to the spatially uniform stationary state grow slowly it is possible to make a prediction about the direction the travelling strip moves in for some initial conditions. Finally, in §4.4, we solve the viscoelastic model numerically and investigate the effect of the Deborah number (the ratio of the elastic relaxation timescale to the observation timescale) and adhesion strength. We demonstrate that the model exhibits steady travelling-wave solutions which are qualitatively similar to the one peak solutions of the minimal model. The bell-shaped dependence on adhesion strength is also retained in our viscoelastic model.

4.1 Model formulation

Viscoelastic substances can respond to displacements both as elastic solids, by resisting deformation, and as fluids by resisting rate of deformation. Typically these responses happen on different timescales, although the modeller is particularly fortunate if the response timescales are well separated. Many different constitutive laws exist for different types

of viscoelastic materials. The basic idea in constructing a mathematical description of a viscoelastic material is to couple fluid elements and elastic elements in series, parallel or combinations of these to create a viscoelastic element with the correct properties [80]. Modellers must make appropriate choices based on rheological experiments. Unfortunately, characterising cell rheology is particularly difficult because cells can change their material properties in response to mechanical tests, altering the very properties experimentalists are attempting to measure [112]. As discussed in [112], a single type of cell can be usefully modelled in a number of apparently conflicting ways depending on the lengthscales, timescales and cell behaviours that are being investigated.

Experimental evidence suggesting that the actomyosin network in cells is short-time (seconds) elastic and long-time viscous has been discussed in §1.1.4 and §2.1.1. Therefore our viscoelastic model should behave as an elastic solid on short timescales and as a viscous fluid on long timescales. We focus on the simplest possible viscoelastic constitutive law with this property. In particular, we will restrict our model to have only one viscoelastic relaxation timescale and we will employ an upper-convected Maxwell model to describe the rheology of the actin network. These modelling assumptions are supported by [88] who use a convected Maxwell model to describe the actin network, although their governing equations are more complicated because the rheology is coupled to a polarization field for the actin fibres. The lamellipod is described as an upper-convected Maxwell fluid in [131]. Both these models are single phase. For further justification of our modelling assumptions we appeal to models developed in [94] and [121]. They describe a hydrated collagen network as a two-phase fluid, where the collagen network is modelled as an upper-convected Maxwell fluid. In [94] the model predictions are compared to measurements from a simple experimental set-up and found to be in good agreement.

4.1.1 Mass and momentum conservation

We continue to work in the one-dimensional, reactive, two-phase flow framework used for the minimal model in Chapter 2. Continuity of the network and solution phases is given by (2.1), where θ is the network volume fraction, u is the network velocity and v is the solution velocity. The force balance on the solution is as stated in (2.2b), whilst the force balance on the network becomes

$$-\theta \frac{\partial p}{\partial x} - \frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x}(\theta \sigma) = H(u - v) + \beta u, \quad (4.1)$$

where $\sigma(x, t)$ is now the network stress and all other variables are as they were in the minimal model. Following [121], we employ the upper-convected Maxwell constitutive equation for the network stress by setting

$$\frac{1}{2G} \left(\frac{\partial \sigma}{\partial t} + u \frac{\partial \sigma}{\partial x} - 2\sigma \frac{\partial u}{\partial x} \right) + \frac{\sigma}{2\mu_n} = \frac{\partial u}{\partial x} + \left(\frac{\nu}{1-2\nu} \right) \frac{\partial u}{\partial x}, \quad (4.2)$$

where G is the shear modulus, μ_n is the shear viscosity and ν is the Poisson ratio of the network. Summing (2.2b) and (4.1) we find the force balance on the two-phase mixture is given by

$$\frac{\partial \Sigma}{\partial x} = \beta u, \quad (4.3)$$

where Σ , the total mixture stress, is given by

$$\Sigma = -p - \Psi + \theta \sigma, \quad (4.4)$$

cf. (2.3) and (2.4). To close the system we require constitutive laws for J , Ψ , H , β , G , μ_n and ν . Wherever possible we shall treat J , Ψ , H and β as prescribed arbitrary functions of θ . Where it is necessary to specify the constitutive laws, for example in numerical simulations, we shall use the same expressions for J , Ψ , H and β as in the minimal model, given by (2.5), (2.7) and (2.9b,c). Following our ‘simplest problem first’ philosophy, we assume throughout this chapter that G , μ_n and ν are constant, although in reality they could depend on a number of things including: the network volume fraction θ ; the network stress; cross-linking molecules in the network; or biochemical signalling molecules in the cell. We note that assuming μ_n is constant is equivalent to imposing a constitutive law of the form (2.9a) in the minimal model and postpone further comparison of the viscoelastic and viscous models until §4.1.7 and §4.2.5.

4.1.2 Boundary and initial conditions

In order to close the viscoelastic model described in §4.1.1 we must impose boundary and initial conditions. We seek to solve the governing equations (2.1), (2.2b), (4.1) and (4.2) on the strip $a(t) \leq x \leq b(t)$, which is aligned with the direction of cell motion, so that as in Chapter 2 the strip moves and $a(t)$ and $b(t)$ must be determined as part of the solution. Following §2.1.3 we impose no flux of network or solution and no mixture stress at the ends of the strip

$$\text{at } x = a(t), \quad \theta(u - \dot{a}) = 0, \quad (1 - \theta)(v - \dot{a}) = 0, \quad \Sigma = 0, \quad (4.5a-c)$$

$$\text{at } x = b(t), \quad \theta(u - \dot{b}) = 0, \quad (1 - \theta)(v - \dot{b}) = 0, \quad \Sigma = 0. \quad (4.6a-c)$$

The system also requires initial conditions

$$a(0) = 0, \quad b(0) = L; \quad \text{for } 0 < x < L, \quad \theta(x, 0) = \theta^*(x), \quad \sigma(x, 0) = \sigma^*(x); \quad (4.7a-c)$$

where L is the initial length of the domain and θ^* and σ^* are the initial network volume fraction and stress respectively.

4.1.3 Reduced system

Introducing the mixture velocity defined in (2.13) we can eliminate the pressure p and the solution velocity v , as in §2.1.4, to obtain the governing equations

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad -\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x}(\theta \sigma) = \alpha(u - V) + \beta u, \quad (4.8a, b)$$

and (4.2), subject to

$$u(a(t), t) = V(t), \quad u(b(t), t) = V(t), \quad (4.9a, b)$$

where $a(t)$ is given by (2.18). Finally we obtain the solvability condition for $V(t)$ by integrating the mixture force balance (4.3) and applying the no stress boundary conditions (4.5c) and (4.6c), to obtain the global force balance given in (2.20).

4.1.4 Nondimensionalization

We nondimensionalize the variables as follows

$$x = Lx^\dagger, \quad t = Tt^\dagger, \quad u = \frac{L}{T}u^\dagger, \quad V = \frac{L}{T}V^\dagger, \quad \sigma = \frac{2\mu_1}{T}\sigma^\dagger, \quad a = La^\dagger, \quad b = Lb^\dagger, \quad (4.10)$$

where L is the initial length of the strip and T is the timescale for network turnover. Noting the form of the viscous term in (4.2) the scaling for the network viscosity is chosen so that

$$\mu_n = \mu_1 \left(\frac{1 - 2\nu}{1 - \nu} \right). \quad (4.11)$$

The magnitude of the network viscosity is contained in μ_1 , with a correction for the network Poisson ratio. We rescale the remaining constitutive equations

$$J = \frac{1}{T}J^\dagger, \quad \Psi = \frac{2\mu_1}{T}\Psi^\dagger, \quad \alpha = \frac{2\mu_1}{L^2}\alpha^\dagger, \quad \beta = \frac{2\mu_1}{L^2}\beta^\dagger, \quad (4.12)$$

where a dagger denotes a dimensionless quantity.

4.1.5 Travelling frame

As in the minimal model we move to a frame that travels with the strip at speed $V(t)$. We make the transformation given in (2.24) along with

$$\sigma^\dagger(x^\dagger, t^\dagger) = \hat{\sigma}(\hat{x}, t^\dagger), \quad (4.13)$$

in the nondimensional versions of (2.18), (2.20), (4.2) and (4.7)–(4.9). After dropping hats and daggers, the governing equations to be solved on $0 \leq x \leq 1$ for $t > 0$ are given by

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad (4.14)$$

$$-\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x}(\theta \sigma) = \alpha u + \beta(u + V), \quad (4.15)$$

$$D \left(\frac{\partial \sigma}{\partial t} + u \frac{\partial \sigma}{\partial x} - 2\sigma \frac{\partial u}{\partial x} \right) + \sigma = \frac{\partial u}{\partial x}, \quad (4.16)$$

where $D = \mu_1(1-2\nu)/(GT(1-\nu))$, the dimensionless ratio of the network's elastic relaxation timescale to the network turnover timescale. These are subject to the boundary and initial conditions

$$\text{for } t > 0, \quad u(0, t) = u(1, t) = 0; \quad \text{for } 0 \leq x \leq 1, \quad \theta(x, 0) = \theta^*(x), \quad \sigma(x, 0) = \sigma^*(x), \quad (4.17)$$

and the solvability condition

$$\int_0^1 \beta(u + V) dx = 0. \quad (4.18)$$

The nondimensional constitutive laws for J , Ψ , α and β are the same as those used in the minimal model, restated here for convenience

$$J(\theta) = \theta - \theta_E, \quad \Psi'(\theta) = \Psi^* \frac{(\theta - \theta_L)(\theta - \theta_R)}{(1 - \theta)}, \quad (4.19a, b)$$

together with

$$\alpha(\theta) = \alpha^* \theta / (1 - \theta), \quad \beta(\theta) = \beta^* \theta. \quad (4.20a, b)$$

However, the expressions for the dimensionless parameters have been modified as follows

$$\Psi^* = \frac{\Psi_0 T}{2\mu_1}, \quad \alpha^* = \frac{H_0 L^2}{2\mu_1}, \quad \beta^* = \frac{\beta_0 L^2}{2\mu_1}. \quad (4.21a-c)$$

In addition to these three dimensionless parameters and the dimensionless network parameters θ_L , θ_E and θ_R , the viscoelastic model has one more parameter than the viscous model in Chapter 2, namely the dimensionless Deborah number D .

4.1.6 Dimensionless parameters

The Deborah number D is the ratio of the elastic relaxation timescale of the network, to the timescale of observation [80], where we choose to observe the cell on a timescale T . This is reasonable because T is the order of minutes and the cells crawl on the order of microns to tens of microns per minute. When D is small the network behaves as a viscous fluid and when D is large the network behaves as an elastic solid. We note that setting $D = 0$, $\nu = 0$ and $\mu_1 = \mu_0/2$ in (4.14)–(4.21) we recover the minimal model of (2.25)–(2.31) in which the network is purely viscous. As a point of clarification, in Chapter 2 μ represents an effective viscosity which includes the dependence of viscosity on the network volume fraction. In this chapter, we see explicitly that viscous terms are weighted by the volume fraction in the expression for σ , the network stress.

Estimates for the viscoelastic relaxation timescale of the actin–myosin network of at most a few seconds can be found in [12, 95, 154]. If we take, as an upper bound, $\mu_n/G = 6$ seconds, this leads to a value for our Deborah number of $D = 0.1$. We note that in our one-dimensional framework, the shear and bulk contributions to the viscosity cannot be distinguished, as shown by (4.11). Therefore, in the absence of reliable estimates for the Poisson ratio of the actin network, ν , and noting the large range of experimental values for μ_0 in Table 2.1, we consider a reasonable range of values for μ_1 in (4.20) to be 10^{-4} – 1 kPa min. Typical values for Ψ^* , α^* and β^* are then the same as in Chapter 2.

4.1.7 Potential hodograph transformation

The potential hodograph transformation of the governing equations in §2.1.8.1 was useful in displaying which terms in the model were stabilising or destabilising and also which terms can be understood as nonlocal. We perform a similar analysis here, seeking similar insight, into the nature of the viscoelastic governing equations (4.14)–(4.16). Following §2.1.8.1, we introduce an integrated mass variable

$$\rho = \int_0^{\chi(\rho,t)} \theta(\xi, t) d\xi, \quad \chi(0, t) = 0. \quad (4.22a, b)$$

Next we transform all the dependent variables

$$\theta(\chi(\rho, t), t) = \Theta(\rho, t), \quad u(\chi(\rho, t), t) = U(\rho, t), \quad \sigma(\chi(\rho, t), t) = \Lambda(\rho, t), \quad (4.23a-c)$$

and introduce ϕ and I as defined in (2.37) to obtain after some manipulation

$$\frac{\partial U}{\partial \rho} = \frac{\partial \phi}{\partial t} + \frac{\partial I}{\partial \rho}, \quad (4.24)$$

$$U = A(\phi) \frac{\partial \phi}{\partial \rho} + B(\phi) \frac{\partial}{\partial \rho} \left(\frac{\Lambda}{\phi} \right) - C(\phi) V, \quad (4.25)$$

$$\frac{1}{D\phi} \frac{\partial U}{\partial \rho} = \frac{\partial \Lambda}{\partial t} + \frac{I}{\phi} \frac{\partial \Lambda}{\partial \rho} + \left(\frac{1}{D} - \frac{2}{\phi} \frac{\partial U}{\partial \rho} \right) \Lambda, \quad (4.26)$$

where

$$A(\phi) = \frac{\Psi'(1/\phi)}{\phi^3 (\alpha(1/\phi) + \beta(1/\phi))}, \quad B(\phi) = \frac{1}{\phi (\alpha(1/\phi) + \beta(1/\phi))}, \quad C(\phi) = \frac{\beta(1/\phi)}{(\alpha(1/\phi) + \beta(1/\phi))}. \quad (4.27a-c)$$

It is not clear how (4.24)–(4.26) can be combined to eliminate U and Λ , so we simplify the problem by considering the no polymerization/depolymerization case where $J = 0$. This means that $I = 0$ and χ becomes a Lagrangian variable. The transformed viscoelastic stress balance becomes

$$\frac{\partial \Lambda}{\partial t} + E\Lambda = F, \quad (4.28)$$

where

$$E(\rho, t) = \frac{1}{D} - \frac{2}{\phi} \frac{\partial \phi}{\partial t}, \quad F(\rho, t) = \frac{1}{D\phi} \frac{\partial \phi}{\partial t}, \quad (4.29a,b)$$

which has the solution

$$\begin{aligned} \Lambda = & \left[\exp \left(\int_0^t -E(\rho, \eta) d\eta \right) \right] \int_0^t \exp \left(- \left(\int_0^\xi -E(\rho, \eta) d\eta \right) \right) F(\rho, \xi) d\xi \\ & + \Lambda^*(\rho) \exp \left(\int_0^t -E(\rho, \eta) d\eta \right), \end{aligned} \quad (4.30)$$

where $\Lambda^*(\rho) = \Lambda(\rho, 0)$ is the initial condition for Λ obtained from σ^* . After manipulating we find that

$$\Lambda = \phi^2 e^{-t/D} \left(\frac{\Lambda^*}{\phi^{*2}} + \frac{1}{D} \int_0^t \frac{e^{\xi/D}}{\phi^3} \frac{\partial \phi}{\partial \xi} d\xi \right), \quad (4.31)$$

where $\phi^*(\rho) = \phi(\rho, 0)$ is obtained from θ^* , the initial condition for the network volume fraction. Now combining (4.24) and (4.25), along with $I = 0$, we see that

$$\frac{\partial \phi}{\partial t} = \frac{\partial}{\partial \rho} \left(A(\phi) \frac{\partial \phi}{\partial \rho} + B(\phi) \frac{\partial}{\partial \rho} \left(\frac{\Lambda}{\phi} \right) - C(\phi) V \right). \quad (4.32)$$

Finally we can substitute for Λ using (4.31) to obtain an integro-partial-differential equation for ϕ , viz.

$$\frac{\partial \phi}{\partial t} = \frac{\partial}{\partial \rho} \left(A(\phi) \frac{\partial \phi}{\partial \rho} + B(\phi) \frac{\partial}{\partial \rho} \left(\phi e^{-t/D} \left(\frac{\Lambda^*}{\phi^{*2}} + \frac{1}{D} \int_0^t \frac{e^{\xi/D}}{\phi^3} \frac{\partial \phi}{\partial \xi} d\xi \right) \right) - C(\phi) V \right), \quad (4.33)$$

which depends on one extra unknown, $V(t)$, which would be determined by a transformed version of the integral constraint (4.18).

When the network volume fraction is in the contractile (swelling) regime $A < 0$ ($A > 0$). Therefore, as in the viscous model, the potential hodograph transform is informative in identifying that contraction of the network can be viewed as a backwards diffusion term. The key difference between (2.42) and (4.33) is that here we see the effect of the viscoelastic network rheology as a term which is nonlocal in time. We also see that the timescale over which the network's flow history effects the current network configuration decreases as $D \rightarrow 0$. In fact in the limit $D \rightarrow 0$, if we assume that $1/\phi$ and the first three time derivatives of ϕ are bounded, then integrating by parts reveals that (4.33) becomes

$$\frac{\partial \phi}{\partial t} = \frac{\partial}{\partial \rho} \left(A(\phi) \frac{\partial \phi}{\partial \rho} + B(\phi) \frac{\partial}{\partial \rho} \left(\frac{1}{\phi^2} \frac{\partial \phi}{\partial t} \right) - C(\phi) V \right). \quad (4.34)$$

Note that this is in agreement with the potential hodograph transformed equation (2.42) which describes the viscous model for $J = 0$, once we account for the difference in definition of viscosity between the two models. Having gained this understanding of the nature of the governing equations we proceed to consider a stability analysis.

4.2 Linear stability analysis

Here we consider the stability of the model's spatially uniform steady state. We investigate how the model responds to a small perturbation of the network volume fraction and network stress about their respective equilibrium values. We seek insight into the polarization of motile cells and the determination of crawling direction. We wish to understand how the results of the linear stability analysis of the viscous model presented in §2.2 are modified by prescribing a viscoelastic rheology for the actin network.

4.2.1 Unbounded domain

On an infinite domain $-\infty < x < \infty$, the spatially uniform steady state $u = u_E$, $V = V_E$, $\theta = \theta_E$, $\sigma = \sigma_E$ satisfies (4.14)–(4.16) if and only if $J_E = 0$, $\sigma_E = 0$ and $\alpha_E u_E + \beta_E (u_E + V_E) = 0$, where we use our usual notation of a subscript E on a function to indicate that the function is evaluated at the equilibrium network volume fraction. We consider a small, order $\varepsilon \ll 1$, perturbation to the steady state by seeking a solution to (4.14)–(4.16) of the form

$$u \sim u_E + \varepsilon u_1 + \dots, \quad V \sim V_E + \varepsilon V_1 + \dots, \quad \theta \sim \theta_E + \varepsilon \theta_1 + \dots, \quad \sigma \sim \varepsilon \sigma_1 + \dots \quad (4.35a-d)$$

At $O(\varepsilon)$ we obtain the following linearized system of equations

$$\frac{\partial \theta_1}{\partial t} + u_E \frac{\partial \theta_1}{\partial x} + \theta_E \frac{\partial u_1}{\partial x} = -J'_E \theta_1, \quad (4.36)$$

$$-\Psi'_E \frac{\partial \theta_1}{\partial x} + \theta_E \frac{\partial \sigma_1}{\partial x} = \alpha_E u_1 + \beta_E (u_1 + V_1) + \left(\alpha'_E u_E + \beta'_E (u_E + V_E) \right) \theta_1, \quad (4.37)$$

$$D \left(\frac{\partial \sigma_1}{\partial t} + u_E \frac{\partial \sigma_1}{\partial x} \right) + \sigma_1 = \frac{\partial u_1}{\partial x}, \quad (4.38)$$

where a prime ' on a function denotes differentiation of that function with respect to its argument θ . Letting $P = \alpha'_E u_E + \beta'_E (u_E + V_E)$ we can eliminate u_1 and σ_1 to obtain the third-order partial differential equation

$$\begin{aligned} & \left[D \left(\frac{\partial}{\partial t} + u_E \frac{\partial}{\partial x} \right) + 1 \right] \left(\frac{\alpha_E + \beta_E}{\theta_E} \left(J'_E \theta_1 + u_E \frac{\partial \theta_1}{\partial x} + \frac{\partial \theta_1}{\partial t} \right) - P \frac{\partial \theta_1}{\partial x} - \Psi'_E \frac{\partial^2 \theta_1}{\partial x^2} \right) \\ &= \frac{\partial^2}{\partial x^2} \left(J'_E \theta_1 + u_E \frac{\partial \theta_1}{\partial x} + \frac{\partial \theta_1}{\partial t} \right). \end{aligned} \quad (4.39)$$

The highest order derivatives are $\partial^3 \theta_1 / \partial x^3$ and $\partial^3 \theta_1 / \partial x^2 \partial t$ along with a second time derivative $\partial^2 \theta_1 / \partial t^2$. If we consider $\theta_1 \propto \text{Re}[\exp(ikx + \Omega(k)t)]$ for real k , then a perturbation with wavenumber k grows at the rate $\text{Re}[\Omega(k)]$, where Ω is given by

$$\Omega^2 + b\Omega + c = 0, \quad (4.40)$$

for $D \neq 0$ and $\alpha_E + \beta_E \neq 0$, with coefficients given by

$$b = J'_E + \frac{1}{D} + 2iku_E - \frac{Pik\theta_E}{\alpha_E + \beta_E} + \frac{\theta_E \Psi'_E k^2}{\alpha_E + \beta_E} + \frac{\theta_E k^2}{D(\alpha_E + \beta_E)}, \quad (4.41)$$

$$\begin{aligned} c = & \frac{J'_E}{D} + ik \left(J'_E u_E + \frac{u_E}{D} - \frac{P\theta_E}{D(\alpha_E + \beta_E)} \right) + ik^3 \left(\frac{u_E \theta_E}{\alpha_E + \beta_E} \right) \left(\frac{1}{D} + \Psi'_E \right) \\ & + k^2 \left(\frac{P\theta_E u_E}{\alpha_E + \beta_E} - u_E^2 + \frac{\theta_E}{D(\alpha_E + \beta_E)} [\Psi'_E + J'_E] \right). \end{aligned} \quad (4.42)$$

4.2.2 Characterising growth rates for a stationary base state

The coefficients of the characteristic equation are dramatically simplified by setting $u_E = V_E = 0$. When we introduce the boundary conditions this stationary steady state will be selected. Motivated by this, we proceed to analyse this case. We characterize the growth rates of perturbations to a stationary strip. They are governed by (4.40) where the coefficients are now given by

$$b(k) = J'_E + \frac{1}{D} + \frac{\theta_E}{\alpha_E + \beta_E} \left[\Psi'_E + \frac{1}{D} \right] k^2, \quad c(k) = \frac{J'_E}{D} + \frac{\theta_E (\Psi'_E + J'_E)}{D(\alpha_E + \beta_E)} k^2. \quad (4.43a,b)$$

A perturbation with wavenumber k has the growth rates $Re(\Omega_{\pm}(k))$, where

$$\Omega_{\pm}(k) = \frac{-b \pm \sqrt{b^2 - 4c}}{2}. \quad (4.44)$$

Manipulating the discriminant $\Delta = b^2 - 4c$ we find that

$$\Delta = \left(\frac{1}{D} - J'_E\right)^2 + \frac{2\theta_E}{\alpha_E + \beta_E} \left(\frac{1}{D} - J'_E\right) \left(\frac{1}{D} - \Psi'_E\right) k^2 + \left(\frac{\theta_E}{\alpha_E + \beta_E}\right)^2 \left(\frac{1}{D} + \Psi'_E\right)^2 k^4. \quad (4.45)$$

Assuming that the equilibrium volume fraction is chosen so that the kinetics drive the network volume fraction into the contractile regime, then $\Psi'_E < 0$. If $DJ'_E < 1$, then the discriminant is positive, there exist two distinct real roots for all wavenumbers and the largest growth rate is given by the positive choice of root in (4.44). If alternatively, $DJ'_E > 1$, then the k^2 coefficient in the discriminant is negative and the discriminant could be negative for some wavenumbers. Since the coefficient of the k^4 term in (4.45) is positive, the discriminant will be positive for sufficiently large k . If $\Delta(k) < 0$, then the growth rate of the perturbation is given by

$$Re[\Omega(k)] = -\frac{b}{2}. \quad (4.46)$$

The ratio DJ'_E , that compares the timescale for elastic relaxation of the network to the timescale for network turnover, has been identified as important in classifying the growth rate: we postpone a discussion of this observation until the end of the section.

In order to construct the shape of the growth rate curves we first note that if $DJ'_E < 1$, then

$$\Omega_+(0) = -J'_E, \quad \Omega_-(0) = -\frac{1}{D}, \quad (4.47a, b)$$

whereas, if $DJ'_E > 1$, then

$$\Omega_+(0) = -\frac{1}{D}, \quad \Omega_-(0) = -J'_E. \quad (4.48a, b)$$

To aid our analysis of the growth rates we introduce the notation $b = b_1 + b_2 k^2$, $c = c_1 + c_2 k^2$.

To look for extrema we set $d\Omega/dk^2 = 0$, which implies

$$b_2 = \pm \frac{(b_1 b_2 + b_2^2 k^2 - 2c_2)}{\sqrt{\Delta}}. \quad (4.49)$$

Since this can't be satisfied if $\Delta < 0$ (except trivially by $b_2 = c_2 = 0$), we proceed to consider $\Delta > 0$ and square both sides of (4.49) and rearrange to obtain

$$b_1 b_2 c_2 - b_2^2 c_1 - c_2^2 = 0, \quad (4.50)$$

which has no k dependence, implying that the growth rate curves have no extrema. Therefore, if $DJ'_E < 1$, then the growth rate curves are monotonic. However, if $DJ'_E > 1$, there is an interval of wavenumbers, k , for which $\Delta < 0$ and the real part of the growth rate may have discontinuous derivatives at either end of this interval.

Now we turn our attention to the growth rate curves for large k . Expanding (4.44) for large k we see that

$$\Omega_{\pm} = -\frac{b_1}{2} - \frac{b_2 k^2}{2} \pm \frac{|b_2| k^2}{2} \left(1 + \frac{b_1}{b_2 k^2} - \frac{2c_2}{b_2^2 k^2} + O(1/k^4) \right), \quad (4.51)$$

so, if $b_2 > 0$, then $\Omega_+ \rightarrow -c_2/b_2$ and $\Omega_- \sim -b_2 k^2$ as $k \rightarrow \infty$. Alternatively, if $b_2 < 0$, then $\Omega_+ \sim -b_2 k^2$ and $\Omega_- \rightarrow -c_2/b_2$ as $k \rightarrow \infty$.

We are now in a position to characterize the growth rates in terms of the parameter values. First we introduce

$$\Psi_a = -\frac{1}{D}, \quad \Psi_b = -J'_E, \quad (4.52a,b)$$

noting that $\Psi_a, \Psi_b < 0$, and also by choice of equilibrium network volume fraction $\Psi'_E < 0$. We consider $DJ'_E < 1$, where the elastic relaxation timescale is smaller than the network turnover timescale and note that this implies $\Psi_a < \Psi_b$ and $\Delta > 0$. The growth rate is monotonic, a spatially uniform perturbation is stable (see (4.47a)) and there exist three regimes based on the size of Ψ'_E . Firstly, if $\Psi'_E < \Psi_a$ then Ω_+ is positive and unbounded for large k , implying that the system is ill-posed. Secondly, if $\Psi_a < \Psi'_E < \Psi_b$ then Ω_+ is initially negative and grows monotonically to plateau at Ω_{∞} , where

$$\Omega_{\infty} = -\frac{c_2}{b_2} = \frac{1}{D} \left(\frac{\Psi_b - \Psi'_E}{\Psi'_E - \Psi_a} \right), \quad (4.53)$$

and $\Omega_{\infty} > 0$. This is the interesting case, similar to the minimal model, where all wavenumbers greater than some critical wavenumber, k_c are unstable but grow at a bounded rate. We can use (4.43b), (4.44) and (4.52b) to show that

$$k_c^2 = -\frac{J'_E(\alpha_E + \beta_E)}{\theta_E(\Psi'_E - \Psi_b)}. \quad (4.54)$$

Thirdly, if $\Psi_b < \Psi'_E$ then Ω_+ is initially negative and grows monotonically to Ω_{∞} , which is negative, so all wavenumbers are stable. Figure 4.1 depicts the roots of (4.44) in all three cases.

If $DJ'_E > 1$, so that the elastic relaxation timescale is longer than the network turnover timescale, we note that $\Psi_b < \Psi_a$ and again work through the three cases for Ψ'_E . We find

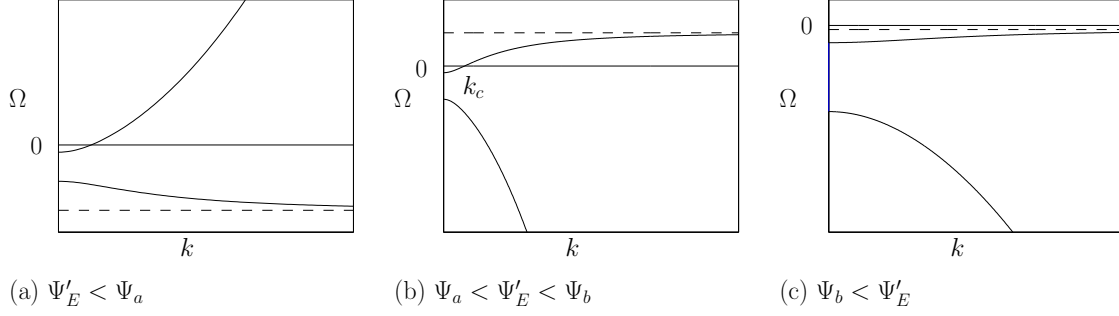


Figure 4.1: The roots of (4.44) are plotted as a function of k for $DJ'_E < 1$ for three cases distinguished by the size of Ψ'_E . In all three cases $\Omega_+(0) = -J'_E$, $\Omega_-(0) = -1/D$ and Ω_∞ , given by (4.53), is marked as a dashed line.

that in both the case where $\Psi'_E < \Psi_b$ and $\Psi_b < \Psi'_E < \Psi_a$ the growth rate, Ω_+ , increases proportionally to k^2 for large wavenumbers and the problem is ill-posed. In the final case, where $\Psi_a < \Psi'_E$, Ω_+ is negative at $k = 0$ (see (4.48a)) and tends to $\Omega_\infty < 0$ as $k \rightarrow \infty$, so that all wavenumbers are stable. All three of these situations are shown in Figure 4.2, along with the corresponding smaller root of (4.44).

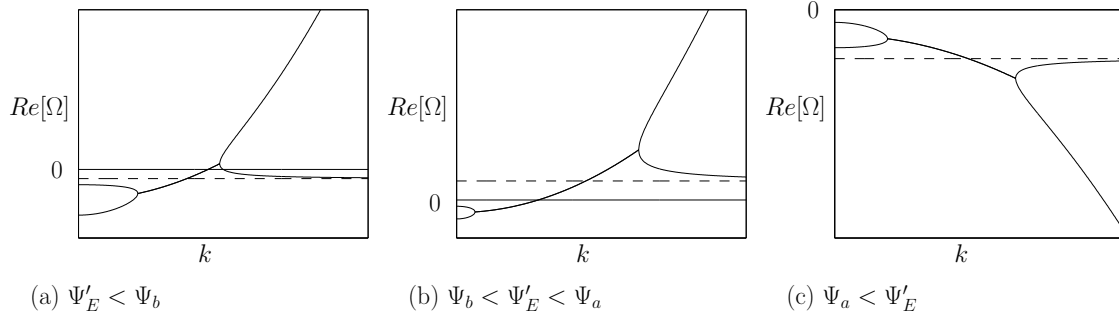


Figure 4.2: The real part of the roots of (4.44) are plotted as a function of k for $DJ'_E > 1$ for three cases distinguished by the size of Ψ'_E . In all three cases $\Omega_+(0) = -1/D$, $\Omega_-(0) = -J'_E$ and Ω_∞ , given by (4.53), is marked as a dashed line.

In the borderline case where $DJ'_E = 1$, we note that $\Psi_a = \Psi_b$ and the roots of (4.44) are

$$\Omega = -J'_E, \quad \Omega = -J'_E - \frac{\theta_E}{\alpha_E + \beta_E} (\Psi'_E - \Psi_a) k^2. \quad (4.55a,b)$$

If $\Psi'_E < \Psi_a$, then the second roots grows proportionally to k^2 and the problem is ill-posed. If $\Psi'_E > \Psi_a$, then both roots are negative for all k and the stationary steady state is stable.

In summary, for the problem to not be ill-posed we require $\Psi'_E > \Psi_a$; for the growth

rate to plateau to a positive value we require $\Psi'_E < \Psi_b$; for both these conditions to hold we need $\Psi_a < \Psi_b$, which is only possible if $DJ'_E < 1$, *i.e.* the elastic relaxation timescale is smaller than the network turnover time. This is a reasonable constraint on the parameter values for our model, *cf.* [26]. If the elastic relaxation timescale was long in comparison to the network turnover timescale, it would suggest a different, long-time elastic rheology for the actin network. This would require a different type of viscoelastic model. Thus the stability analysis demonstrates that in order for the model to not be ill-posed we must pick parameters consistent with the short-time-elastic assumption that underpins our upper-convected Maxwell constitutive law for the network stress. If the parameters are chosen so that $\Psi_a < \Psi'_E < \Psi_b$ then the maximum growth rate has the same form as that in the minimal model with $\Omega_\infty > 0$: *cf.* Figure 2.2(b) and Figure 4.1(b). We note that the plots in Figure 4.2 for $DJ'_E > 1$ display an interval in k where the roots of (4.44) are complex conjugate pairs. If we consider just the parameter sub-regime $\Psi_a < \Psi'_E$ that leads to a well-posed model (see Figure 4.2(c)), the stability analysis predicts that for a range of wavenumbers, perturbations to the stationary steady state will travel as they decay in amplitude. This could represent actin ruffles that travel over lamellipodia as described in §1.1.3.

4.2.3 Effect of boundary conditions

Now we restrict our stability analysis to the domain $0 \leq x \leq 1$ and ensure our perturbations satisfy the boundary conditions $u(0, t) = u(1, t) = 0$. Substituting (4.35) into (4.14)–(4.16) and imposing the boundary conditions on u we obtain (4.36)–(4.38) with $u_E = V_E = 0$, at $O(\varepsilon)$. We seek solutions of the form $\theta_1 = \theta_E + \varepsilon \hat{\theta}_1(x)e^{\omega t}$, $u_1 = \varepsilon \hat{u}_1(x)e^{\omega t}$, $\sigma_1 = \varepsilon \hat{\sigma}_1(x)e^{\omega t}$ and $V = \varepsilon \hat{V}_1 e^{\omega t}$, which after dropping hats and eliminating θ_1 and σ_1 , leads to a second-order, constant-coefficient, linear, differential equation for u_1 forced by V_1 , namely

$$\left[\frac{\theta_E \Psi'_E}{J'_E + \omega} + \frac{\theta_E}{D\omega + 1} \right] u_1'' - (\alpha_E + \beta_E) u_1 = \beta_E V_1, \quad (4.56)$$

for $J'_E + \omega \neq 0$ and $D\omega + 1 \neq 0$. (We note from the previous section that $J'_E + \omega = 0$ and $D\omega + 1 = 0$ only at $k = 0$.) We see that the viscoelastic formulation has produced a governing equation for perturbations to the stationary steady state which has the same form as that found in the minimal model (2.57) with modified coefficients. This equation must be solved subject to the boundary conditions $u_1(0) = u_1(1) = 0$ and the linearized

version of (4.18) given by

$$\langle u_1 + V_1 \rangle = 0, \quad (4.57)$$

where, here and throughout this chapter,

$$\langle f \rangle = \int_0^1 f \, dx. \quad (4.58)$$

For $V_1 = 0$, we find the following solutions

$$u_1^\pm = Q \sin(n\pi x), \quad \theta_1^\pm = -\frac{Q\theta_E n\pi}{\omega_n^\pm + J'_E} \cos(n\pi x), \quad (4.59)$$

$$\sigma_1^\pm = \frac{Qn\pi}{D\omega_n^\pm + 1} \cos(n\pi x), \quad (4.60)$$

where the real part is understood here and throughout this chapter, Q is some arbitrary complex constant, $\omega_n^\pm = \Omega_\pm(n\pi)$ and (4.57) forces us to choose n an even integer. Again we see that small perturbations to the network volume fraction or network stress of the stationary strip that are symmetric about $x = 1/2$ do not cause the strip to start moving. There are also solutions

$$u_1^\pm = \frac{\beta_E V_1}{\alpha_E + \beta_E} \left(\frac{\cos(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)} - 1 \right), \quad (4.61)$$

$$\theta_1^\pm = \frac{\beta_E \theta_E V_1 \lambda_n}{(\omega_{(\lambda_n)}^\pm + J'_E)(\alpha_E + \beta_E)} \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad (4.62)$$

$$\sigma_1^\pm = -\frac{\beta_E V_1 \lambda_n}{(D\omega_{(\lambda_n)}^\pm + 1)(\alpha_E + \beta_E)} \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad (4.63)$$

corresponding to $V_1 \neq 0$, where $\omega_{\lambda_n}^\pm = \Omega_\pm(\lambda_n)$ and λ_n is given by

$$\frac{\alpha_E}{\beta_E} + \frac{2}{\lambda_n} \tan\left(\frac{\lambda_n}{2}\right) = 0, \quad (4.64)$$

such that $(2n - 1)\pi < \lambda_n < 2\pi n$. This demonstrates that small perturbations to the stationary strip that break the symmetry, about $x = 1/2$, of the network volume fraction or network stress lead to a travelling strip. The viscoelastic model retains the feature of the minimal model that motion requires a break in symmetry, which is biologically relevant in understanding how cells polarize their network in order to crawl.

4.2.4 Solution of the linearized initial value problem

We consider how the stationary strip responds to a small perturbation of its network volume fraction and network stress. We seek a solution to the linearized initial value problem by

writing arbitrary initial conditions for θ_1 and σ_1 as sums over the modes found in §4.2.3 as follows

$$\theta_1 = \frac{a_0(t)}{2} + \sum_{n \text{ even}} a_n(t) \cos(n\pi x) + \sum_n b_n(t) \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad (4.65)$$

$$\sigma_1 = \frac{c_0(t)}{2} + \sum_{n \text{ even}} c_n(t) \cos(n\pi x) + \sum_n d_n(t) \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad (4.66)$$

where λ_n is given by (4.64). The initial conditions for a_n , b_n , c_n and d_n are given by the initial conditions for θ and σ , where we have expanded $\theta^* \sim \theta_E + \varepsilon\theta_1^* + \dots$ and $\sigma^* \sim \varepsilon\sigma_1^* + \dots$, so that

$$a_n(0) = 2 \int_0^1 \theta_1^*(\zeta) \cos(n\pi\zeta) d\zeta, \quad b_n(0) = \frac{2\lambda_n \cos(\lambda_n/2)}{\lambda_n - \sin(\lambda_n)} \int_0^1 \theta_1^*(\zeta) \sin(\lambda_n(\zeta - 1/2)) d\zeta, \quad (4.67a, b)$$

$$c_n(0) = 2 \int_0^1 \sigma_1^*(\zeta) \cos(n\pi\zeta) d\zeta, \quad d_n(0) = \frac{2\lambda_n \cos(\lambda_n/2)}{\lambda_n - \sin(\lambda_n)} \int_0^1 \sigma_1^*(\zeta) \sin(\lambda_n(\zeta - 1/2)) d\zeta. \quad (4.68a, b)$$

Substituting (4.65) into (4.39), with $u_E = V_E = 0$, and using the orthogonality of the modes to collect like terms, we obtain

$$D\ddot{a}_n + \left(\frac{D(\Psi'_E + 1)\theta_E n^2 \pi^2}{\alpha_E + \beta_E} + DJ'_E + 1 \right) \dot{a}_n + \left(\frac{(J'_E + \Psi'_E)\theta_E n^2 \pi^2}{\alpha_E + \beta_E} + J'_E \right) a_n = 0 \quad (4.69)$$

for $n > 0$ and even, along with

$$D\ddot{b}_n + \left(\frac{D(\Psi'_E + 1)\theta_E \lambda_n^2}{\alpha_E + \beta_E} + DJ'_E + 1 \right) \dot{b}_n + \left(\frac{(J'_E + \Psi'_E)\theta_E \lambda_n^2}{\alpha_E + \beta_E} + J'_E \right) b_n = 0 \quad (4.70)$$

for $n > 0$ an integer and

$$D\ddot{a}_0 + (DJ'_E + 1)\dot{a}_0 + J'_E a_0 = 0. \quad (4.71)$$

For $D \neq 0$, these second-order, linear differential equations for a_n and b_n can be written as

$$\ddot{a}_n + b(n\pi)\dot{a}_n + c(n\pi)a_n = 0, \quad \ddot{b}_n + b(\lambda_n)\dot{b}_n + c(\lambda_n)b_n = 0, \quad (4.72a, b)$$

where b and c are the coefficients for the growth rate quadratic and are defined in (4.43). Noting that we have only one initial condition for each a_n and b_n , we differentiate (4.37) once with respect to x , combine it with (4.38) and set $u_E = V_E = 0$ to obtain an expression relating θ_1 to σ_1 . Into this expression we substitute (4.65) and (4.66) and appeal to orthogonality to equate like terms to find:

$$\dot{a}_n = \frac{\theta_E^2 n^2 \pi^2}{\alpha_E + \beta_E} c_n - \left(J'_E + \frac{\theta_E \Psi'_E n^2 \pi^2}{\alpha_E + \beta_E} \right) a_n \quad (4.73)$$

for $n > 0$ and even;

$$\dot{b}_n = \frac{\theta_E^2 \lambda_n^2}{\alpha_E + \beta_E} d_n - \left(J'_E + \frac{\theta_E \Psi'_E \lambda_n^2}{\alpha_E + \beta_E} \right) b_n \quad (4.74)$$

for $n > 0$ an integer; and

$$\dot{a}_0 = -J'_E a_0. \quad (4.75)$$

The first two expressions provide initial conditions for $\dot{a}_n(0)$ and $\dot{b}_n(0)$ in terms of the known $a_n(0)$, $b_n(0)$, $c_n(0)$ and $d_n(0)$. The third equation can be solved immediately to give

$$a_0(t) = a_0(0) e^{-J'_E t}, \quad (4.76)$$

which we note is compatible with (4.71). Having found sufficient initial conditions we solve for a_n and b_n as follows

$$a_n(t) = \left(\frac{\dot{a}_n(0) - a_n(0) \Omega_-(n\pi)}{\Omega_+(n\pi) - \Omega_-(n\pi)} \right) e^{\Omega_+(n\pi)t} + \left(\frac{a_n(0) \Omega_+(n\pi) - \dot{a}_n(0)}{\Omega_+(n\pi) - \Omega_-(n\pi)} \right) e^{\Omega_-(n\pi)t}, \quad (4.77)$$

$$b_n(t) = \left(\frac{\dot{b}_n(0) - b_n(0) \Omega_-(\lambda_n)}{\Omega_+(\lambda_n) - \Omega_-(\lambda_n)} \right) e^{\Omega_+(\lambda_n)t} + \left(\frac{b_n(0) \Omega_+(\lambda_n) - \dot{b}_n(0)}{\Omega_+(\lambda_n) - \Omega_-(\lambda_n)} \right) e^{\Omega_-(\lambda_n)t}, \quad (4.78)$$

where $\Omega_{\pm}$ is given by (4.44). These coefficients can now be reassembled in (4.65) to determine the response of the network volume fraction, θ_1 , to a small perturbation of the network in terms of the model parameters. Using (4.73) and (4.74), we find c_n and d_n in terms of a_n and b_n , so we also have an expression for σ_1 in terms of the initial conditions and model parameters. We proceed to calculate an expression for u_1 and then V_1 .

Taking (4.36) with $u_E = 0$ and substituting in the series expression for θ_1 , (4.65), where all the coefficients are now known, we can integrate once with respect to x and apply the boundary conditions $u_1(0) = u_1(1) = 0$ to obtain the following expression:

$$u_1 = \sum_n \frac{J'_E b_n + \dot{b}_n}{\theta_E \lambda_n} \left(\frac{\cos(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)} - 1 \right) - \sum_{n \text{ even}} \left(\frac{J'_E a_n + \dot{a}_n}{\theta_E n \pi} \right) \sin(n\pi x). \quad (4.79)$$

Finally, we use (4.57) to find that

$$V_1 = \left(1 + \frac{\alpha_E}{\beta_E} \right) \sum_n \frac{J'_E b_n + \dot{b}_n}{\theta_E \lambda_n}. \quad (4.80)$$

The speed V_1 , which the strip crawls at in response to a small perturbation of the stationary strip, can be written solely in terms of the b_n modes. The b_n modes in turn depend on the d_n modes via the initial condition $\dot{b}_n(0)$, as seen from (4.74). There is no dependence on the a_n or c_n modes. This demonstrates explicitly that any motion of the strip due to small perturbations of the network volume fraction and network stress is a function only

of the modes which are asymmetric about $x = 1/2$. This suggests that in order to ‘pick’ a direction and start moving cells must polarize their actin network, either by altering the actin volume fraction or stressing the network in a way that breaks the cell’s symmetry. When the parameters are chosen so that the model is well-posed and there are some unstable wavenumbers, we are in the case depicted in Figure 4.1(b) where the growth rate plateaus to Ω_∞ . This means that for large n all the modes grow at a comparable rate and we cannot isolate a dominant mode on exiting the linear regime. So for the same reasons as in the viscous model of Chapter 2, we are unable to predict a preferred direction for the cell solely from the model parameters. The direction of motion depends on the parameters used and the combination and relative amplitude of modes contained in the asymmetric part of the initial conditions. These results are verified by comparison to numerical simulation in §4.4.1.

4.2.5 Viscous limit

We might expect to recover the minimal model of Chapter 2 as the small Deborah number limit of our viscoelastic model. In order to understand further how the stability analysis of this model is related to that of the minimal model, and to provide a partial check on our calculations, we consider briefly the limit $D \rightarrow 0$. This small Deborah number limit restricts us to the regime where $DJ'_E < 1$. In this regime $\Omega_-(0) = -1/D$ and $\Omega_-(k)$ is monotonically decreasing. Therefore, as $D \rightarrow 0$, every point on this growth rate curve tends to negative infinity. In some sense we lose this root and return to the purely viscous case where there is only one solution to the growth rate equation. Also as $D \rightarrow 0$, $\Psi_a \rightarrow -\infty$, so remembering that problem is ill-posed only for $\Psi'_E < \Psi_a$, we see that increasingly extreme values Ψ'_E are required for the problem to be ill-posed, *cf.* the viscous case which is always at least weakly well-posed. Now assuming $\Psi'_E > \Psi_a$, so that the problem is at least weakly well-posed, then as $D \rightarrow 0$,

$$\Omega_\infty \rightarrow -(J'_E + \Psi'_E), \quad (4.81)$$

which is in agreement with the maximum growth rate from the viscous model, (2.54), once we recall that viscosity is defined differently in the two models. To compare easily note that for the suggested constitutive laws $\mu_E = \theta_E$ in Chapter 2.

That $D \rightarrow 0$ is a singular limit of the viscoelastic model can be seen clearly from (4.69) and (4.70). When $D = 0$ in these the order of the initial value problem is reduced and there is no need for an initial condition on the network stress.

4.3 Small growth rate limit

It is not possible to carry out a traditional weakly nonlinear analysis, because there is no dominant wavenumber to excite. However, further analytical progress can be made in an analogous way by working in the parameter regime where the growth rate plateaus if we set the height of that plateau to be small. Specifically we define $\varepsilon = \Omega_\infty$ and insist that $\varepsilon \ll 1$. Rearranging (4.53) we see that ε now parametrizes the swelling and contractile pressure so that

$$\frac{\partial \Psi}{\partial \theta}(\theta_E; \varepsilon) = -\frac{J'_E + \varepsilon}{D\varepsilon + 1}. \quad (4.82)$$

In order to retain $k_c = O(1)$, (4.54) indicates that we must rescale $\alpha = \varepsilon \hat{\alpha}$ and $\beta = \varepsilon \hat{\beta}$, so that we are considering a weak adhesion and weak inter-phase drag limit. As for the small growth rate analysis in §2.3, we are working in a slightly contrived parameter regime where a cell has been placed on a surface to which it adheres only weakly.

The small growth rate problem reveals three important timescales over which the cell responds to a small perturbation. The easiest to anticipate is the long timescale over which the cell polarizes and the profile of the actin network, network velocity and network stress develop to be far from their equilibrium values. We can reasonably anticipate that this will happen on a long timescale, because the parameters are such that the growth rate of the perturbations are small. With the knowledge of the small growth rate analysis in §2.3, it is also reasonable to predict that there will be a short-time problem, where we see the mean value of the network volume fraction tend to θ_E , the equilibrium volume fraction. The total amount of network in the travelling strip relaxes to the equilibrium amount on the timescale for polymerization and depolymerization. Perhaps harder to predict *a priori* is an initial response, where the cell rapidly redistributes its network and the network stress on the elastic relaxation timescale. The need for all three of these timescales will be elucidated below.

4.3.1 Large time problem $t = O(1/\varepsilon)$

Since the growth rate is small we anticipate that any small perturbation will develop over a long timescale. So, we rescale $\tau = \varepsilon t = O(1)$ and substitute into (4.14)–(4.16) and (4.18) the expansions $\theta \sim \theta_E + \varepsilon \theta_1 + \varepsilon^2 \theta_2 + \dots$, $\sigma \sim \varepsilon \sigma_1 + \varepsilon^2 \sigma_2 + \dots$, $u \sim \varepsilon u_1 + \varepsilon^2 u_2 \dots$ and $V \sim \varepsilon V_1 + \varepsilon^2 V_2 + \dots$ as $\varepsilon \rightarrow 0$. The spatially uniform steady state satisfies the governing

equations at $O(1)$, while at $O(\varepsilon)$ we find that the perturbation is governed by

$$\theta_E \frac{\partial u_1}{\partial x} = -J'_E \theta_1, \quad J'_E \frac{\partial \theta_1}{\partial x} + \theta_E \frac{\partial \sigma_1}{\partial x} = 0, \quad \sigma_1 = \frac{\partial u_1}{\partial x}, \quad V_1 = -\langle u_1 \rangle, \quad (4.83a-d)$$

which must be solved subject to the boundary conditions $u_1(0, \tau) = u_1(1, \tau) = 0$. We note first that integrating (4.83a,c) with respect to x and applying the boundary conditions shows that both the network stress and the perturbation to the network volume fraction have zero mean on the long timescale, with

$$\langle \theta_1 \rangle = 0, \quad \langle \sigma_1 \rangle = 0. \quad (4.84a,b)$$

Secondly, we can eliminate u_1 between (4.83a) and (4.83c) to see that at all late times the perturbation to the equilibrium network volume fraction is proportional to the network stress, with

$$J'_E \theta_1 + \theta_E \sigma_1 = 0. \quad (4.85)$$

Differentiating this expression once with respect to x we obtain (4.83b), which represents a the balance between the swelling/contractile pressure and the network stress, indicating that we have too few equations to close the problem at this order and must obtain a solvability condition at next order. At $O(\varepsilon^2)$, we find that

$$\frac{\partial \theta_1}{\partial \tau} + \theta_E \frac{\partial u_2}{\partial x} + \frac{\partial}{\partial x}(\theta_1 u_1) = -\frac{J''_E}{2} \theta_1^2 - J'_E \theta_2, \quad (4.86)$$

$$\frac{\partial}{\partial x} \left(J'_E \theta_2 - \frac{\Psi''_E \theta_1^2}{2} \right) + (1 - DJ'_E) \frac{\partial \theta_1}{\partial x} + \frac{\partial}{\partial x}(\theta_E \sigma_2 + \theta_1 \sigma_1) = (\alpha_E + \beta_E) u_1 + \beta_E V_1, \quad (4.87)$$

$$D \left(\frac{\partial \sigma_1}{\partial \tau} + u_1 \frac{\partial \sigma_1}{\partial x} - 2\sigma_1 \frac{\partial u_1}{\partial x} \right) + \sigma_2 = \frac{\partial u_2}{\partial x}. \quad (4.88)$$

where we have introduced the notation $\Psi''_E := \partial^2 \Psi / \partial \theta^2(\theta_E, 0)$ and dropped the hats on α and β . We use (4.88) to eliminate σ_2 from (4.87) and then eliminate θ_2 and u_2 by combining the resulting equation and (4.86). Finally, we use (4.83a,c) to obtain

$$\frac{\partial}{\partial x} \left[(1 - DJ'_E) \frac{\partial}{\partial x} \left(\frac{\partial u_1}{\partial \tau} + u_1 \frac{\partial u_1}{\partial x} - u_1 \right) - a_E \left(\frac{\partial u_1}{\partial x^2} \right)^2 \right] = b_E (\alpha_E u_1 + \beta_E (u_1 + V_1)), \quad (4.89)$$

where

$$a_E = 1 + \frac{J''_E \theta_E}{2J'_E} + \frac{\theta_E \Psi''_E}{2J'_E} - 3DJ'_E, \quad b_E = \frac{J'_E}{\theta_E}. \quad (4.90)$$

Equation (4.89) is a nonlinear, third-order, partial differential equation for u_1 depending on one additional function of time, V_1 , which is determined by (4.83d). It should be solved subject to the boundary conditions $u_1(0, \tau) = u_1(1, \tau) = 0$ and initial conditions that can

be obtained from suitable initial conditions for θ_1 by integrating (4.83a). We do not pursue a solution of this equation further, since it is sufficiently complicated to require numerical simulation at which point we may as well solve the full nonlinear problem, which we do in §4.4. The integral constraint (4.83d) allows us to write the velocity of the crawling strip at a given time as a function of the network volume fraction at that time:

$$V_1(\tau) = -\langle u_1 \rangle = \frac{J'_E}{\theta_E} \int_0^1 \int_0^x \theta_1(\zeta, \tau) d\zeta dx. \quad (4.91)$$

This long-time analysis does not apply to arbitrary initial conditions: it is restricted to initial conditions that satisfy (4.84) and (4.85). This motivates us to consider the short-time problem in the anticipation that arbitrary initial conditions for the network volume fraction and network stress will relax over a short timescale to variables that are consistent with this long-time analysis.

4.3.2 Small time problem $t = O(1)$

We consider $t = O(1)$ and substitute into (4.14)–(4.16) and (4.18) the expressions $\theta \sim \theta_E + \varepsilon \hat{\theta}_1 + \dots$, $\sigma \sim \varepsilon \hat{\sigma}_1 + \dots$, $u \sim \varepsilon \hat{u}_1 \dots$ and $V \sim \varepsilon \hat{V}_1 + \dots$ as $\varepsilon \rightarrow 0$. At $O(\varepsilon)$ we find

$$\frac{\partial \hat{\theta}_1}{\partial t} + \theta_E \frac{\partial \hat{u}_1}{\partial x} = -J'_E \hat{\theta}_1, \quad J'_E \frac{\partial \hat{\theta}_1}{\partial x} + \theta_E \frac{\partial \hat{\sigma}_1}{\partial x} = 0, \quad D \frac{\partial \hat{\sigma}_1}{\partial t} + \hat{\sigma}_1 = \frac{\partial \hat{u}_1}{\partial x}, \quad \hat{V}_1 = -\langle \hat{u}_1 \rangle. \quad (4.92a-d)$$

We can immediately integrate (4.92a,c) once with respect to x and apply the zero Dirichlet boundary conditions for $\hat{u}_1$, then solve the resulting ordinary differential equations to find that

$$\langle \hat{\theta}_1 \rangle = \langle \hat{\theta}_1^* \rangle e^{-J'_E t}, \quad \langle \hat{\sigma}_1 \rangle = \langle \hat{\sigma}_1^* \rangle e^{-t/D}, \quad (4.93)$$

where a star identifies initial conditions. We see that the mean values of the perturbation to the network volume fraction and the network stress decay to zero over this timescale and provide variables that are consistent with (4.84). Next, we integrate (4.92b) twice with respect to x and find that

$$J'_E \hat{\theta}_1 + \theta_E \hat{\sigma}_1 = J'_E \langle \hat{\theta}_1 \rangle + \theta_E \langle \hat{\sigma}_1 \rangle \quad (4.94)$$

for all t . Since we know that the mean values decay this indicates that as we exit the small time limit our network volume fraction and stress variables will be consistent with (4.85). It

is also possible to manipulate (4.92a–c) to isolate $\hat{\theta}_1$ and $\hat{\sigma}_1$ in turn and solve the resulting partial differential equations to find that

$$\hat{\theta}_1 = \hat{\theta}_1^* + \langle \hat{\theta}_1^* \rangle \left(e^{-J'_E t} - 1 \right), \quad \hat{\sigma}_1 = \hat{\sigma}_1^* + \langle \hat{\sigma}_1^* \rangle \left(e^{-t/D} - 1 \right), \quad (4.95a, b)$$

so on this timescale the x dependence of network volume fraction and stress is unaltered, both of the perturbation profiles simply translate to have zero mean. Substituting (4.95a) into (4.92a) and using (4.92d) we can calculate that the network velocity and speed of the travelling strip are given by

$$\hat{u}_1 = -\frac{J'_E}{\theta_E} \left(\int_0^x \hat{\theta}_1^*(\zeta) d\zeta - x \langle \hat{\theta}_1^* \rangle \right), \quad \hat{V}_1 = \frac{J'_E}{\theta_E} \left(\int_0^1 \int_0^x \hat{\theta}_1^*(\zeta) d\zeta dx - \frac{1}{2} \langle \hat{\theta}_1^* \rangle \right). \quad (4.96a, b)$$

On this timescale the network velocity and the velocity of the travelling strip are determined by the initial conditions and take the same form as in the minimal viscous model (*c.f.* (2.94)). The speed of the travelling strip is constant and the small growth rate limit enables us to say a little more about the early direction of motion. In the case where, loosely speaking, one side of the strip has a higher than mean network volume fraction and the other side has a lower than mean network volume fraction, the side with the higher network volume fraction will become the rear of the travelling strip. We also note that (4.96b) is consistent with (4.91). The short-time analysis provides appropriate initial conditions for the long-time problem that satisfy (4.84) and (4.85). However, it will not work for arbitrary initial conditions. Initial conditions for this small time problem must satisfy (4.94), to resolve this limitation we consider an even shorter timescale.

4.3.3 Very small time problem $t = O(\varepsilon)$

We consider a very short timescale in which there is fast flow of the network in order to modify arbitrary initial network volume fraction and stress profiles to comply with (4.94). We make the scalings $t = \varepsilon \tilde{t}$, where $\tilde{t} = O(1)$ and substitute into (4.14)–(4.16) and (4.18) the expansions $\theta \sim \theta_E + \varepsilon \tilde{\theta}_1 + \dots$, $\sigma \sim \varepsilon \tilde{\sigma}_1 + \dots$, $u \sim \tilde{u}_1 + \dots$ and $V \sim \tilde{V}_1 + \dots$ as $\varepsilon \rightarrow 0$. At leading order, we obtain

$$\frac{\partial \tilde{\theta}_1}{\partial \tilde{t}} + \theta_E \frac{\partial \tilde{u}_1}{\partial x} = 0, \quad D \frac{\partial \tilde{\sigma}_1}{\partial \tilde{t}} = \frac{\partial \tilde{u}_1}{\partial x}, \quad \tilde{V}_1 = -\langle \tilde{u}_1 \rangle, \quad (4.97a-c)$$

$$J'_E \frac{\partial \tilde{\theta}_1}{\partial x} + \theta_E \frac{\partial \tilde{\sigma}_1}{\partial x} = (\alpha_E + \beta_E) \tilde{u}_1 + \beta_E \tilde{V}_1. \quad (4.98)$$

We integrate (4.97*a,b*) and apply the boundary conditions $\tilde{u}_1(0, \tilde{t}) = \tilde{u}_1(1, \tilde{t}) = 0$ to see that

$$\frac{\partial}{\partial \tilde{t}} \langle \tilde{\theta}_1 \rangle = 0, \quad \frac{\partial}{\partial \tilde{t}} \langle \tilde{\sigma}_1 \rangle = 0, \quad (4.99a,b)$$

so the average network volume fraction and network stress is fixed on this timescale, but may be re-distributed within the strip. We eliminate $\tilde{\sigma}_1$, $\tilde{\theta}_1$ and $\tilde{V}_1$ between (4.97) and (4.98) and rearrange to find

$$\frac{\partial \tilde{u}_1}{\partial \tilde{t}} = A \frac{\partial^2 \tilde{u}_1}{\partial x^2} + B \int_0^1 \frac{\partial \tilde{u}_1}{\partial \tilde{t}} dx, \quad (4.100)$$

where

$$A = \frac{\theta_E(1 - DJ'_E)}{D(\alpha_E + \beta_E)}, \quad B = \frac{\beta_E}{\alpha_E + \beta_E}, \quad (4.101)$$

and we note that both these constants are positive for parameter values where the growth rate plateaus.

Below we shall show that $\tilde{u}_1 \rightarrow 0$ as $\tilde{t} \rightarrow \infty$, which implies that $\tilde{V}_1 \rightarrow 0$ and so the right hand side of (4.98) vanishes for large $\tilde{t}$. Therefore, arbitrary initial conditions for θ and σ relax on this very short timescale to profiles that satisfy (4.94). We note that $\tilde{u}_1$ and $\tilde{V}_1$ tending to zero is consistent with the small time analysis where the leading order terms in the network and travelling strip velocities are $O(\varepsilon)$. In order to show that $\tilde{u}_1 \rightarrow 0$ as $\tilde{t} \rightarrow \infty$ we multiply (4.100) by $\tilde{u}_1$, write the second spatial derivative term as a divergence minus a square, integrate with respect to x from 0 to 1, apply the boundary conditions and manipulate to obtain

$$\frac{1}{2} \frac{d}{d\tilde{t}} \left[\int_0^1 \tilde{u}_1^2 dx - B \left(\int_0^1 \tilde{u}_1 dx \right)^2 \right] = -A \int_0^1 \left(\frac{\partial \tilde{u}_1}{\partial x} \right)^2 dx. \quad (4.102)$$

We introduce a new variable

$$w = \tilde{u}_1 - \int_0^1 \tilde{u}_1 dx, \quad (4.103)$$

and substitute for $\tilde{u}_1^2$ in (4.102) to find that

$$\frac{1}{2} \frac{d}{d\tilde{t}} \left[\int_0^1 w^2 dx + (1 - B) \left(\int_0^1 \tilde{u}_1 dx \right)^2 \right] = -A \int_0^1 \left(\frac{\partial \tilde{u}_1}{\partial x} \right)^2 dx, \quad (4.104)$$

where we note that $1 - B$ is strictly positive. The terms inside the square brackets in (4.104) are greater than or equal to zero. If the right-hand-side of (4.104) is nonzero then it is strictly negative which implies that the positive terms on the left-hand-side decrease to zero, ensuring that the right-hand-side tends to zero as $\tilde{t} \rightarrow \infty$. The integral on the

right-hand-side is zero only when $\partial\tilde{u}_1/\partial x = 0$ throughout the strip (assuming continuity). Therefore as $\tilde{t} \rightarrow \infty$, $\tilde{u}_1$ tends to a constant, which is selected as zero by applying the boundary conditions.

The small growth rate analysis has enabled us to say a little more about the direction of motion of the travelling strip. It has also revealed three timescales for the cell responses. First we see a rapid redistribution of network on the elastic timescale. Then polymerization and depolymerization push the total amount of network in the cell towards the equilibrium volume fraction without altering the shape of the network distribution. This occurs on the order one timescale and is associated with a small constant velocity of the travelling strip. Finally the perturbation grows and the speed of the travelling strip grows on the long timescale. Unfortunately the governing equation obtained on this long timescale, (4.89), is too complicated to tackle analytically, so we proceed to present numerical solutions to the full nonlinear problem in the next section. The analysis here is verified against a numerical simulation in Appendix B (Figure B.3).

4.4 Numerical simulations

4.4.1 Description and validation of the scheme

We now proceed to solve numerically the two-phase, reactive, poroviscoelastic flow model stated in (4.14)–(4.20), to gain further insight into the effects of the actin network viscoelasticity. We modify the code used in §2.4 to solve the additional equation for the evolution of the network stress. We also use the PETSc run time options ‘`-snes_atol 1.0e-10 -pc_type lu`’. The first option slightly slackens the error tolerance for solving the nonlinear system at each timestep. The second option causes the solver to use an LU preconditioner and then GMRES to solve the linear system arising at each Newton-like iteration [7, 8, 9].

To ensure that our code converges as the spatial and temporal discretization is refined we solve the same problem on a sequence of grids: specifically we fix the timestep at 10^{-3} and double the number of space elements from 200 to 64000. The spatial convergence of the code is order $3/2$. The temporal convergence of the code was shown to be first order by solving the same problem on a grid with 800 space elements for a range of timesteps doubling from 1.25^{-4} to 4×10^{-3} . Graphs demonstrating both the temporal and spatial convergence can be found in Figure B.2 in Appendix B. In order to further validate the

viscoelastic code we compare the numerical solution of a suitable initial value problem to the solution predicted by the linear stability analysis of §4.2.4. The two are in excellent agreement at early times as shown in Figure 4.3, further details are given in the next section.

4.4.2 Results

Figure 4.3 displays the solution to an initial value problem where the initial conditions are a small perturbation to the spatially uniform, stationary strip. We see in Figure 4.3(a) that the velocity of the crawling strip increases initially and subsequently decays to a constant positive value in an oscillatory manner. The scale masks an initial transient in the speed of the travelling strip seen clearly in Figure 4.3(b). The linear prediction is able to match this non-monotonic behaviour because there are two roots to the growth rate equation (4.44) and at very early times we can observe the effect of the negative exponential in b_n , (see (4.78)). The simulation was run on a grid with five thousand spatial elements and a timestep of 10^{-3} . Comparing the computed velocity of the travelling strip to that obtained by running the same simulation on a grid with half the number of spatial elements and the same timestep, or the same number of spatial elements and a timestep of 2×10^{-3} , yielded a relative error in V less than 1.1×10^{-3} .

The spatio-temporal behaviour of the network volume fraction θ , the network velocity u and the network stress σ for this initial value problem is plotted in Figure 4.4. From these simulated variables we also calculate the nondimensional force exerted on the substrate by the crawling strip according to

$$F = \beta(\theta)(u + V). \quad (4.105)$$

We see in Figure 4.4(a) that initially the volume fraction is everywhere close to the equilibrium value, then two peaks in the volume fraction develop, one at the rear of the travelling strip and one in the interior. These steepen and the interior peak travels backwards and coalesces with the peak at the rear. Subsequently, smaller regions of high actin network form in the strip interior and travel backwards. The size of these ripples decreases with time. By $t = 60$ these ripples have all but disappeared. The network velocity in Figure 4.4(b) is initially close to zero. Later it is negative in the bulk, displaying retrograde flow, apart from in a small region associated with the moving actin peak, where the flow is forwards. If we consider a fixed $x = 0.5$ we see that after the actin peak has moved to the back of the crawling strip, the network velocity displays oscillations in time associated with

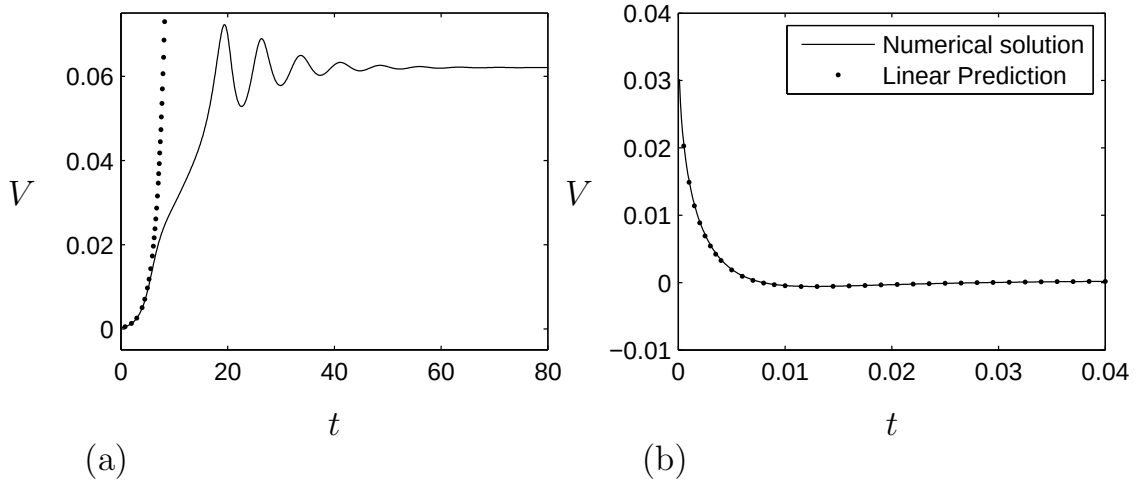


Figure 4.3: Velocity of the crawling strip, V , as a function of time calculated numerically (solid line) and compared to the linear prediction (dots) given by (4.80). A zoomed in plot of (a) showing the very early time behaviour is shown in (b). Parameters used are $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$, $\beta^* = 3$ and $D = 0.1$ along with the initial conditions $\theta^* = \theta_E + \varepsilon \cos(3\pi x)$ and $\sigma^* = \varepsilon \cos(\pi x)$ for $\varepsilon = 10^{-4}$. Other variables of interest are plotted for the same initial value problem in Figures 4.4 and 4.5.

the ripples seen in the network volume fraction plot. At late times the amplitude of these oscillations decays. The network stress depicted in Figure 4.4(c) resembles a photographic negative for the network volume fraction picture, indicating that for these parameters at least the network volume fraction and stress are intimately linked. Regions of high network volume fraction are associated with regions of negative network stress. Finally, the force exerted on the substrate by the crawling strip is displayed in Figure 4.4(d). We see that the force changes rapidly across the interior peak in the actin network. At later times the force exerted on the substrate is in the direction of motion at both ends of the travelling strip and opposing the direction of motion in the centre of the strip. The ripples in the network volume fraction, velocity and stress profiles result in the oscillations in the speed of the travelling strip about the travelling-wave speed.

As the speed of the travelling strip tends to a constant value we obtain a steady, travelling-wave solution as the long-time limit of the unsteady problem. The maximum relative difference between the travelling-strip velocity V and the average of the travelling-strip velocity for $t \in [80, 100]$ is less than 1.9×10^{-4} . The steady profiles obtained are plotted in Figure 4.5. The shape of the profiles for the network volume fraction, the network and solution velocities, hydrodynamic pressure and the mixture stress are qualitatively the same

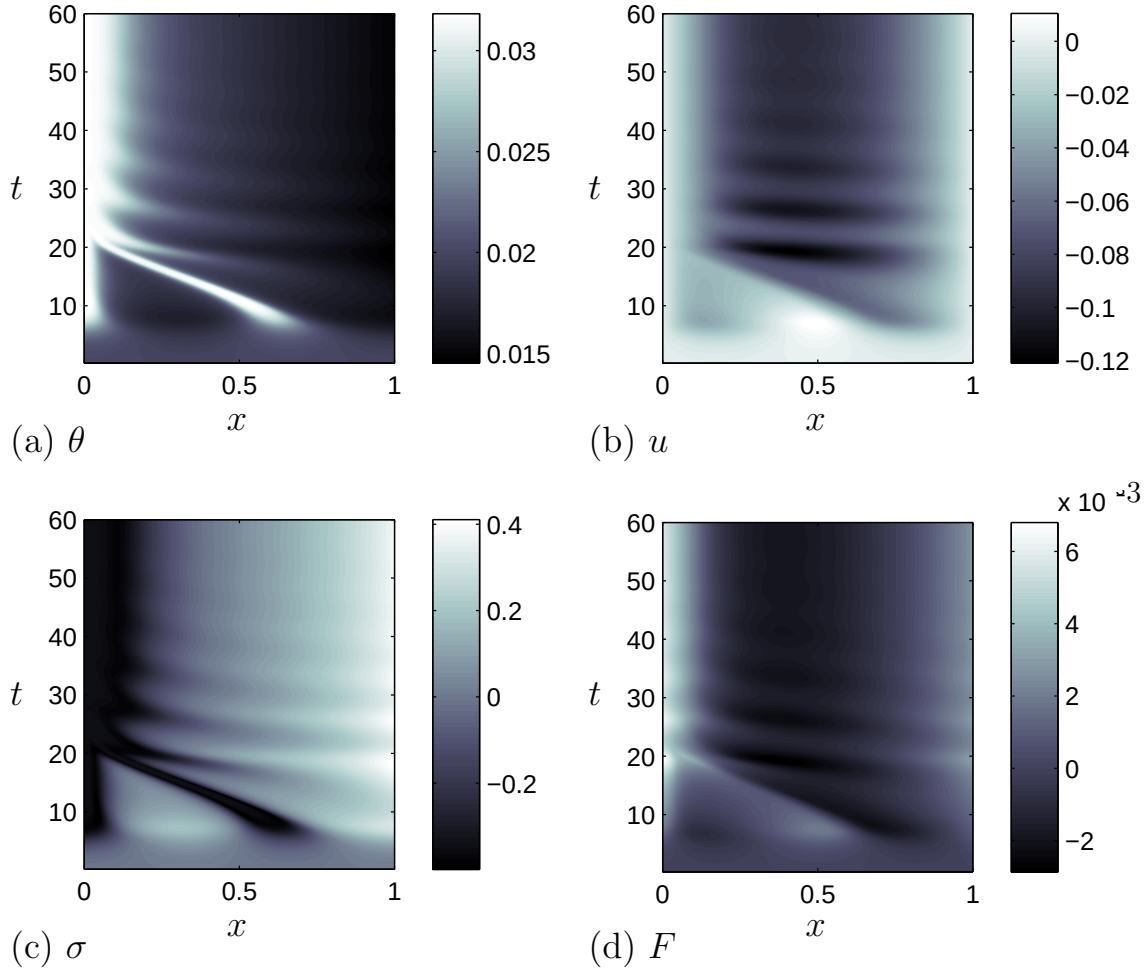


Figure 4.4: Graphs depicting the variation in space and time of (a) the network volume fraction, (b) the network velocity, (c) the network stress and (d) the force exerted on the substrate by the crawling strip. The associated crawling strip velocity is plotted in Figure 4.3 and the parameters and initial conditions used are stated in the caption to that Figure.

as those seen for the travelling-wave, one-peak solutions of the viscous model, *cf.* Figure 2.8. The force exerted on the substrate is also qualitatively the same as that found for the one peak solutions in the viscous model *cf.* Figure 2.7. The extra information accessible in the viscoelastic formulation is the network stress displayed in Figure 4.5(c). We see that regions of high network volume fraction are associated with a negative network stress and regions of low network volume fraction are associated with a positive network stress. The network stress is not monotonic: it has a local minimum in the strip interior, just in front of the peak in network volume fraction at the rear of the travelling strip. This simulation suggests that viscoelastic model behaves similarly to the minimal model of Chapter

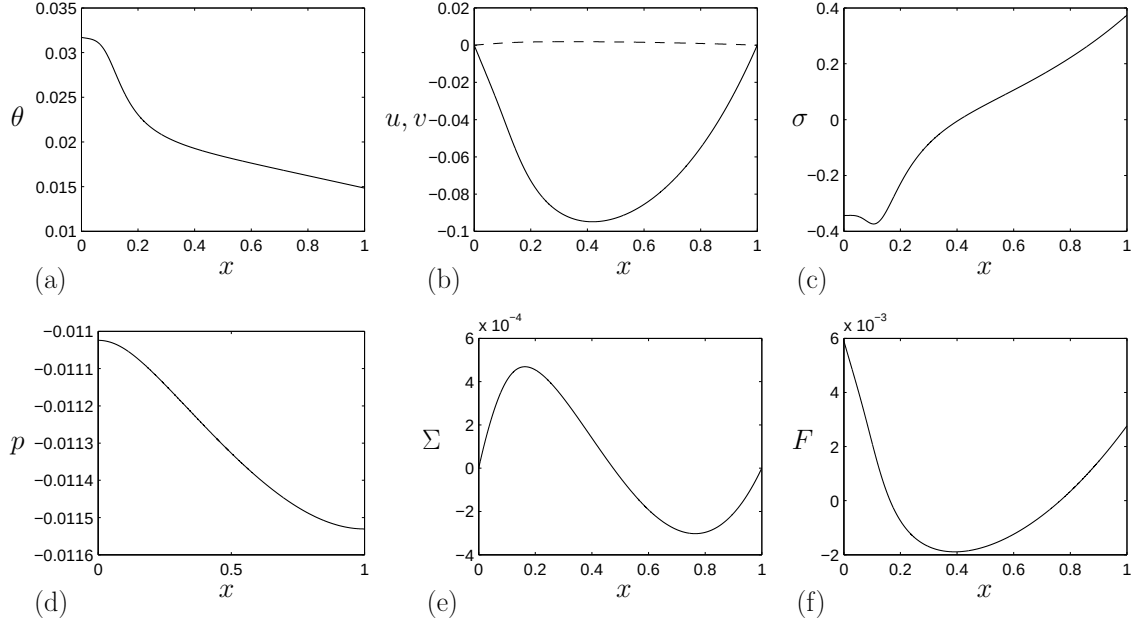


Figure 4.5: Steady travelling-wave profiles for (a) the network volume fraction, (b) the network velocity (solid line) and solution velocity (dashed line), (c) the network stress, (d) the hydrodynamic pressure, (e) the mixture stress and (f) the force exerted on the substrate by the crawling strip. These are obtained from the initial value problem simulation at $t = 100$. Parameters and initial conditions are as stated in the caption to Figure 4.3.

2. We further investigate the relationship between the two models in the next section by considering the effect of the Deborah number.

4.4.3 Effect of Deborah number

The Deborah number quantifies to what extent the network behaves as a viscous fluid or an elastic solid over our timescale of observation. As $D \rightarrow 0$ the network becomes increasingly viscous. We numerically simulate the solution to an initial value problem where the initial condition is a small perturbation to the spatially uniform steady state and plot the travelling-strip velocity in Figure 4.6. We also plot the solution to a corresponding initial value problem for the viscous model of Chapter 2, although we cannot draw a direct comparison, because the minimal model has no initial condition for the network stress. We see that in all the simulations the velocity of the travelling strip initially increases, then settles to a constant value in an oscillatory manor. For increasing Deborah number the travelling-strip speed peaks slightly sooner at a smaller value. The steady, travelling speed attained as the long-time limit of the unsteady problem is slower for larger Deborah

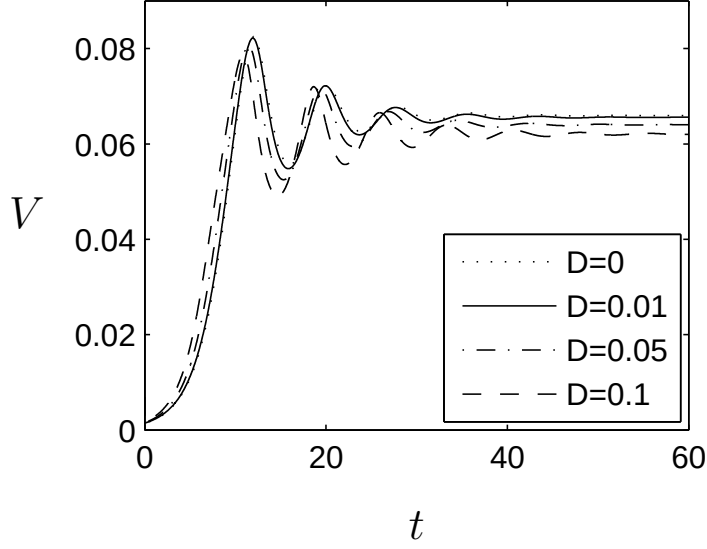


Figure 4.6: Velocity of the travelling strip as a function of time for different Deborah numbers. Parameters used for the minimal model ($D = 0$) calculation are $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$ and $\beta^* = 3.0$ with the initial condition $\theta^* = \theta_E + \varepsilon \cos(\pi x)$ where $\varepsilon = 10^{-4}$. For the viscoelastic simulations we have in addition $\sigma^* = \varepsilon \cos(\pi x)$ and $D = 0.01$, $D = 0.05$ or $D = 0.1$.

number. A small Deborah number, $D = 0.01$ appears to return us almost to the viscous model.

Figure 4.7 displays the travelling-wave profiles obtained as the long-time limit of the initial value problem in Figure 4.6. The first observation is that an order of magnitude change in Deborah number has no qualitative impact on the travelling-wave solutions. We see in Figure 4.7(b) that the retrograde flow of the network near the centre of the strip is slightly slower for a larger Deborah number. A larger Deborah number also seems to result in a slightly steeper peak in the actin network volume fraction, as illustrated in Figure 4.7(a). The viscoelastic simulations in this section were run on grids with 800 or 1600 spatial elements and timesteps of 1×10^{-3} or 2×10^{-3} and the worst relative error in crawling speed V between different grids was less than 1.4×10^{-3} .

4.4.4 Effect of adhesion strength

In order to see if the viscoelastic model retains the same bell-shaped dependence of speed on adhesion strength as the minimal model of Chapter 2, we computed steady, travelling-wave solutions by continuation in β^* as for the minimal model. Figure 4.8 displays crawling

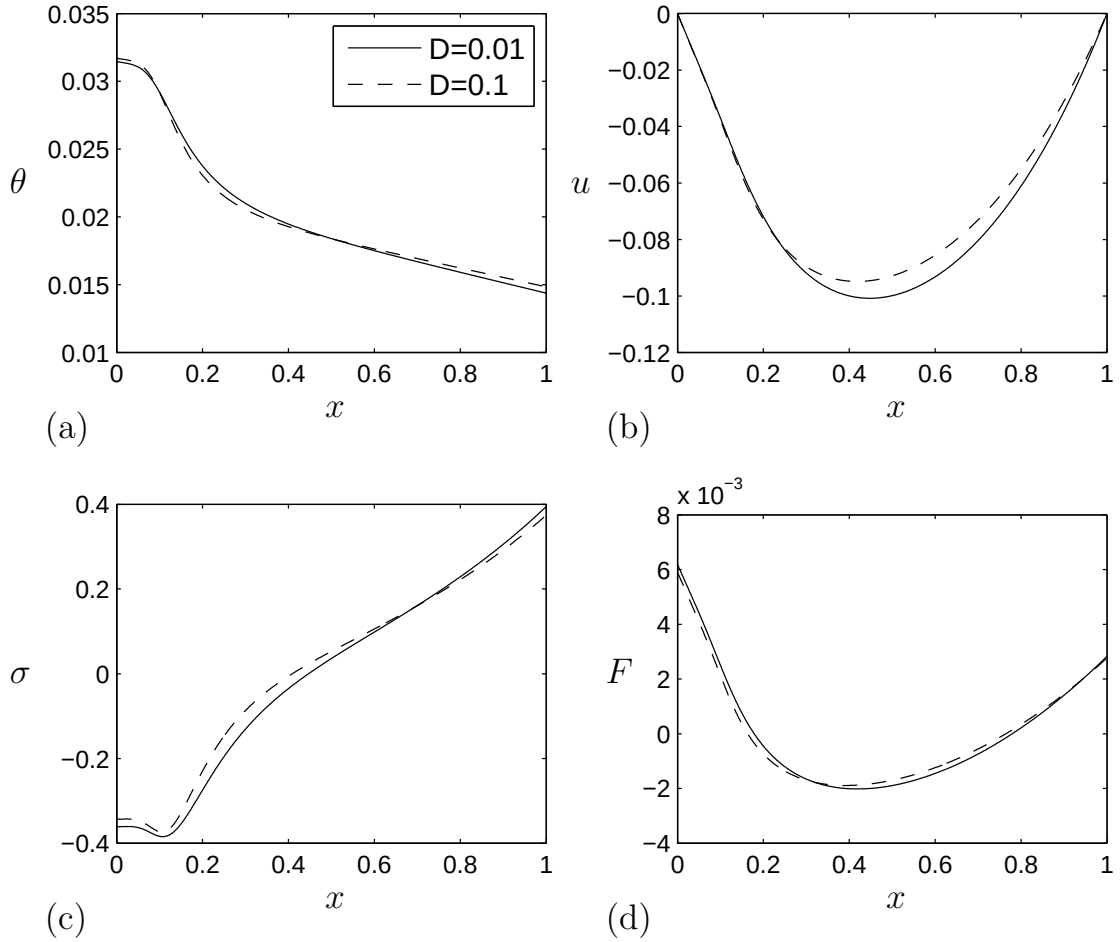


Figure 4.7: Comparison of the steady, travelling-wave profiles for two of the initial value problems in Figure 4.6 with $D = 0.01$ (solid line) and $D = 0.1$ (dashed line). We plot (a) the network volume fraction, (b) the network velocity, (c) the network stress and (d) the force exerted on the substrate by the travelling strip, found with the unsteady code at $t = 100$.

speeds for travelling-wave solutions that have one peak in their actin network at the rear of the travelling strip. The viscoelastic model does display a bell-shaped dependence of travelling speed on adhesion strength. We note that for a given adhesion strength, the viscoelastic model predicts a slightly smaller crawling speed than the minimal model. The viscoelastic model also suggests a marginally smaller optimal adhesion strength. The viscoelastic solutions have all been computed on two grids, one with five thousand and one with ten thousand spatial elements. The maximum relative error in the travelling speed, V , computed on these grids is less than 2×10^{-4} . The maximum relative difference between V and the average value of V computed over the final twenty nondimensional time units is

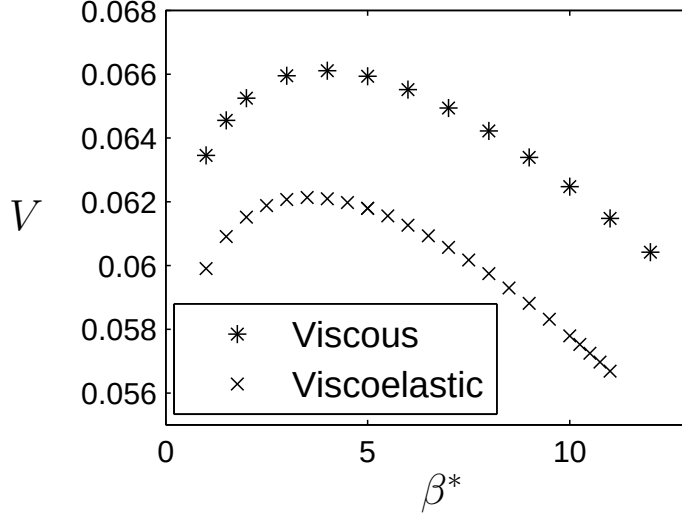


Figure 4.8: Travelling speed of steady travelling-wave solutions as a function of adhesion strength for the viscoelastic model and the viscous model of Chapter 2. Parameters used are $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$ and $D = 0.1$. The viscous data points are taken from Figure 2.9.

less than 3.3×10^{-5} .

4.4.5 Remarks

Some numerical simulations with stronger cell-substrate adhesion ($\beta^* \sim 20$, not shown here) suggested that there may be steady, travelling-wave solutions with an interior peak in the actin network, qualitatively similar to the two-peak solutions of the minimal model. The simulations generated a crawling velocity for the strip with small oscillations about a constant value. However, these simulations (not shown) were not sufficiently resolved to conclude whether these are true oscillations or numerical artefacts. The degree of spatial resolution required to properly resolve the interior peak in the actin network, which appears to be associated with a sharp peak in the network stress profile, makes these calculations extremely costly. We believe the very fine discretization is also leading to a poorly conditioned matrix arising as part of the numerical solver. These issues could potentially be resolved with a spatially adaptive routine, but since the one peak solutions are more biologically relevant and they display so little qualitative difference to those obtained from the viscous model we shall not pursue this matter further in this thesis.

4.5 Chapter summary

In this chapter we have generalised the viscous model of Chapter 2 by modelling the cell's actin network as an upper-convected Maxwell fluid. This was in response to concerns that a purely viscous model could not adequately capture the behaviour of a crawling cell over the relevant timescales. We formulated the new viscoelastic model in §4.1, reduced the system in §4.1.3 and transformed to a travelling frame in §4.1.5. The extra parameters introduced in this formulation are discussed in §4.1.6. To determine which terms drive the crawling strip, and to display explicitly the history dependence introduced by the viscoelastic formulation, we presented a potential hodograph transform of the governing equations in §4.1.7. This was compared to the viscous model in the case where there is no polymerization or depolymerization of the network.

We have also presented a linear stability analysis. In §4.2.1 we consider perturbations to a spatially uniform steady state in order to linearize the governing equations. How the growth rate of such a perturbation depends on its wavenumber is calculated in §4.2.2 in the case where the spatially uniform steady state that we perturb is stationary. We show that for a particular parameter regime the largest growth rate plateaus to a positive constant as in the viscous model. However, the viscoelastic formulation has introduced an additional root to the growth rate equation that is important in understanding the early time behaviour of the linearized initial value problem presented in §4.2.4. The growth rate characterization highlights the need for a sensible parameter choice; the viscoelastic model is ill-posed when the viscoelastic relaxation timescale is longer than the network turnover timescale. In §4.2.3 we find the modes selected by the boundary conditions with calculations very similar to those used for the viscous model. The solution of the linearized initial value problem in §4.2.4 demonstrates that the viscoelastic model retains the biologically relevant features of the viscous model: motion of the travelling strip requires a break in symmetry of the initial conditions and the final crawling velocity depends on both the frequency and the relative amplitude of the modes that form the initial perturbation. We show, as validation of our analysis, that we retain the results of the minimal model in the appropriate viscous limit in §4.2.5.

The small growth rate analysis presented in §4.3 reveals that both a short timescale analysis and a very short timescale analysis are required to connect arbitrary initial conditions with the set of equations that governing the late-time evolution of the perturbation.

On the first, very fast timescale we see rapid redistribution of the network volume fraction and stress until the perturbation of the network stress is proportional to the perturbation of the network volume fraction plus some function which depends only on time. Over the next intermediate timescale this function tends to zero as the perturbations to the network volume fraction and the network stress translate to have zero mean. During this stage the travelling strip moves at a constant velocity and the network velocity varies across the strip, but has no time dependence, at leading order. If, after the rapid re-distribution of network, we can identify one side of the cell as having a greater than mean actin volume fraction then this side will become the rear of the cell. The velocity of the network and the travelling strip evolve on the long timescale according to (4.89) and (4.91) respectively.

In §4.4 we proceed to present numerical solutions of the full nonlinear governing equations. The code is first verified by checking convergence and comparison to the linear stability predictions in §4.4.1. We present in §4.4.2 a solution to the initial value problem in which the initial conditions are a small perturbation to the spatially uniform strip. We present graphs to show how the network volume fraction, network velocity, network stress and the force exerted on the substrate by the travelling strip all evolve to a constant profile as the velocity of the travelling strip tends to a constant. The long-time steady, travelling-wave profiles are qualitatively the same as the one-peak solutions of the viscous model. The similarities to the viscous model are further highlighted by the investigation of the effect of the Deborah number in §4.4.3. Finally in §4.4.4 we demonstrate that the viscoelastic model also displays the biologically observed bell-shaped dependence of velocity on adhesion strength.

In summary, this chapter demonstrates that incorporating the short-time elastic behaviour into our model for cell crawling has some quantitative, but no real qualitative, effect. This justifies our assertion in Chapter 2 that over the timescales relevant to cell crawling it is reasonable to treat the actin network as purely viscous, at least in the first instance. We have demonstrated that viscoelasticity of the actin network can be readily incorporated into the reactive, two-phase, flow framework, which will be useful for future models seeking to make quantitative predictions when more of the parameter values are known more accurately. We shall now return to our viscous formulation and consider a hierarchy of more sophisticated models for the effect of myosin on the dynamics of the actin network in Chapter 5.

Chapter 5

Modelling the effect of myosin

A major simplification in the viscous model of Chapter 2 is the assumption that myosin is uniformly distributed through the network phase, so that the swelling or contraction of the network can be understood purely in terms of the volume fraction of the network. In this chapter we relax this assumption by explicitly tracking the distribution of myosin in the cell. The first advantage of this generalization is that we can formulate different constitutive equations for the swelling/contractile pressure, Ψ , that depend both on the local volume fraction and the local concentration of myosin bound to the network. The network contraction can be modelled as a process driven by the network volume fraction, myosin concentration or a combination of the two. The second advantage of modelling myosin explicitly is that it provides us with an extra piece of information to compare to biological observations.

To date, authors have included myosin in their crawling cell models in a variety of ways. In [100], [116] and [153] the distribution of free myosin is assumed to be spatially uniform, while the distribution of bound myosin varies with space and time, and the model accounts for binding and unbinding of myosin to the network. This approach is taken one step further in [133], where the diffusion of free myosin and the binding/unbinding rates are assumed to be fast, so that the concentration of bound myosin is proportional to the local cytoskeleton density multiplied by the local myosin binding rate. A similar approach is adopted in [67], where the authors introduce a third myosin species and distinguish between bound, inactivated and bound, activated myosin. They model the concentration of free myosin as spatially uniform and have constant binding/unbinding rates. Their activation rate for the bound myosin molecules and the actin density are both prescribed functions of distance along the one-dimensional cell. So, the concentration of bound, activated myosin is a

prescribed, time independent function. All of the models in the literature discussed so far in this chapter are single phase models. In the two-phase model presented in [4], the authors present a coupled advection–reaction–diffusion system for free and bound myosin. However, they only consider the limit in which the free myosin concentration is spatially uniform, there is no advection of myosin and the binding/unbinding is at equilibrium. Therefore, the concentration of bound myosin is given as a function of the network volume fraction. A single phase model presented in [11] proposes that the rate of unbinding/rebinding of myosin to the network is sufficiently rapid to justify an advection–diffusion equation for bound myosin. An advection–diffusion equation is also used to model bound myosin in [62] within the two-phase flow framework. A single-phase viscoelastic model in [131] considers a model in which the concentration of bound myosin satisfies an advection–reaction equation and the free myosin satisfies a reaction–diffusion equation.

There is a need to understand how these different myosin models are related, but more importantly what assumptions they rely on and in which situations such assumptions are valid. In §5.1, we couple a fairly general set of myosin equations to our minimal viscous model. In §5.2, we identify a number of biologically plausible sub-models. In §5.3, we explore the stability of the stationary steady state for some of our sub-models and identify which models are ill-posed, weakly well-posed or well-posed. Informed by the linear stability analysis we select two models to study further, which we shall call the kinetic model and the diffusion model. We claim these models exhibit many of the different types of features observed in more general myosin models and compute numerical solutions of the governing equations in §5.4. Numerical simulations indicated a boundary layer structure in the travelling-wave solution of the diffusion model in the strong cell–substrate adhesion limit. In §5.5 we analyse the boundary layer structure using matched asymptotic expansions and compare our asymptotic and numerical solutions. Finally the results of the chapter are summarised in §5.6.

5.1 Model formulation

5.1.1 Myosin transport

We formulate our model as before in a strip moving on a flat surface, where the location of the strip $a(t) < x < b(t)$ must be found as part of the solution. We retain the equations governing the polymerization, depolymerization and flow of the actin network, (2.1)–(2.2),

that were used in the viscous model in Chapter 2. We supplement these with the following equations to describe the transport and binding dynamics of myosin:

$$\frac{\partial}{\partial t}(m_b\theta) + \frac{\partial}{\partial x}(m_b\theta(u + u_c)) = k_{\text{on}}m_f - k_{\text{off}}m_b, \quad (5.1)$$

$$\frac{\partial}{\partial t}(m_f(1 - \theta)) + \frac{\partial}{\partial x}(m_f(1 - \theta)v) = \frac{\partial}{\partial x} \left(D_{f0}(1 - \theta) \frac{\partial m_f}{\partial x} \right) - k_{\text{on}}m_f + k_{\text{off}}m_b, \quad (5.2)$$

where m_b is the concentration of myosin bound to the actin network per unit volume of network and m_f is the concentration of free myosin per unit volume of solution. Bound myosin is assumed to be advected with the network (at velocity u) and is also assumed to be able to move relative to the network with velocity u_c in order to model the crawling of myosin along actin filaments. The free myosin is advected with the solution (at velocity v) and diffuses through the solution phase, with diffusivity D_{f0} . The reaction terms containing k_{on} and k_{off} describe myosin binding to and unbinding from the actin network. The myosin equations are coupled to the minimal viscous model equations both through the explicit appearance of θ , u and v in the myosin equations and via the constitutive laws, as we shall now describe.

5.1.2 Constitutive laws

The polymerization/depolymerization rate of actin, J , the myosin binding and unbinding rates, k_{on} and k_{off} , and the inter-phase drag, H , are assumed for simplicity to depend only on the local network volume fraction, θ . The myosin crawling velocity, u_c , the swelling and contractile pressure, Ψ , the network viscosity, μ , and the cell–substrate adhesion strength, β , are assumed to depend on the network volume fraction θ and the concentration of bound myosin m_b . We shall assume for simplicity that the diffusivity of unbound myosin in solution, D_{f0} , is constant.

Where specific forms of the constitutive laws are required, for instance in our numerical simulations, we shall use those stated in (2.5) and (2.9) for J , μ , H and β . We adopt the simplest law that makes sense for the binding rate of myosin. We suppose that the rate of binding is proportional to both the volume fraction of network and the concentration of free unbound myosin. Similarly, we assume the unbinding rate to be proportional to the volume fraction of solution and bound myosin, so that

$$k_{\text{on}} = K^+\theta(1 - \theta), \quad k_{\text{off}} = K^-\theta(1 - \theta), \quad (5.3a,b)$$

where K^+ and K^- are positive rate constants. Tracking the concentration of bound myosin allows us to investigate different biophysical mechanisms for driving network contraction. We can view the actin network as a passive polymer network undergoing hydration (swelling), with myosin acting to reduce this swelling, resulting in an effective contraction of the network. We follow [4] in choosing a constitutive law of the form

$$\Psi = -S_0 \ln(1 - \theta) - C_0 \theta m_b, \quad (5.4)$$

where the first term represents swelling of the network and the second represents myosin-driven contraction. We note that the size of the S_0 and C_0 can be chosen so that $\partial\Psi/\partial\theta$ is either positive, or negative or changes sign as the network volume fraction varies, meaning that depending on the parameter values chosen the network volume fraction may drive the network to swell or contract. In contrast, $\partial\Psi/\partial m_b$ is always negative, so myosin always acts to contract the network.

The final constitutive assumption required is an expression for u_c . As discussed in §1.1.4 bound myosin crawls towards the barbed ends of actin filaments. However, our model does not track the orientation of actin filaments. In the absence of more information we assume that the actin network is randomly oriented and formulate a constitutive assumption of the form

$$u_c = -E_0(m_b, \theta) \frac{\partial m_b}{\partial x}. \quad (5.5)$$

This constitutive law assumes that motion on a randomly oriented network is comparable to Brownian motion in free space, so that myosin crawling can be modelled with a diffusive term. In reality the directed crawling of myosin will be more complicated, but this is a simple starting point. Different forms for E will be discussed in §5.2.

5.1.3 Boundary and initial conditions

As well as suitable constitutive laws, boundary and initial conditions are required to close our system. We impose no flux of network or solution out at the ends of the travelling strip, and no mixture stress Σ at either end of the strip, for Σ defined in (2.4). We also impose no flux of free or bound myosin out for the travelling cell, so that

$$\text{at } x = a(t) \quad u = \dot{a}, \quad v = \dot{a}, \quad \Sigma = 0, \quad u_c = 0, \quad \frac{\partial m_f}{\partial x} = 0, \quad (5.6a-e)$$

$$\text{at } x = b(t) \quad u = \dot{b}, \quad v = \dot{b}, \quad \Sigma = 0, \quad u_c = 0, \quad \frac{\partial m_f}{\partial x} = 0, \quad (5.7a-e)$$

where a dot denotes differentiation with respect to time. We also impose the initial conditions

$$a(0) = 0, \quad b(0) = L, \quad \theta(x, 0) = \theta^*(x), \quad m_b(x, 0) = m_b^*(x), \quad m_f(x, 0) = m_f^*(x). \quad (5.8a-e)$$

5.1.4 Reduced system

As in the minimal model we introduce the mixture velocity $V(t)$ and eliminate v and p to obtain (2.16), (5.1) and

$$\frac{\partial}{\partial t}(m_f(1 - \theta)) + \frac{\partial}{\partial x}(m_f(V - \theta u)) = \frac{\partial}{\partial x} \left(D_{f0}(1 - \theta) \frac{\partial m_f}{\partial x} \right) - k_{\text{on}} m_f + k_{\text{off}} m_b. \quad (5.9)$$

We also integrate the mixture force balance (2.3) and apply the zero mixture stress boundary conditions, (5.7c) and (5.8c) to obtain the solvability condition (2.20). We note that summing (5.1) and (5.9), integrating and applying the no-flux boundary conditions we obtain

$$M_0 = \int_a^b m_b \theta + m_f(1 - \theta) dx, \quad (5.10)$$

where M_0 , the total amount of myosin in the travelling strip, is conserved.

5.1.5 Nondimensionalization

We make the same nondimensionalization as detailed in §2.1.5 for the viscous model. We scale our new variables as follows

$$m_b = M_0 m_b^\dagger, \quad m_f = M_0 m_f^\dagger, \quad u_c = \frac{\chi L}{T} u_c^\dagger, \quad (5.11)$$

so that we normalise the total concentration of myosin in the cell to unity and χ is a dimensionless constant included so that we can consider $u^\dagger$ and $u_a^\dagger$ to be of the same order with any difference in order of magnitude absorbed into χ . The constitutive laws and remaining parameter are scaled as follows

$$k_{\text{on}} = \frac{1}{T} k_{\text{on}}^\dagger, \quad k_{\text{off}} = \frac{1}{T} k_{\text{off}}^\dagger, \quad D_{f0} = \frac{L^2}{T} D_f^\dagger. \quad (5.12)$$

5.1.6 Travelling frame

As in the minimal model, we move to a frame that travels with the strip at velocity $V(t)$ by making the transformation (2.24), along with

$$m_b^\dagger(x^\dagger, t^\dagger) = \hat{m}_b(\hat{x}, \hat{t}), \quad m_f^\dagger(x^\dagger, t^\dagger) = \hat{m}_f(\hat{x}, \hat{t}), \quad u_c^\dagger(x^\dagger, t^\dagger) = \hat{u}_c(\hat{x}, \hat{t}). \quad (5.13a,b)$$

After dropping hats and daggers we obtain the following governing equations

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad (5.14)$$

$$-\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x} \left(\mu \frac{\partial u}{\partial x} \right) = \alpha u + \beta(u + V), \quad (5.15)$$

$$\frac{\partial}{\partial t}(m_b \theta) + \frac{\partial}{\partial x}(m_b \theta(u + \chi u_c)) = k_{\text{on}} m_f - k_{\text{off}} m_b, \quad (5.16)$$

$$\frac{\partial}{\partial t}(m_f(1 - \theta)) - \frac{\partial}{\partial x}(m_f \theta u) = \frac{\partial}{\partial x} \left(D_f(1 - \theta) \frac{\partial m_f}{\partial x} \right) - k_{\text{on}} m_f + k_{\text{off}} m_b \quad (5.17)$$

for $t > 0$, $0 < x < 1$, subject to the boundary conditions

$$u = 0, \quad u_c = 0, \quad \frac{\partial m_f}{\partial x} = 0, \quad (5.18a-c)$$

at $x = 0$ and $x = 1$, and the initial conditions

$$\theta(x, 0) = \theta^*(x), \quad m_b(x, 0) = m_b^*(x), \quad m_f(x, 0) = m_f^*(x), \quad (5.19a-c)$$

for $0 < x < 1$. We also require the solvability condition

$$V = -\frac{\langle \beta u \rangle}{\langle \beta \rangle}. \quad (5.20)$$

Dimensionless versions of the constitutive laws are stated here for ease of reference

$$J(\theta) = \theta - \theta_E, \quad \Psi(\theta) = -S^* \ln(1 - \theta) - C^* \theta m_b, \quad (5.21a,b)$$

$$\mu(\theta) = \theta, \quad \alpha(\theta) = \alpha^* \theta / (1 - \theta), \quad \beta(\theta) = \beta^* \theta, \quad (5.22a-d)$$

$$k_{\text{on}} = k_{\text{on}}^* \theta(1 - \theta), \quad k_{\text{off}} = k_{\text{off}}^* \theta(1 - \theta), \quad u_c = -E(m_b, \theta) \frac{\partial m_b}{\partial x}, \quad (5.23a-c)$$

where, in addition to θ_E and χ , the dimensionless parameters are

$$S^* = \frac{S_0 T}{\mu_0}, \quad C^* = \frac{C_0 M_0 T}{\mu_0}, \quad \alpha^* = \frac{H_0 L^2}{\mu_0}, \quad \beta^* = \frac{\beta_0 L^2}{\mu_0}, \quad (5.24a-d)$$

$$k_{\text{on}}^* = k^+ T, \quad k_{\text{off}}^* = k^- T, \quad D_f = \frac{D_{f0} T}{L^2}, \quad (5.25a-c)$$

The first set of parameter groupings, (5.24), represent the ratio of swelling pressure, contractile pressure, network-solution and network-substrate drag to the viscous forces in the actin network respectively. The next set of constants, (5.25), compare the typical timescale for network turnover to the timescales for myosin binding, myosin unbinding and diffusion of free myosin through the cytosol.

5.1.7 Dimensionless parameters

Appropriate values for some of the nondimensional parameters have been discussed in §2.1.7, namely $\theta_E = 0.02$, $\alpha^* = 4 \times 10^{-5} - 4 \times 10^3$ and $\beta^* = 2 - 2 \times 10^7$. We shall continue to use $T = 1\text{min}$ as the typical timescale for the network turnover and $L = 20\mu\text{m}$ as the typical lengthscale for a crawling cell. By comparing the form of the swelling/contractile pressure stated in (5.21b) to that used in the minimal model, and insisting the maximum pressure is similar, we estimate $C^* = 0 - 2.5 \times 10^4$. A comparable constitutive law is used in [45] and there the swelling and contractile pressures are similar in size, we take $S^* = 0 - 2.5 \times 10^4$.

There is a distinct lack of useful quantitative information available in the literature for estimating myosin binding, unbinding or diffusion rates. Larripa and Mogilner stated in 2006 that myosin kinetic rates were unknown [100]. Verkhovsky *et al.* [148] estimate the dissociation rate of myosin aggregates to be of the order of 3min^{-1} , while the unbinding rate is claimed to be less than $6 \times 10^{-1}\text{min}^{-1}$ in [43]; however, this measurement is taken *in vitro* and it is unclear what type of myosin was used in the experiment. We therefore consider $k_{\text{off}}^* = 0.1 - 10$ to be an appropriate parameter range, and, in the absence of binding rate information we take $k_{\text{on}}^* = 0.1 - 10$ also. The best guidance for the size of the diffusion coefficient we have found in the literature is $4.3 \times 10^3 \mu\text{m}^2\text{min}^{-1}$, which is the diffusion coefficient for heavy meromyosin over actin in the presence of ATP [16]. The first caveat is that heavy meromyosin is a sub-unit of the myosin II molecule we are primarily interested in. The second caveat is that diffusion over actin in the presence of ATP suggests this diffusion coefficient amalgamates pure diffusion through the solution and ATP-funded ‘crawling’ along actin fibres. Due to our lack of confidence in this parameter estimate we suggest $D_{f0} = 10^2 - 10^5 \mu\text{m}^2\text{min}^{-1}$ as a suitable parameter range, which leads to an estimate of $D_f = 2.5 \times 10^{-1} - 2.5 \times 10^2$. In [146] it is reported that retrograde flow of actin occurs at velocities of the order of $1\mu\text{mmin}^{-1}$, while [2] found typical crawling velocities for myosin V molecules along actin filaments to be an order of magnitude larger. However, this is somewhat unsatisfactory as an estimate for myosin crawling velocities because again it refers to a different type of myosin whose role is to transport cargo along filaments. We suggest it is likely that myosin II crawls much more slowly, in part because it gets accumulated into aggregates, so we propose $\chi = 0 - 10$.

This discussion of parameter values serves to highlight the number of parameters we have little confidence in estimating. Furthermore, the system is now much more complicated

than those we have previously considered in this thesis. Attempting a stability analysis of perturbations to the equilibrium volume fraction and equilibrium bound and unbound myosin concentrations in the full system (not shown) leads to a complicated system which provides little insight. In the next section we shall probe the range of stated parameter values by considering possible limits of the myosin equations. We aim to highlight the key features exhibited by the model in different parameter regimes.

5.2 Sub-models

Since the range of parameter values proposed for D_f , k_{on}^* , k_{off}^* and χ encompass values that could be considered small or big we present in this section various model reductions obtained for different parameter limits of (5.16) and (5.17). We compare these models to those used in the literature.

5.2.1 Rapid diffusion of free myosin

We consider first the effect of very fast diffusion of free myosin through the cytosol. For $D_f \gg 1$, with the remaining parameters of interest order one, at leading order (5.17) becomes

$$\frac{\partial}{\partial x} \left(D_f (1 - \theta) \frac{\partial m_f}{\partial x} \right) = 0. \quad (5.26)$$

After employing the no-flux boundary condition (5.18c), we see that at leading order the free myosin is distributed uniformly throughout the solution phase, so that m_f depends only on time. The myosin equations (5.16) and (5.17) thus reduce to

$$\frac{\partial}{\partial t} (m_b \theta) + \frac{\partial}{\partial x} (m_b \theta (u + \chi u_c)) = k_{\text{on}} m_f - k_{\text{off}} m_b, \quad (5.27)$$

where m_f is given by

$$m_f(t) \int_0^1 (1 - \theta) dx = 1 - \int_0^1 m_b \theta dx. \quad (5.28)$$

We have a reaction–advection equation for bound myosin and an integral constraint for the spatially uniform concentration of free myosin. Note that if we substitute (5.23c), our randomly–aligned–filaments constitutive assumption for u_c , into (5.27), then it becomes a reaction–advection–diffusion equation for m_b that may be viewed as the multiphase generalization of the myosin transport equation presented in [100].

A singular sub-limit of this regime is obtained by assuming myosin crawls slowly along actin fibres, so that $\chi \ll 1$, in which case (5.27) becomes

$$\frac{\partial}{\partial t}(m_b\theta) + \frac{\partial}{\partial x}(m_b\theta u) = k_{\text{on}}m_f - k_{\text{off}}m_b, \quad (5.29)$$

at leading order. Thus, in this particular regime bound myosin is advected with the actin filaments and transferred between the bound state and a spatially uniform reservoir of free myosin.

5.2.2 Slow reaction kinetics

We now consider the limit obtained when the binding and unbinding rates of myosin are slow. We consider $k_{\text{on}} \ll 1$ and $k_{\text{off}} \ll 1$, whilst the other parameters of interest are $O(1)$. At leading order, the myosin transport equations (5.16) and (5.17) decouple. Since none of our constitutive laws depends on the concentration of free myosin, it decouples from the problem entirely. Using the constitutive assumption for randomly aligned filaments, (5.23c), we obtain the following advection–diffusion equation for the concentration of bound myosin

$$\frac{\partial}{\partial t}(m_b\theta) + \frac{\partial}{\partial x}(m_b\theta u) = \chi \frac{\partial}{\partial x} \left(m_b\theta E \frac{\partial m_b}{\partial x} \right), \quad (5.30)$$

where the diffusion coefficient is a function of θ and m_b . If we select $E = 1/m_b$ then we obtain the advection–diffusion equation for myosin previously studied in [62]. This form of the constitutive function E could be justified by assuming that myosin crawling slows as the concentration of myosin increases due to aggregation of myosin molecules or myosin molecules blocking others on the same or neighbouring filaments.

In the slow myosin crawling sub-limit, $\chi \ll 1$, we recover an advection equation for the concentration of bound myosin

$$\frac{\partial}{\partial t}(m_b\theta) + \frac{\partial}{\partial x}(m_b\theta u) = 0. \quad (5.31)$$

This is exactly the form of the myosin equation employed by Liu in [105].

5.2.3 Rapid reaction kinetics

We now turn our attention to the opposite extreme and consider what form the myosin equations take when the binding and unbinding rates are very fast. We consider $k_{\text{on}}^* = O(1/\delta)$ and $k_{\text{off}}^* = O(1/\delta)$, where $\delta \ll 1$ and the remaining parameters are of $O(1)$. The

first order problem does not provide enough information to close the system, and we must proceed to the next order: in anticipation of this we introduce expansions of the form

$$m_b \sim m_{b0} + \delta m_{b1} + \dots, \quad m_f \sim m_{f0} + \delta m_{f1} + \dots \quad (5.32)$$

At leading order the binding and unbinding kinetics are in equilibrium (there is no net binding or unbinding of myosin to the actin network) and the concentrations of free and bound myosin satisfy

$$k_{\text{on}} m_{f0} = k_{\text{off}} m_{b0}. \quad (5.33)$$

Note that if k_{on} and k_{off} each have a different dependence on the network volume fraction, then the ratio of free to bound myosin can vary spatially along the strip. At next order we obtain partial differential equations for the first order myosin concentration with reaction terms that depend on the higher order corrections

$$\frac{\partial}{\partial t}(m_{b0}\theta) + \frac{\partial}{\partial x}(m_{b0}\theta(u + \chi u_a)) = k_{\text{on}} m_{f1} - k_{\text{off}} m_{b1}, \quad (5.34)$$

$$\frac{\partial}{\partial t}(m_{f0}(1 - \theta)) - \frac{\partial}{\partial x}(m_{f0}\theta u) = \frac{\partial}{\partial x} \left(D_f(1 - \theta) \frac{\partial m_{f0}}{\partial x} \right) - k_{\text{on}} m_{f1} + k_{\text{off}} m_{b1}. \quad (5.35)$$

These can be summed to eliminate m_{b1} and m_{f1} . Using (5.33) we can eliminate m_{f0} to obtain an equation solely for m_{b0}

$$\begin{aligned} \frac{\partial}{\partial t} \left(m_{b0} \left(\theta + \frac{k_{\text{off}}}{k_{\text{on}}} (1 - \theta) \right) \right) + \frac{\partial}{\partial x} \left[m_{b0}\theta \left(u \left(1 - \frac{k_{\text{off}}}{k_{\text{on}}} \right) + \chi u_c \right) \right] \\ = \frac{\partial}{\partial x} \left[D_f(1 - \theta) \frac{\partial}{\partial x} \left(\frac{k_{\text{off}}}{k_{\text{on}}} m_{b0} \right) \right]. \end{aligned} \quad (5.36)$$

This is an advection–diffusion equation for the bound myosin, albeit with some exotic coefficients that depend on the ratio of the myosin binding and unbinding rates and the local network volume fraction. Using (5.23c) we see two alternative biological mechanisms that appear in the governing equations as diffusion of bound myosin through the network. The first depends on the assumption of rapid binding/unbinding rates and represents myosin unbinding, diffusing through the solution phase, then re-binding at a new site on the actin network. The second represents myosin crawling along actin fibres.

There are a number of interesting parameter sub-limits to be discussed here. The simplest arises if we assume slow crawling of myosin and slow diffusion of free myosin, which yields an advection equation for bound myosin with modified coefficients

$$\frac{\partial}{\partial t} \left(m_{b0} \left(\theta + \frac{k_{\text{off}}}{k_{\text{on}}} (1 - \theta) \right) \right) + \frac{\partial}{\partial x} \left[m_{b0}\theta u \left(1 - \frac{k_{\text{off}}}{k_{\text{on}}} \right) \right] = 0. \quad (5.37)$$

Somewhat surprisingly it appears that rapid binding and unbinding when there is little diffusion of free myosin or crawling of bound myosin leads to a situation where myosin can be advected against the flow of the actin network if $k_{\text{off}}/k_{\text{on}} > 1$.

Both the binding and unbinding rates are assumed to be fast in this section, but we could consider within this regime what happens when the binding rate is fast compared to the unbinding rate. This would be equivalent to stating that the myosin spends more time bound to the network than free in solution. If we neglect $k_{\text{off}}/k_{\text{on}}$ terms in (5.36) then we recover the advection–diffusion equation for slow kinetics given by (5.30). We could also obtain an advection–diffusion equation by an alternative route with slow crawling myosin by considering $k_{\text{off}}/k_{\text{on}}$ small and D_f of the order of $k_{\text{on}}/k_{\text{off}}$ modelling fast diffusion of free myosin through the solution, which leads to

$$\frac{\partial}{\partial t} (m_{b0}\theta) + \frac{\partial}{\partial x} (m_{b0}\theta u) = \frac{\partial}{\partial x} \left[D_f(1-\theta) \frac{\partial}{\partial x} \left(\frac{k_{\text{off}}}{k_{\text{on}}} m_{b0} \right) \right], \quad (5.38)$$

a fairly simple advection–diffusion equation where diffusion of bound myosin is solely due to the diffusion of free myosin through the solution phase. We note there remains an interesting nonlinear diffusion coefficient which depends on the constitutive assumptions for k_{on} and k_{off} .

5.2.4 Rapidly crawling myosin

Now we consider the effect of modelling myosin crawling along actin filaments as fast in conjunction with our randomly–aligned–fibres constitutive assumption. When $\chi \gg 1$, and the remaining parameters are of order unity, the crawling term dominates and the boundary condition (5.18b) ensures that bound myosin is spread uniformly throughout the actin network and everywhere proportional to the network volume fraction. This is consistent with assumptions made in our minimal model presented in Chapter 2.

5.2.5 Rapid reaction kinetics and rapid diffusion of free myosin

The final limit we consider is that which arises when the free myosin diffuses quickly through the solution and the myosin binding and unbinding kinetics are also rapid. We consider $k_{\text{on}} \gg 1$, $k_{\text{off}} \gg 1$ and $D_f \gg 1$ in (5.16) and (5.17). At leading order we see that

$$k_{\text{on}} m_f = k_{\text{off}} m_b, \quad k_{\text{on}} m_f = \frac{\partial}{\partial x} \left(D_f(1-\theta) \frac{\partial m_f}{\partial x} \right) + k_{\text{off}} m_b, \quad (5.39a,b)$$

so as in §5.2.1 we find that m_f depends only on time and is given by (5.28). Substituting into (5.39a) we find that

$$m_f(t) = \frac{1}{\langle 1 - \theta + k_{\text{on}}\theta/k_{\text{off}} \rangle}, \quad m_b(x, t) = \frac{k_{\text{on}}}{k_{\text{off}}\langle 1 - \theta + k_{\text{on}}\theta/k_{\text{off}} \rangle}. \quad (5.40a, b)$$

Hence, the concentration of bound myosin per unit volume of network is a function of the network volume fraction and the model parameters. This is very similar to the myosin equations implemented by Alt in *et al.* [4].

This section enables us to understand a range of models available in the literature as different limits of the same myosin equations. We have also uncovered some new possible myosin models that are simpler than (5.16) and (5.17). In the next section we continue to explore the implications of the assumptions that generate these sub-models by considering their effect on the stability of the spatially uniform steady state to small perturbations.

5.3 Effect of myosin modelling on stability and posedness

In this section we will present the results of linear stability analyses for a number of the different limits discussed above. We show how myosin-driven contraction provides a new mechanism for destabilising the steady state. We also identify which of the sub-models we consider have ill-posed regions in parameter space. We model myosin as contracting the network, so that the derivative of Ψ with respect to m_b , evaluated at the equilibrium values, is negative.

5.3.1 Slow reaction kinetics

Probably the simplest sub-model (besides that which recovers our minimal model in §5.2.4) is obtained in §5.2.2 when the myosin binding and unbinding rates are assumed slow. For clarity we rewrite (5.30) as

$$\frac{\partial}{\partial t}(m_b\theta) + \frac{\partial}{\partial x}(m_b\theta u) = \frac{\partial}{\partial x} \left(D_m\theta \frac{\partial m_b}{\partial x} \right), \quad (5.41)$$

where D_m can in general be a function of θ and m_b . We write it in this form to emphasize how the combination of slow binding kinetics and randomly aligned actin filaments leads to a formulation representing advection and diffusion of bound myosin through the actin network. This general form of equation is also obtained in sublimits of the rapid kinetics limit, see §5.2.3. We treat D_m as a constant in the subsequent stability analysis without

loss of generality. Furthermore, since the free myosin concentration does not appear in this limit, it is convenient to renormalise the concentration of bound myosin such that

$$\int_0^1 m_b \theta \, dx = \theta_E, \quad (5.42)$$

so that the equilibrium myosin concentration per unit volume of network is one. Linear stability analysis has already been carried out for an equivalent model in [62] and for $D_m = 0$ in [105]. Below we summarise the findings.

We consider small perturbations, proportional to $\varepsilon \exp(ikx + \Omega t)$, where $0 < \varepsilon \ll 1$, about the steady state $\theta = \theta_E$, $u = 0$, $m_b = 1$ on an unbounded domain. We obtain the quadratic equation $A\Omega^2 + B\Omega + C = 0$ for Ω in terms of the wavenumber, k , of the perturbation, where

$$A(k) = \alpha_E + \beta_E + \mu_E k^2, \quad (5.43)$$

$$B(k) = D_m A k^2 + A J'_E + \Psi_{\theta E} \theta_E k^2, \quad (5.44)$$

$$C(k) = J'_E D_m A k^2 + J'_E \Psi_{mE} k^2 + D_m \Psi_{\theta E} \theta_E k^4. \quad (5.45)$$

The derivatives of the swelling/contractile stress with respect to the network volume fraction and the concentration of bound myosin are written as Ψ_θ and Ψ_m respectively. The subscript E on any function indicates it is evaluated at the equilibrium network volume fraction and equilibrium bound myosin concentration. The $(k = 0)$ perturbation is neutrally stable and there are two real roots to the growth rate equation, since $\Psi_{mE} < 0$. If $D_m = 0$, then both roots are monotonic in k and plateau to a constant value for large k . The larger root of the growth rate equation plateaus to a positive value, so all wavenumbers are unstable. The maximum growth rate is given by

$$\frac{-\Psi_{\theta E} \theta_E - \mu_E J'_E + \sqrt{(\Psi_{\theta E} \theta_E + \mu_E J'_E)^2 - 4 \Psi_{mE} J'_E \mu_E}}{2 \mu_E}, \quad (5.46)$$

which increases as $\Psi_{\theta E}$ decreases and as Ψ_{mE} decreases. We see that myosin-driven contraction of the network is sufficient to destabilise the steady state, and that volume-fraction-driven swelling/contraction of the network is a stabilising phenomenon for $\Psi_{\theta E} > 0$ and destabilising for $\Psi_{\theta E} < 0$. For nonzero D_m the roots are not necessarily monotonic: the smaller root decays proportionally to $-D_m k^2$ as $k \rightarrow \infty$, whilst the larger root plateaus to

$$-\frac{\mu_E J'_E + \theta_E \Psi_{\theta E}}{\mu_E}. \quad (5.47)$$

In [62] a perturbation is shown to be unstable if and only if $C(k) < 0$. So there exist parameter regimes with $\Psi_{mE} < 0$ for which the growth rate is positive for small k , has a local maximum and plateaus for large k . If $\Psi_{\theta E} < -\mu_E J'_E / \theta_E$, the growth rate is positive as $k \rightarrow \infty$ and the model is weakly well-posed. Alternatively, if $\Psi_{\theta E} > -\mu_E J'_E / \theta_E$, large wavenumbers are stable, so coupling an advection diffusion equation for bound myosin has stabilised the linearized minimal model at large wavenumbers.

We proceed to report the results of the linear stability analyses carried out on the bounded domain in [62] and [105]. Substituting perturbations of the form $u = \varepsilon u_1(x)e^{\omega t}$, $\theta = \theta_E + \varepsilon \theta_1(x)e^{\omega t}$, $m_b = 1 + \varepsilon m_1(x)e^{\omega t}$ and $V = \varepsilon V_1 e^{\omega t}$, into (5.14), (5.15), (5.20) and (5.41) results at $O(\varepsilon)$ in

$$Qu_1'''' - 2Ru_1'' + Su_1 = -\beta_E \omega(\omega + J'_E)V_1, \quad (5.48)$$

a fourth-order ordinary differential equation where dash denotes differentiation with respect to x , and

$$Q(\omega) = D_m [\theta_E \Psi_{\theta E} + \mu_E(\omega + J'_E)], \quad (5.49)$$

$$R(\omega) = \frac{1}{2} [(\omega + J'_E)(D_m(\alpha_E + \beta_E) + \mu_E \omega) + \omega \theta_E \Psi_{\theta E} + J'_E \Psi_{mE}], \quad (5.50)$$

$$S(\omega) = \omega(\omega + J'_E)(\alpha_E + \beta_E). \quad (5.51)$$

The boundary conditions are $u_1(0) = u_1(1) = 0$ along with

$$u_1''(0) = u_1''(1) = \frac{\beta_E(\omega + J'_E)V_1}{\theta_E \Psi_{\theta E} + \mu_E(\omega + J'_E)}. \quad (5.52)$$

Clearly when $D_m = 0$ the order of the problem reduces and we do not require the second pair of boundary conditions. Both cases share considerable similarities with the stability analysis of the minimal model. They each have two families of solutions; a set where θ_1 and m_1 are symmetric about $x = 1/2$ corresponding to $V_1 = 0$ and an asymmetric set for $V_1 \neq 0$. In the case where $D_m = 0$, the wavenumbers used to construct the asymmetric modes are selected by a solvability condition obtained from the linearized integral constraint. In the case where $D_m \neq 0$, the analysis is far more involved, the perturbation to the network velocity has the form

$$u_1 = \frac{\beta_E V_1}{\alpha_E + \beta_E} \left(\frac{U_1 \cos(k_1(x - 1/2))}{\cos(k_1/2)} + \frac{U_2 \cos(k_2(x - 1/2))}{\cos(k_2/2)} - 1 \right) \quad (5.53)$$

where the real part is understood, U_1 and U_2 are constants determined by the boundary conditions and

$$k_1 = \sqrt{\frac{-R + \sqrt{R^2 - QS}}{Q}}, \quad k_2 = \sqrt{\frac{-R - \sqrt{R^2 - QS}}{Q}}, \quad (5.54a,b)$$

which are in general complex. Imposing the linearized version of the integral constraint (5.20) yields

$$\text{Re} \left[\frac{U_1}{k_1} \tan \left(\frac{k_1}{2} \right) + \frac{U_2}{k_2} \tan \left(\frac{k_2}{2} \right) + \frac{\alpha_E}{2\beta_E} \right] = 0, \quad (5.55)$$

which we note is stated incorrectly in [62]. At first glance this looks very similar to the stability analysis in §2.2.2 where equation (2.62) selects a wavenumber in each 2π interval. However, since k_1 and k_2 are given in terms of ω by (5.54), equation (5.55) should be understood as a condition to select growth rates. The growth rate with the largest real part is then used to determine k_1 and k_2 and hence the form of the perturbation that grows fastest. It has not been possible to fully characterize the roots of (5.55). We have found real solutions, one of which is used in Appendix B to verify this stability analysis against the numerical solutions. We have also found complex ω , suggesting that small perturbations to the equilibrium network volume fraction may travel as they grow, providing a possible mechanism for the experimentally observed ruffling of actin networks.

5.3.2 Fast diffusion of free myosin with slow crawling bound myosin

We now present the results of a linear stability analysis in the limit obtained when free myosin diffuses rapidly through the solution phase, as described in (5.14), (5.15), (5.20), (5.27) and (5.28) in §5.2.1. A spatially uniform steady state is given by $\theta = \theta_E$, $u = 0$, $V = 0$, $m_b = M_b$ and $m_f = M_f$, where

$$M_b = \frac{k_{\text{on}E}}{k_{\text{off}E}(1 - \theta_E) + k_{\text{on}E}\theta_E}, \quad M_f = \frac{k_{\text{off}E}}{k_{\text{off}E}(1 - \theta_E) + k_{\text{on}E}\theta_E}, \quad (5.56a,b)$$

and as before a subscript E indicates a function is evaluated at the values corresponding to our spatially uniform steady state. We consider here the slow crawling sub-limit by substituting perturbations of the form $u \sim \varepsilon u_1(x)e^{\omega t} + \dots$, $V \sim \varepsilon V_1 e^{\omega t} + \dots$, $\theta \sim \theta_E + \varepsilon \theta_1(x)e^{\omega t} + \dots$, $m_b \sim M_b + \varepsilon m_{b1}(x)e^{\omega t} + \dots$ and $m_f \sim M_f + \varepsilon m_{f1}e^{\omega t} + \dots$ into (5.14), (5.15), (5.20), (5.28) and (5.29) for $\varepsilon \ll 1$. At $O(\varepsilon)$ we find

$$\omega \theta_1 + \theta_E u'_1 = -J'_E \theta_1, \quad -\Psi_{\theta E} \theta'_1 - \Psi_{mE} m'_{b1} + \mu_E u''_1 = \alpha_E u_1 + \beta_E (u_1 + V_1), \quad (5.57a,b)$$

$$\omega \theta_E m_{b1} + \omega M_b \theta_1 + M_b \theta_E u'_1 = (k'_{\text{on}E} M_f - k'_{\text{off}E} M_b) \theta_1 + k_{\text{on}E} m_{f1} - k_{\text{off}E} m_{b1}, \quad (5.58)$$

$$V_1 = -\langle u_1 \rangle, \quad (M_b - M_f)\langle \theta_1 \rangle + (1 - \theta_E)m_{f1} = -\theta_E\langle m_{b1} \rangle, \quad (5.59a,b)$$

which must be solved subject to initial conditions for θ_1 and m_{b1} and the boundary conditions $u(0, t) = u(1, t) = 0$, where as previously $'$ denotes differentiation with respect to x and the angular brackets indicate the integral over the travelling strip.

Manipulating (5.57)–(5.59), we can eliminate θ_1 , m_{b1} and m_{f1} to obtain a second-order, ordinary differential equation for u_1 forced by V_1 . As in the minimal model there are two families of solutions. Symmetric (non-flat) perturbations of the network volume fraction and bound myosin concentration are associated with $V_1 = 0$ and are of the form

$$u_1^\pm = G \sin(n\pi x), \quad \theta_1^\pm = -\frac{G\theta_E n\pi}{\omega_n^\pm + J'_E} \cos(n\pi x), \quad m_{f1}^\pm = 0, \quad (5.60a-c)$$

$$m_{b1}^\pm = -\left(\frac{G\theta_E n\pi}{\omega_n^\pm + J'_E}\right) \left(\frac{M_b J'_E + M_f k'_{\text{on}E} - M_b k'_{\text{off}E}}{\omega_n^\pm \theta_E + k_{\text{off}E}}\right) \cos(n\pi x), \quad (5.61a,b)$$

for some arbitrary constant G and n an even integer and have the growth rate $Re(\omega_n^\pm)$, where $\omega_n^\pm = \omega_\pm(n\pi)$ is defined below. Alternatively, asymmetric eigensolutions that cause the strip to travel are of the form

$$u_1^\pm = \frac{\beta_E V_1}{\alpha_E + \beta_E} \left(\frac{\cos(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)} - 1 \right), \quad \theta_1^\pm = \frac{\beta_E V_1 \theta_E \lambda_n \sin(\lambda_n(x - 1/2))}{(\omega_{(\lambda_n)}^\pm + J'_E)(\alpha_E + \beta_E) \cos(\lambda_n/2)}, \quad (5.62a,b)$$

$$m_{b1}^\pm = \frac{\beta_E V_1 \theta_E \lambda_n (k'_{\text{on}E} M_f - k'_{\text{off}E} M_b + M_b J'_E)}{(\omega_{(\lambda_n)}^\pm \theta_E + k_{\text{off}E})(\omega_{(\lambda_n)}^\pm + J'_E)(\alpha_E + \beta_E)} \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad m_{f1} = 0, \quad (5.63a,b)$$

where the λ_n are selected by

$$\frac{\alpha_E}{\beta_E} + \frac{2}{\lambda_n} \tan\left(\frac{\lambda_n}{2}\right) = 0, \quad (5.64)$$

and the growth rate of the asymmetric perturbations is $Re(\omega_{(\lambda_n)}^\pm)$, where $\omega_{(\lambda_n)}^\pm = \omega_\pm(\lambda_n)$.

Now we shall characterize the growth rate as a function of the model parameters. The growth satisfies a quadratic equation, with roots

$$\omega_\pm(k) = \frac{-B \pm \sqrt{B^2 - 4AC}}{2A}, \quad (5.65)$$

where

$$A(k) = \theta_E(\alpha_E + \beta_E + \mu_E k^2) = \theta_E \tilde{A}, \quad (5.66)$$

$$B(k) = \tilde{A}(k_{\text{off}E} + J'_E \theta_E) + \theta_E^2 \Psi_{\theta E} k^2, \quad (5.67)$$

$$C(k) = \tilde{A} J'_E k_{\text{off}E} + (J'_E M_b - k'_{\text{off}E} M_b + k'_{\text{on}E} M_f) \theta_E \Psi_{mE} k^2 + k_{\text{off}E} \theta_E \Psi_{\theta E} k^2, \quad (5.68)$$

and we have introduced $\tilde{A}(k)$ for brevity. Perturbations with zero wavenumber are stable, while as $k \rightarrow \infty$ both roots of the growth rate equation tend to a constant. Now we consider the discriminant $\Delta = B^2 - 4AC$ which can be written as

$$\Delta = \left[\tilde{A}(k_{\text{off}E} - J'_E \theta_E) - \theta_E^2 \Psi_{\theta E} k^2 \right]^2 + 4 \tilde{A} \theta_E^2 \Psi_{mE} k^2 (M_b(k'_{\text{off}E} - J'_E) - k'_{\text{on}E} M_f). \quad (5.69)$$

In considering the sign of the second term we note that $\tilde{A}$, θ_E , M_b and M_f are all positive and for sensible choices of constitutive equation $J'_E > 0$ and $\Psi_{mE} < 0$. Determining the sign of $M_b(k'_{\text{off}E} - J'_E) - k'_{\text{on}E} M_f$, without specifying constitutive equations for k_{on} and k_{off} is not as simple. There are no local extrema of the growth rate curves, so if Δ is positive for all k then both roots of the growth rate equation are monotonic in k .

We now consider different mechanisms by which the steady state can be unstable to small perturbations. The steady state will be unstable if $B < 0$: this is the case for k greater than some critical wavenumber when $\Psi_{\theta E} < -\mu_E(k_{\text{off}E} + J'_E \theta_E)/\theta_E^2$, which holds for sufficiently negative $\Psi_{\theta E}$. This describes the situation in which the network is able to contract at its equilibrium volume fraction even in the absence of myosin. The steady state is also unstable when $C < 0$. For a given wavenumber we could make $C < 0$ by picking either $\Psi_{\theta E}$ to be large and negative, or by picking Ψ_{mE} to be large and negative, if $M_b J'_E - M_b k'_{\text{off}E} + M_f k'_{\text{on}E} > 0$. In this case sufficiently strong myosin-driven contraction is enough to destabilise the steady state even when $\Psi_{\theta E} > 0$. A further biologically interesting observation from (5.68) is that the destabilising effect of myosin contraction can be disabled by reaction rate laws such that $M_b J'_E - M_b k'_{\text{off}E} + M_f k'_{\text{on}E} < 0$. If we suppose that both $k'_{\text{on}E}$ and $k'_{\text{off}E}$ are positive, which seems plausible since increasing the amount of actin network increases both the available bound myosin and binding sites, then sufficiently fast unbinding of myosin acts to stabilise the steady state. It is certainly biologically plausible that contraction driven by gradients in bound myosin concentration is reduced if myosin rapidly unbinds and spreads through the solution.

In order to make more concrete statements about when the steady state is unstable we adopt the following form of the reaction rates

$$k_{\text{on}} = K^+ f(\theta), \quad k_{\text{off}} = K^- f(\theta), \quad (5.70a,b)$$

to simplify the classification, where f can be any differentiable function and K^+, K^- are constants. Note that the constitutive equations proposed in (5.3) are of this form. The

simplification arises because $M_b k'_{\text{off}E} = M_f k'_{\text{on}E}$. The discriminant, (5.69), becomes

$$\Delta = \left[\tilde{A}(k_{\text{off}E} - J'_E \theta_E) - \theta_E^2 \Psi_{\theta E} k^2 \right]^2 - 4 \tilde{A} \theta_E^2 \Psi_{mE} M_b J'_E k^2, \quad (5.71)$$

which is always positive since $\Psi_{mE} < 0$, so there are two real roots to the growth rate equation for every k . We first identify two parameter groups, namely

$$\Psi_B = -\frac{\mu_E(k_{\text{off}E} + J'_E \theta_E)}{\theta_E^2}, \quad \Psi_C = -\frac{k_{\text{off}E}(\mu_E J'_E + \theta_E \Psi_{\theta E})}{M_b \theta_E J'_E}, \quad (5.72a, b)$$

and split the discussion into two cases dependent on the size of $\Psi_{\theta E}$. If $\Psi_{\theta E} > \Psi_B$, then $\omega_- < 0$ for all k . If $\Psi_{mE} < \Psi_C$, then $\omega_+ > 0$ and the steady state is unstable for $k > k_c$, where $C(k_c) = 0$, so that

$$k_c^2 = -\frac{J'_E k_{\text{off}E}(\alpha_E + \beta_E)}{\Psi_{mE} \theta_E M_b J'_E + k_{\text{off}E}(\mu_E J'_E + \theta_E \Psi_{\theta E})}. \quad (5.73)$$

Note that the condition on Ψ_{mE} can be satisfied by making Ψ_{mE} very large and negative (strong, myosin-driven contraction of the network), note also that it is a softer condition to satisfy when $\Psi_{\theta E}$ is negative. If $\Psi_{mE} = \Psi_C$, then ω_+ tends to zero as $k \rightarrow \infty$ and the steady state is neutrally stable. Finally, if $\Psi_{mE} > \Psi_C$ then ω_+ tends to a negative constant as $k \rightarrow \infty$ and the steady state is stable for all k . Now we consider the case where $\Psi_{\theta E} < \Psi_B$ and manipulate the expression for Ψ_C to show that when this inequality holds $\Psi_C > 0$, which in turn implies that $\Psi_{mE} < \Psi_C$. For sufficiently large k the steady state will be unstable, and it remains to determine the critical wavenumber. We define k_b such that $B(k_b) = 0$, so that

$$k_b^2 = -\frac{(\alpha_E + \beta_E)(k_{\text{off}E} + J'_E \theta_E)}{\theta_E^2 \Psi_{\theta E} + \mu_E(k_{\text{off}E} + J'_E \theta_E)}, \quad (5.74)$$

and substitute this into (5.68) to find that

$$C(k_b) = \frac{\alpha_E + \beta_E}{\theta_E^2(\Psi_B - \Psi_{\theta E})} \left[k_{\text{off}E}^2 \theta_E \Psi_{\theta E} + J'_E M_b \Psi_{mE} \theta_E (k_{\text{off}E} + \theta_E J'_E) \right] < 0. \quad (5.75)$$

Therefore, since C and B are both linear in k^2 the critical wavenumber in this regime is k_c and perturbations with $k > k_c$ are unstable. Figure 5.1 displays some example growth rate curves.

Next we proceed to consider the linearized initial value problem in order to investigate how the stationary strip responds to a small perturbation. We substitute $u \sim \varepsilon u_1(x, t) + \dots$, $V \sim \varepsilon V_1(t) + \dots$, $\theta \sim \theta_E + \varepsilon \theta_1(x, t) + \dots$, $m_b \sim M_b + \varepsilon m_{b1}(x, t) + \dots$ and $m_f \sim M_f +$

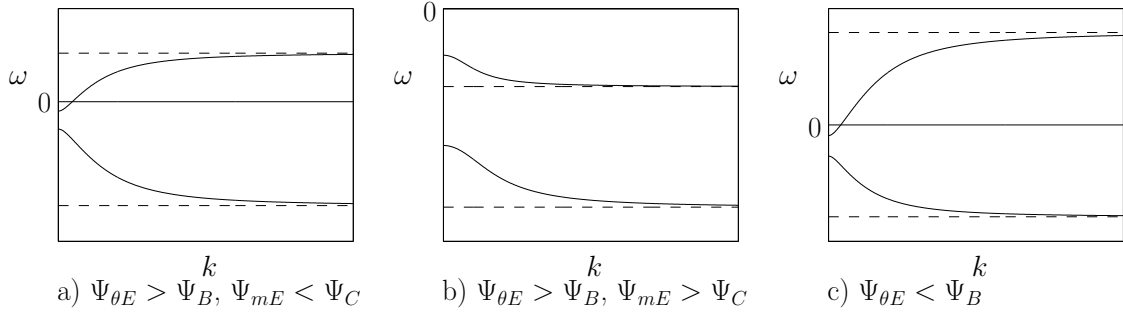


Figure 5.1: Diagrams showing both solutions to the growth rate equation (5.65) for different parameter values and the constitutive assumption (5.70). Dashed lines indicate the large wavenumber asymptote and in both (a) and (c) the larger root crosses the $\omega = 0$ axis at $k = k_c$.

$\varepsilon m_{f1}(t) + \dots$ into (5.14), (5.15), (5.20), (5.28) and (5.29) for $\varepsilon \ll 1$ and obtain the linearized equations

$$\dot{\theta}_1 + \theta_E u'_1 = -J'_E \theta_1, \quad -\Psi_\theta \theta'_1 - \Psi_m m'_{b1} + \mu_E u''_1 = \alpha_E u_1 + \beta_E (u_1 + V_1), \quad (5.76a,b)$$

$$\theta_E \dot{m}_{b1} + M_b \dot{\theta}_1 + M_b \theta_E u'_1 = (k'_{\text{on}E} M_f - k'_{\text{off}E} M_b) \theta_1 + k_{\text{on}E} m_{f1} - k_{\text{off}E} m_{b1}, \quad (5.77)$$

$$V_1 = -\langle u_1 \rangle, \quad (M_b - M_f) \langle \theta_1 \rangle + (1 - \theta_E) m_{f1} = -\theta_E \langle m_{b1} \rangle, \quad (5.78a,b)$$

where dots denote differentiation with respect to time. We construct solutions as sums over the modes found in the stability analysis above

$$\theta_1 = \frac{a_0(t)}{2} + \sum_{n \text{ even}} a_n(t) \cos(n\pi x) + \sum_n b_n(t) \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad (5.79)$$

$$m_{b1} = \frac{c_0(t)}{2} + \sum_{n \text{ even}} c_n(t) \cos(n\pi x) + \sum_n d_n(t) \frac{\sin(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)}, \quad (5.80)$$

where $a_n(0)$, $b_n(0)$, $c_n(0)$ and $d_n(0)$ are all set by the initial conditions for θ and m_b . Solving the initial value problem then follows closely the method set out in §4.2.4. After manipulating (5.76)–(5.78), substituting in the series solutions and exploiting the orthogonality of the modes we obtain a second order differential equation for a_n and b_n for $n > 0$, which can be solved to give

$$a_n(t) = \left(\frac{\dot{a}_n(0) - a_n(0)\omega_-(n\pi)}{\omega_+(n\pi) - \omega_-(n\pi)} \right) e^{\omega_+(n\pi)t} + \left(\frac{a_n(0)\omega_+(n\pi) - \dot{a}_n(0)}{\omega_+(n\pi) - \omega_-(n\pi)} \right) e^{\omega_-(n\pi)t}, \quad (5.81)$$

$$b_n(t) = \left(\frac{\dot{b}_n(0) - b_n(0)\omega_-(\lambda_n)}{\omega_+(\lambda_n) - \omega_-(\lambda_n)} \right) e^{\omega_+(\lambda_n)t} + \left(\frac{b_n(0)\omega_+(\lambda_n) - \dot{b}_n(0)}{\omega_+(\lambda_n) - \omega_-(\lambda_n)} \right) e^{\omega_-(\lambda_n)t}. \quad (5.82)$$

The initial values $\dot{a}_n(0)$ and $\dot{b}_n(0)$ are found from the initial profile of bound myosin via

$$\Psi_\theta \theta_E n^2 \pi^2 a_n + \Psi_m \theta_E n^2 \pi^2 c_n + \mu_E n^2 \pi^2 (J'_E a_n + \dot{a}_n) = -(\alpha_E + \beta_E)(J'_E a_n + \dot{a}_n), \quad (5.83)$$

$$\Psi_\theta \theta_E \lambda_n^2 b_n + \Psi_m \theta_E \lambda_n^2 d_n + \mu_E \lambda_n^2 (J'_E b_n + \dot{b}_n) = -(\alpha_E + \beta_E)(J'_E b_n + \dot{b}_n). \quad (5.84)$$

These expressions also allow us to calculate $c_n(t)$ for $n > 0$ and $d_n(t)$ from (5.81) and (5.82). The expressions for a_0 and c_0 are a special case that require more attention. From substituting the series expressions into the linearized equations and resolving terms we find that

$$\theta_E \dot{c}_0 + k_{\text{off}E} c_0 - (M_b J'_E + k'_{\text{on}E} M_f - k'_{\text{off}E} M_b) a_0 = 2k_{\text{on}E} m_{f1}, \quad (5.85)$$

$$J'_E a_0 + \dot{a}_0 = 0, \quad (M_b - M_f) a_0 + 2(1 - \theta_E) m_{f1} = -\theta_E c_0. \quad (5.86a, b)$$

Equation (5.86a) can be solved immediately to find

$$a_0(t) = a_0(0) e^{-J'_E t}, \quad (5.87)$$

so we see that the total amount of network in the travelling strip relaxes to the equilibrium volume fraction on the timescale for network turnover. Next we eliminate m_{f1} between (5.85) and (5.86b) and find

$$(1 - \theta_E) \theta_E \dot{c}_0 + (k_{\text{off}E}(1 - \theta_E) + k_{\text{on}E} \theta_E) c_0 = G_1 a_0 \quad (5.88)$$

where $G_1 = k_{\text{on}E} M_f - k_{\text{on}E} M_b + (1 - \theta_E)(J'_E M_b - k'_{\text{off}E} M_b + k'_{\text{on}E} M_f)$. If we let $G_2 = G_1 / \theta_E (1 - \theta_E)$ and $G_3 = (k_{\text{off}E}(1 - \theta_E) + k_{\text{on}E} \theta_E) / \theta_E (1 - \theta_E)$, then the solution for c_0 is given by

$$c_0(t) = \frac{a_0(0) G_2}{G_3 - J'_E} e^{-J'_E t} + \left(c_0(0) - \frac{a_0(0) G_2}{G_3 - J'_E} \right) e^{-G_3 t}. \quad (5.89)$$

The key point is that both J'_E and G_3 are positive, so c_0 decays to zero as $t \rightarrow \infty$ and the total amount of bound myosin in the strip always tends to the equilibrium value, M_b . We find m_{f1} by substituting a_0 and c_0 into (5.86b).

Thus, perturbations to the network volume fraction and myosin concentration are known. Now we can integrate (5.76a) once with respect to x and apply the boundary conditions $u_1(0, t) = u_1(1, t) = 0$ to obtain

$$u_1 = \sum_n \frac{J'_E b_n + \dot{b}_n}{\theta_E \lambda_n} \left(\frac{\cos(\lambda_n(x - 1/2))}{\cos(\lambda_n/2)} - 1 \right) - \sum_{n \text{ even}} \left(\frac{J'_E a_n + \dot{a}_n}{\theta_E n \pi} \right) \sin(n \pi x). \quad (5.90)$$

Finally, we use (5.78a) to find that

$$V_1 = \left(1 + \frac{\alpha_E}{\beta_E}\right) \sum_n \frac{J'_E b_n + \dot{b}_n}{\theta_E \lambda_n}. \quad (5.91)$$

As in the minimal model we see clearly that only asymmetric perturbations to either the network volume fraction or bound myosin concentration contribute to the velocity of the travelling strip. We conclude that coupling a model for myosin to the minimal model in this way does not have a great effect on the stability properties of the steady state. We retain the same monotonic, plateauing growth rate that means there is no single dominant mode which grows fastest; a break in symmetry is required to cause the stationary strip to begin to move and the model is always at least weakly well-posed. The addition of the myosin model does contribute an extra root to the growth rate equation which is always stable; we may anticipate the effect of this could be seen at very short timescales as in the viscoelastic model of Chapter 4. This analysis does reveal the destabilising effect of network volume fraction and myosin-driven contraction of the network and that rapid unbinding of myosin has a stabilising effect.

5.3.3 Fast diffusion of free myosin

Now we turn our attention to the more general form of the limit presented in §5.2.1. Free myosin still diffuses rapidly through the solution phase, so that m_f depends only on time, but we allow the bound myosin to crawl along actin filaments. Using our assumption, (5.23c) of randomly-aligned filaments, (5.27) becomes

$$\frac{\partial}{\partial t} (m_b \theta) + \frac{\partial}{\partial x} (m_b \theta u) = \frac{\partial}{\partial x} \left(m_b \theta F \frac{\partial m_b}{\partial x} \right) + k_{\text{on}} m_f - k_{\text{off}} m_b, \quad (5.92)$$

where we have absorbed the constant χ into the function F , which in general can depend on both the network volume fraction and the concentration of bound myosin. The governing equations for this section are (5.14), (5.15), (5.20), (5.28) and (5.92), which must be solved subject to initial conditions for θ and m_b and boundary conditions $u = 0$ and $\partial m_b / \partial x = 0$ at $x = 0$ and $x = 1$. The stationary, spatially uniform steady state is given by $\theta = \theta_E$, $u = 0$, $V = 0$, $m_b = M_b$ and $m_f = M_f$, where M_b and M_f are as stated in (5.56). We investigate a perturbation of the steady state by substituting $u \sim \varepsilon u_1(x) e^{\omega t} + \dots$, $V \sim \varepsilon V_1 e^{\omega t} + \dots$, $\theta \sim \theta_E + \varepsilon \theta_1(x) e^{\omega t} + \dots$, $m_b \sim M_b + \varepsilon m_{b1}(x) e^{\omega t} + \dots$ and $m_f \sim M_f + \varepsilon m_{f1} e^{\omega t} + \dots$ as $\varepsilon \rightarrow 0$ into the governing equations, to find at $O(\varepsilon)$, that

$$\omega \theta_1 + \theta_E u'_1 = -J'_E \theta_1, \quad -\Psi_{\theta E} \theta'_1 - \Psi_{m E} m'_{b1} + \mu_E u''_1 = \alpha_E u_1 + \beta_E (u_1 + V_1), \quad (5.93a, b)$$

$$(\omega\theta_E + k_{\text{off}E})m_{b1} + M_b\theta_E u_1' = M_b\theta_E F_E m_{b1}'' + (k_{\text{on}E}'M_f - k_{\text{off}E}'M_b - \omega M_b)\theta_1 + k_{\text{on}E}m_{f1}, \quad (5.94)$$

$$V_1 = -\langle u_1 \rangle, \quad (M_b - M_f)\langle \theta_1 \rangle + (1 - \theta_E)m_{f1} = -\theta_E\langle m_{b1} \rangle, \quad (5.95a,b)$$

After eliminating the other variables we find a fourth-order ordinary differential equation for u_1 , forced by V_1 of the form $Qu_1'''' - 2Ru_1'' + Su_1 = -S\beta_EV_1/(\alpha_E + \beta_E)$ where

$$Q = F_E M_b \theta_E \left[\Psi_{\theta E} \theta_E + \mu_E (J_E' + \omega) \right], \quad (5.96)$$

$$R = \frac{1}{2} \left[\Psi_{mE} \theta_E (k_{\text{on}E}' M_f - k_{\text{off}E}' M_b) + (k_{\text{off}E} + \theta_E \omega) \left(\Psi_{\theta E} \theta_E + \mu_E (J_E' + \omega) \right) \right. \\ \left. + \theta_E M_b F_E (\alpha_E + \beta_E) (\omega + J_E') + J_E' M_b \theta_E \Psi_{mE} \right] \quad (5.97)$$

$$S = (J_E' + \omega) (k_{\text{off}E} + \theta_E \omega) (\alpha_E + \beta_E). \quad (5.98)$$

which must be solved subject to $u_1(0) = u_1(1) = 0$ and

$$u_1''(0) = u_1''(1) = \frac{\beta_E V_1 (\omega + J_E')}{\theta_E \Psi_{\theta E} + \mu_E (\omega + J_E')}. \quad (5.99)$$

The solution then follows that presented in §5.3.1. There are two families of solutions for $k > 0$. The first correspond to $V_1 = 0$ and $u_1 = \sin(n\pi x)$ for n an even integer and represent symmetric disturbances to the network volume fraction and bound myosin concentration. The second set corresponding to asymmetric perturbations and $V_1 \neq 0$ have solutions of the form (5.53), where k_1 and k_2 are given by (5.54) and we require the solvability condition (5.55) to select the growth rate.

It is not clear how to write arbitrary initial conditions as a sum over eigenmodes as we did in the previous section. So, we proceed to consider the stability of a perturbation on an unbounded domain to identify stabilising and destabilising features of this sub-model. We consider perturbations to the steady state proportional to $\varepsilon \exp(ikx + \omega t)$, where the wavenumber k is real, and thereby obtain a quadratic equation for the growth rate, $A\omega^2 + B\omega + C = 0$, where

$$A(k) = \theta_E \left[\alpha_E + \beta_E + \mu_E k^2 \right] = \theta_E \tilde{A}, \quad (5.100)$$

$$B(k) = (k_{\text{off}E} + J_E' \theta_E + F_E M_b \theta_E k^2) \tilde{A} + \Psi_{\theta E} \theta_E^2 k^2, \quad (5.101)$$

$$C(k) = (J_E' k_{\text{off}E} + F_E J_E' M_b \theta_E k^2) \tilde{A} + k_{\text{off}E} \Psi_{\theta E} \theta_E k^2 \\ + (J_E' M_b - k_{\text{off}E}' M_b + k_{\text{on}E}' M_f) \Psi_{mE} \theta_E k^2 + F_E M_b \Psi_{\theta E} \theta_E^2 k^4. \quad (5.102)$$

For simplicity we shall restrict the following discussion to the case where the binding/unbinding rates are of the form given in (5.70) so that $M_b k_{\text{off}E}' = M_f k_{\text{on}E}'$. The discriminant,

$\Delta = B^2 - 4AC$, is thus

$$\Delta = \left(\tilde{A} \left[k_{\text{off}E} - \theta_E J'_E + \theta_E F_E M_b k^2 \right] - \theta_E^2 \Psi_{\theta E} k^2 \right)^2 - 4 \Psi_{mE} \tilde{A} \theta_E^2 k^2 J'_E M_b, \quad (5.103)$$

which is positive, so there are two real roots for all k . We note first that perturbations are stable for $k = 0$ and that

$$\omega \rightarrow -F_E M_b k^2, \quad \omega \rightarrow -\frac{\mu_E J'_E + \theta_E \Psi_{\theta E}}{\mu_E}, \quad (5.104a, b)$$

as $k \rightarrow \infty$. If F is chosen so that $F_E > 0$, then it models myosin molecules walking randomly along randomly aligned filaments and we have a diffusion term, albeit with some complicated coefficient. Choosing F so that $F_E < 0$ leads to an ill-posed model. This seems to describe myosin molecules walking towards each other along actin filaments and aggregating. This seems somewhat biologically implausible, so we shall assume $F_E > 0$.

For $F_E > 0$ and the linearized model is at least weakly well-posed. Then, if $\Psi_{\theta E} < -\mu_E J'_E / \theta_E$, the larger root of the growth rate equation plateaus to a positive constant for large k and perturbations are unstable if their wavenumber exceeds some critical value. Biologically, the destabilising mechanism at work is volume fraction dependent contraction of the actin network. If instead $F_E > 0$ and $\Psi_{\theta E} > -\mu_E J'_E / \theta_E$, then the larger root of the growth rate equation tends to a negative value for large k and the steady state is stable to perturbations with a sufficiently large wavenumber. In this case, we note that $B > 0$ for all k and look for k such that $C(k) < 0$. We note that $C(k)$ is quadratic in k^2 , with

$$C = \left[k_{\text{off}E} (\Psi_{\theta E} \theta_E + J'_E \mu_E) + M_b J'_E \theta_E ((\alpha_E + \beta_E) F_E + \Psi_{mE}) \right] k^2 + F_E M_b \theta_E (J'_E \mu_E + \Psi_{\theta E} \theta_E) k^4 + (\alpha_E + \beta_E) J'_E k_{\text{off}E} \quad (5.105)$$

and for the parameter regime currently under consideration the constant term and the coefficient of the k^4 term are positive. Thus, there will be an interval of wavenumbers for which the steady state is unstable only if the k^2 coefficient is sufficiently negative. This can be achieved via a large negative Ψ_{mE} identifying myosin-driven contraction of the network as another destabilising mechanism. Illustrative examples are provided in Figure 5.2.

Our stability analysis has identified myosin-driven network contraction and volume-fraction-driven network contraction as mechanisms for a perturbation to the steady state to grow. In contrast to the minimal model, we have been unable to find families of solutions to the linearized problem on the bounded domain, so we do not pursue a solution to the linearized initial value problem.

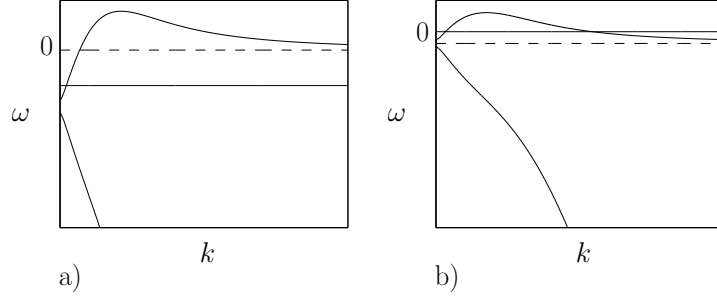


Figure 5.2: Diagrams showing both roots of the growth rate equation with coefficients (5.100)–(5.102), subject to (5.70) for different parameter values. The dashed line indicates the large wavenumber asymptote stated in (5.104b). In (a) $F_E > 0$ and $\Psi_{\theta E} < -\mu_E J'_E / \theta_E$ and in (b) $F_E > 0$ and $\Psi_{\theta E} > -\mu_E J'_E / \theta_E$. In both graphs $\Psi_{mE} < 0$.

5.3.4 Rapid reaction kinetics with slow crawling myosin

We now consider the stability of the steady state in a sub-limit of the rapid reaction kinetics limit derived in §5.2.3. We choose to consider the case where myosin crawling is so slow that we can set $\chi = 0$ in (5.36). We further simplify the system by assuming the binding and unbinding rates are of the form stated in (5.70), and introduce the parameter $\kappa = K^- / K^+$ so that (5.36) becomes

$$\frac{\partial}{\partial t} (m_b (\theta + \kappa(1 - \theta))) + \frac{\partial}{\partial x} (m_b \theta u (1 - \kappa)) = \frac{\partial}{\partial x} \left[D_f (1 - \theta) \kappa \frac{\partial}{\partial x} (m_b) \right], \quad (5.106)$$

which along with (5.14), (5.15) and (5.20) make up the governing equations. It follows that that $m_b (\theta + \kappa(1 - \theta))$ is conserved, specifically our choice of nondimensionalization ensures that

$$\int_0^1 m_b (\theta + \kappa(1 - \theta)) \, dx = 1. \quad (5.107)$$

The governing equations must be solved subject to initial conditions for m_b and θ and the boundary conditions $u = 0$ and $\partial m_b / \partial x = 0$ at $x = 0$ and $x = 1$. We wish to understand how perturbations to the spatially uniform steady state, $u = 0$, $V = 0$, $\theta = \theta_E$, $m_b = m_E$ evolve in time, where $m_E = 1 / (\theta_E + \kappa(1 - \theta_E))$. We substitute a perturbation of the form $\theta \sim \theta_E + \varepsilon \theta_1(x) e^{\omega t} + \dots$, $u \sim \varepsilon u_1(x) e^{\omega t} + \dots$, $V \sim \varepsilon V_1 e^{\omega t} + \dots$, $m_b \sim m_E + \varepsilon m_1(x) e^{\omega t} + \dots$ as $\varepsilon \rightarrow 0$ into the governing equations, to obtain at $O(\varepsilon)$,

$$\omega \theta_1 + \theta_E u'_1 = -J'_E \theta_1, \quad V_1 = -\langle u_1 \rangle, \quad (5.108a,b)$$

$$-\Psi_{mE} m'_{b1} - \Psi_{\theta E} \theta'_1 + \mu_E u''_1 = \alpha_E u_1 + \beta_E (u_1 + V_1), \quad (5.109)$$

$$\omega(\theta_E + \kappa(1 - \theta_E))m_{b1} + \omega(1 - \kappa)m_E\theta_1 + (1 - \kappa)m_E\theta_E u_1' = D_f\kappa(1 - \theta_E)m_{b1}''. \quad (5.110)$$

After eliminating the other variables we find that u_1 satisfies the fourth-order ordinary differential equation $Qu_1'''' - 2Ru_1'' + Su_1 = -S\beta_EV_1/(\alpha_E + \beta_E)$, where

$$Q = D_f\kappa(1 - \theta_E)\left(\Psi_{\theta E}\theta_E + \mu_E(J_E' + \omega)\right) \quad (5.111)$$

$$R = \frac{1}{2}\left[(1 - \kappa)m_E\Psi_{mE}\theta_E J_E' + \left(\Psi_{\theta E}\theta_E + \mu_E(J_E' + \omega)\right)K_E\omega + (\alpha_E + \beta_E)(1 - \theta_E)(J_E' + \omega)D_f\kappa\right] \quad (5.112)$$

$$S = (\alpha_E + \beta_E)(J_E' + \omega)K_E\omega, \quad (5.113)$$

and we have introduced the notation $K_E = \theta_E + \kappa(1 - \theta_E)$. The boundary conditions are $u_1(0) = u_1(1) = 0$ and

$$u_1''(0) = u_1''(1) = \frac{\beta_E(\omega + J_E')V_1}{\theta_E\Psi_{\theta} + \mu_E(J_E' + \omega)}, \quad (5.114)$$

so we see the form of the problem is exactly that found in the previous section. There are two families of solutions, symmetric modes corresponding to $V_1 = 0$ and asymmetric modes corresponding to $V_1 \neq 0$. The asymmetric solutions are constructed with two ‘wavenumbers’ as defined by (5.54) and the dominant growth rate is determined as the solution of (5.55) which has the largest real part.

We turn our attention to characterizing the growth rate as a function of the parameters on an unbounded domain. We consider a small perturbation to the steady state that is proportional to $\varepsilon \exp(ikx + \omega t)$ for real k and find that the growth rate equation is $A\omega^2 + B\omega + C = 0$, where

$$A(k) = (\alpha_E + \beta_E + \mu_E k^2)K_E = \tilde{A}K_E, \quad (5.115)$$

$$B(k) = \left[J_E'K_E + D_f\kappa(1 - \theta_E)k^2\right]\tilde{A} + K_E\theta_E\Psi_{\theta E}k^2, \quad (5.116)$$

$$C(k) = D_f\kappa(1 - \theta_E)J_E'\tilde{A}k^2 + D_f\kappa(1 - \theta_E)\theta_E\Psi_{\theta E}k^4 + J_E'\theta_E(1 - \kappa)m_E\Psi_{mE}k^2. \quad (5.117)$$

We note first that $k = 0$ perturbations are neutrally stable and that

$$\omega \rightarrow -\frac{D_f\kappa(1 - \theta_E)k^2}{K_E}, \quad \omega \rightarrow -\frac{J_E'\mu_E + \theta_E\Psi_{\theta E}}{\mu_E}, \quad (5.118a,b)$$

as $k \rightarrow \infty$. The expression in (5.118a) is negative for all sensible parameter choices, so the linearized version of this sub-model is always at least weakly well-posed. We see that if $\Psi_{\theta E} < -\mu_E J_E'/\theta_E$ then the steady state is unstable to perturbations with a sufficiently

large wavenumber. We shall now consider what parameter values permit the steady state to be unstable when $\Psi_{\theta E} > -\mu_E J'_E / \theta_E$. In this case $B > 0$ for all k , so the steady state will be unstable only when both the discriminant $\Delta = B^2 - 4AC$ is positive and $C < 0$. The discriminant can be written

$$\Delta = \left[\tilde{A} \left(J'_E K_E - D_f \kappa (1 - \theta_E) k^2 \right) + \Psi_{\theta E} \theta_E K_E k^2 \right]^2 - 4(1 - \kappa) \Psi_{mE} \tilde{A} K_E J'_E m_E \theta_E k^2, \quad (5.119)$$

so we see that the sign of the second term depends on the size of κ . We can write $C = c_1 k^2 + c_2 k^4$ where

$$c_1 = J'_E \left(D_f \kappa (\alpha_E + \beta_E) (1 - \theta_E) + (1 - \kappa) m_E \Psi_{mE} \theta_E \right), \quad (5.120)$$

$$c_2 = D_f \kappa (1 - \theta_E) (J'_E \mu_E + \Psi_{\theta E} \theta_E). \quad (5.121)$$

Remembering that we are considering the case where c_2 is positive, if $c_1 > 0$ then $C > 0$ for all k and the steady state is stable, whereas if $c_1 < 0$ then $C < 0$ for k^2 in the interval $(0, -c_1/c_2)$. For c_1 to be negative we require

$$(1 - \kappa) \Psi_{mE} < -\frac{D_f \kappa (\alpha_E + \beta_E) (1 - \theta_E)}{m_E \theta_E}, \quad (5.122)$$

so that $(1 - \kappa) \Psi_{mE} < 0$, which implies $\Delta > 0$ and hence the steady state is unstable to perturbations with wavenumber $k < \sqrt{-c_1/c_2}$. Examples of growth rate curves are shown in Figure 5.3.

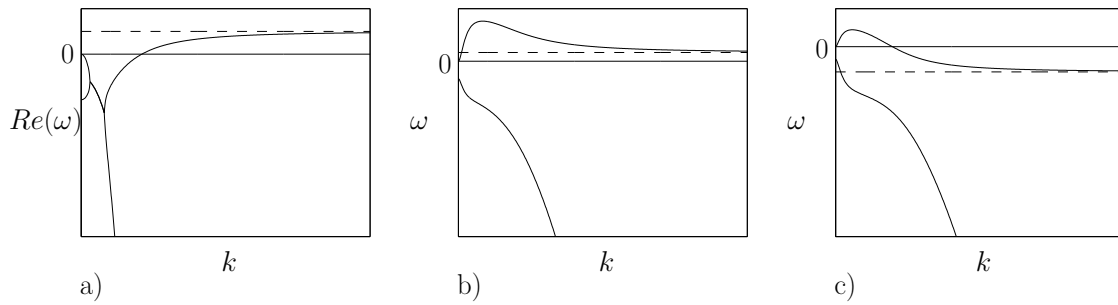


Figure 5.3: Diagrams showing both roots of the growth rate equation for different parameter values. The dashed line indicates the large wavenumber asymptote stated in (5.118b). In (a) $\Psi_{\theta E} < -\mu_E J'_E / \theta_E$ and $\kappa > 1$, in (b) $\Psi_{\theta E} < -\mu_E J'_E / \theta_E$ and $\kappa < 1$ in (c) $\Psi_{\theta E} > -\mu_E J'_E / \theta_E$ and $\kappa < 1$. In all three graphs $\Psi_{mE} < 0$.

The inequality (5.122) can be satisfied with a sufficiently large and negative Ψ_{mE} representing strong myosin-driven contraction of the actin network when $\kappa < 1$, *i.e.* when

myosin binding is faster than unbinding. If $\kappa > 1$, representing a situation in which myosin unbinds from the network more rapidly than it binds, then the inequality cannot be satisfied for $\Psi_{mE} < 0$. So we see rapid unbinding switching off the effect of myosin-driven contraction. Equation (5.122) also highlights the stabilising effect of diffusion of free myosin: as D_f gets larger Ψ_{mE} must be larger and more negative for the inequality to hold.

5.3.5 Remarks

The above stability analyses have revealed numerous differences between the stability properties of the different sub-models. Key themes emerge and we are now in a position to make some summarising statements about the effect of coupling myosin equations to the minimal model. In all the examples we have considered, both volume-fraction-driven and myosin-driven contraction of the network may destabilise the steady state. Aggregation of bound myosin via crawling can also destabilise the stationary steady state, but this leads to an ill-posed problem. Diffusion terms, whether they enter the governing equations by describing myosin crawling or diffusion of free myosin, are stabilising. It is only when such terms are present that we have been able to find parameter regimes with a local maximum in the growth rate curve. This permits the existence of a dominant mode and scenarios where the steady state is unstable to perturbations whose wavenumbers belong to a finite interval, but stable as $k \rightarrow \infty$. Rapid unbinding of myosin can also disable the destabilising effect of myosin-driven contraction in some situations.

5.4 Numerical Simulations

Based on the linear stability analyses in the previous section, we proceed to investigate numerically two sub-models that we select to illustrate the different mechanisms at work in the myosin equations. The first, that we shall term henceforth the ‘kinetic’ model, has the governing equations (5.14), (5.15), (5.20), (5.28) and (5.29), which we solve subject to initial conditions for θ and m_b and the boundary conditions $u(0, t) = u(1, t) = 0$. The constitutive laws are those stated in §5.1.6. The second model that we shall consider is given by (5.14), (5.15), (5.20) and (5.41), where initial conditions for θ and m_b are chosen to satisfy (5.42) and the boundary conditions are $u = 0$ and $\partial m_b / \partial x = 0$ at $x = 0$ and $x = 1$. We term this model the ‘diffusion’ model and the constitutive laws are as stated in §5.1.6 unless otherwise specified.

5.4.1 Description and validation of the scheme

We use a modified version of the code used in §2.4 to solve the minimal model, further details are given in Appendix B. The convergence of the code used is demonstrated in Figures B.4 and B.6 in Appendix B. We further validate the code against the linear stability predictions made in §5.3.1 and §5.3.2. Graphs demonstrating the excellent agreement between theory and code are presented in Figures B.5 and B.7 of Appendix B.

5.4.2 The kinetic model

We start our numerical investigation by solving an initial value problem to see how the stationary strip responds to a small perturbation. In Figure 5.4 we present the velocity of the strip for two different initial conditions. The solid line shows a velocity that is initially small and then increases smoothly before settling to a constant velocity, in an oscillatory manner. In the second simulation, shown as a dashed line, the velocity is slow for longer, before increasing more steeply and exhibiting a larger amplitude variation before settling to the same steady state.

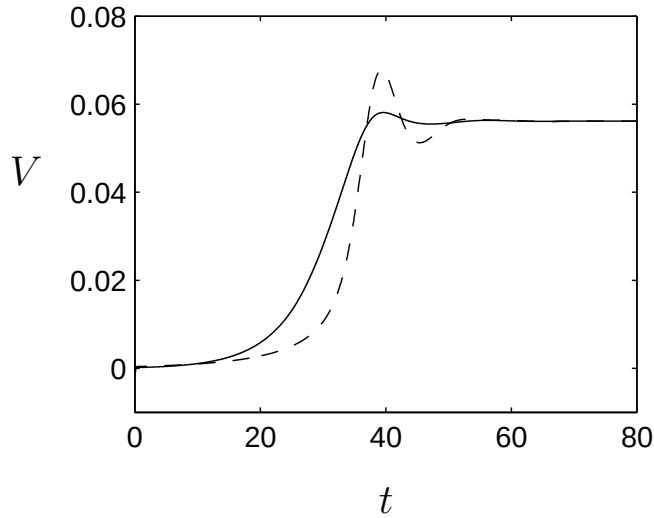


Figure 5.4: Crawling velocity, V , of the strip for two initial value problems. The initial conditions for the solid line are $\theta^* = \theta_E + \varepsilon \cos(3\pi x)$ and $m_b^* = 0.6 + \varepsilon(5 + \cos(\pi x))$ and the initial conditions for the dashed line are $\theta^* = \theta_E + \varepsilon \cos(\pi x)$ and $m_b^* = 0.5 - \varepsilon \cos(3\pi x)$. In both cases $\varepsilon = 10^{-4}$, $\theta_E = 0.02$, $S^* = 55$, $C^* = 80$, $\alpha^* = 0.4$, $\beta^* = 3$, $k_{\text{on}}^* = 10$ and $k_{\text{off}}^* = 15$. Profiles of the spatially dependent variables associated with this simulation are shown in Figures 5.5 and 5.6.

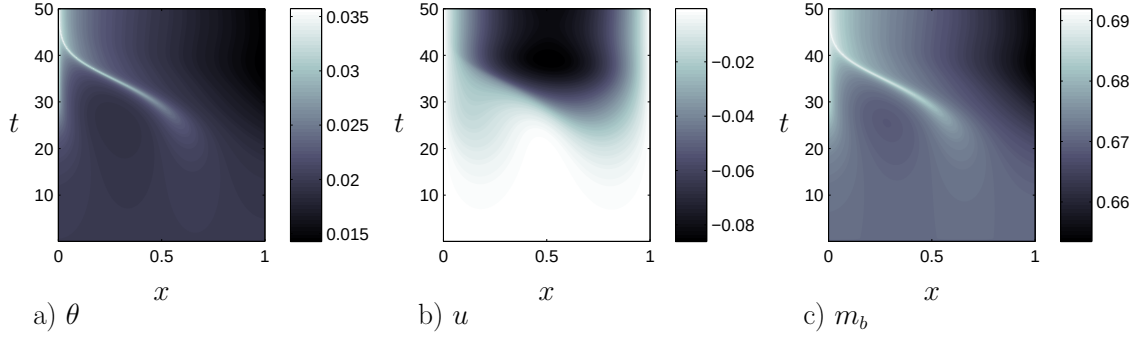


Figure 5.5: Spatio-temporal plots of the evolution of: (a) network volume fraction; (b) network velocity and (c) bound myosin concentration; as solutions to the initial value problem shown as the solid line in Figure 5.4.

The spatio-temporal evolution of the network volume fraction, network velocity and myosin concentration per unit volume of actin network are plotted in Figure 5.5. We see that initially the perturbation grows slowly; then around $t = 25$, we see a peak develop in the actin network. This peak is swept to the rear of the travelling strip. Coincident with the formation of a peak in the actin network is the formation of a peak in concentration of bound myosin per unit volume of network. This peak travels with the actin peak to the rear of the travelling strip, so we see an accumulation of myosin driving contraction of the network, which in turn drags more myosin into the peak. Figure 5.5 (b) displays retrograde flow of the actin network.

Whilst the greyscale plots are useful for displaying the evolution of the solution variables, they do not display the solution profile that clearly. In Figure 5.6 we plot the travelling wave profiles, computed as the long-time solution from our unsteady code, for θ , u and m_b , along with some other variables calculated from them. The network profile has the same key characteristics as the one peak solutions of the minimal model (*cf.* Figure 2.8). The actin volume fraction peaks at the rear of the cell and is lowest at the front; it also covers a similar range of values, but the actin peak at $x = 0$ does not have a ‘shoulder’. The network and solution velocities are very like those obtained in the minimal model and the viscoelastic model: the network flows backwards and the solution flows forwards at comparable speeds to those seen in the minimal model. The mixture stress and force exerted on the substrate, given by

$$F = \beta(u + V), \quad (5.123)$$

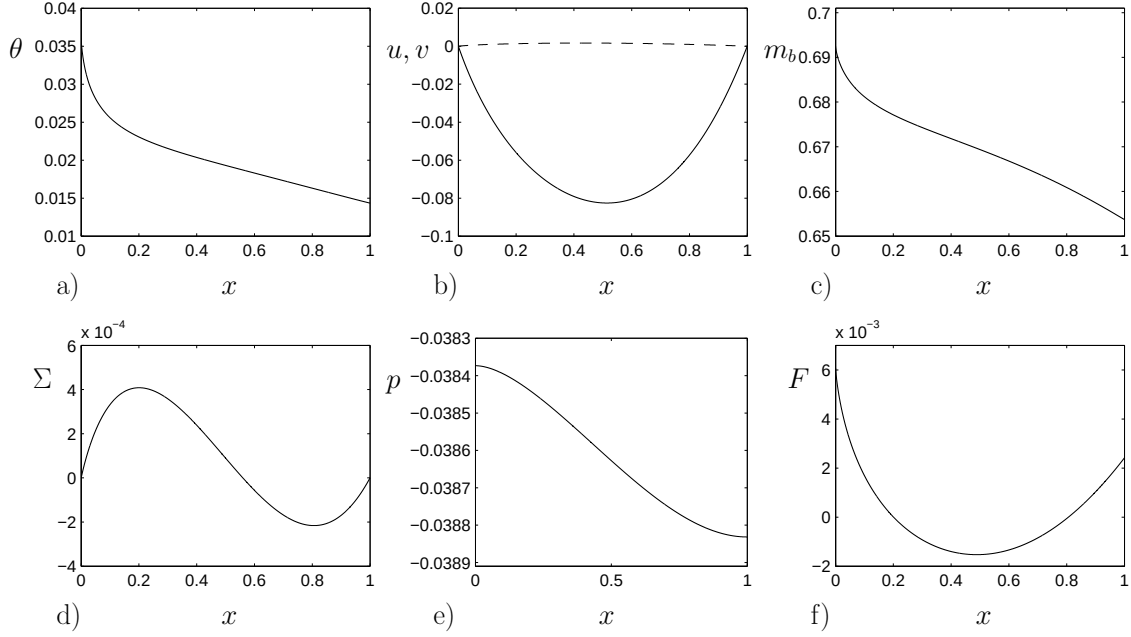


Figure 5.6: Steady travelling-wave profiles from the initial value problem shown in Figure 5.4 at $t = 120$. We plot (a) network volume fraction; (b) network velocity (solid) and solution velocity (dashed); (c) bound myosin concentration; (d) mixture stress; (e) pressure and (f) force exerted on the substrate.

are also of a similar size and shape to the minimal model and viscoelastic model profiles. The pressure is high at the rear of the strip and low at the front, and there is a similar pressure drop over the strip to that seen in the minimal and viscoelastic models. The bound myosin profile is very similar in shape to that of the network volume fraction.

The simulations shown in Figures 5.4—5.6 were computed on grids with five thousand or ten thousand spatial elements and with a timestep of either 1×10^{-3} or 2×10^{-3} . The largest relative error in V was less than 1.8×10^{-2} . The relative distance of V from its mean value between $t = 100$ and $t = 120$ was less than 2.1×10^{-6} and the largest relative error for the θ or m_b travelling-wave profiles across different grids was less than 1.8×10^{-3} , so we can be confident the solution has settled to a steady travelling-wave solution. The concentration of free myosin per unit volume of solution tends very rapidly to a constant that agrees, to six significant figures, with the value for M_f given in (5.56b).

Next we turn our attention to the effect that adhesion strength has on the speed and shape of the travelling wave solutions. Figure 5.7 shows that the kinetic myosin model retains the bell-shaped dependence of speed on adhesion strength seen experimentally and

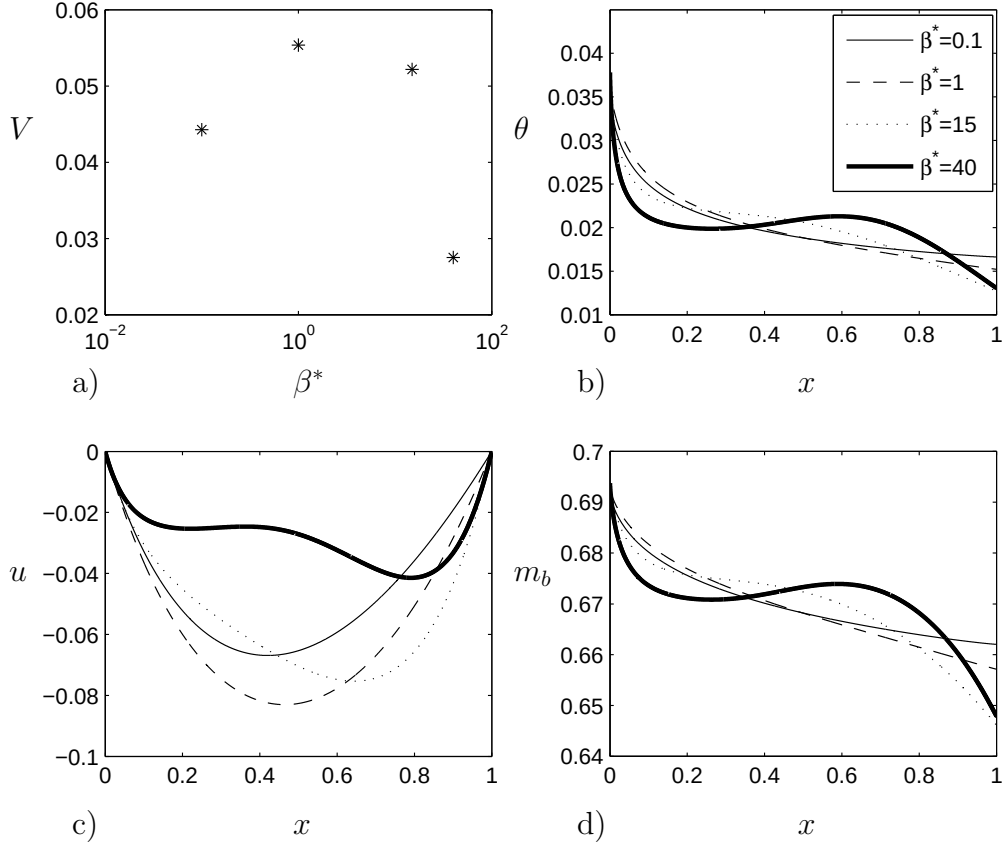


Figure 5.7: (a) depicts the effect of adhesion strength, β^* , on the speed of the travelling strip. The remaining graphs show the steady state profile of (b) the network volume fraction, (c) the network velocity and (d) the concentration per unit volume of bound myosin. Parameters used are $\theta_E = 0.02$, $S^* = 55$; $C^* = 80$, $\alpha^* = 0.04$, $k_{\text{on}}^* = 10$, $k_{\text{off}}^* = 15$ and β^* as indicated in the key.

in our previous models. For small β^* , the network volume fraction and myosin concentration are monotonic, increasing as we move backwards along the travelling strip. As the adhesion strength increases, the profile seems to ‘buckle’ representing a local maximum of network and myosin in the strip interior, although this is very different in shape to the network peaks observed in the minimal model. For all the simulations the network exhibits retrograde flow. In the strong adhesion simulations there is a region near the front of the cell where the network flows more quickly feeding the localised actin bulge. In the transient solutions localised regions of high actin emerge in the cell interior and steepen as they travel backwards. For larger values of β^* there are more of these peaks in the transient solutions, but we have found no properly resolved simulation with peaks travelling forwards and no evidence of multi-peak travelling-wave profiles. However, we cannot exclude the possibility

that they exist, particularly for larger values of β^* .

All of the simulations in Figure 5.7 were run on grids with five thousand and ten thousand spatial elements. The largest relative error between solutions computed on different grids was less than 1.2×10^{-2} for the travelling-strip speed, 3.5×10^{-3} for the network volume fraction and 2.8×10^{-4} for the myosin concentration. The difference between V and its mean value over the final twenty nondimensional time units was less than 5.8×10^{-4} .

Finally we consider the effect of the binding and unbinding rates. A few preliminary simulations were run with parameter values within the ranges set out in §5.1.7 to find parameters that led to biologically plausible solutions. Throughout this section we investigate myosin-driven contraction, because this is the new mechanism introduced by explicit modelling of myosin concentration. We ensure the parameters are such that $\Psi_{\theta E} > 0 > \Psi_{mE}$. Many of the preliminary simulations showed sharp increases in network volume fraction, network volume fractions substantially greater than those seen experimentally and numerical oscillations in the network volume fraction that were unphysical. The simulations were instructive in demonstrating that myosin-driven network contraction must be balanced by competing effects in the model, if we are to obtain biologically plausible solutions. The options for restricting network accumulation are network swelling and increasing the ratio of the rate of unbinding to binding of myosin. We note that the balance of swelling and contractile forces at equilibrium in (5.4) depends not only on the relative sizes of S^* and C^* , but also on k_{on}^* and k_{off}^* via their control of M_b .

Solutions which travel at reasonable speeds with biologically plausible network volume fractions have been found as displayed in this chapter and the search to find such values was by no means comprehensive, so it seems reasonable to assume that many suitable combinations of parameters exist. However, our simulations were quite sensitive to the values of k_{on}^* and k_{off}^* . In Figure 5.8 we show the profiles of travelling wave solutions for two simulations. The first (solid line) travels with velocity $V = 0.0562$ (to three significant figures) and has a maximum network volume fraction of around 0.03 which is one and a half times the equilibrium volume fraction. For the second simulation (dashed line) k_{on}^* was increased from ten to eleven and k_{off}^* was increased from fifteen to sixteen. The effect on the travelling-wave solution is quite dramatic, the strip now travels about three times as fast ($V = 0.187$, to three significant figures) and at the rear of the cell the network volume fraction reaches 0.1.

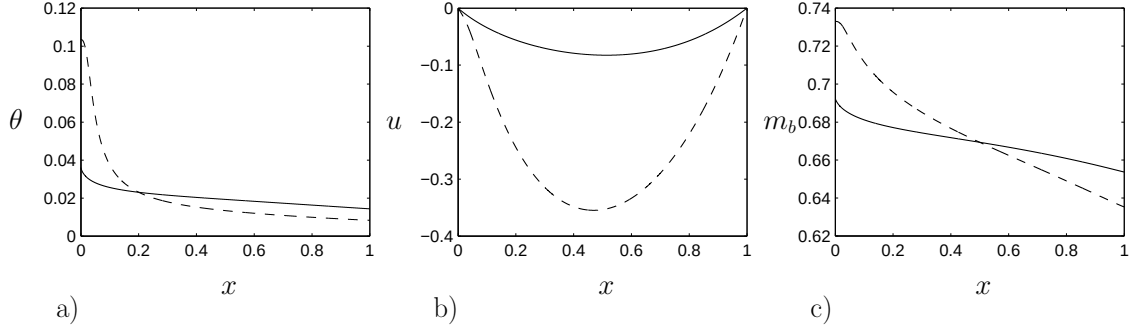


Figure 5.8: Simulations to show the effect of binding and unbinding rates for myosin on the steady travelling-wave profiles. The solid lines have $k_{\text{on}}^* = 10$ and $k_{\text{off}}^* = 15$, the dashed lines have $k_{\text{on}}^* = 11$ and $k_{\text{off}}^* = 16$, the other parameter values are $\theta_E = 0.02$, $S^* = 55$, $C^* = 80$, $\alpha^* = 0.4$ and $\beta^* = 3$.

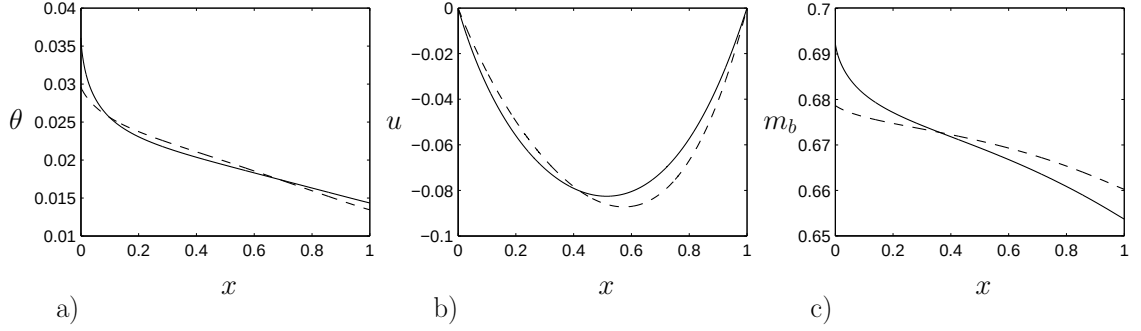


Figure 5.9: Simulations to demonstrate the effect of parameter values on the profile of the travelling-wave solutions. Parameters used are $\theta_E = 0.02$, $S^* = 55$, $C^* = 80$, $\alpha^* = 0.4$, $\beta^* = 3$, $k_{\text{on}}^* = 10$, and $k_{\text{off}}^* = 15$ (solid line) and $\theta_E = 0.02$, $S^* = 53.32$, $C^* = 80$, $\alpha^* = 0.4$, $\beta^* = 3$, $k_{\text{on}}^* = 20$, and $k_{\text{off}}^* = 30$ (dashed line).

In Figure 5.9 we compare the travelling-wave profile for two simulations with substantially different myosin binding and unbinding rates. We reprint the simulation with $k_{\text{on}}^* = 10$ and $k_{\text{off}}^* = 15$ (solid line) and compare it to a simulation for $k_{\text{on}}^* = 20$ and $k_{\text{off}}^* = 30$ (dashed line), where the swelling strength, S^* , has been reduced. In fact for the solid line case ($\theta_E = 0.02$, $S^* = 55$, $C^* = 80$, $\alpha^* = 0.4$, $\beta^* = 3$, $k_{\text{on}}^* = 10$, $k_{\text{off}}^* = 15$), $\Psi_{mE} - \Psi_C = -0.0969$ to three significant figures, where Ψ_C is defined in (5.72b) and $\Psi_{mE} < \Psi_C$ means the steady state is unstable. For the dashed line case $\Psi_{mE} - \Psi_C = -0.0958$ to three significant figures. The resulting travelling-wave profiles are qualitatively very similar. Note that if we had simply doubled k_{on}^* and k_{off}^* , without reducing the swelling strength then $\Psi_{mE} - \Psi_C = 1.41$ to three significant figures, and the steady state would be stable.

5.4.3 The diffusion model

Now we present selected numerical solutions to the diffusion model. In Figure 5.10 we plot the velocity the cell crawls at in response to two different initial conditions, both of which are a small perturbation to the stationary steady state. In both cases the speed is initially small, before growing and finally plateauing to a constant as the strip settles into a steady travelling-wave configuration. We see that different initial conditions can cause the strip to move in opposite directions, but ultimately they attain the same speed. We also note for these two particular examples that, in contrast to many of the previous models discussed in this thesis, the development of the velocity is monotonic and there is no oscillation about the travelling-wave velocity.

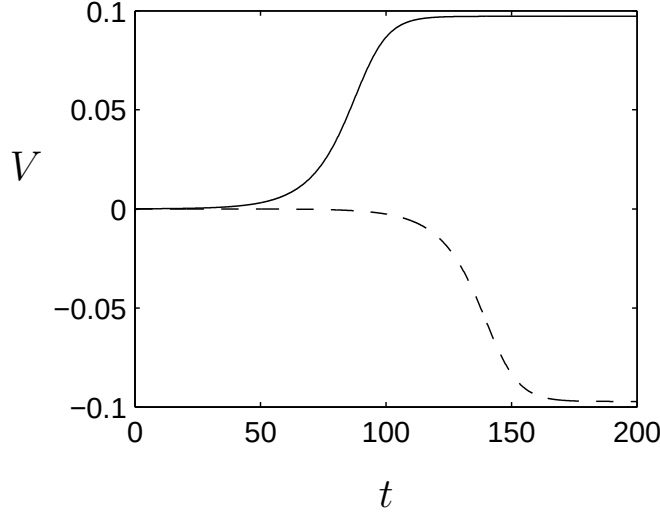


Figure 5.10: Crawling velocity of the strip for the initial value problem with initial conditions $\theta^* = \theta_E + \varepsilon \cos(\pi x)$, $m_b^* = 1.0 + \varepsilon \cos(5\pi x)$ (solid line) and $\theta^* = \theta_E + \varepsilon \cos(5\pi x)$, $m_b^* = 1$ (dashed line). Parameters used are $\varepsilon = 10^{-4}$, $\theta_E = 0.02$, $S^* = 50$, $C^* = 27$, $\alpha^* = 0.4$, $\beta^* = 5$ and $D_m = 0.1$. The other solution variables associated with the solid line simulation are plotted in Figures 5.11 and 5.12.

As for the kinetic model we plot both the spatio-temporal evolution of the solution variables and the travelling-wave profile for the solution variables and other physical quantities calculated from them in Figures 5.11 and 5.12 respectively. In contrast to the solutions of the kinetic model we see no peaks of actin or myosin in the cell interior. The shape of the myosin profile closely matches the shape of the actin profile. The profiles for the network and solution velocity, the mixture stress, the pressure and the force exerted on the substrate

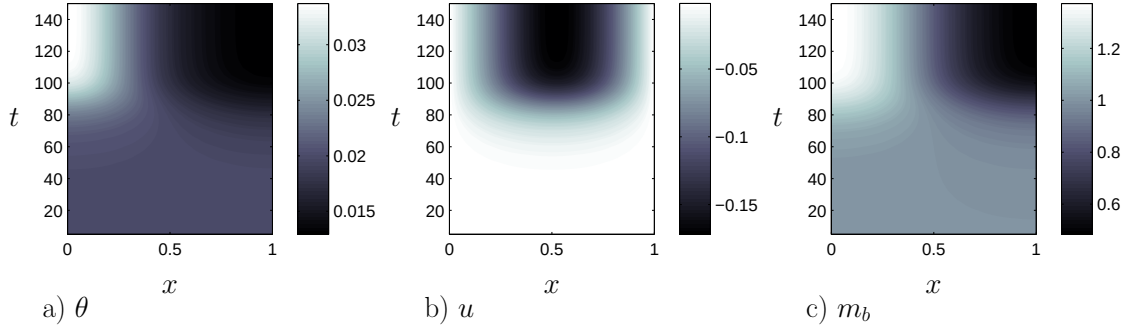


Figure 5.11: Spatio-temporal evolution of (a) network volume fraction, (b) network velocity and (c) concentration of bound myosin per unit volume of actin network in response to a small perturbation of the steady state. Corresponding velocity of the travelling strip is plotted as the solid line in Figure 5.10.

are all similar to those seen in the kinetic model.

All the simulations plotted in Figures 5.10 to 5.12 were calculated on grids with one thousand or two thousand spatial elements and a temporal step size of 10^{-3} or 2×10^{-3} . The worst relative error between the cell velocities computed on those grids over the duration of the simulation was less than 4.1×10^{-4} . The largest relative error between the travelling-wave θ and m_b profiles computed on different grids was less than 4.3×10^{-6} . Finally the relative difference between V and the average value of V over the final twenty nondimensional time units was less than 10^{-15} , so we can be confident the simulations have settled to a steady travelling-wave solution.

Next we consider the effect of adhesion strength on the travelling-wave solution in Figure 5.13. The trend displayed by the diffusion model is a departure from that seen throughout the rest of this thesis. Whilst for strong adhesion the speed of the travelling strip decays with increasing β^* , for weak adhesion the speed of the travelling-wave solution appears to tend to a nonzero constant value as β^* decreases. For the weak adhesion strengths simulated there is very little change in the solution profiles commensurate with the similar travelling speeds. Increasing the adhesion strength yields shallower actin and myosin profiles and slower retrograde flow of the network. We postpone further discussion of how the travelling strip behaves in the strong adhesion limit for the next section.

All the adhesion simulations in Figure 5.13 were run on grids with one thousand and two thousand spatial elements. The largest relative errors in the travelling-wave velocity, network volume fraction and myosin concentration between the grids were 2.5×10^{-5} , $1.5 \times$

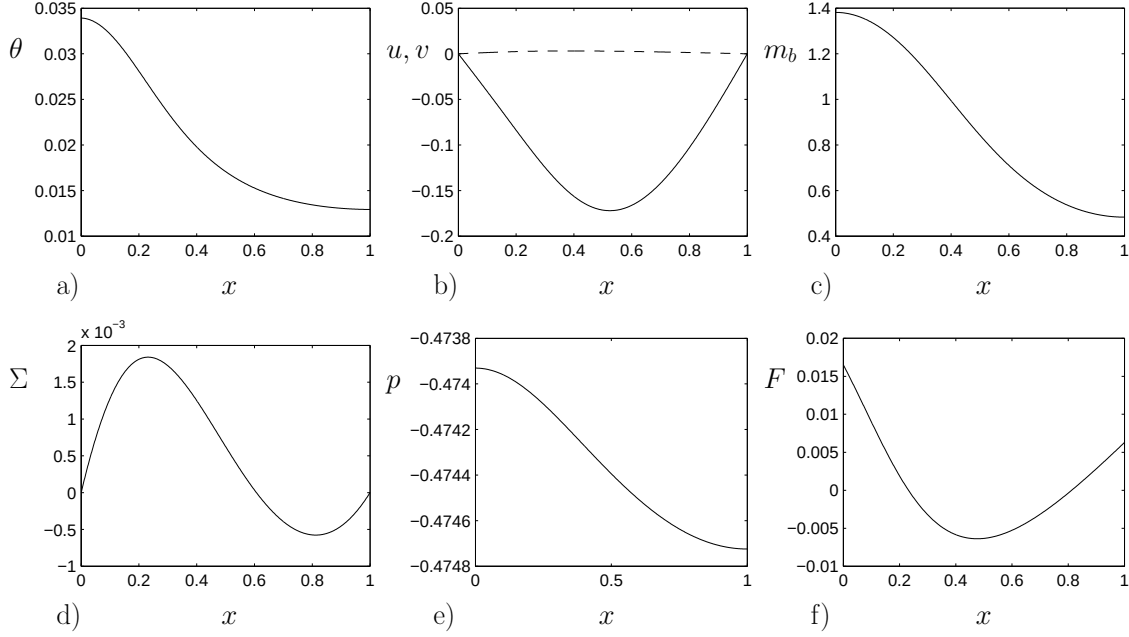


Figure 5.12: Steady travelling-wave profiles for the initial value problem in Figure 5.10 for (a) network volume fraction, (b) network velocity (solid line) and solution velocity (dashed line), (c) concentration of bound myosin per unit volume of network, (d) mixture stress, (e) pressure and (f) force exerted on the substrate, plotted at $t = 300$.

10^{-5} and 2.4×10^{-5} , respectively. The maximum relative difference between V and its average value over the final twenty nondimensional time units was 2.8×10^{-8} .

The key feature that separates this model from those previously discussed is the diffusion of bound myosin through the network phase, so we investigate the effect of the diffusion coefficient D_m in Figure 5.14. The travelling speed of the cell displays a bell-shaped dependence of the diffusion coefficient, so there exists a value of D_m for which the cell crawls fastest. The actin and myosin and network velocity profiles plotted for different values of D_m provide some insight into this. The network exhibits retrograde flow, so myosin which is bound to the network is swept towards the rear of the cell. When diffusion is slow compared to advection (small D_m) the myosin cannot diffuse forwards quickly enough to avoid being confined near the rear of the cell. Myosin-driven contraction of the actin network is restricted to the rear of the cell. For larger D_m values the trend observed is that, as D_m decreases we see higher actin volume fractions in a thinner region at the cell rear, faster retrograde flow of the network and faster travelling-strip speeds. At small D_m values the actin peak at the rear of the travelling strip becomes very narrow and decreasing D_m no

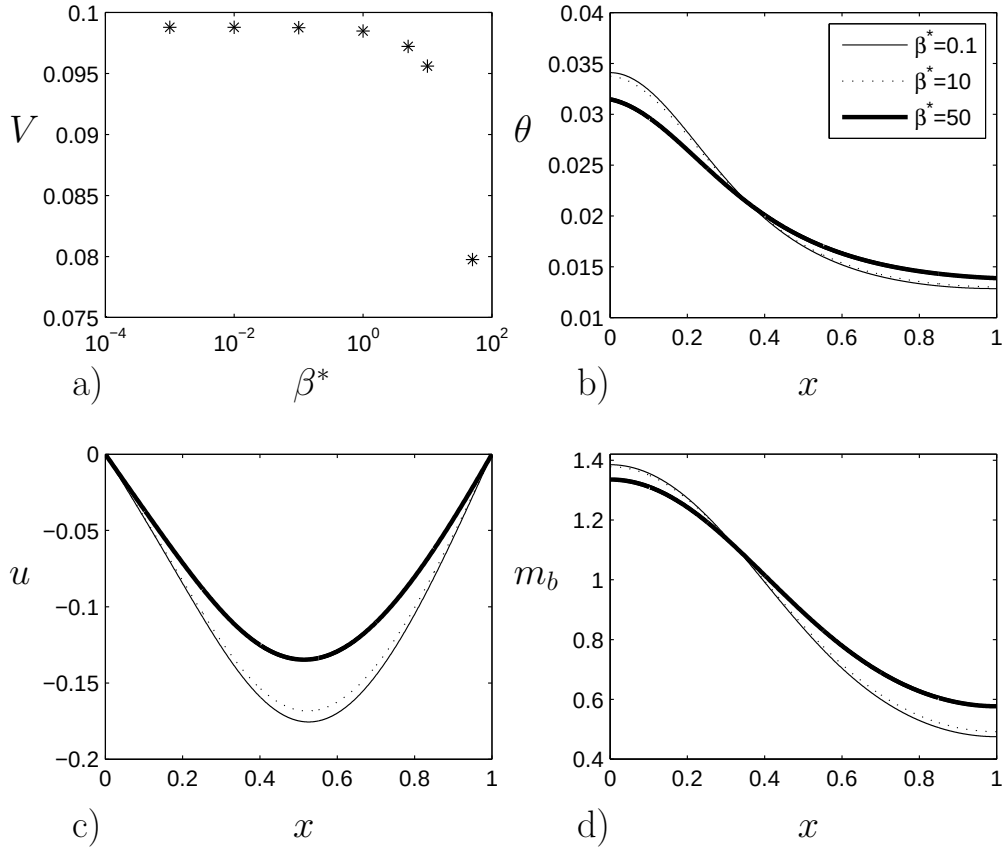


Figure 5.13: Graphs to show the effect of adhesion strength β^* on the form of the travelling-wave solution, including (a) travelling-strip velocity, (b) actin profile, (c) network velocity and (d) concentration of bound myosin per unit volume of actin network. Parameters used are $\theta_E = 0.02$, $S^* = 50$, $C^* = 27$, $\alpha^* = 0.4$ and $D_m = 0.1$ along with β^* values ranging from $\beta^* = 0.001$ to $\beta^* = 50$.

longer results in a faster travelling-strip speed. At the smallest value of diffusion coefficient simulated, $D_m = 0.01$, the maximum network volume fraction in the strip is 0.36, which is larger than biologically plausible.

All of the simulations plotted in Figure 5.14 were calculated on grids with one thousand and two thousand spatial elements. The relative error in the travelling-wave speed was less than 1.7×10^{-4} , the relative error in the network volume fraction was less than 4.5×10^{-3} . The largest relative difference between V and its mean over the final twenty nondimensional time units was 4.5×10^{-6} .

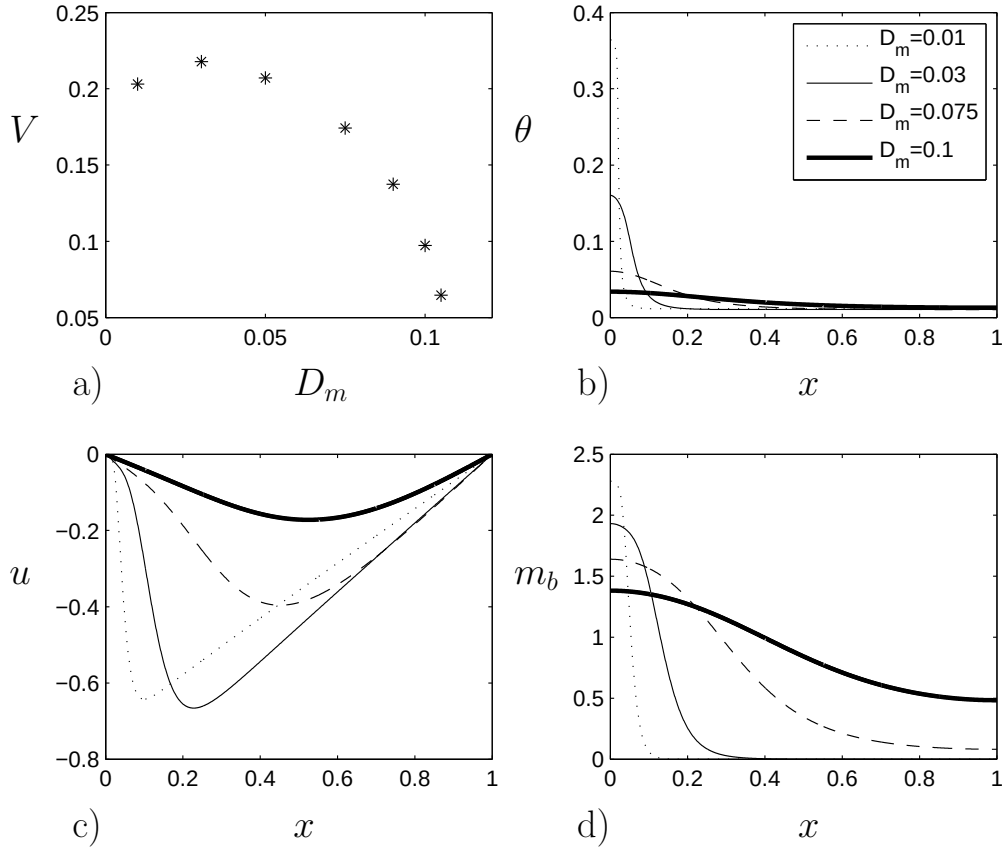


Figure 5.14: Graphs to show the effect of the myosin diffusion coefficient D_m on the form of the travelling wave solution including (a) the speed of the travelling strip, (b) the network volume fraction, (c) the network velocity and (d) the concentration of bound myosin per unit volume of actin network. Parameters used are $\theta_E = 0.02$, $S^* = 50$, $C^* = 27$, $\alpha^* = 0.4$ and $\beta^* = 5$, with values for the myosin diffusion coefficient D_m between $D_m = 0.01$ and $D_m = 0.105$.

5.5 Strong adhesion limit of the diffusion model

Previous models discussed in this thesis revealed steady travelling-wave solutions with interior peaks in the actin network at large β^* values. For comparison we would like to understand what happens in the strong adhesion limit of the diffusion model. However, when we consider the stability of the model by finding real roots to (5.55) in the regime with $\Psi_{\theta E} > 0$, increasing β^* seems to shift the roots towards smaller ω values and the stationary steady state becomes stable at quite moderate values of β^* . For the parameters used in Figure 5.14, the steady state is stable for $\beta^* = 125$. We considered a different parameter regime with $\Psi_{\theta E} < 0$ and $\Psi_{mE} < 0$ in which contraction of the network was both

volume fraction and myosin driven which led to the stationary steady state being unstable for larger β^* values. However, this introduced a new problem: it was difficult to find parameter values for which the network did not form regions of implausibly high volume fraction. This identified a need to limit the contractile ability of the network, so we introduce a new constitutive law for the swelling/contractile pressure of the network. Motivated by [97], we set

$$\Psi(\theta, m_b) = -S^* \log(1 - \theta) - \frac{C^* m_b \theta^2}{(1 + \theta/\theta_{\text{sat}})^2}. \quad (5.124)$$

We note that, while the swelling term is unchanged from (5.21b), the contractile term has been modified, in such a way that the contractile strength of the network is reduced for $\theta > \theta_{\text{sat}}$. Biologically we are stating that above a certain volume fraction, cross-linking and crowding of the network reduce the ability of myosin molecules to contract the network. We have already compared our new constitutive equation with m_b constant to the constitutive equation for the minimal model in Figure 2.1(b) in §2.1.2.

5.5.1 Strong adhesion boundary layer analysis

Numerical simulations for large β^* , with our new constitutive law (5.124) replacing (5.21b), indicate the existence of steady travelling-wave solutions with a boundary layer structure. (An example solution is shown in Figure 5.16.) Here we investigate this limit analytically. To aid our intuition in understanding the appropriate scalings for this problem we work in the lab frame, where for $t > 0$ and $a(t) < x < 1 + a(t)$,

$$\frac{\partial \theta}{\partial t} + \frac{\partial}{\partial x}(\theta u) = -J, \quad -\frac{\partial \Psi}{\partial x} + \frac{\partial}{\partial x} \left(\mu \frac{\partial u}{\partial x} \right) = \alpha(u - V) + \beta u, \quad (5.125a, b)$$

$$\frac{\partial}{\partial t}(m_b \theta) + \frac{\partial}{\partial x}(m_b \theta u) = \frac{\partial}{\partial x} \left(D_m \theta \frac{\partial m_b}{\partial x} \right), \quad \int_a^{1+a} \beta u \, dx = 0, \quad (5.126a, b)$$

are the governing equations which must be solved subject to $u = V$ and $\partial m_b / \partial x = 0$ at $x = a$ and $x = 1 + a$.

We suppose $\beta = \hat{\beta}/\varepsilon$, where $\varepsilon \ll 1$ and anticipate that strong adhesion will generate a region of slow moving network in the bulk of the cell, with boundary layers at each end of the cell driving the cell motion. In the cell bulk we scale $u = \varepsilon u^\circ$, where u° denotes the outer variable. The variables θ and m_b are not rescaled, but we write $\theta = \theta^\circ$ and $m_b = m^\circ$ for clarity to indicate that they are outer variables. We scale $V = \varepsilon^{1/2} \hat{V}$ for reasons that

will later become apparent. After dropping $O(\varepsilon^{1/2})$ terms we obtain

$$\frac{\partial \theta^o}{\partial t} = -J(\theta^o), \quad u^o = -\frac{1}{\hat{\beta}(\theta^o, m^o)} \frac{\partial \Psi}{\partial x}(\theta^o, m^o), \quad \frac{\partial}{\partial t}(m^o \theta^o) = D_m \frac{\partial}{\partial x} \left(\theta^o \frac{\partial m^o}{\partial x} \right), \quad (5.127a-c)$$

subject to initial conditions for m^o and θ^o , where we suppress subscripts identifying θ^o , u^o and m^o as leading order variables. Since these leading-order outer equations will not allow us to satisfy the boundary conditions on u we seek boundary layer solutions at $x = a(t)$ and $x = 1 + a(t)$. Boundary conditions for the outer variables will be set by matching to the solutions in the boundary layers.

We begin with the boundary layer near $x = a$. We substitute

$$u(x, t) = \varepsilon^\gamma u^L(X^L, t), \quad V(t) = \varepsilon^\gamma \hat{V}(t), \quad x = a(t) + \varepsilon^\lambda X^L, \quad (5.128a-c)$$

along with $\theta(x, t) = \theta^L(X^L, t)$ and $m_b(x, t) = m^L(X^L, t)$, into (5.125) and (5.126a), where γ and λ will be determined by a dominant balance on the network force balance equation. The governing equations become

$$\frac{\partial \theta^L}{\partial t} + \varepsilon^{\gamma-\lambda} \frac{\partial}{\partial X^L} (\theta^L (u^L - \hat{V})) = -J, \quad (5.129)$$

$$\frac{\partial}{\partial t} (m^L \theta^L) + \varepsilon^{\gamma-\lambda} \frac{\partial}{\partial X^L} (m^L \theta^L (u^L - \hat{V})) = \varepsilon^{-2\lambda} D_m \frac{\partial}{\partial X^L} \left(\theta^L \frac{\partial m^L}{\partial X^L} \right), \quad (5.130)$$

$$-\varepsilon^{-\lambda} \frac{\partial \Psi}{\partial X^L} + \varepsilon^{\gamma-2\lambda} \frac{\partial}{\partial X^L} \left(\mu \frac{\partial u^L}{\partial X^L} \right) = \varepsilon^\gamma \alpha (u^L - \hat{V}) + \varepsilon^{\gamma-1} \hat{\beta} u^L, \quad (5.131)$$

subject to

$$u^L = \hat{V}, \quad \frac{\partial m^L}{\partial X^L} = 0 \quad \text{on } X^L = 0. \quad (5.132a,b)$$

We must retain the swelling/contractile pressure and the adhesion terms in (5.131), because this is the dominant balance we will require in order to match to the outer solution. The viscosity term is required to enable us to satisfy the boundary condition. So we balance the swelling and contractile stress, viscosity and adhesion in (5.131) and find that $\gamma = \lambda = 1/2$. Neglecting $O(\varepsilon)$ and $O(\varepsilon/D_m)$ terms we obtain, at leading order

$$\frac{\partial \theta^L}{\partial t} + \frac{\partial}{\partial X^L} (\theta^L (u^L - \hat{V})) = -J, \quad \frac{\partial}{\partial X^L} \left(\theta^L \frac{\partial m^L}{\partial X^L} \right) = 0, \quad (5.133a,b)$$

$$-\frac{\partial \Psi}{\partial X^L} + \frac{\partial}{\partial X^L} \left(\mu \frac{\partial u^L}{\partial X^L} \right) = \hat{\beta} u^L, \quad (5.134)$$

subject to boundary conditions (5.132) and initial conditions for θ^L and m^L , where we suppress subscripts identifying u^L , θ^L and m^L as leading-order variables. From (5.132b)

and (5.133b) we see m^L is depends only on time in the boundary layer. Matching with the outer solution is only possible if

$$\theta^L \rightarrow \theta^o|_{x=a}, \quad u^L \rightarrow 0 \quad \text{as } X^L \rightarrow \infty, \quad (5.135a,b)$$

and $m^L = m^o|_{x=a}$. Note that we expect to impose the far-field condition on θ^L only when $\hat{V} > 0$, *i.e.* when the characteristics of (5.133a) flow into the domain.

A similar analysis holds in the right-hand boundary layer at $x = 1 + a$. We substitute into (5.125) and (5.126a) the scalings

$$u(x, t) = \varepsilon^{1/2} u^R(X^R, t), \quad V(t) = \varepsilon^{1/2} \hat{V}(t), \quad x = 1 + a(t) + \varepsilon^{1/2} X^R, \quad (5.136a-c)$$

along with $\theta(x, t) = \theta^R(X^R, t)$ and $m_b(x, t) = m^R(X^R, t)$, to obtain at leading order the boundary layer equations

$$\frac{\partial \theta^R}{\partial t} + \frac{\partial}{\partial X^R}(\theta^R(u^R - \hat{V})) = -J(\theta^R), \quad \frac{\partial}{\partial X^R} \left(\theta^R \frac{\partial m^R}{\partial X^R} \right) = 0, \quad (5.137a,b)$$

$$-\frac{\partial \Psi}{\partial X^R} + \frac{\partial}{\partial X^R} \left(\mu \frac{\partial u^R}{\partial X^R} \right) = \hat{\beta} u^R \quad (5.138)$$

for $X^R < 0$, where we suppress subscripts identifying θ^R , u^R and m^R as leading order variables. The boundary conditions are

$$u^R = \hat{V}, \quad \frac{\partial m^R}{\partial X^R} = 0 \quad \text{on } X^R = 0. \quad (5.139a,b)$$

Again we note m^R is independent of x and matching with the outer solution requires that

$$\theta^R \rightarrow \theta^o|_{x=1+a}, \quad u^R \rightarrow 0 \quad \text{as } X^R \rightarrow -\infty, \quad (5.140a,b)$$

and $m^R = m^o|_{x=1+a}$; note that we expect to impose the far-field condition on θ^R only when $\hat{V} < 0$.

To recap, we have equations and boundary conditions for the leading-order versions of m_b , θ , u and V in two inner boundary layer regions and an outer region that connects them. It remains to impose the integral constraint (5.126b) and check that we have the correct number of boundary conditions to close the problem at leading-order. To answer these questions we need to investigate the far-field behaviour of the inner solutions.

5.5.2 Steady-state boundary layer analysis

To simplify the problem and motivated by our interest in the travelling-wave solutions we consider the steady state problem, in which the outer problem, (5.127), implies

$$\theta^o = \theta_E, \quad m^o = m_0 + m_1(x - a), \quad u^o = -\frac{m_1}{\hat{\beta}(\theta_E, m^o)} \frac{\partial \Psi}{\partial m}(\theta_E, m^o), \quad (5.141a-c)$$

where m_0 and m_1 are constants, yet to be determined. The system of equations, (5.133) and (5.134) governing the left boundary layer becomes, for $X^L > 0$

$$\frac{d}{dX^L}(\theta^L(u^L - \hat{V})) = -J, \quad -\frac{\partial \Psi}{\partial \theta^L} \frac{d\theta^L}{dX^L} + \frac{d}{dX^L} \left(\mu \frac{du^L}{dX^L} \right) = \hat{\beta}u^L, \quad (5.142a,b)$$

with $m^L = m_0$, subject to $u^L \rightarrow 0$ as $X^L \rightarrow \infty$ and $u^L = \hat{V}$ at $X^L = 0$. Matching to the outer solution also requires that $\theta^L \rightarrow \theta_E$ as $X^L \rightarrow \infty$.

We consider the far-field expansion, $\theta^L = \theta_E + \tilde{\theta}^L$ and $u^L = \tilde{u}^L$, where the $\tilde{\theta}^L$ and $\tilde{u}^L$ are small as $X^L \rightarrow \infty$. Linearizing, we obtain

$$-\hat{V} \frac{d\tilde{\theta}^L}{dX^L} + \theta_E \frac{d\tilde{u}^L}{dX^L} = -J'_E \tilde{\theta}^L, \quad -\Psi_{\theta^L}(\theta_E, m_0) \frac{d\tilde{\theta}^L}{dX^L} + \mu_E \frac{d^2 \tilde{u}^L}{dX^{L2}} = \hat{\beta}(\theta_E, m_0) \tilde{u}^L \quad (5.143a,b)$$

as $X^L \rightarrow \infty$, where, as before, a subscript E indicates a variable is evaluated at the equilibrium volume fraction θ_E and to simplify matters we assume the network viscosity, μ , depends only on θ . We introduce a new variable $w^L = d\tilde{u}^L/dX^L$ and formulate the problem as a first-order system, $d\mathbf{u}^L/dX^L = \mathcal{A}\mathbf{u}^L$ where $\mathbf{u}^L = (\tilde{\theta}^L, \tilde{u}^L, w^L)^T$. The eigenvalues of the 3×3 matrix $\mathcal{A}$, which determine the behaviour of our solutions in the far-field, satisfy the cubic polynomial

$$\xi^3 + A\xi^2 + B\xi + C = 0, \quad (5.144)$$

where

$$A = -\frac{(\mu_E J'_E + \theta_E \Psi_\theta)}{\mu_E \hat{V}}, \quad B = -\frac{\hat{\beta}}{\mu_E}, \quad C = \frac{\hat{\beta} J'_E}{\mu_E \hat{V}}, \quad (5.145a-c)$$

with Ψ_θ and $\hat{\beta}$ evaluated at $(\theta^L, m^L) = (\theta_E, m_0)$. To investigate the roots of (5.144) we write

$$(\xi - \xi_1 - i\xi_4)(\xi - \xi_2 + i\xi_4)(\xi - \xi_3) = 0, \quad (5.146)$$

for ξ_1, ξ_2, ξ_3 and ξ_4 all real and note that

$$B = \xi_1 \xi_2 + \xi_2 \xi_3 + \xi_3 \xi_1, \quad C = -\xi_1 \xi_2 \xi_3 \quad \text{if } \xi_4 = 0, \quad (5.147a,b)$$

$$B = \xi_1^2 + \xi_4^2 + 2\xi_1 \xi_3, \quad C = -\xi_3(\xi_1^2 + \xi_4^2) \quad \text{if } \xi_4 \neq 0. \quad (5.148a,b)$$

We restrict our attention to the case where $\hat{V} > 0$, but note the following arguments would hold in the right-hand boundary layer for $\hat{V} < 0$. We see from (5.145c) that $C > 0$ for $\hat{V} > 0$, so either precisely one or three of the eigenvalues have negative real part. If all the eigenvalues had negative real part then $B > 0$, but (5.145b) implies $B < 0$ for all parameter values. Therefore, there are two growing modes and one decaying mode as $X^L \rightarrow \infty$. We reject the two growing modes, because they are physically unrealistic and will not allow us to match to the outer solution. We find

$$u^L = E_1 e^{\xi_3 X^L}, \quad \theta^L = \theta_E + \tilde{E}_1 e^{\xi_3 X^L}, \quad (5.149a,b)$$

as $X^L \rightarrow \infty$ where E_1 and $\tilde{E}_1$ are related and ξ_3 is the eigenvalue of $\mathcal{A}$ with negative real part, which is known in terms of θ_E , m_0 and $\hat{V}$. There is only one undetermined constant E_1 in this far-field expansion.

At steady state, the boundary layer equations on the right are $m^R = m_0 + m_1$,

$$\frac{d}{dX^R}(\theta^R(u^R - \hat{V})) = -J, \quad -\frac{\partial \Psi}{\partial \theta^R} \frac{d\theta^R}{dX^R} + \frac{d}{dX^R} \left(\mu \frac{du^R}{dX^R} \right) = \hat{\beta} u^R, \quad (5.150a,b)$$

subject to $u^R \rightarrow 0$ as $X^R \rightarrow -\infty$ and $u^R = \hat{V}$ at $X^R = 0$. Matching to the outer solution requires that $\theta^R \rightarrow \theta_E$ as $X^R \rightarrow -\infty$. Carrying out the same linearization argument in the far-field, we recover (5.143) where L and m_0 are replaced by R and $m_0 + m_1$ respectively. The arguments from the left-hand boundary layer concerning the signs of eigenvectors of the linearized system still hold, albeit with different values in $\mathcal{A}$. There are still exactly two eigenvectors with positive real part. The difference here is that we must consider whether the modes grow or decay as $X^R \rightarrow -\infty$. Therefore the far-field expansion in the right boundary layer is

$$u^R = E_2 e^{\xi_1 X^R} + E_3 e^{\xi_2 X^R}, \quad \theta^R = \theta_E + \tilde{E}_2 e^{\xi_1 X^R} + \tilde{E}_3 e^{\xi_2 X^R} \quad (5.151a,b)$$

for $\xi_4 = 0$. Alternatively when $\xi_4 \neq 0$

$$\begin{aligned} u^R &= F_2 e^{\xi_1 X^R} \cos(\xi_4 X^R) + F_3 e^{\xi_1 X^R} \sin(\xi_4 X^R), \\ \theta^R &= \theta_E + \tilde{F}_2 e^{\xi_1 X^R} \cos(\xi_4 X^R) + \tilde{F}_3 e^{\xi_1 X^R} \sin(\xi_4 X^R), \end{aligned} \quad (5.152a,b)$$

where E_i and $\tilde{E}_i$ or F_i and $\tilde{F}_i$ ($i = 2, 3$) are related and $\xi_1 + i\xi_4$ and $\xi_2 - i\xi_4$ are the eigenvalues of the matrix $\mathcal{A}$ in the right-hand boundary layer, that have positive real part. There are two undetermined constants in the far-field expansion for the right-hand boundary layer.

We are now in a position to evaluate the integral condition, which we anticipate will be needed to close the system. We introduce a new parameter δ such that $\varepsilon^{1/2} \ll \delta \ll 1$ and write (5.126b) as

$$\left(\int_a^{a+\delta} + \int_{a+\delta}^{1+a-\delta} + \int_{1+a-\delta}^{1+a} \right) \beta u \, dx = 0. \quad (5.153)$$

Using the same scalings as previously and the steady boundary layer equations (5.142b) and (5.150b), we find

$$\int_a^{a+\delta} \beta u \, dx \sim \int_0^{\delta\varepsilon^{-1/2}} \hat{\beta} u^L \, dX^L = \left[-\Psi(\theta^L, m^L) + \mu(\theta^L) \frac{\partial u^L}{\partial X^L} \right]_{X^L=0}^{X^L=\delta\varepsilon^{-1/2}}, \quad (5.154)$$

$$\int_{1+a-\delta}^{1+a} \beta u \, dx \sim \int_{-\delta\varepsilon^{-1/2}}^0 \hat{\beta} u^R \, dX^R = \left[-\Psi(\theta^R, m^R) + \mu(\theta^R) \frac{\partial u^R}{\partial X^R} \right]_{X^R=-\delta\varepsilon^{-1/2}}^{X^R=0}. \quad (5.155)$$

Using the steady outer solution, (5.141c), for u^o implies

$$\int_{a+\delta}^{1+a-\delta} \beta u \, dx \sim \int_{a+\delta}^{1+a-\delta} \hat{\beta} u^o \, dx = [-\Psi(\theta^o, m^o)]_{a+\delta}^{1+a-\delta}. \quad (5.156)$$

After expanding these terms and summing the contribution from all three integrals, we let $\delta \rightarrow 0$. Neglecting exponentially small terms and those of linear or higher order in δ , the integral constraint (2.20) becomes

$$\Psi(\theta^L(0), m_0) - \mu(\theta^L(0)) \frac{du^L}{dX^L}(0) - \Psi(\theta^R(0), m_0 + m_1) + \mu(\theta^R(0)) \frac{du^R}{dX^R}(0) = 0. \quad (5.157)$$

In order to understand whether or not we have enough information to close the system (5.141), (5.142) and (5.150) without going to higher order in our asymptotic analysis, we count the degrees of freedom in the system. In order to do this we must consider the order of our system, the number of parameters that have to be determined as part of the solution and the number of degrees of freedom.

In each of the boundary layers, (5.142) and (5.150) is a third order system of equations and we have the extra condition (5.157) for $\hat{V}$. We know that the total amount of myosin is conserved, so an analysis similar to that leading to (5.157) applied to the lab frame version of (5.42) gives

$$\int_a^{1+a} m_0 + m_1(x-a) \, dx = 1, \quad (5.158)$$

where we have suppressed the subscript identifying a as a leading order variable. We see that $m_0 = 1 - m_1/2$, thus, the leading-order problem has two parameters $\hat{V}$ and m_1 , which must be determined as part of the solution. We know from the far-field expansions (5.149) and (5.151) or (5.152) that there is one degree of freedom associated with $X^L \rightarrow \infty$ and

that there are two degrees of freedom associated with $X^R \rightarrow -\infty$. To complete the degree of freedom count, we must consider local expansions at $X^L = 0$ and $X^R = 0$ to find the number of undetermined constants associated with these boundaries.

In the left boundary layer, near $X^L = 0$, we consider series expansions of the form

$$u^L = \sum_{k=0}^{\infty} u_k (X^L)^k, \quad \theta^L = \sum_{k=0}^{\infty} \theta_k (X^L)^k \quad (5.159a,b)$$

as $X^L \rightarrow 0$. Substituting these expansions into (5.142a) and equating coefficients for the first few terms in X^L we see that

$$O(1): \quad \theta_1(u_0 - \hat{V}) + \theta_0 u_1 = -J(\theta^o), \quad (5.160)$$

$$O(X^L): \quad 2\theta_2(u_0 - \hat{V}) + 2\theta_1 u_1 + 2\theta_0 u_2 = -J_\theta \theta_1, \quad (5.161)$$

$$O((X^L)^2): \quad 3\theta_3(u_0 - \hat{V}) + 3\theta_2 u_1 + 3\theta_1 u_2 + 3\theta_0 u_3 = -J_\theta \theta_2 - \frac{1}{2} J_{\theta\theta} \theta_1^2, \quad (5.162)$$

and so on. Then substituting the series expansions into (5.142b) and equating coefficients we find

$$O(1): \quad -\Psi_\theta \theta_1 + \mu_\theta \theta_1 u_1 + 2\mu u_2 = \hat{\beta} u_0, \quad (5.163)$$

$$O(X^L): \quad -2\Psi_\theta \theta_2 - \Psi_{\theta\theta} \theta_1^2 + \mu_{\theta\theta} \theta_1^2 u_2 + 2\mu_\theta \theta_2 u_1 + 4\mu_\theta \theta_1 u_2 + 6\mu u_3 = \hat{\beta} u_1 + \hat{\beta}_\theta \theta_1 u_0, \quad (5.164)$$

and so on, where J , Ψ , μ and $\hat{\beta}$ are evaluated at (θ_0, m_0) . The boundary condition on $X^L = 0$ gives $u_0 = \hat{V}$. For $i \geq 1$, θ_i and u_{i+1} are given, in terms of lower indexed coefficients, by two simultaneous equations: the equation at $O((X^L)^i)$ in the expansion of (5.142a) and at $O((X^L)^{i-1})$ in the expansion of (5.142b). So all the coefficients are determined if we know θ_0 and u_1 , which are related by the condition (5.160). Therefore, there is only one degree of freedom in the local expansions of θ^L and u^L as $X^L \rightarrow 0$. The same argument holds in the right boundary layer. We have a total of seven degrees of freedom ($\hat{V}$, m_1 , one at $X^L \rightarrow \infty$, one at $X^L \rightarrow 0$, two at $X^R \rightarrow \infty$ and one at $X^R \rightarrow 0$) which agrees with the order of the governing equations (5.142), (5.150) and the condition (5.157). The coupled problem is correctly specified.

5.5.3 Steady-state numerical solution for strong adhesion limit

Our boundary layer analysis has simplified the governing equations substantially in the limit of strong cell-substrate adhesion. However, the resulting problem still requires numerical solution. A potential plan for such a numerical scheme is as follows. First make a guess for

$\hat{V}$ and m_1 , then solve the left-hand boundary layer equations as an initial value problem by shooting in the direction of decreasing X^L . Next use the value of θ^L at $X^L = 0$ and the integral constraint condition (5.157) to calculate θ^R and du^R/dX^R at $X^R = 0$. Then solve the right-hand-side boundary layer equations as an initial value problem in the direction of decreasing X^R . If we had made the correct guess for $\hat{V}$ and m_1 the solution of the initial value problem will be such that $\theta^R \rightarrow \theta_E$ and $u^R \rightarrow 0$ as $X^R \rightarrow -\infty$. At a large negative X^R we could use $\theta^R - \theta_E$ and u^R as residuals for minimisation over the choices of $\hat{V}$ and m_1 . The appeal of this approach is that we solve computationally fast, initial value problems. This is important, because we may need to solve the boundary layer equations many times over the course of the minimisation. Unfortunately this approach will never work in practice. We know from the form of (5.151) that the problem is very stiff. Numerical integration towards large negative X^R will pick up some component of the eigenvector associated with the negative eigenvalue, which will grow and swamp the true solution. Furthermore it is unclear how to construct appropriate criteria to select the two constants in the far-field expansion (5.151) or (5.152) in order to be able to shoot towards $X^R = 0$.

Unable to solve the coupled boundary layer problem numerically at present, we take a somewhat pragmatic approach to verifying the boundary layer structure. We use our unsteady code discussed in §5.4 to solve the full system numerically and find a steady travelling-wave solution with β^* large. We then scale the travelling-wave speed to obtain $\hat{V}$ and fit a line to the myosin profile in the bulk of the cell to extract an approximation for m_1 . We use these values to solve the boundary layer equations.

Starting with the left-hand boundary layer equations we note that (5.142) is an autonomous system, so the equations are invariant under a translation in X^L . The domain is semi-infinite, so we truncate the domain and use our far-field expansion (5.149) to approximate u^L , θ^L and du^L/dX^L at some large X^L . This requires picking a value for E_1 in (5.149), but varying this constant is equivalent to varying the X^L we start from. So, we exploit our freedom to translate X^L by picking a small constant E_1 and $X^L = 0$ in (5.149) and integrating back until $u^L = \hat{V}$. We save the X^L value where $u^L = \hat{V}$ and translate the domain by this distance so that $u^L = \hat{V}$ at the new $X^L = 0$ position. It is important to ensure that the value of E_1 is sufficiently small that our solution does not depend on the choice of E_1 .

To solve the right-hand boundary layer equations (5.150) we work on the truncated

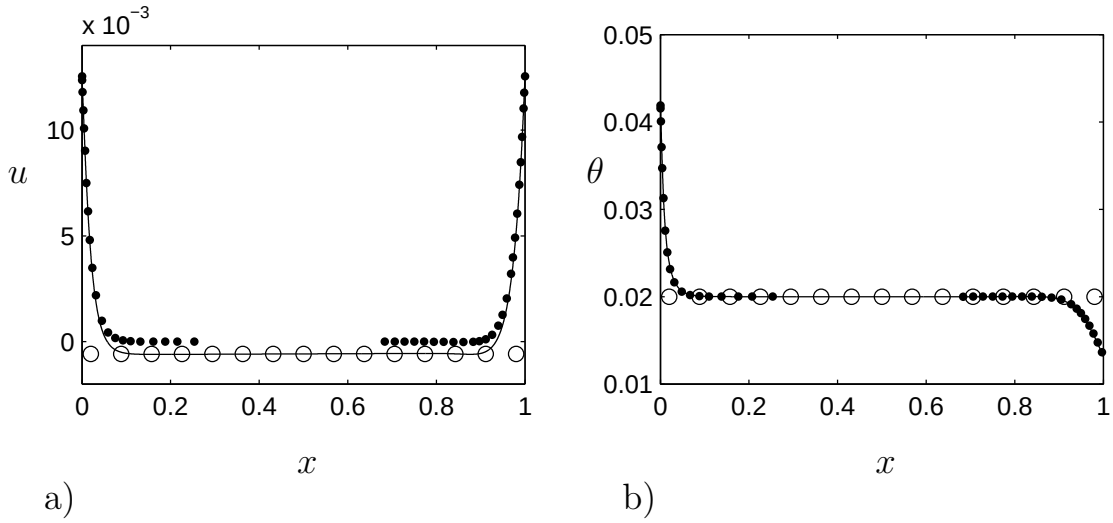


Figure 5.15: Graphs to show the agreement between the full numerical solution (solid line), the boundary layer solutions (dots) and the outer solution (open circles) for (a) the network velocity in the lab frame and (b) the network volume fraction. Parameters used are $\theta_E = 0.02$, $S^* = 5$, $C^* = 500$, $\alpha^* = 0.4$; $D = 0.1$, $\theta_{\text{sat}} = 0.04$ and $\beta^* = 1000$ corresponding to $\varepsilon = 10^{-3}$. Calculation of the inner and outer solutions relies on extracting m_1 and $\hat{V}$ from the full numerical solution.

domain $X^R \in [-M, 0]$ for some large M subject to the boundary conditions $u^R = \hat{V}$ at $X^R = 0$ and $u^R = 0$ at $X^R = -M$. We use the MATLAB solver `fsolve`, to minimise a residual, where each call to the residual function calculates θ^R as the solution to the initial value problem, (5.150a), integrating by the implicit Euler method from $X^R = 0$ to $X^R = -M$, given the current guess for u^R . Then the residual function uses θ^R to solve for u^R as the solution of a finite element, boundary value problem formulation of (5.150b). We take $u^R \equiv 0$ as the initial guess for u^R . This method is not as quick as the shooting method in the left-hand boundary layer, because we must compute approximations for θ^R and u^R at every iteration of the solver.

Figure 5.15 shows the agreement between a solution to the full numerical problem and solutions to the outer and inner solutions of the boundary layer analysis, calculated using m_1 and $\hat{V}$ extracted from the solution to the full numerical problem. These results are for a large β^* corresponding to $\varepsilon = 10^{-3}$. Figure 5.16 shows the agreement between a solution to the full numerical problem and a composite solution constructed from the inner and outer solutions, following [76]. From these solutions we find the left-hand-side of (5.157) evaluates to -8.90×10^{-4} which is certainly small. The solution to the full numerical problem used in this section was calculated on grids with 3200 and 6400 spatial elements.

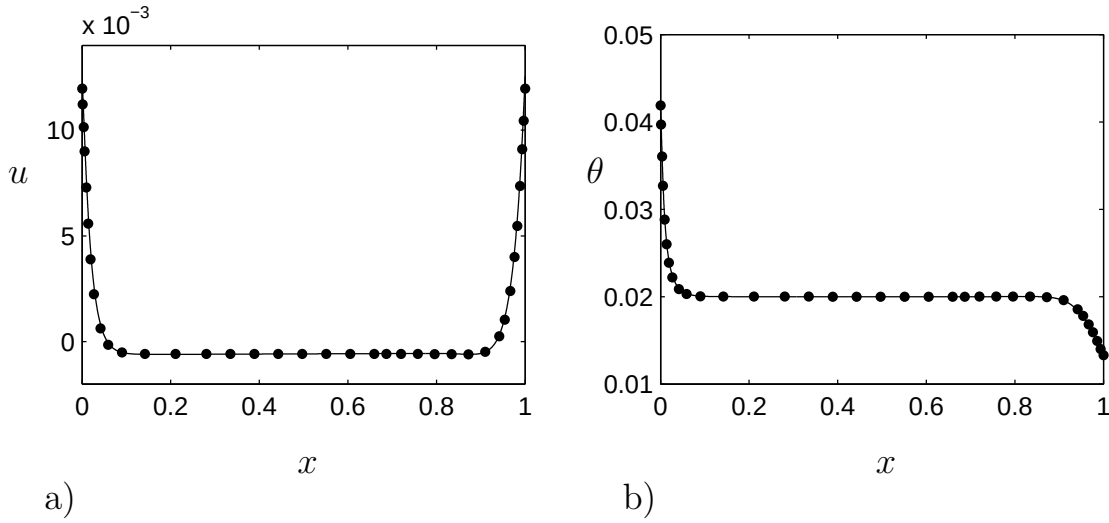


Figure 5.16: Graphs to show the agreement between the full numerical solution (solid line) and the composite solution (dots) constructed from the inner and outer solutions in Figure 5.15. Parameters as in Figure 5.15.

At steady state, the relative error in V between the two grids is less than 3.0×10^{-5} , the relative error in θ is less than 9.3×10^{-4} and the relative error in m_b is less than 2.1×10^{-6} . Over the final twenty dimensionless time units the relative difference in V from its mean is less than 2.0×10^{-15} .

5.6 Chapter summary

In this chapter, we have coupled equations describing the transport, binding and unbinding of myosin to our minimal model of a crawling cell. We have found estimates for the sizes of the dimensionless parameters in the model, which revealed quite a range of possible values. In light of this uncertainty we considered different sub-models of the myosin equations. The analysis indicated that we can obtain a diffusive term in the equation governing the transport of bound myosin, either by allowing myosin to crawl along the actin fibres, or via diffusion of free myosin in solution when binding kinetics are fast. It also enables us to understand models presented in the literature as different limits of the same more general governing equations and provides two-phase analogues of models that we have only found in the literature in one-phase models.

We then present the results of linear stability analyses for the model obtained in the slow-reaction-kinetics limit. We calculate the linear stability of the stationary steady state

in three limits. Firstly, where diffusion of free myosin is fast and crawling of bound myosin is slow, secondly, where diffusion of free myosin is fast, and thirdly, where reaction kinetics are fast and crawling is slow. In the cases where we found a complete set of modes, we also solved the linearized initial value problem. This section demonstrates that myosin-driven contraction of the network is destabilising and depending on the sign of $\partial\Psi/\partial\theta$ the volume fraction can drive the network to contract (destabilising) or swell (stabilising). We are able to obtain an unstable steady state when $\partial\Psi/\partial m_b < 0$ even when the parameters are such that $\partial\Psi/\partial\theta > 0$. Diffusive terms are stabilising, and we mainly see the effect of a diffusive term at large wavenumbers. The inclusion of such terms allows us to formulate models that will be unstable for some interval of wavenumbers, but stable at high wavenumber. Rapid unbinding of myosin can act to stabilise the stationary steady state.

We chose two simple sub-models as examples to improve our understanding of the types of behaviour to expect from coupling myosin models to the minimal model. The first, termed the kinetic model, has a spatially uniform distribution of free myosin through the solution phase and an advection-reaction equation governing the concentration of bound myosin per unit volume of actin network. In the second model, the diffusion model, free myosin decouples from the problem and we have an advection-diffusion equation governing the bound myosin. Both models exhibit steady travelling-wave solutions attained as the long-time behaviour of an initial value problem. The profiles for θ and u associated with each are similar to the one-peak solutions of the minimal model. They exhibit retrograde flow of the network and network collects at the rear of the cell. The concentration of myosin per unit volume fraction of network is highest at the rear of the cell in line with the experimental observations discussed in §1.1.4. Whilst the transient solutions are dependent on the precise form of the initial conditions, the form of the steady, travelling-wave solution appears to be unaffected by initial conditions, up to the direction of motion. The kinetic model exhibits the biologically observed bell-shaped dependence on adhesion strength. Solutions of the kinetic model are quite sensitive to the binding and unbinding rates for myosin. The speed of the travelling-wave solutions for the diffusion model do not exhibit the experimentally observed bell-shaped dependence on adhesion strength for the parameter values we considered. Whilst the speed decreases for large β^* it appears to plateau to a constant as $\beta^* \rightarrow 0$.

The constitutive law originally used in this chapter allowed us to consider myosin-driven

contraction as a new mechanism for driving the cell crawling. Unfortunately, it is much harder to control possible ranges for the network volume fraction with this constitutive law, which led us to introduce a second constitutive law. It is more complicated, but allows contraction of the network to be impaired if the volume fraction exceeds a given value. Finally, using this constitutive law, we investigated the strong adhesion limit of the diffusion model and found the solutions have a boundary layer structure, and that the velocity of the travelling strip scales with $1/\sqrt{\beta^*}$ as $\beta^* \rightarrow \infty$. The predictions of the boundary layer analysis were verified against the numerical solution of the full problem and found to be in excellent agreement.

The next chapter is a departure from the two-phase flow models we have discussed so far in this thesis. We instead turn our attention to hydrodynamical descriptions of cells spreading on flat surfaces.

Chapter 6

Image analysis and model comparisons for cell spreading

In the previous chapters we have focused on modelling the crawling motion of well-spread cells on flat surfaces. In this chapter we shift our focus slightly and consider another stage of self-propelled cell motion, namely cell spreading on a flat substrate. We select cell spreading as a simple system for understanding aspects of cell motility because the geometry of a spreading cell is typically near axisymmetric in the early stages of spreading. It also seems reasonable to assume that there may be some early timescale over which simple hydrodynamical models hold before the more complicated physics associated with motility has to be accounted for. Such an assumption can be justified by Frisch and Thoumine's work, [61], comparing experimental observations of cell spreading to a partially wetting droplet.

The aims of this chapter are twofold. Firstly, we seek to develop an image analysis code capable of extracting quantitative information about cell shape from the experimental data obtained by our collaborator, Dr. J. Ratcliffe, (Division of Materials, Mechanics and Structures at the University of Nottingham.) Secondly, we consider to what extent a spreading cell can be modelled using ideas from fluid dynamics. The question is interesting in its own right, but also has implications for the justification of any fluid based models of cell motility. In §6.1 we describe the confocal microscope images of spreading cells *in vitro*. We detail in §6.2 how the cell shape can be extracted from the images with an image processing routine implemented in MATLAB. We proceed to test the image analysis code on images of beads in §6.3. In §6.4 we review a number of existing fluid-based models of cell spreading that can be found in the literature, before demonstrating, in §6.5, how cell data obtained from the image analysis could be compared to the different models.

6.1 Experimental data

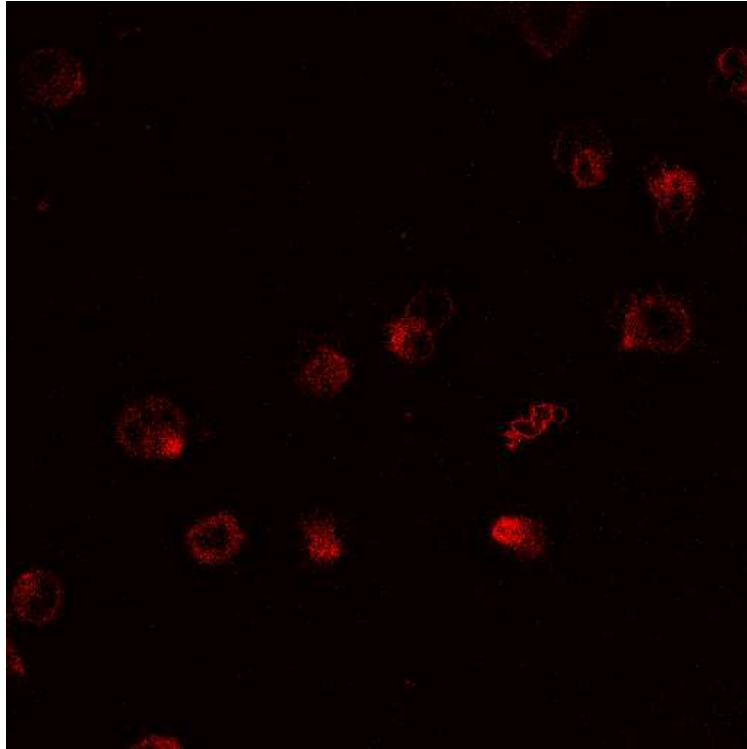


Figure 6.1: A microscope image of Human Osteosarcoma cells on a microscope plate, obtained by Dr. J. Ratcliffe, an experimental collaborator and reproduced here with his permission. The cells are stained red. The image represents a square of length $238\mu\text{m}$.

Our experimental collaborator, Dr. J. Ratcliffe, has obtained confocal microscope images of Human Osteosarcoma cells. The confocal microscope captures images of the spreading cells in planes parallel to the microscope slide. A key advantage to this technique is that it provides three-dimensional information about the shape of the cell. We term these images slices and we typically have 10 – 15 image slices of a given cell at each time point. The cells are imaged at a set interval, usually around five minutes, over a period of up to three hours. The resolution of the microscope used is around 500nm. Prior to imaging, the cells are stained with LavaCell [34]. This molecule stains cell membranes and lipophilic organelles and has a red fluorescence. A typical image is shown in Figure 6.1. There are about twelve cells on this slide. The dark space seen inside some of the cells is the cell nucleus, which is not stained by LavaCell.

There are limitations on the data that can be gathered. Focusing to a specified height above the microscope slide and taking the image require some time, so demanding finer

slices leads to larger time intervals. The laser used to excite the LavaCell stain, which makes it fluoresce, photobleaches the stain; furthermore, continued exposure to the laser is harmful to the cells. Both of these facts effectively impose a maximum number of images that can be taken of one slide of cells. If we wish to image over longer time periods, we must reduce the laser strength and image less frequently to protect the cells from photobleaching. We also note that reducing the laser strength reduces the signal to noise ratio in the image, making it more difficult to detect the cells.

6.2 Image segmentation for cells

The key image processing task is to locate the cells and separate them from the background: this is termed segmentation. The segmentations can then be analysed to provide useful information about the three-dimensional shape of the cell. Our experimental collaborator developed a MATLAB script that used the image processing toolbox in MATLAB to segment cells and stack the outlines. However, the segmentation was often not a very close representation of the cell and the code could only deal with one cell at a time. Each cell had to be isolated manually by cropping the images. Here we present a substantially improved image segmentation algorithm, again using the image processing toolbox in MATLAB.

MATLAB handles greyscale images as a matrix of pixel values between 0 and 1, where 1 represents a white pixel and zero represents a black pixel. In seeking to separate the light cells from the dark background, the simplest thing to do would be to pick a threshold value between 0 and 1 and set all the pixels above this value to white and all those below it to black. This is a technique called thresholding. The significant amount of noise in the image (see *e.g.* Figure 6.1) means that the segmentations obtained by thresholding are far from satisfactory, so we proceed to discuss ways of smoothing the image to reduce the effect of the noise.

6.2.1 Image smoothing

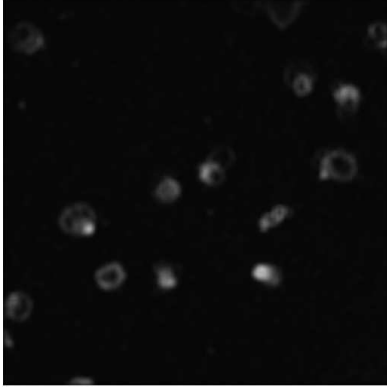
Edges in an image are associated with large changes in pixel intensity over a small number of pixels. Unfortunately noise can be described similarly. Smoothing the image, to remove some of the noise, can aid edge detection. One of the simplest ways to smooth an image is with a low pass filter. The filtered pixel value at a point is the weighted average of the

intensities in a matrix of pixels (say of size 5×5) of which the filtered pixel is the central value. Various weighting functions are available [109].

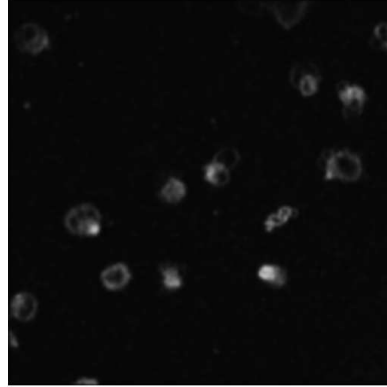
A useful family of low pass filters is available in the MATLAB toolbox, based on the Gaussian function

$$f(x, y) = ke^{-\frac{x^2+y^2}{2\sigma^2}}, \quad (6.1)$$

where k and σ are constant, with σ the standard deviation, which tells us how narrow the Gaussian peak is. The values in a filter matrix can be chosen to approximate this Gaussian function, with larger values of σ chosen to increase the amount of blurring of the image. In a filter, k is chosen so that the entries in the filter sum to unity. The effect of this type of smoothing can be seen in Figure 6.2(a). The main disadvantage of using a Gaussian filter is that edges get blurred as well as the noise, so we lose valuable information about the position of cell edges.



(a) Gaussian filter



(b) Nonlinear diffusion

Figure 6.2: The results of applying smoothing techniques to the image shown in Figure 6.1. In (a) we use a 10×10 pixel Gaussian filter with $\sigma = 5$ and in (b) we use nonlinear diffusion with $c = 0.5$ and 10 timesteps as discussed in the text.

An alternative to filtering that seeks to preserve information about edges in the image is described in [124]. The idea is to use a nonlinear diffusion operator to smooth the image, where the conductivity coefficient depends on the image. We present the diffusion equation for a continuous function, but the method we implement is discrete, with each pixel being a given point in the discretization. We write

$$\frac{\partial I}{\partial t} = \nabla \cdot (g \nabla I), \quad (6.2)$$

where I is the pixel intensity, g is the conductivity coefficient and $\nabla = (\partial/\partial x, \partial/\partial y)$ is the gradient operator, with x, y being co-ordinates in the plane of the image. We aim to smooth within regions of similar pixel intensity, without blurring across boundaries between such regions, by taking g to be small near edges when the pixel gradients are large. Specifically we choose

$$g(\nabla I) = e^{-(|\nabla I|/c)^2}, \quad (6.3)$$

where c is some constant. We use a finite difference implementation of this nonlinear diffusion equation taken from MATLAB Central, [106], which uses the scheme described in [64]. There are a number of parameters associated with the implementation, including the size of the timestep, which is picked to ensure stability. The choice of c and the number of timesteps required must be chosen to suit the image. Experiments with our data have shown that between 10 and 15 timesteps and $c = 0.5$ produce good results. This number of timesteps corresponds to around one hundredth of the diffusive timescale near to the cells. Increasing c means that only stronger edges are detected. Too few timesteps means the picture is not smoothed, but too many means that all the detail is lost. Figure 6.2(b) shows an image smoothed with this nonlinear diffusion method.

6.2.2 Edge detection

Having improved the clarity of the cell edges in our images by smoothing, we consider how to find them. Ideally an edge is a curve across which there is a clear jump in pixel intensity, but the data displays softer variations in pixel intensity which the brain can interpret as an edge. This suggests that we could define an edge where there is a big enough jump in pixel intensity between neighbouring pixels. This would be a very bad definition, because it is far too local a measure. We could find pairs of pixels with an ‘edge’ between them, but there is no guarantee that these ‘edges’ would be more than a pixel or two in length. A much better approach, as discussed in [109], is to consider edges to be large gradients in pixel intensity. It makes sense to define the edge to be where the pixel gradient is a local maximum. Since it is often easier numerically to find a root than it is to find a maximum, we work with the second derivative as shown in Figure 6.3.

In order to locate edges, defined as large gradients in pixel intensity, we can compute the discrete approximation to the Laplacian of the pixel intensity. Then the edges are the curves that separate regions where the Laplacian is positive from where the Laplacian is

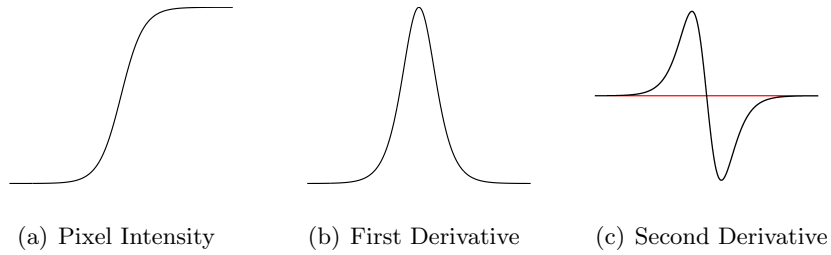


Figure 6.3: (a) a one-dimensional variation in pixel intensity, (b) the derivative of the pixel intensity, where we define the edge to correspond to the maximum, (c) the second derivative, where we define the edge to correspond to the zero. Note that the red line indicates zero.

negative. In practice these curves are interpolated onto the pixels, so that we obtain a set of edge pixels. A nice feature of this method is that all the edges are closed contours or intersect the boundary of the image. MATLAB's image processing toolbox has an edge finding function which implements a Gaussian filter, followed by a Laplacian filter (a LoG filter) and then finds the zero crossings (the curves across which the Laplacian changes sign). The problem with this function is that it finds 'edges' between slightly different regions of the background as well as the cell edges, as illustrated in Figure 6.4. The condition of finding zero crossings can be relaxed to combat this, so that the function returns an edge only if the local difference in pixel value is larger than some threshold. However, we then lose weaker parts of the cell outlines.

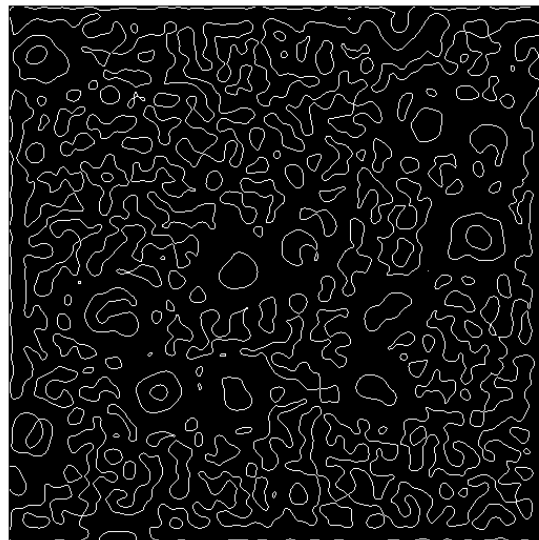


Figure 6.4: The zero crossings of a LoG filtered image. The Gaussian filter has a standard deviation of 6 pixels and all the zero crossings are shown.

These techniques can be used in conjunction with other in-built MATLAB functions as follows. First we smooth the image with nonlinear diffusion, then we apply a LoG filter to obtain the zero crossings. We are able to use a small standard deviation in the Gaussian filter and avoid excessive blurring of edges, because the image has already undergone some smoothing. Next we return to the original image and apply a threshold to obtain a binary image. We use MATLAB image processing tools to dilate the white areas in the binary image by a few pixels and then change any black areas that are enclosed with white pixels to white. We then remove from the binary image any white regions that are too small to be cells or too close to the border of the image to be properly analysed. This results in a binary mask: an image that is white near what we think are cells and black everywhere else. Potential edge pixels are identified as the previously obtained zero crossings that are also white in the mask. The mask and cell outlines generated by this process for Figure 6.4 are shown in Figure 6.5.

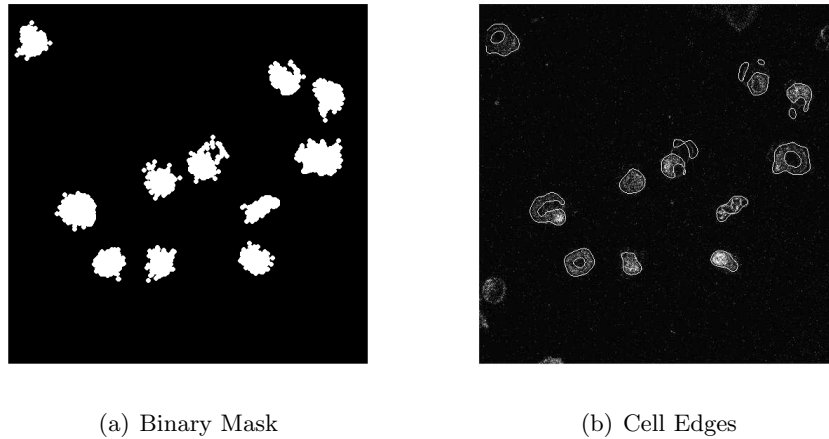


Figure 6.5: (a) the binary mask used to select edges from Figure 6.4 and (b) the edges selected.

In cells where the nucleus is clearly visible we also detect the edge of the nucleus. Again we utilise in-built MATLAB functions to fill these edges in order to remove items too small to be cells and discard the nuclei outlines to locate just the cell outlines as shown in Figure 6.6. The filled cell images are used to determine the three-dimensional connectivity to cells in other slices, enabling us to stack the outlines. There are however some problems with this approach. If a cell's nucleus is very close to the edge of the cell the outline can be very weak. In such a scenario the edge detection can fail, because we find part of the cell outline and part of the nucleus outline and obtain an horseshoe shaped 'cell'. Examples of this can

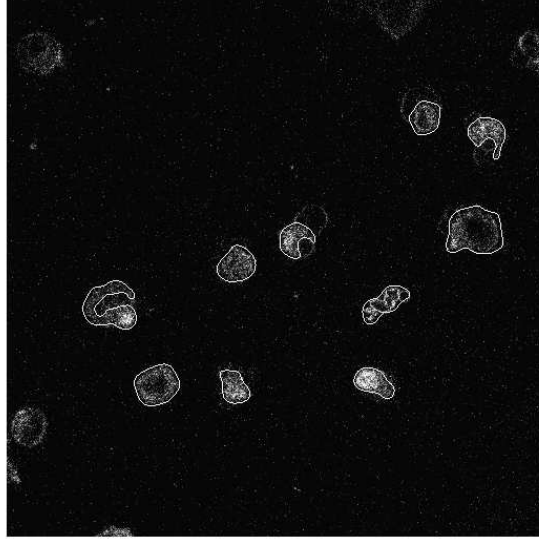


Figure 6.6: The cell outlines as detected by a LoG filter, a binary mask and post-segmentation cleaning of the image with in-built MATLAB functions.

be seen in Figure 6.6.

Initial attempts to analyse the cell data showed that the horseshoe shaped outlines were quite common, so we consider an alternative edge detection method, namely active contours. There are many variations to the active contours method, but the underlying principal is the same. We start with a (typically closed) curve, drawn over the image and evolve it according to a function which depends on the shape of the curve and the pixel intensities along the curve. We hope to pick a function that moves the curve towards the cell outlines. Following [90], for a curve $\mathbf{c}(s, t)$ depending on time t and parametrized by $s \in [0, 1]$, we define an energy

$$E[\mathbf{c}](t) = \int_0^1 E_{int}(\mathbf{c}(s, t)) + E_{image}(\mathbf{c}(s, t)) + E_{con}(\mathbf{c}(s, t)) ds. \quad (6.4)$$

Movement of the curve over the image is driven by minimising the energy $E[\mathbf{c}]$. The internal energy of the contour is given by,

$$E_{int}(\mathbf{c}(s, t)) = \frac{a_1}{2} \left| \frac{\partial \mathbf{c}(s, t)}{\partial s} \right|^2 + \frac{a_2}{2} \left| \frac{\partial^2 \mathbf{c}(s, t)}{\partial s^2} \right|^2. \quad (6.5)$$

where a_1 and a_2 are positive constants. These terms can be thought of as representing the elasticity and bending stiffness of the curve. This part of the energy prevents extreme elongation and curvature of the contour. The E_{image} term is designed to move the contour

towards edges and the E_{con} term can be used to further control how the contour moves. We use a calculus of variations argument to see that the contour that minimises the energy satisfies

$$-\frac{\partial}{\partial s}\left(a_1\frac{\partial \mathbf{c}}{\partial s}\right) + \frac{\partial^2}{\partial s^2}\left(a_2\frac{\partial^2 \mathbf{c}}{\partial s^2}\right) + \nabla E_{image}(\mathbf{c}) + \nabla E_{con}(\mathbf{c}) = 0. \quad (6.6)$$

We reformulate the problem by introducing a time derivative, so that we evolve the contour to a steady state corresponding to a local energy minimum, by solving the partial differential equation

$$\frac{\partial \mathbf{c}}{\partial t} - \frac{\partial}{\partial s}\left(a_1\frac{\partial \mathbf{c}}{\partial s}\right) + \frac{\partial^2}{\partial s^2}\left(a_2\frac{\partial^2 \mathbf{c}}{\partial s^2}\right) = -\nabla E_{image}(\mathbf{c}) - \nabla E_{con}(\mathbf{c}), \quad (6.7)$$

subject to initial conditions set by the initial contour. Now we see that the image energy acts like the potential in a steepest descent problem. Following [37] we consider

$$\frac{\partial \mathbf{c}}{\partial t} - \frac{\partial}{\partial s}\left(a_1\frac{\partial \mathbf{c}}{\partial s}\right) + \frac{\partial^2}{\partial s^2}\left(a_2\frac{\partial^2 \mathbf{c}}{\partial s^2}\right) = a_3\mathbf{n} - a_4\frac{\nabla P}{|\nabla P|}. \quad (6.8)$$

The ∇E_{con} term is $a_3\mathbf{n}$ where a_3 is a constant and $\mathbf{n}$ is the outward pointing normal to the curve. The ∇E_{image} term is the final term, where a_4 is a positive constant and $P = -|\nabla I|^2$. The normal term is called a balloon force. If for instance, we know the initial contour lies inside the region we wish to segment, a positive choice of a_3 encourages the contour to ‘inflate’ like a balloon outwards to find the required edge. This term does not depend on the pixel intensities and should be set to zero when near the minimising contour. The ∇E_{image} term moves the contour towards regions of high pixel intensity gradients. It is normalised to ensure weaker edges are not drowned out by the forces associated with the internal energy.

The key benefits of the active contours method are that it takes into account the pixel intensities, whilst allowing the topology to be constrained so that the method always returns a closed curve. This can help in finding edges that become quite weak, i.e. edges that the brain detects largely by continuation of the stronger parts of the edge. The key disadvantages of this method are the requirement of a good initial contour, which is close to the desired contour, and the need to choose suitable values for the parameters.

The active contours method implemented is as follows. We take each time point at which the cells were imaged in turn and first smooth the images with nonlinear diffusion as in the first method and save the smoothed images. Then we obtain a binary mask as detailed in the first method, which serves as a crude approximation of where the cells are. We stack these binary masks and use the MATLAB function `bwlabeln` to identify connected regions, *i.e.*

the cells. We work through the data, one cell at a time. We load a cropped, smoothed image for each slice in turn. The cropping is automated, based on the maximum and minimum pixel locations generated by the binary mask. For each image we scale the pixel intensities linearly to improve contrast to aid visualisation. Next we calculate the initialisation for the active contour method by thresholding and then dilating, filling, removing small objects and finding the perimeter of the object with in-built MATLAB functions. Generating an initialisation was the stage which was most likely to fail or provide unsatisfactory results in development. If the initialisation reaches the edge of the cell's cropped box, then it is deleted when cleaning the edge of the image. This cleaning is necessary because another cell may intrude at the corners of a cell's box. So, if the list of pixel numbers in the initial guess is empty we have a second attempt at generating an initialisation, with a slightly higher threshold value and slightly less image dilation. If this also fails to provide a non-empty initialisation, we use an ellipse, centred on the image with the same aspect ratio as the cropped region around the cell. The code prints warnings to the console each time it requires a second or third attempt at generating an initialisation. At this stage the initial outline calculated could be a horseshoe shape, so we use a MATLAB function to take a convex hull of the points in the initialisation. We order the points by increasing angle in polar co-ordinates and pass the initialisation to the active contour code.

We use an active contours code based on one provided by our collaborator Dr. J. Lee (King's College, London), which he wrote following closely the implementation described in [47]. The first thing it does is calculate a representation of the initialisation with, say 50 equally spaced points. If we discretize (6.8) with N equally spaced points on the contour at time k and label these points $\mathbf{c}_i^k$ for $i = 1 \dots N$ we find

$$\left(\frac{1}{\Delta t}I + A\right)\mathbf{c}_i^k = \frac{1}{\Delta t}\mathbf{c}_i^{k-1} + a_3\mathbf{n}_i^{k-1} - a_4\frac{\nabla P}{|\nabla P|}(\mathbf{c}_i^k), \quad (6.9)$$

where

$$A\mathbf{c}_i^k = -\frac{a_1}{h_k^2}\left(\mathbf{c}_{i+1}^k - 2\mathbf{c}_i^k + \mathbf{c}_{i-1}^k\right) + \frac{a_2}{h_k^4}\left(\mathbf{c}_{i+2}^k - 4\mathbf{c}_{i+1}^k + 6\mathbf{c}_i^k - 4\mathbf{c}_{i-1}^k + \mathbf{c}_{i-2}^k\right), \quad (6.10)$$

and h_k is the arc length distance between the $\mathbf{c}_i^k$. In this formulation the distance between points changes at each timestep, requiring the recalculation of A and the inverse of $I/\Delta t + A$. Instead [47] works with a 'normalised-length copy' of the contour, $\tilde{\mathbf{c}}_i^{k-1} = \mathbf{c}_i^{k-1}/h_{k-1}$. They calculate how much each point moves at each timestep via

$$\mathbf{d}_i^k = \left[\frac{1}{\Delta t}I + A\right]^{-1}\left[\frac{1}{\Delta t}\tilde{\mathbf{c}}_i^{k-1} + a_3\mathbf{n}_i^{k-1} - a_4\frac{\nabla P}{|\nabla P|}(\mathbf{c}_i^k)\right] - \tilde{\mathbf{c}}_i^{k-1}, \quad (6.11)$$

and update the points by setting $\mathbf{c}_i^k = \mathbf{c}_i^{k-1} + \mathbf{d}_i^k$. In this version $h_k = 1$ in A , so the inverse of the matrix need be calculated only once. At each timestep it is necessary to re-distribute the points to have equal spacing around the contour. This is done in Dr. J. Lee's implementation by cubic interpolation. We stop evolving the contour either when it has converged or when it reaches 400 iterations. A contour is said to have converged when it has moved less than some tolerance during the last twenty iterations.

The weightings given to different terms do effect the segmentations that are possible. However by experimenting with different values we have found that values of around $a_1 = 0.7, a_2 = 0.5, a_3 = 0, a_4 = 0.5, \Delta t = 1/8$ work well for our images, as illustrated in Figure 6.7. By far the parameter which has most effect on the final contour is the threshold used to generate the initialisation. This is because if the contour moves over edge-like structures it will probably get stuck there. For example if part of the initialisation starts within a dark patch inside the cell, it is unlikely to be able to move past the edge of that dark region resulting in failed segmentations as in Figure 6.7. The most robust way to avoid this was to choose a threshold such that the initial contour lies outside the cell and set $a_3 \leq 0$, to encourage the contour to shrink in towards the cell like a deflating balloon. Contours initialised too far outside the cell are also likely to fail.

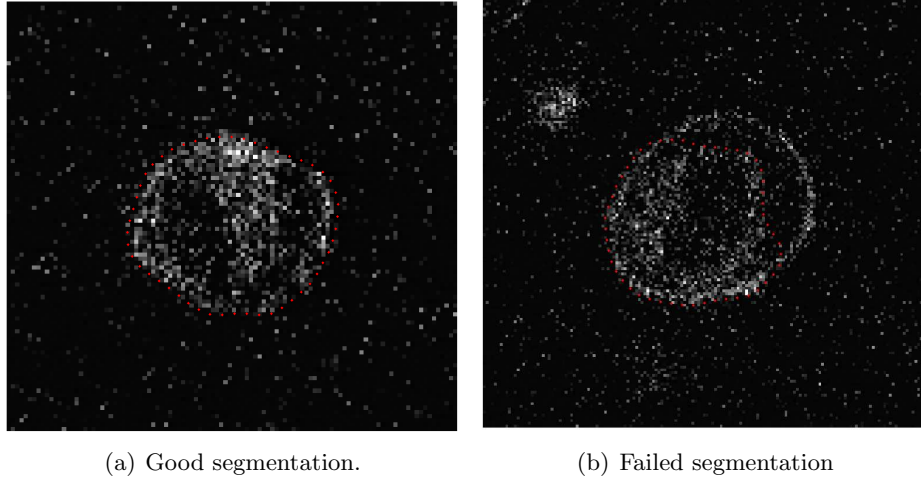


Figure 6.7: The active contours are made up of the red dots. In (a) we see the active contour code working well, with an initialisation threshold of 0.15, the contour converged in 108 iterations. In (b) we see the result of a poor initialisation threshold 0.3, where the contour gets stuck outlining a dark region within the cell, this result required 125 iterations.

To combat these issues we use two different thresholds to generate two initial contours for each image and save an image of both the resulting segmentations, along with the number

of iterations required and the co-ordinates of the points on the curves. We have written a MATLAB GUI (displayed in Figure 6.8) so that both segmentations can be compared side by side and rapidly checked by eye. If either segmentation is acceptable, the user clicks to select the preferred segmentation and the associated data points are automatically added to a file for the correct cell. This file will eventually contain co-ordinates in three dimensions for points on the surface of the cell. If neither segmentation is acceptable the user has three buttons to choose from. They can click to reject both segmentations, because the image is such that the code cannot be reasonably expected to segment the image, in which case no data is added to the co-ordinate file. Alternatively they can click to reject both

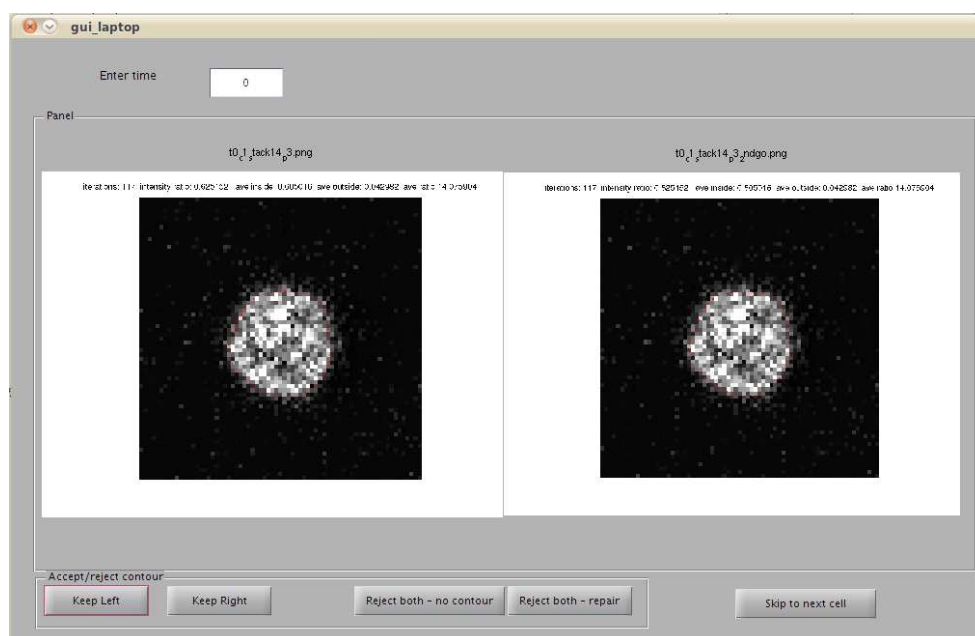


Figure 6.8: This is a screenshot of the GUI written to allow the rapid comparison and checking of cell segmentations.

segmentations, because they have failed (probably due to a poor initialisation). No data is added to the co-ordinate file, but identifying details of the image are added to a ‘repair’ list, so that the user can return to these individually and create a better initialisation, with a different choice of threshold. In both of these cases and when segmentations are accepted the GUI automatically displays the next pair of images, be they the next slice in the same cell or the first slice in the next cell. The final button is for when the current images indicate that the segmented object is not a single, adhered cell. Clicking it skips to images of the next cell and no co-ordinate file is saved for the current object. As an example of how quickly it allows a person to review the code’s segmentations, the segmentations from

images taken at one time point, over eighteen slices, with approximately twenty-five cells in the field of view were checked using the GUI in twelve minutes.

As written the code for the active contours method is best suited to segmenting cells that are convex. This is adequate for most spreading cells. At later time points when the cells extend pseudopodia, ordering the points by angle in polar co-ordinates or taking a convex hull are no longer sensible ways to generate an initial contour. The active contours method is still valuable in these cases, though the initialisation requires more care. Figure 6.9 shows the sort of segmentations that can be achieved by reducing the value of the elasticity and bending stiffness constants and not taking a convex hull of the cell outline when generating the initialisation.

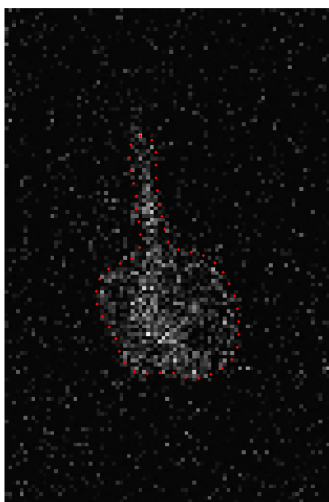


Figure 6.9: An active contour segmentation, shown in red, of a cell with pseudopodia, which converged after 105 iterations. The elasticity and stiffness parameters of the active contour have been reduced to $a_1 = a_2 = 0.2$.

6.2.3 Alternative approaches

There are of course many other possible methods for image segmentation. The level sets method can be thought of as a generalisation of the active contours method. Instead of moving a curve, we move some surface defined over the whole image, given by say $C(x, y, t)$. The segmentation is then defined by a contour on this curve, typically the set of (x, y) such that $C(x, y, t) = 0$ at some end time point. A celebrated feature of this method is its ability to evolve not only the location, but also the topology of a segmentation. For instance it may be possible to segment all the cells on one slide in one go, without knowing *a priori*

how many cells there are. However, the control our active contours method gives us over the topology of our segmentation is one of its strengths.

We used a piece of software called CREASEG [48] that allows the user to segment an image with six different level set algorithms, namely the methods by Bernard [14], Caselles [31], Chan–Vese [32], Lankton [99], Li [104] and Shi [140]. We also trialled Lankton’s implementation of the Chan–Vese method [98]. None of these was found to improve on the segmentation obtained with our active contours method. Finally we note three–dimensional segmentations are also possible. The code obtained from [98] is able to segment three–dimensional structures from a stack of images. Again this was not found to improve the segmentation for our cell images. Some of the slices that were not successfully segmented were found to disrupt the segmentation in the neighbouring slices.

6.3 Image analysis for beads

In an attempt to quantify the errors associated with imaging the cells and the image processing procedure discussed above, our experimental collaborators imaged $5\mu\text{m}$ diameter, fluorescent, polystyrene beads. We processed these images as described above. The data points are stacked with the correct spacing between each slice and we calculate the best fit sphere. (A detailed description of the best fit optimization is given below for the cell data.) Figure 6.10 shows data points from a typical bead and the best fitting sphere which has radius $2.53\mu\text{m}$ to three significant figures. The relative error in radius size is 1.3% to two significant figures. However, the image displays a distortion, the points appear stretched in the vertical direction and to a lesser extent squashed in the horizontal directions. There are thirteen slices in the stack, with a $0.5\mu\text{m}$ separation, so the confocal images display cross–sections of the $5\mu\text{m}$ diameter bead over at least a $6\mu\text{m}$ range. This indicates that a considerable amount of distortion occurs during the image capturing, masking errors introduced by the image processing. The distortion is a function of the optical properties of the microscope set–up and can be approximated according to [15].

The bead images highlight another complication in obtaining the cell data. In the central slices the bead images resemble an annulus, since only the surface of the bead fluoresces. The beads are said to be edge stained. Classically objects viewed under microscopes are considered edge or volume stained, the latter meaning that the whole object fluoresces. The distinction has implications for the image segmentation. The edge of an edge–stained object

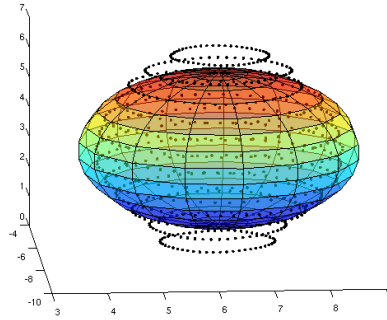


Figure 6.10: Data points obtained from image processing confocal microscope images of $5\mu\text{m}$ diameter beads and the best fitting sphere, coloured for visualization. Axes are in μm .

is located where the intensity is maximal, whereas the edge of a volume-stained object is located where the intensity gradient is maximal. Our image processing procedure treats the cells as volume-stained objects, since we use a membrane stain which stains both the cell membrane and lipophilic organelles within the cell. The unknown staining distribution within the cell makes it less than straightforward to use the bead distortion as a calibration for the distortion we must now assume is present in our cell images. Time constraints mean we shall not resolve these issues associated with the capturing of confocal cell images here. However, we proceed to demonstrate how the data generated from our image processing routine could be used to analyse cell shape and inform models of cell spreading.

6.4 Models for cell spreading

The comparisons between cell data and models carried out in this section are preliminary and presented as a ‘proof of concept’. The limitations of the confocal data currently available to us prevent us from drawing conclusions about the validity of competing models. We first briefly review a number of models found in the literature, starting with the simplest possible assumption that a cell surface has constant mean curvature in §6.4.1 and continuing to discuss dynamic models in §6.4.2 and §6.4.3.

6.4.1 Constant mean curvature

We shall consider droplets that are sufficiently small for the effects of gravity to be neglected, the typical lengthscale of the droplet will be assumed to be smaller than the capillary lengthscale, $\sqrt{\gamma/\rho g}$. At the interface between two static fluids a force balance yields the

boundary conditions

$$p^- - p^+ = \gamma\kappa, \quad \nabla_s \gamma = \mathbf{0}, \quad (6.12a,b)$$

where p^+ and p^- are the hydrostatic pressures in the fluid each side of the interface, γ is the surface tension, κ is the mean curvature (positive if the centre of curvature is on the $-$ side of the interface) and ∇_s is the surface gradient operator [33]. Now considering the specific case of modelling a cell as a droplet, we can set the pressure outside the droplet $p^+ = 0$, without loss of generality and note that the pressure inside the droplet is constant. This implies that the mean curvature is constant.

A calculus of variations argument demonstrates that for a partially wetting droplet with a prescribed volume and contact set the configuration that minimises the surface area of the droplet is a surface with constant mean curvature [42]. For a circular contact set, the surface that minimises surface area for a given enclosed volume is a spherical cap. Whilst it may be appropriate to assume that if the cells spread sufficiently slowly their surfaces will have almost constant mean curvature, the model provides no information about the dynamics of cell spreading.

6.4.2 Surface tension dominated droplet spreading

In this section we consider modelling a spreading cell as a viscous droplet spreading on a surface. Frisch and Thoumine's model for cell spreading, [61], models a fibroblast as a viscous droplet with viscosity μ , macroscopic equilibrium contact angle α_e and surface tension γ_c . The cell's ability to adhere to the substrate is modelled by an adhesion energy w_a . The model assumes the droplet spreads as a spherical cap until the contact angle achieves its equilibrium value, which is given by

$$w_a = \gamma_c(1 + \cos \alpha_e), \quad (6.13)$$

according to Young's equation [61]. They use a contact line law from [21], which models the flow near the contact line, in a droplet with small contact angle, as a wedge flow. They assume the friction between the droplet and the substrate is due to shear flow and neglect any contributions from the tip of the wedge. However, the treatment of the singularity arising at the tip is *ad hoc*. We will consider instead the more sophisticated model of Hocking and Rivers of a spreading viscous droplet, which allows us to deal systematically with large contact angles [78]. Frisch and Thoumine compare their model to cell data, but

they only measure the contact radius and this is done from above the cell, so at early times the true contact radius maybe obscured by the cell bulk.

The Hocking and Rivers model relies on the following assumptions: the Reynolds number is small¹ so that Stokes flow equations are appropriate; the lengthscales are small enough that surface tension is dominant and gravity can be neglected²; any effect of the surrounding fluid can be safely ignored and the flow is axisymmetric. The no-slip boundary condition at a substrate leads to singularities in the stress at the contact line [83]. Hocking and Rivers regularize this by imposing instead a Navier-slip condition, which allows the droplet to spread. They consider the case where Υ^* , the typical slip length, is small compared to R^* , the radius of a sphere with the same volume as the droplet. Thus, $\Upsilon^*/R^* = \Upsilon \ll 1$ is a small parameter.

Hocking and Rivers also assume a constant microscopic equilibrium contact angle α_s . This means that when we look on the scale of the behaviour at the contact line, *i.e.* the slip length, the free surface of the droplet makes an angle α_s with the substrate, as shown in Figure 6.11. The angle α_s is a property of the fluid and the substrate. The macroscopic contact angle $\alpha(t)$ is the contact angle we see when we look on the lengthscale of the cell. This angle is typically the one observed experimentally and has an initial value π when the droplet first makes contact with the substrate and the contact radius is zero. The droplet spreads towards a steady configuration with $\alpha(t) \rightarrow \alpha_s$ as $t \rightarrow \infty$.

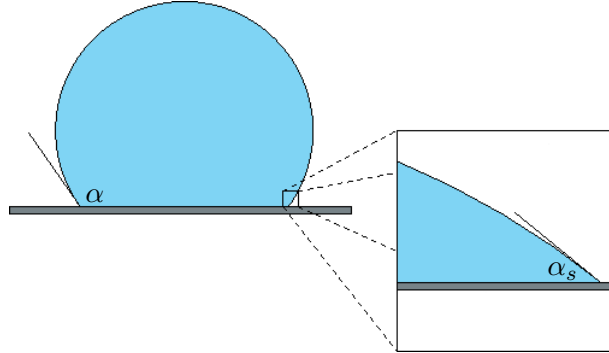


Figure 6.11: A diagram identifying the macroscopic (α) and microscopic (α_s) contact angles.

Hocking and Rivers calculate the leading-order approximation to the contact angle and

¹Taking values from [61], appropriate scales for fibroblasts are: cell radius $R^* = 8 \times 10^{-6}$ m, cell density $\rho_c = 1.06 \times 10^3 \text{ kg m}^{-3}$, cell viscosity $\mu = 10^4 \text{ kg m}^{-1} \text{ s}^{-1}$ and spreading speed $U = 10^{-9} \text{ m s}^{-1}$, so that $Re = \rho_c R^* U / \mu < 10^{-15}$.

²Again taking values from [61], the cortical tension of a fibroblast is approximately $\gamma = 2 \times 10^{-4} \text{ kg s}^{-2}$ assuming the medium the cell spreads in has approximately the same density as water $\rho_m = 10^3 \text{ kg m}^{-3}$, the capillary length $l_c = \sqrt{\gamma / (\rho_c - \rho_m) g} > 5 \times 10^{-4} \text{ m}$.

contact radius for a droplet that spreads on the timescale $T^* = \mu R^* / \delta \gamma$, where μ is the fluid viscosity, γ is the constant surface tension and $\delta = 1 / \ln(1/\Upsilon)$ is a small parameter, that arises from the matching described below. They formulate the problem as an axisymmetric Stokes flow in cylindrical polar co-ordinates. The droplet has the previously discussed Navier-slip and no-flux boundary conditions on the substrate. At the free surface they impose kinematic and dynamic boundary conditions. For the latter, pressure in the surrounding medium is set to zero without loss of generality. The surface tension is constant so there is no tangential stress on the boundary. The condition which drives the droplet spreading is that the microscopic contact angle is α_s . Initial conditions are also required to close the problem, when the droplet first touches down onto the substrate the contact angle $\alpha = \pi$ and the contact radius $a = 0$. Hocking and Rivers found the solution of this problem requires three asymptotic regions: an outer region corresponding to the macroscopic contact angle; an inner region which allows us to impose the microscopic contact angle; and an intermediate region to match between the two solutions. The asymptotic regions are shown in Figure 6.12.

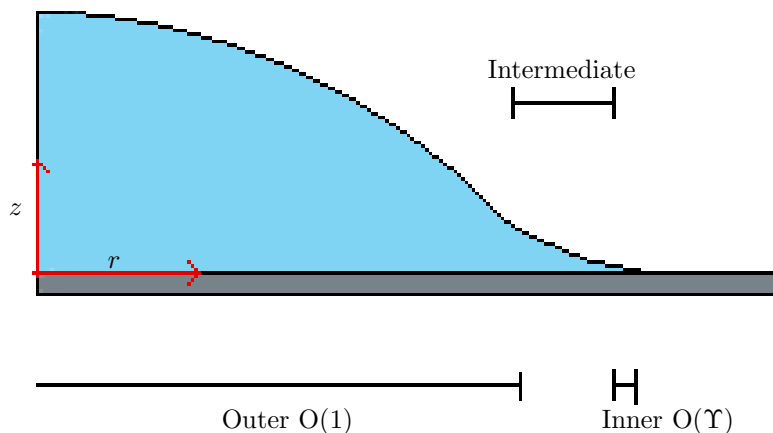


Figure 6.12: A diagram identifying the three asymptotic regions used by Hocking and Rivers, where the origin for r and z is where the drop's axis of symmetry meets the surface. In the outer region r and z are of $O(1)$. In the inner region $a - r$ and z are of $O(\Upsilon)$.

Hocking and Rivers first address the outer problem, away from the contact line. The choice of timescale means the droplet spreads slowly enough that to leading-order the pressure in the droplet is spatially uniform and so the curvature of the droplet is constant. Therefore, as discussed in §6.4.1, the leading-order outer solution for the shape of the droplet is a spherical cap. The angle that this spherical cap makes with the substrate, $\alpha_0(t)$, is the leading-order approximation to the macroscopic contact angle $\alpha(t)$. Since the

bulk of the droplet is in this outer region, to leading-order we can relate $\alpha_0(t)$ and $a_0(t)$, the leading-order approximation to the contact radius, by noting that the volume is conserved. The relation is

$$\frac{\pi a_0^3}{3V} = \frac{(1 + \cos \alpha_0)^2}{\sin \alpha_0 (2 + \cos \alpha_0)}, \quad (6.14)$$

where V is the constant volume of the droplet. (Their choice of R^* as the lengthscale to nondimensionalize against ensures that $V = 4\pi/3$.) Hocking and Rivers also calculate the flow inside the droplet and how it modifies the shape of the free surface at next order, we omit this from our discussion for brevity.

Hocking and Rivers rescale their variables to consider the inner region, which is within an $O(\Upsilon)$ distance from the contact line. They again find that the pressure and the curvature of the free surface are both constant to leading-order, so the free surface must either be flat or part of a circle. It must meet the substrate with angle α_s and it must extend to match to the outer solution. Therefore, the leading-order approximation to the surface is flat and the leading-order flow, near the contact line, is confined to a wedge. Expressing the free surface in terms of a local slope, they find it is not possible to match the inner and outer solutions unless $\alpha(t) = \alpha_s$ for all t , which necessitates a third, intermediate region.

Hocking and Rivers introduce an intermediate variable and solve the leading order Stokes equations between the free boundary and the substrate, matching to both the inner and outer solutions. The resulting problem is an ordinary differential equation for the contact radius:

$$\frac{da_0}{dt} = \frac{G(\alpha_0) - G(\alpha_s)}{2}, \quad (6.15)$$

where

$$G(\beta) = \int_0^\beta \frac{\tilde{\beta} - \sin \tilde{\beta} \cos \tilde{\beta}}{\sin \tilde{\beta}} d\tilde{\beta}, \quad (6.16)$$

This depends on the contact angle α_0 , but (6.14) provides a relation between a_0 and α_0 . Differentiating (6.14) implies

$$\frac{da_0}{dt} = -\frac{a_0}{\sin \alpha_0 (2 + \cos \alpha_0)} \frac{d\alpha_0}{dt}, \quad (6.17)$$

so we obtain from (6.14) an ordinary differential equation for $\alpha_0(t)$, *viz.*

$$\frac{d\alpha_0}{dt} = \frac{F(\alpha_0)(G(\alpha_0) - G(\alpha_s))}{2}, \quad (6.18)$$

where $F(\alpha_0) = -\sin \alpha_0 (2 + \cos \alpha_0) / a_0(\alpha_0)$.

To help understand what this model predicts for a spreading droplet, we calculate an example numerical solution in §6.4.2.1. We also consider the scaling law it predicts for the speed at which a droplet spreads at late times in the fully wetting case $\alpha_s = 0$. Expanding (6.18) for late times, *i.e.* $\alpha_0 \ll 1$ we find that

$$\frac{d\alpha_0}{dt} \propto \alpha_0^{13/3}. \quad (6.19)$$

Solving this we obtain the late-time behaviour for α_0 . Written in terms of the contact radius, via (6.14), this implies that

$$a_0 \sim t^{1/10}, \quad (6.20)$$

for late times, when the droplet is well spread. Note that this is within the range of scalings reported by [59] for the late-time spreading behaviour of cells.

6.4.2.1 Numerical solution

In order to solve numerically (6.18) we must calculate the integral

$$G(\alpha_0) - G(\alpha_s) = \int_{\alpha_s}^{\alpha_0} \frac{\tilde{\beta}}{\sin \tilde{\beta}} - \cos \tilde{\beta} d\tilde{\beta}, \quad (6.21)$$

which has a singularity at $\tilde{\beta} = \pi$. If we wish to consider the evolution of the drop from when it touches down onto the substrate (initial conditions $\alpha_0(0) = \pi$) we must instead take $\alpha_0 \sim \pi - \epsilon$, for $\epsilon \ll 1$, at some small time t . We now calculate the small-time asymptotic behaviour to find how t and ϵ are related. Expanding (6.14) shows that to first-order $a_0 \sim \epsilon$ when $\alpha_0 \sim \pi - \epsilon$. Careful treatment of (6.21) shows that $G(\pi - \epsilon) - G(\alpha_s) \sim -\pi \ln \epsilon$ as $\epsilon \rightarrow 0$. For small times (6.15) yields

$$\frac{d\epsilon}{dt} = \frac{-\pi \ln \epsilon}{2}, \quad (6.22)$$

so we write $t = 2I(-\ln(\epsilon))/\pi$, where I is the exponential integral

$$I(z) = \int_z^\infty \frac{e^{-s}}{s} ds, \quad (6.23)$$

which we could evaluate numerically with the trapezium rule. Alternatively, using an asymptotic approximation from [1] for (6.23), we see that for small ϵ and small t , $t \sim -2\epsilon/(\pi \log \epsilon)$. An explicit Euler method is used to solve (6.18) numerically, subject to our asymptotic initial conditions. The integral $G(\alpha_0) - G(\alpha_s)$ is calculated once initially: at all subsequent time points we compute the integral $G(\alpha_0) - G(\alpha_s)$ by subtracting off the

contribution to the integral between the current value of α_0 and α_0 at the previous timestep. All the integrals are evaluated with the trapezium rule. The numerical solution generated is in agreement with a code written independently by Dr. J. Oliver for the same problem, which uses MATLAB's in-built routines for quadrature and solving initial value problems. Figure 6.13 displays an example solution for a droplet spreading, with equilibrium contact angle $\alpha_s = \pi/8$.

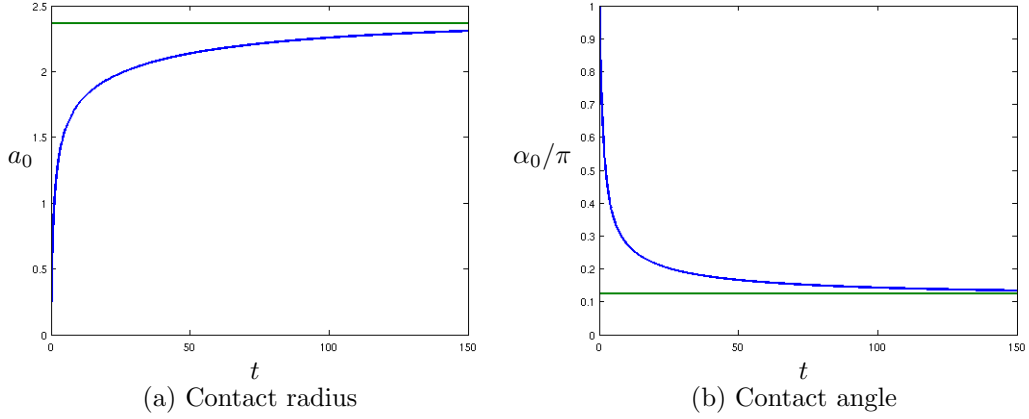


Figure 6.13: Numerical solutions to the leading-order Hocking and Rivers problem with $\alpha_s = \pi/8$. The contact radius is plotted in (a) where t and a_0 are nondimensional and (b) shows the contact angle in radians as a function of nondimensional time t . The green lines show the static equilibrium values that are approached as $t \rightarrow \infty$.

6.4.3 Membrane bound droplet spreading

Another type of fluid based model proposed and compared to cell data in the literature is that of Cuvelier *et al.* [39]. As in the Hocking and Rivers model the authors assume cell spreading is axisymmetric, but now the viscous droplet is bound by a more viscous shell, as illustrated in Figure 6.14. The cell's actin cortex is represented as a layer of Newtonian fluid, rather than a membrane with a prescribed surface tension. The model is based on balancing the adhesion energy gained as bonds form between the cell and the substrate, with the energy dissipated by the deforming cell. We introduce some notation: a the contact radius; d the thickness of the cortical shell, which is assumed to be constant; and $\mathbf{u}$ the velocity of the fluid in the cortical shell, so that the viscous dissipation is given by

$$\int_V \mu_c \left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right)^2 dV, \quad (6.24)$$

The image originally presented here cannot be made freely available via ORA because of copyright. The image can be found in Figure 3(A) of [39].

Figure 6.14: A diagram of a cell as modelled by Cuvelier *et al.*, based on the diagrams in [39].

where μ_c is the cortex viscosity and we integrate over the volume V where deformation is happening. The rate of energy gained by adhesion is proportional to

$$Ja \frac{da}{dt}, \quad (6.25)$$

where J is the adhesion energy per unit area. The authors assume cell spreading occurs in two stages. Initially when the contact radius is comparable to the length of filaments in the actin cortex, they hypothesise that most of the deformation is restricted to within the actin cortex, near the contact region. The actin cortex in contact with the substrate is fixed ($\mathbf{u} = 0$) and the interior of the actin cortex flows with speeds comparable to the velocity of the contact line. Approximating the flow velocity gradient as the difference in velocity divided by the size of the region, the viscous dissipation is argued to be proportional to

$$\mu_c \left(\frac{1}{d} \frac{da}{dt} \right)^2 a^2 d. \quad (6.26)$$

Setting the expressions for energy gain and viscous dissipation proportional to each other implies a scaling for the contact radius

$$a \sim t^{1/2}. \quad (6.27)$$

At later times when the contact radius exceeds the length of cortical filaments and the whole cell deforms and flattens, we know the flow is still zero on the substrate, so the expression for viscous dissipation is proportional to

$$\mu_i \left(\frac{1}{h} \frac{da}{dt} \right)^2 a^2 h, \quad (6.28)$$

where μ_i is the cell viscosity inside the cell and h is the height of the cell. To relate h to a , [39] introduces R_0 , the initial radius of the spherical cell, before it starts settling, and note that the constant R_0^3 is proportional to $a^2 h$. Equating the energy gain with the viscous dissipation then implies that

$$a \sim t^{1/4}. \quad (6.29)$$

6.5 Comparisons to cell data

In order to qualitatively assess how well the model of constant mean curvature captures the shape of spreading cells we can compare the cell data points obtained with the image processing regime to mean curvature surfaces. We use the the base contour from the image processing as the contact set and calculate mean curvature surfaces for different estimates of the cell volume, with a piece of software called Surface Evolver available from [19] and described in [18]. Fluid surfaces are discretized as a triangular mesh, which the user can refine and the software can evolve the mesh to minimise the surface area. Figure 6.15 displays an example Surface Evolver mesh and the corresponding cell data points. A considerable drawback to this approach is that the optimisation problem for guessing the correct cell volume would be computationally quite expensive.

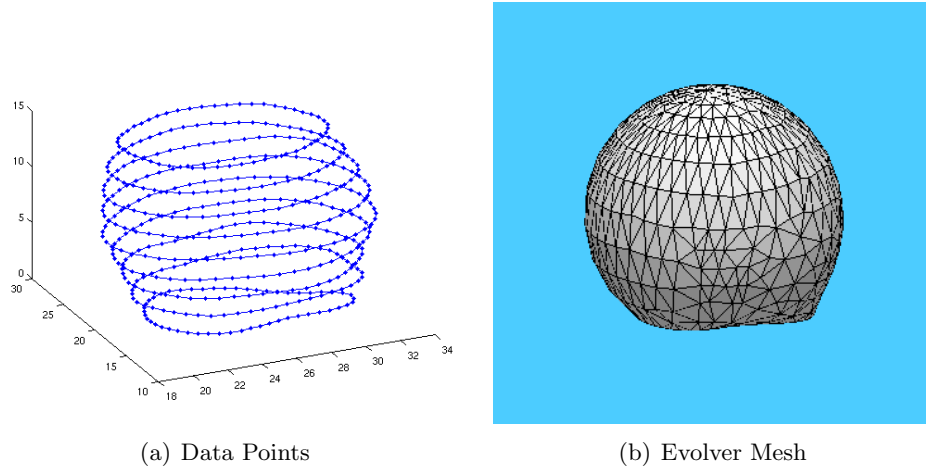


Figure 6.15: (a) cell data points obtained from confocal microscope images by image processing, dimensions are in μm . (b) shows the surface generated by Surface Evolver for the contact set in (a) and a guessed volume.

Now we demonstrate how cell data obtained from the confocal microscope images can be compared to the Hocking and Rivers model for cell spreading. The outer solution is a spherical cap, so we fit a spherical cap through the cell data and consider the evolution of

the contact radius and contact angle of the spherical cap. Initially we use the nonlinear least squares solver in MATLAB to find the co-ordinates (X, Y, Z) of the sphere centre and the sphere radius R , that define the sphere that best fits the data points in a single stack. In other words we find the parameters that minimise the residual vector whose i th component is the distance of the i th data point from the spherical cap

$$\sqrt{(x_i - X)^2 + (y_i - Y)^2 + (z_i - Z)^2} - R, \quad (6.30)$$

where (x_i, y_i, z_i) are the co-ordinates of the i th data point from the image processing of a given cell at a given time point. The MATLAB solver requires a reasonable initial guess for (X, Y, Z) and R , which can be easily constructed as the centre of mass of the data points and their average distance from this point, respectively. An example fit can be seen in Figure 6.16.

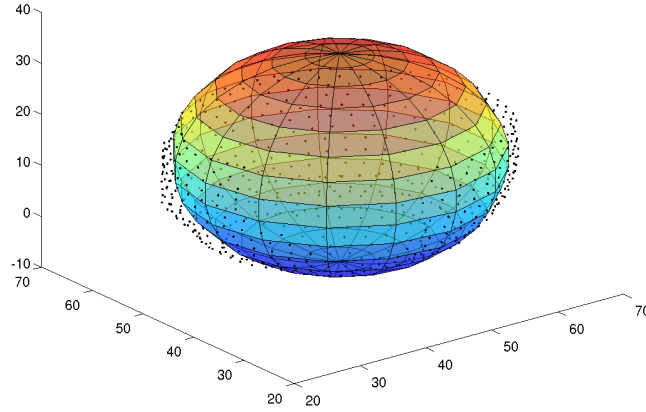


Figure 6.16: A cloud of data points obtained from confocal microscope images by image processing and the least squares sphere that best fits them, coloured to aid visualisation. Dimensions are in μm .

The cap's contact radius, a^* , contact angle, α^* , height, h and volume, V^* , can be calculated from R , the radius of the cap, and Z , the height of cap's centre above the plane. For completeness we include the formulae here:

$$a^* = \sqrt{2Rh - h^2}, \quad \alpha^* = \frac{\pi}{2} + \tan^{-1} \left(\frac{h - R}{a^*} \right), \quad (6.31a,b)$$

$$h = Z + R, \quad V^* = \frac{\pi a^{*3} (1 - \cos \alpha^*) (2 + \cos \alpha^*)}{3 \sin \alpha^* (1 + \cos \alpha^*)}. \quad (6.32a,b)$$

We calculate these properties of the cap at each time point for a given cell.

Plots displaying these values for one cell can be seen in Figure 6.17. One advantage of this fitting method is the evaluation of the model it provides through the least squares residuals. We obtain a vector containing the distance of each point from the best fitting spherical cap. The square of the Euclidean norm of this vector is the sum over the data points of the squared distances. Dividing this sum by the number of data points and taking the square root, then dividing by R gives a root mean squared measure of the relative error in the fit with each data point having equal weight. We calculate a second, ‘worse case scenario’ measure of the relative error as the largest absolute value in our residual vector divided by R , so this depends only on the data point furthest from the fitted sphere. Whilst we shall mainly want to think about the first error it seems prudent to keep an eye on how far away from the spherical cap outlying points can be found.

It would now be possible to estimate parameters in the Hocking and Rivers model, using say the average volume of the fitted spheres, and solve the first-order problem numerically to compare the data points for the contact angle and radius to the model. However, the considerable variation in the volume of the fitted spherical cap, illustrated in Figure 6.17 is a cause for concern. Cell volume is thought to be approximately constant during cell spreading. Data indicating that fibroblasts spread with constant volume is presented in [144]. The ratio of largest fitted volume to smallest fitted volume is approximately 3.5, which corresponds to a ratio of approximately 1.5 in the radii of the fitted spherical caps. Since the radius is a fitting parameter, it is probably a more appropriate measure of the variation. This variation in fitted volumes prompts an alternative approach. Taking the data for a given cell over all the time intervals we fit for the centre of the cap at time j , (X^j, Y^j, Z^j) and a constant cell volume V . The i th data point, at time j contributes the term

$$\sqrt{(x_i^j - X^j)^2 + (y_i^j - Y^j)^2 + (z_i^j - Z^j)^2} - R^j \quad (6.33)$$

to the residual vector, where R^j is related to the volume V and Z^j , by

$$2R^{j3} + 3R^{j2}Z^j - Z^{j3} = \frac{3V}{\pi}. \quad (6.34)$$

There are three roots to this equation, but identifying the turning points at $R^j = 0$ and $R^j = -Z^j$ indicates there is at most one root R^{j*} such that $R^{j*} > |Z^j|$. This is the root we select to ensure the fitted sphere intersects the plane. Equation (6.34) is solved (using the MATLAB function `fzero`) every time the residual is calculated.

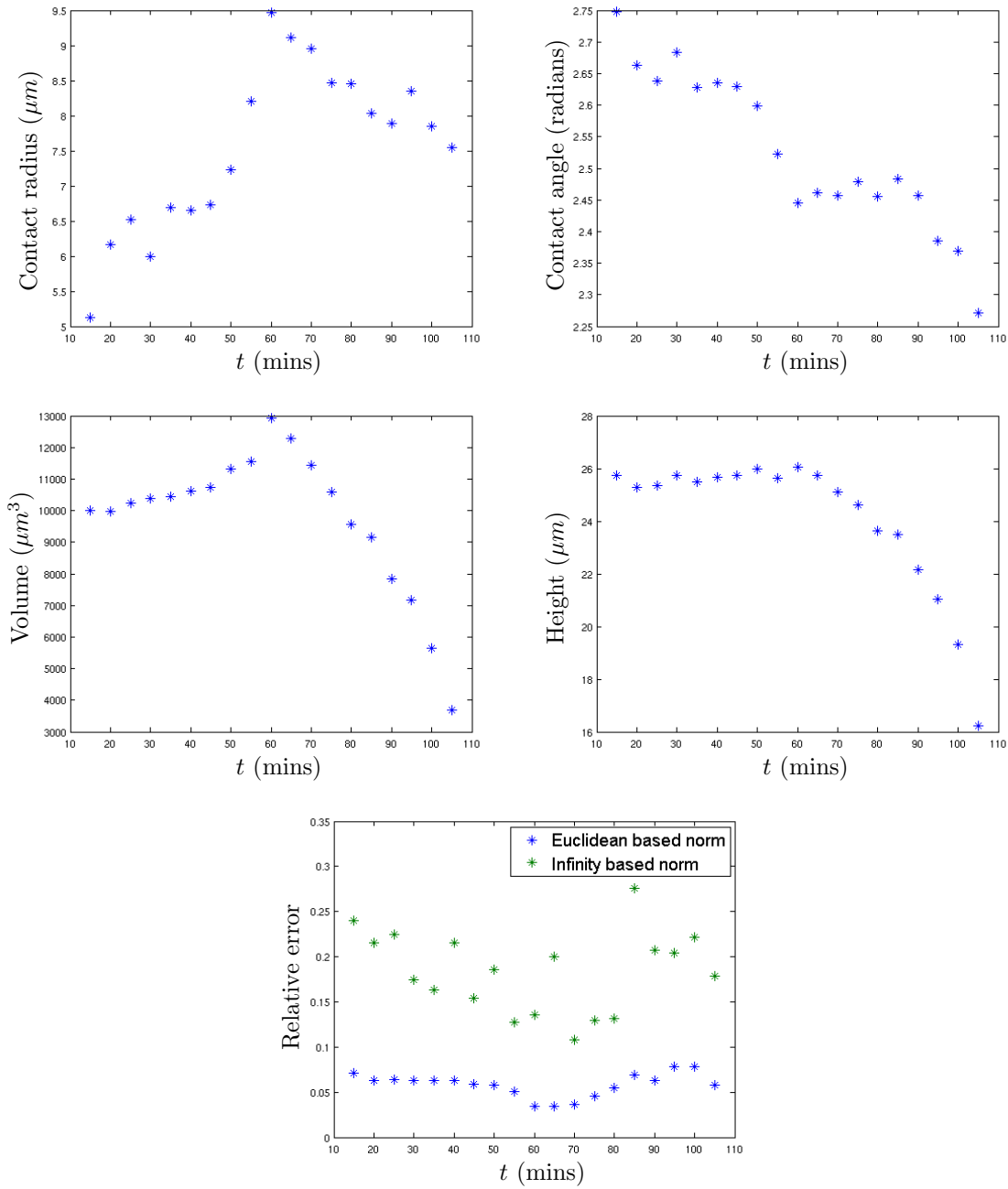


Figure 6.17: A plot of various cell attributes changing over time. See text for an explanation of the fitting algorithm and the errors calculated.

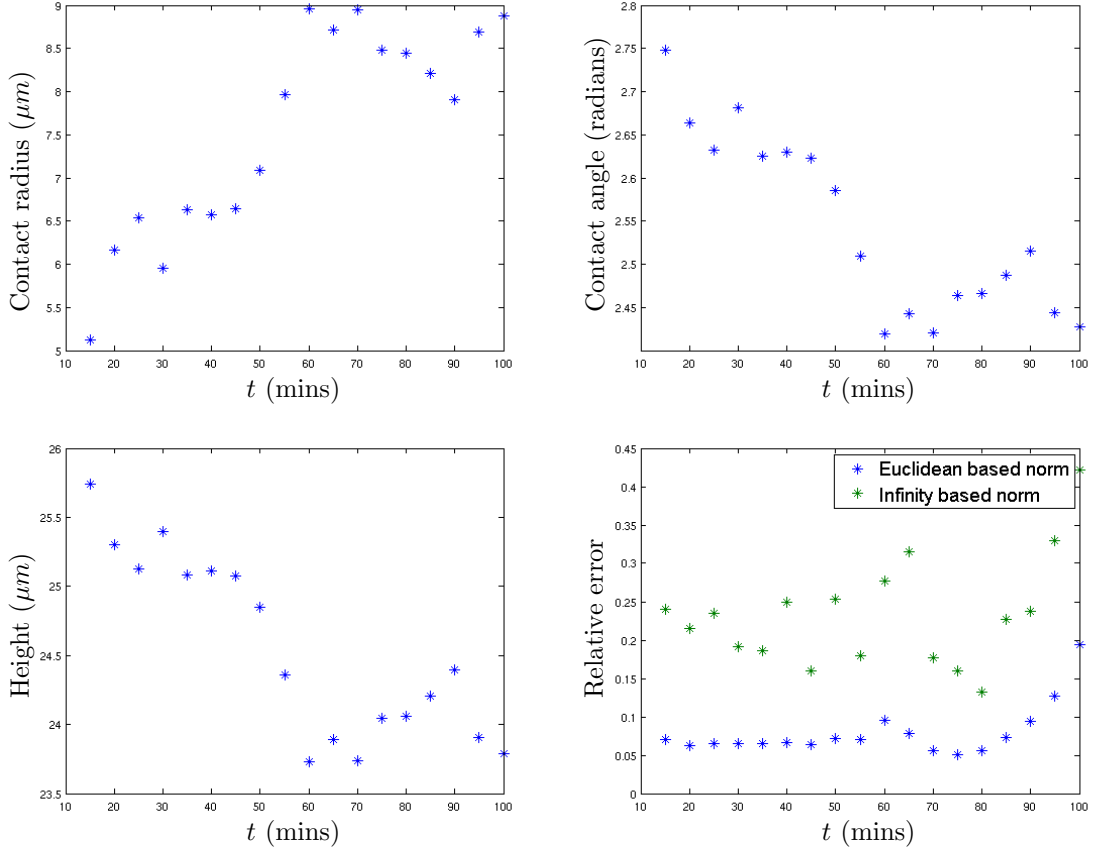


Figure 6.18: A plot of various cell attributes changing over time. Fits calculated for the data as shown in Figure 6.17, with one fewer time point and the same error measures. See text for details of the fitting algorithm.

In Figure 6.18, we present plots of the cell attributes calculated from spherical caps fitted with constant volume. We use the same image processing data as in Figure 6.17. The Euclidean based error norm provides us with another way to compare the cell data to the hydrodynamical model. We hypothesise that if there is a sharp increase in the Euclidean based fitting error, then this could indicate the onset of polarisation and identify a timescale for the breakdown of the purely hydrodynamical model. We notice in Figure 6.18 that, while the Euclidean based error is initially comparable to that in Figure 6.17, it grows rapidly at later times. In fact the fit on the last point is so poor (it generates a sphere that does not intersect the plane) the contact variables are not defined, so the point is omitted. Figure 6.19 shows the error in fits of the same data over different intervals. Notice how the accuracy of the fit deteriorates after inclusion of data from the final time points.

The data presented here is only for one cell. However, we have seen similar variations

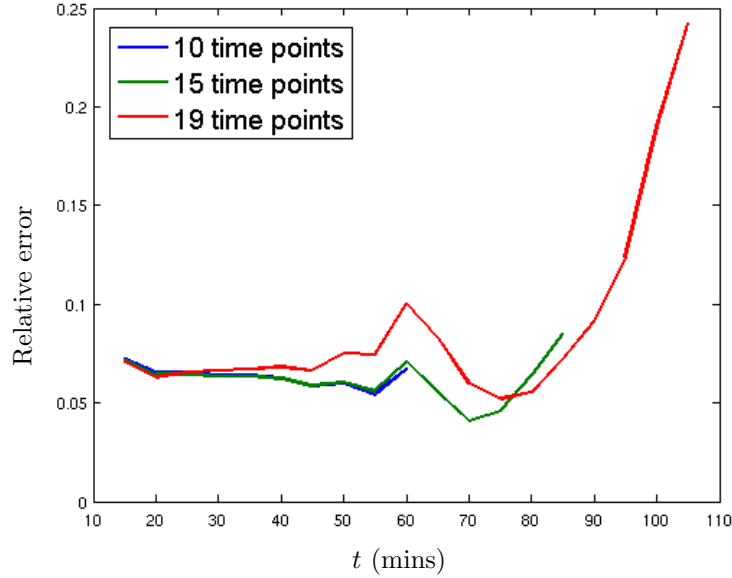


Figure 6.19: A plot showing how the Euclidean based error changes as we fit over more time points.

in volume in the analysis of several other cells. It seems unlikely that cell volumes actually change this much during cell spreading [144]. It remains to be understood whether the variation can be attributed to the vertical distortion of the images. Another possibility is if the base image from the microscopy does not actually capture the bottom of the cell. The fitting of a spherical cap may be converging to a local minima in some cases as opposed to finding the true best fit. This is a potential source of error which we have tried to combat by providing the least squares solver with a good initial guess and inspecting the fits visually. It remains to investigate whether it may be more appropriate to exclude data points in the base stack from the fit, so that we are fitting for just the outer solution.

The membrane bound droplet model does not assume cells spread as spherical caps, only that the contact set is circular. However, whilst the residual errors for the fitted caps are small, we can justify using the contact radius of the spherical cap as a good measure of the cell's contact radius. It is then very simple to plot the evolution of the contact radius on a logarithmic scale and compare the speed of cell spreading to the $a \sim t^{1/2}$ or $a \sim t^{1/4}$ behaviours predicted by the membrane bound droplet model and the $a \sim t^{1/10}$ scaling predicted at late times by the Hocking and Rivers model for $\alpha_s = 0$. Figure 6.20 plots the contact radius for one cell, using the first ten time points from the constant volume fitted spherical caps for the same data as in Figure 6.17 and Figure 6.18. The data is probably

not measured at sufficiently late times in the cell spreading process for the Hocking and Rivers scaling to be applicable, but our purpose here is to demonstrate how data obtained at later stages of cell spreading could be compared to the model predictions. Depending on when and if the hydrodynamical model breaks down a spreading cell may initially be governed by the Hocking and Rivers model, but never reach the $a \sim t^{1/10}$ spreading regime.

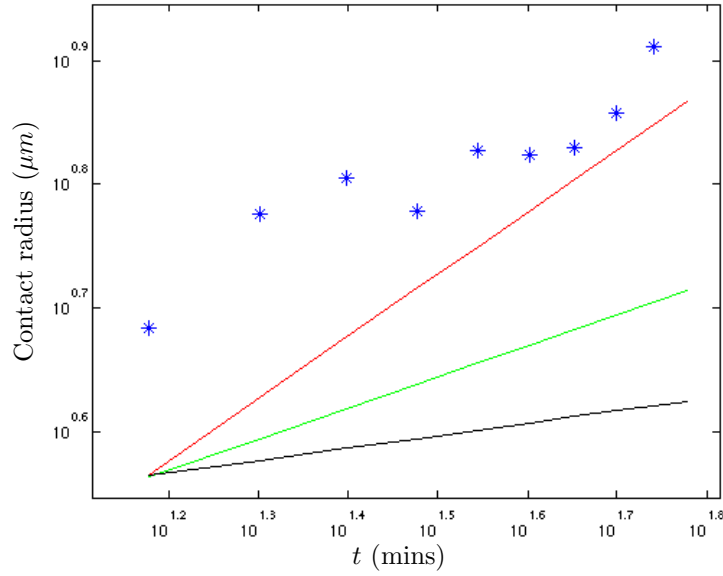


Figure 6.20: A loglog plot of the cell's contact radius varying with time. The red, green and black lines are proportional to $t^{1/2}$, $t^{1/4}$ and $t^{1/10}$ respectively. They are included for comparison.

6.6 Chapter summary

In this chapter we have presented two methods for extracting cell outlines from confocal images. The first uses anisotropic diffusion to smooth the image and a LoG filter to find edges. It is quite quick, but struggles to extract some of the weaker edges. The second, the active contours method, can segment even quite weak cell outlines in a fairly noisy background. Generating sufficiently good initialisations for the code, especially when the cell outlines are far from circular is the most difficult part of the procedure to automate. We have combated this by generating multiple initialisations and writing a GUI to allow the user to rapidly compare the results of different initialisations. Analysing confocal images of beads highlighted a considerable source of potential error in obtaining the confocal images of cells, which is yet to be resolved. It is therefore not possible to draw conclusions about

how cells spread from the data that we have at present. It is also not sensible to draw conclusions from the data of just one cell (although we have observed the trends displayed here in a few other cells). In order to use the cell data as evidence for or against models of cell spreading available in the literature or to develop new models we would require data for more cells, so that the behaviour could be analysed statistically.

The rest of this chapter serves as a demonstration that we have developed the tools to analyse this data when it becomes available and the uses we have for this data in comparing it to various models for cell spreading. We note that the development of the image processing routine is valuable in itself. It is already being used by another researcher within my group to study cell blebbing.

Chapter 7

Discussion

7.1 Key results

The overriding conclusion from our investigation into two-phase flow models for cell motility, is that even in their simplest, stripped-down forms, they exhibit a rich array of behaviours applicable to modelling cell crawling. Next is the observation that the highly nonlinear, coupled partial differential equations which make up the two-phase flow framework are mathematically challenging, but progress in understanding them can be made through linear stability analyses, carefully chosen parameter sub-limits and numerical studies.

Stability analysis of the minimal, two-phase, poroviscous, reactive flow model in Chapter 2 revealed that a small perturbation to the network volume fraction, about the equilibrium volume fraction associated with the stationary steady state, must be asymmetric for the strip to start travelling. The model exhibits a mechanism for polarization of the cell's actin network leading to cell motility. The analysis also shows that the growth rate of a perturbation plateaus to a positive constant at large wavenumber, when the contraction of the network is sufficiently strong. Therefore, the direction of cell motion is dependent on both the frequency and relative amplitude of the initial conditions. Mathematically, the model is weakly well-posed, while biologically it represents a cell that is sensitive to its initial network distribution. Qualitatively distinct families of travelling-wave solution that display a bell-shaped dependence of speed on adhesion strength and co-exist in parameter space were found numerically.

In Chapter 3 we formulated two different ways to model the effect of membrane-regulated network polymerization. The first imposes a constant flux of material out of the solution phase into the network phase at one end of the travelling strip. In the limit of small polymerization, a near-to-equilibrium, stationary, steady state exists, only when

the related minimal model is stable to perturbations of the network volume fraction of all wavenumbers. Numerical solutions revealed that qualitatively distinct families of travelling-wave solutions co-exist in parameter space. The model predicts a bell-shaped dependence of speed on adhesion strength, in which the actin volume fraction has local maxima at the front and rear of the travelling cell in some parameter regimes. This model also displays intriguing travelling-wave solutions that self organise the strip into seemingly distinct regimes. The second membrane-regulated polymerization model enforces polymerization of the network at the front of the strip and depolymerization at the rear, but allows the strip to determine its front and back as part of the solution. It exhibits qualitatively distinct families of travelling-wave solutions, which have high actin network volume fraction at both the front and rear of the cell and a bell-shaped dependence on adhesion strength.

Chapter 4 generalises the minimal model of Chapter 2 by modelling the actin network as a short-time elastic, long-time fluid, viscoelastic material. For a poor choice of parameter values this model is ill-posed. However, when the elastic relaxation timescale of the network is smaller than the network turnover timescale and the contraction of the network is not too strong, the linearized problem shares the same stability structure as the minimal model, with a growth rate that plateaus for large wavenumbers. We have not found any multi-peak travelling-wave solutions in the viscoelastic model, but the single peak solutions display qualitatively the same behaviour as their minimal model counterparts. A small growth rate analysis reveals that the timescales for elastic relaxation, network turnover and cell polarization are nested sequentially in this limit and associated with distinct network responses.

In Chapter 5 we formulated coupled partial differential equations for the transport and binding kinetics of free and bound myosin and identified several parameter limits. We thereby identify a common link between myosin modelling approaches adopted in a range of continuum models in the literature. Stability analyses reveal diffusion of free myosin or undirected crawling of bound myosin can be used to stabilise large wavenumber perturbations. Such analyses also reveal a new mechanism of myosin-driven contraction for destabilizing the stationary steady state. We present numerical simulations for two sub-models. The first describes myosin binding/unbinding kinetics, advection of bound myosin with the network and a spatially uniform concentration of unbound myosin. We found only one family of travelling-wave solutions, the solutions behave very similarly to the one-peak

solutions of the minimal model. The second model describes advection and diffusion of bound myosin. It has a steady travelling speed which tends to a nonzero constant as the adhesion strength tends to zero, while for strong adhesion, the travelling-wave solutions exhibit a boundary layer structure.

Chapter 6 concerns a different strand of research in our endeavours to understand cell motility. We develop two image processing codes to extract the three-dimensional shape of spreading cells from confocal microscope images, obtained by our collaborator Dr. J. Ratcliffe. We review a selection of hydrodynamical models for cell spreading in the literature and explain how these could in principle be compared to experimental data.

7.2 Future research directions

The work contained in this thesis points to a number of different ideas for future research. The dependence of travelling wave speed on adhesion strength displayed in Figure 2.9 for the minimal model only contains stable solutions that were obtained with our unsteady code. It remains to determine whether these branches of stable solutions extend further, and could be found by allowing the code more time to reach a steady state, or whether there are some bifurcation points for the parameter β^* beyond which the stable steady solutions become unstable or do not exist. A complete characterisation of the steady state solutions, including unstable solutions and the domains of attraction for the stable solutions, remains an open problem.

There is also considerable scope for characterizing the transient solutions of the minimal model. We have some simulations in which many peaks in the network volume fraction emerge, grow, move, merge and display what appears to be very complicated behaviour, particularly as the swelling and contractile pressure Ψ^* increases. Previous studies of similar systems have suggested the system may be chaotic. However, whenever we have pursued simulations that hinted at chaos we have found that the complicated behaviour is an artefact of a poorly converged simulation. Of course this does not preclude the existence of chaos in the system, we simply have not found good evidence for it. Future numerical work might attempt a more thorough investigation of parameter space and look for any periodic in time solutions which may exist. These questions can be posed for any of the two-phase models in this thesis, but should probably be first attempted for either the minimal model, because

it has fewest parameters, or the myosin diffusion model, because it is the easiest to solve numerically.

The membrane-regulated polymerization models of Chapter 3 provide some of the best opportunities for biological prediction and comparison to existing experiments. If, instead of having constant polymerization rates K and K^* , we allow them to be a function of the cell's position we can investigate cell crawling in response to chemotactic cues. It may also be possible to design experiments that disrupt the regulation of actin polymerization in a controlled way, which could be interpreted as altering K or K^* , and compare the experimental outcomes to the trends predicted by the model for varying rates of polymerization.

Furthermore, the effect of the substrate could be investigated. As discussed in §1.1.5, there already exists experimental data detailing how cells respond to the adhesiveness of the substrate. If we allow the adhesion strength in our models to depend on the cell's position we could verify the model against existing experiments and make predictions about how a cell might behave on new substrates. It would also be of great interest to formulate the model with a deformable substrate and compare this to the wrinkling experiments of cells crawling on elastic sheets. Such a model could allow for a more quantitative interpretation of the wrinkle patterns in terms of cell traction strength.

The viscoelastic model presented in Chapter 4 emphasizes the importance of timescales in formulating the right material description of a cell. It is possible that instead of a short-time elastic, long-time viscous fluid, the network phase may be better described as an elastic solid with considerable remodelling. It would be interesting to formulate such a model and see whether or not it is equivalent to our two fluid approach on crawling timescales.

Using only one-dimensional models means we cannot use the model to say anything about how a cell's shape changes when it crawls. However, a three-dimensional model would be much harder to analyse both analytically and numerically. A sensible next step in the modelling would be to consider thin-film reductions of the full three-dimensional model. This is a sensible thing to do biologically, because crawling cells typically have a small aspect ratio. Mathematically, it is a sensible approach because it significantly reduces the number of unknowns in the problem, making it more tractable. In the three-dimensional model, cell adhesion may be modelled via a Navier-slip condition and there are two distinct thin-film limits, namely the lubricational and extensional flow limits corresponding to strong and weak cell adhesion, respectively as in [93]. A crucial question to address is whether the

peaks we observe in our one-dimensional solutions manifest as spots or stripes in the higher dimensional problem.

It also remains to understand how to correct the distortion in the confocal microscope images, so that cell data can be generated for enough cells to allow for comparison to the hydrodynamical cell spreading models.

In summary, our work to date has revealed a rich array of behaviours relevant to modelling cell motility in simple two-phase flow models. We have generalised our minimal model to investigate the effects of membrane-regulated actin polymerization, actin network rheology and a hierarchy of modelling assumptions for myosin. Hopefully, it serves as a starting point for future work to further our understanding of two-phase flow models for cell motility.

Appendix A

Details on the two-phase flow formulation

The two-phase flow framework is widely used in the literature to model cell motility, as discussed in §1.2. Here we reproduce the key points from [102] of the multi-phase flow mixture theory on which those models are based. We shall assume for simplicity that the phases in our mixture have equal density. The continuity equation for each phase is given by

$$\frac{\partial \theta_i}{\partial t} + \nabla \cdot (\theta_i \mathbf{v}_i) = \sum_j k_{ij} \theta_j - \left(\sum_j k_{ji} \right) \theta_i, \quad (\text{A.1})$$

where θ_i and $\mathbf{v}_i$ are the volume fraction and the velocity of the i th phase. The rate of transfer of material from phase j to phase i is k_{ij} . There are no voids so

$$\sum_i \theta_i = 1. \quad (\text{A.2})$$

Now we consider the force on each phase, neglecting inertia we write

$$\nabla \cdot (\theta_i \mathbf{\Psi}_i) + \sum_{j \neq i} \mathbf{f}_{ij} = \mathbf{0}, \quad (\text{A.3})$$

where $\mathbf{\Psi}_i$ is the stress tensor in phase i , and $\mathbf{f}_{ij}$ is the force exerted on phase i by phase j . Note that $\mathbf{f}_{ij} = -\mathbf{f}_{ji}$. We must make constitutive assumptions for $\mathbf{\Psi}_i$ and $\mathbf{f}_{ij}$. Following [102], we write

$$\mathbf{f}_{ij} = p_{ij} \theta_j \nabla \theta_i - p_{ji} \theta_i \nabla \theta_j + H_{ij} \theta_i \theta_j (\mathbf{v}_j - \mathbf{v}_i), \quad (\text{A.4})$$

where p_{ij} is the interphase pressure exerted on phase i by phase j and H_{ij} is the coefficient of drag between phases i and j . We require $p_{ij} = p_{ji}$ and $H_{ij} = H_{ji}$. The stress in a phase can be written as

$$\mathbf{\Psi}_i = -p_i \mathbf{I} + \mathbf{\Psi}_{i,d}, \quad (\text{A.5})$$

where p_i is the intraphase pressure and $\Psi_{i,d}$ is the deviatoric part of the stress. Next we introduce a “contact-independent” pressure p , so that the interphase pressure can be written as this plus an extra pressure

$$p_{ij} = p + \psi_{ij}, \quad (\text{A.6})$$

where $\psi_{ij} = \psi_{ji}$ describes the tractions between phase i and phase j . The intraphase pressure can then be written as the common pressure, p , plus a weighted contribution of the ψ_{ij} and pressure due to internally generated traction, ϕ_i , (for example actin contraction of the network) so that

$$p_i = p + \phi_i + \sum_{j \neq i} \theta_j \psi_{ij}. \quad (\text{A.7})$$

Now combining (A.3)–(A.7) and rearranging, we obtain the force balance on the i th phase

$$-\theta_i \nabla p - \nabla (\theta_i \phi_i) + \nabla \cdot (\theta_i \Psi_{i,d}) - \sum_{j \neq i} [\theta_i \theta_j \nabla \psi_{ij} + 2\theta_i \psi_{ij} \nabla \theta_j] + \sum_{j \neq i} H_{ij} \theta_i \theta_j (\mathbf{v}_j - \mathbf{v}_i) = 0. \quad (\text{A.8})$$

In our application, we shall have only two fluids namely the actin network and the cytosol. We assume there is no traction between the phases ($\psi_{ij} = 0$), so that the only forces transmitted between the phases are due to hydrodynamic drag between the phases and the common pressure, which we identify as the hydrodynamic pressure. The force balance on the i th phase reduces to

$$-\theta_i \nabla p - \nabla (\theta_i \phi_i) + \nabla \cdot (\theta_i \Psi_{i,d}) + H_{ij} \theta_i \theta_j (\mathbf{v}_j - \mathbf{v}_i) = 0. \quad (\text{A.9})$$

The force balances in our minimal model are of this form, but in addition the force balance on the network has a extra term to model adhesion to the substrate.

Appendix B

Graphs to validate numerical schemes

For ease of reference we summarise the numerical method used throughout the thesis. It is a fully coupled, fully implicit finite element method with continuous approximation in space and the approximation is linear on each element. The scheme is implemented in C++ and the resulting nonlinear system is solved using the PETSc libraries [7, 8, 9].

B.1 Graphs for Chapter 3

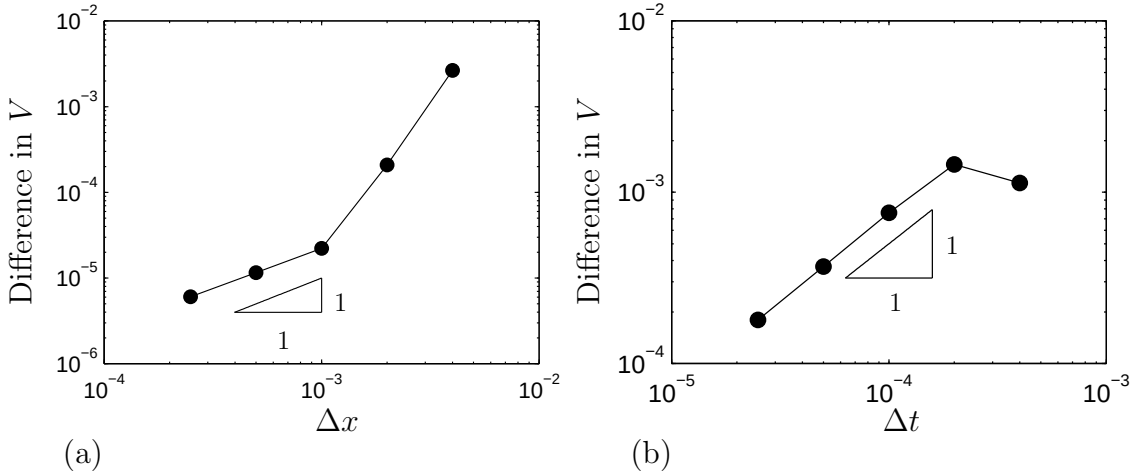


Figure B.1: Rate of spatial (a) and temporal (b) convergence of the velocity dependent polymerization code. Initial conditions are $\theta^*(x) = 3\theta_F/4 - \theta_F/4 \cos(\pi x)$ and the parameters are $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\theta_F = 0.03$, $K^* = 0.01$, $V^* = 1$, $t_R = 50$, $\Psi^* = 15000$, $\alpha^* = 0.4$ and $\beta^* = 3$.

Figure B.1 displays spatial and temporal convergence for the velocity dependent polymerization code used in §3.2. The initial value problem (3.51)–(3.56) was solved with the

same initial conditions and parameters on a sequence of nested grids, then the maximum absolute difference in the velocity of the travelling strip, V , on successive grids was plotted against the grid size. Figure B.1(a) shows the difference in V for each grid decreases as the size of the spatial element decreases, so the code exhibits spatial convergence. The temporal convergence of the code is similarly shown in Figure B.1(b) for sufficiently small timesteps.

B.2 Graphs for Chapter 4

Figure B.2 demonstrates that the code used to simulate the viscoelastic problem described in §4 converges. The initial value problem (4.14)–(4.20) was solved on a sequence of nested grids with the same timestep. The maximum absolute difference between the crawling velocity of the strip, V , calculated on successive grids is plotted against the size of the spatial element Δx in Figure B.2(a). We see that the difference decreases as the spatial grid is refined, so the code converges as the number of spatial elements increases. Temporal convergence is similarly demonstrated in Figure B.2(b) by plotting the difference in crawling velocity of the strip as a function of step size. The problem was simulated on the same spatial grid for a sequence of timesteps. Full details of the initial conditions and parameters used are given in the caption.

Figure B.3 validates the small growth rate analysis in §4.3 by comparing the predictions

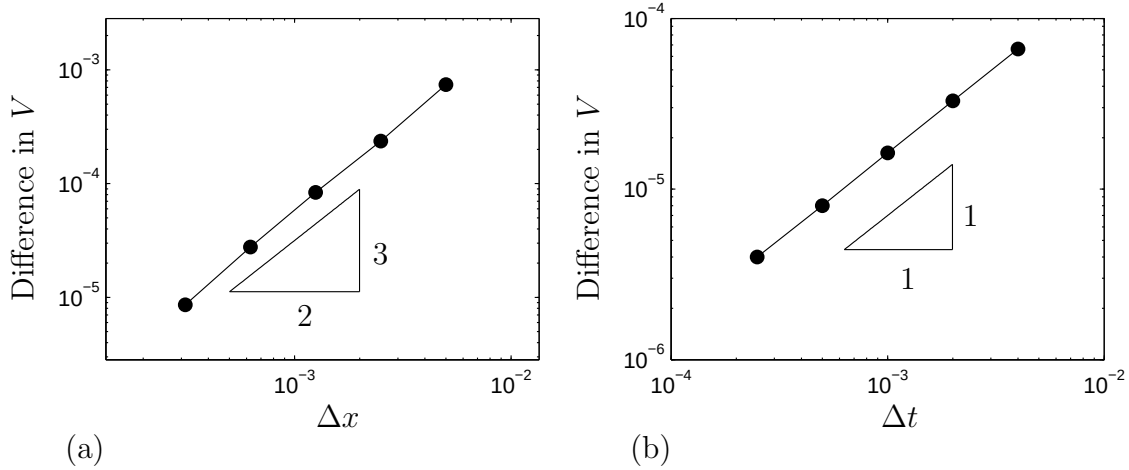


Figure B.2: (a) shows the rate of spatial convergence for the viscoelastic code with initial conditions $\theta^* = \theta_E + 10^{-4} \cos(\pi x)$, $\sigma^* = 10^{-4} \cos(\pi x)$ and the parameter values $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $\Psi^* = 15000$, $\alpha^* = 0.4$, $\beta^* = 3$ and $D = 0.1$. The rate of temporal convergence is shown in (b) for the viscoelastic code with the same initial conditions and parameters as in (a) except that here $\Psi^* = 12000$.

it makes to a numerical simulation for appropriate parameter values as stated in the caption. In Figure B.3(a) we plot $\mu_E J'_E \theta_1$ (solid line) and $-\theta_E \sigma_1$ (dots) at $t = 100$. The excellent agreement shows that (4.85) holds. In Figure B.3(b) we check that (4.91) holds at late times by plotting V_1 (solid line) and the right-hand-side of (4.91) (dots), which are also in excellent agreement. A grid with 1000 spatial elements and a timestep of 10^{-3} was used for these simulations, doubling the timestep or the number of spatial elements produced solutions with a relative difference in V of less than 10^{-5} .

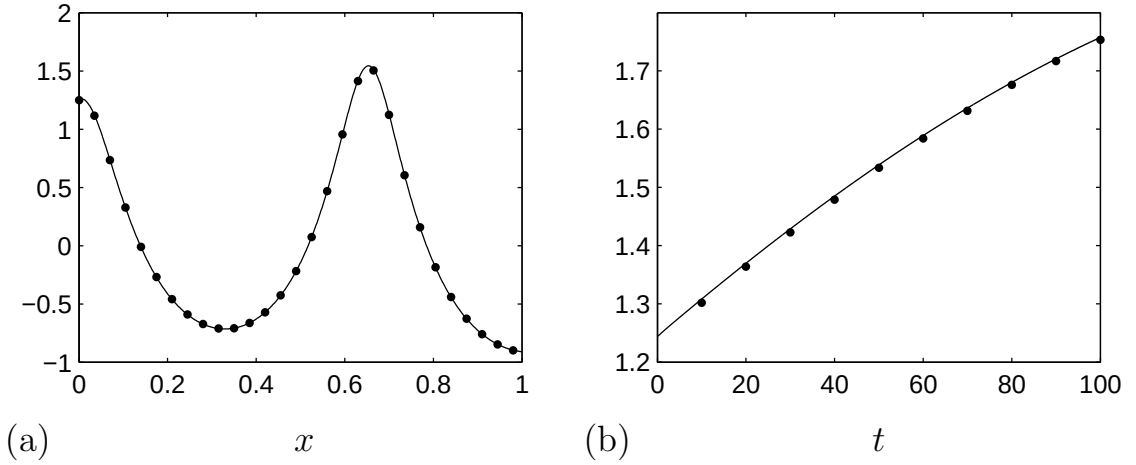


Figure B.3: Graphs validating the small growth rate analysis of the viscoelastic model in §4.3, see text for details. The initial conditions are $\theta^* = \theta_E + \varepsilon(\cos(3\pi x) + 0.25 \sin(\pi x))$, $\sigma^* = \varepsilon \sin(2\pi x)$ and the parameter values are $\varepsilon = 10^{-4}$, $\theta_L = 0.01$, $\theta_E = 0.02$, $\theta_R = 0.03$, $D = 0.08$, $\Psi^* = 9800$, $\alpha^* = 10^{-5}$ and $\beta^* = 8.23 \times 10^{-5}$ (to three significant figures).

B.3 Graphs for Chapter 5

We check the convergence of the code used to simulate solutions to the kinetic myosin model with governing equations (5.14), (5.15), (5.20), (5.28) and (5.29) in §5.4.2. The same problem was simulated on a series of nested grids and the solutions obtained on different grids are compared. To check spatial convergence the timestep was set to 10^{-3} and grids with 50 elements doubling to 6400 elements were used. To check the temporal convergence a grid with 800 elements and timesteps halving from 8×10^{-3} to 5×10^{-4} were used. Figure B.4 shows that the difference between successive simulations decreases as we refine the spatial grid or the timestep. We can be confident that the code converges, but the rate of spatial convergence is quite slow. Before embarking on a large parameter study, it would probably be worth improving the spatial order of accuracy of the code. This was not

attempted here because the convergence was adequate to meet our goal of computing some illustrative examples.

Next we validate the code by comparing the numerical solution for the velocity of the crawling strip to the linear prediction made in (5.91). Figure B.5 demonstrates that the two are in excellent agreement at early times, whilst the perturbation to the stationary steady state is still small and the linearization holds.

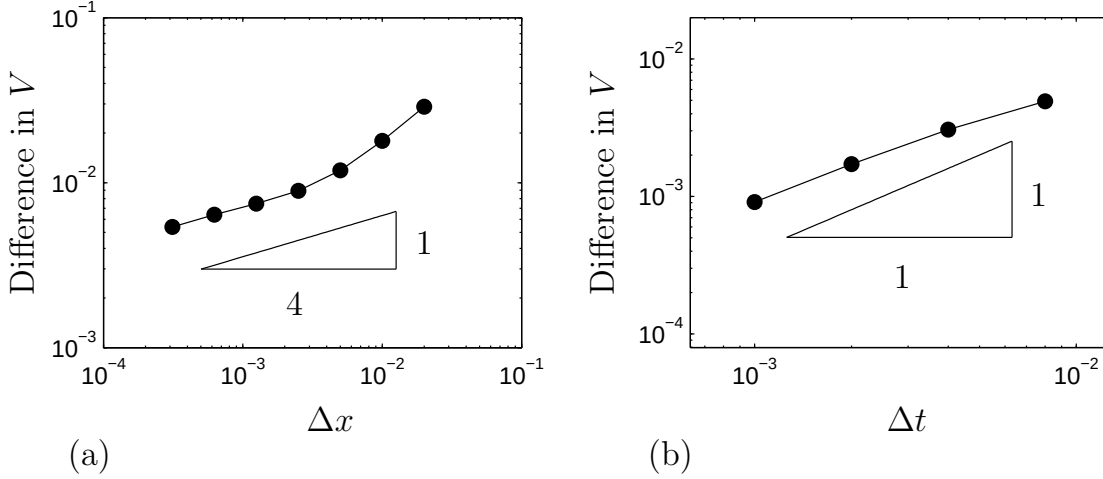


Figure B.4: Graphs to demonstrate (a) the spatial and (b) the temporal convergence of the code to solve the kinetic myosin model in §5.4.2 with initial conditions $\theta^* = \theta_E + 10^{-4} \cos(\pi x)$ and $m_b^* = 0.2 + 10^{-4} \cos(\pi x)$. The parameters are $\theta_E = 0.02$, $S^* = 53$, $C^* = 80$, $\alpha^* = 0.4$, $\beta^* = 3$, $k_{\text{on}}^* = 20$ and $k_{\text{off}}^* = 30$.

Now we turn our attention to the other myosin model that we compute numerical solutions for in §5.4.3, the diffusion model with the governing equations (5.14), (5.15), (5.20) and (5.41). First we check that the code converges by solving the same problem on grids with the number of spatial elements doubling from 100 to 6400 whilst keeping the timestep fixed at 10^{-3} to check the spatial convergence. Then we check the temporal convergence by fixing the number of elements in the grid at 800 and halving the timestep from 1.6×10^{-2} to 5×10^{-4} . Figure B.6 displays how the difference in the solutions computed in successive simulations decreases as the simulations are refined.

Finally we would also like to validate the code for the myosin diffusion model against the linear stability predictions discussed in §5.3.1. We did not solve the linearized initial value problem for arbitrary initial conditions, so instead we carefully select eigenmodes as initial conditions so that we can calculate the growth rate. The network volume fraction and concentration of bound myosin that correspond to the perturbation of the network

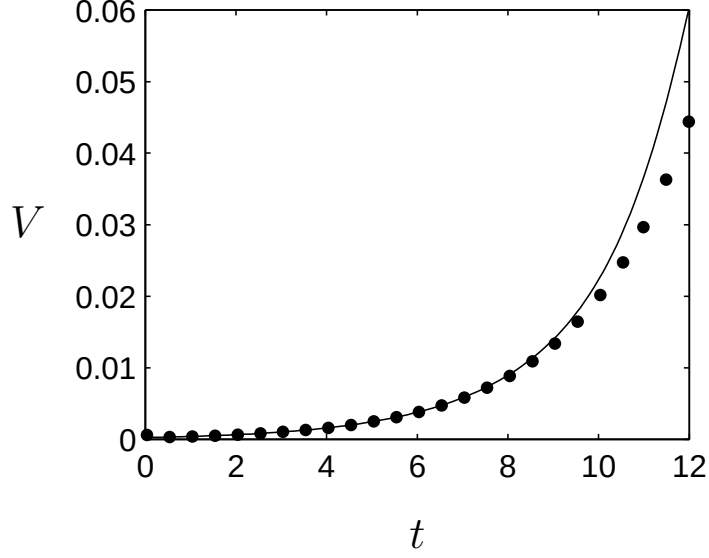


Figure B.5: Graph to show the early time agreement between the numerical solution (solid line) and the linear prediction (dots) for the kinetic myosin model in §5.4.2. The parameters are $\theta_E = 0.02$, $S^* = 53$, $C^* = 80$, $\beta^* = 3$, $\alpha^* = 0.4$, $\varepsilon = 10^{-4}$, $k_{\text{on}}^* = 20$, and $k_{\text{off}}^* = 30$. The initial conditions are $\theta^* = \theta_E + \varepsilon \cos(3\pi x)$ and $m_b^* = M_b + \varepsilon(5 + \cos(\pi x))$.

velocity stated in (5.53) are of the form

$$m_{b1} = -\frac{V_1\beta_E}{\Psi_{mE}} \left(\frac{U_1}{k_1} \left(1 + \frac{k_1^2}{\alpha_E + \beta_E} \left(\mu_E + \frac{\theta_E \Psi_{\theta E}}{\omega + J'_E} \right) \right) \frac{\sin(k_1(x - 1/2))}{\cos(k_1/2)} \right. \\ \left. + \frac{U_2}{k_2} \left(1 + \frac{k_2^2}{\alpha_E + \beta_E} \left(\mu_E + \frac{\theta_E \Psi_{\theta E}}{\omega + J'_E} \right) \right) \frac{\sin(k_2(x - 1/2))}{\cos(k_2/2)} \right) e^{\omega t}, \quad (\text{B.1})$$

$$\theta_1 = \frac{V_1\theta_E\beta_E}{(\omega + J'_E)(\alpha_E + \beta_E)} \left(k_1 U_1 \frac{\sin(k_1(x - 1/2))}{\cos(k_1/2)} + k_2 U_2 \frac{\sin(k_2(x - 1/2))}{\cos(k_2/2)} \right) e^{\omega t}, \quad (\text{B.2})$$

where the real part is understood. We set the parameters as listed in the caption to Figure B.7 and find the largest real ω that satisfies (5.55), which in this case is $\omega = 0.193$ to three significant figures. We use this to calculate k_1 and k_2 and then U_1 and U_2 . To form the initial conditions we set $t = 0$ and $V_1 = 1$ in (B.1) and (B.2), take $\varepsilon = 10^{-4}$ and use $\theta^* = \theta_E + \varepsilon\theta_1$ and $m_b^* = 1 + \varepsilon m_{b1}$. The predicted velocity of the crawling strip is then $V = \varepsilon e^{\omega t}$. Figure B.7 shows this prediction and the numerical solution to be in excellent agreement.

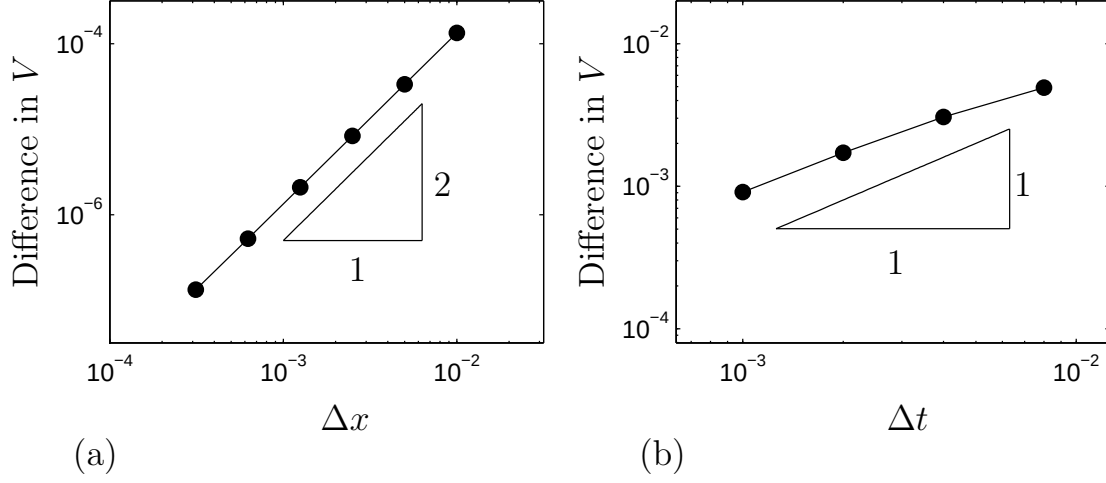


Figure B.6: Graphs to demonstrate (a) the spatial and (b) the temporal convergence of the numerical code for the diffusion myosin model from §5.4.3. The initial conditions are $\theta^* = \theta_E + 10^{-4} \cos(5\pi x)$ and $m_b^* = 1 + 10^{-4} \cos(\pi x)$ and the parameters are $\theta_E = 0.02$, $S^* = 60$, $C^* = 35$, $\alpha^* = 0.04$, $\beta^* = 3$ and $D = 0.1$.

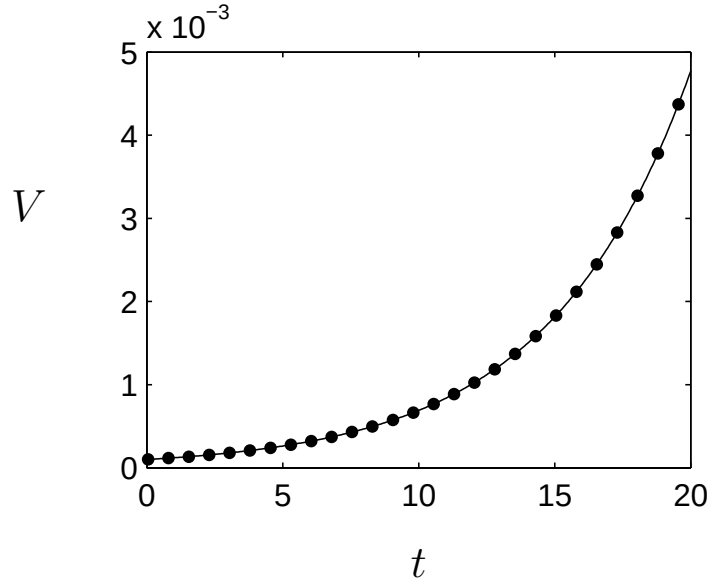


Figure B.7: Graph to demonstrate the agreement at early times between the numerical solution (solid line) and the linear prediction (dots) for the diffusion myosin model from §5.4.3. The parameters used are $\theta_E = 0.02$, $S^* = 60$, $C^* = 35$, $\alpha^* = 0.04$, $\beta^* = 100$ and $D = 0.1$. See text for details of initial conditions.

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