Assessing the efficacy of administration modalities for collagen-based electroactive medical devices for wound healing and skin rejuvenation

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Efstratios Hatzistefanis

Supervised by Prof. Malavika Nair

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1 Introduction

This dissertation investigates the development of collagen-based electroactive constructs for wound healing and skin regeneration, with a focus on how their composition, architecture, and electroactive properties can be tuned to influence fibroblast behaviour and improve regenerative outcomes. The central aim is to establish design principles that integrate structural, biochemical, and electrical cues into a single, biologically compatible platform capable of guiding cell activity and extracellular matrix (ECM) organisation in a controlled, clinically relevant manner.

The motivation for this work stems from the clinical and economic burden of non-healing wounds, extensive burns, and pathological scarring, where standard dressings offer protection but limited active intervention. In such contexts, the ability to direct fibroblast migration, proliferation, and ECM synthesis is critical to accelerating closure, improving tissue quality, and reducing the risk of fibrosis. Collagen is the natural choice for such constructs: as the most abundant protein in the dermis, it offers mechanical stability, intrinsic bioactivity, and a well-understood degradation profile. Its hierarchical structure, integrin-binding domains, and piezoelectric properties make it uniquely suited for incorporation into devices that combine mechanical support with electrical stimulation.

Electrical stimulation (ES) has been shown to enhance dermal fibroblast proliferation, motility, collagen production, and growth factor secretion. Physiologically, endogenous wound fields—arising from transepithelial potentials disrupted by injury—provide directional cues (*galvanotaxis*) that promote re-epithelialisation and coordinated tissue repair.

Replicating or amplifying these cues through engineered devices offers a route to bias healing towards functional closure and away from disordered remodelling. Embedding electroactive capability directly into collagen constructs, whether *via* piezoelectric enhancement or conductive and ionomeric modification, allows for localised, phase-specific stimulation without reliance on bulky external systems.

This research addresses four interlinked challenges:

1. Optimising electroactive parameters — identifying safe and effective field strengths, frequencies, and stimulation durations for *in vivo* fibroblast activation.
2. Balancing degradation with sustained function — tuning construct breakdown to match healing timelines while maintaining mechanical integrity and electrical responsiveness.
3. Integrating scaffold and film characteristics — combining the high porosity and vascularisation potential of scaffolds with the barrier and electrode-interface functions of films.
4. Ensuring long-term bioelectrical stability — maintaining electroactive performance in the mechanically dynamic and biochemically variable wound environment.

The following chapters build the foundation for tackling these challenges. Chapter 2 reviews the skin's bioelectric environment, the structural and functional properties of collagen, and how these can be engineered into scaffolds and films for regenerative applications. It also examines fibroblast responses to collagen constructs with and without ES, highlighting strategies for controlling migration, alignment, ECM deposition, and differentiation. These insights shape the experimental approach, which applies advanced fabrication and functionalisation techniques to create electroactive collagen prototypes. The subsequent chapters present the outcomes of this experimental approach. Chapter 3 reports the fabrication, mechanical, electrical, and biological performance of collagen–taurine scaffolds and films, with *ex vivo* wound and burn models used to assess therapeutic potential. Chapter 4 reports the results of the experiments performed, and Chapter 5 discusses these findings and forms conclusions regarding their suitability as electroactive wound dressings. Finally, Chapter 6 delivers the overall conclusions

and outlines future work to optimise fabrication, validate efficacy *in vivo*, and explore clinical translation.

The overarching objective is to move beyond passive wound coverage towards active, responsive systems that can adapt to the biological and mechanical state of the tissue over time. By integrating mechanical, biochemical, and electrical functionality into a single regenerative platform, the work aims to lay the groundwork for next-generation wound dressings that are not only biocompatible and structurally supportive, but also bioelectrically instructive—guiding repair towards the production of faster, stronger, and more organised tissues.

2 Literature Review

2.1 Skin Repair Applications

2.1.1 Introduction to the Skin and its Bioelectric Environment

The skin is a dynamic, electrically active organ in which epithelial, stromal, and immune processes are choreographed by biochemical gradients, mechanical cues, and endogenous bioelectric fields [1]. Among the resident stromal cells, dermal fibroblasts are pivotal: they deposit, remodel, and apply contractile stresses on their collagen-rich extracellular matrix (ECM), coordinate with keratinocytes and endothelial cells, and respond strongly to exogenous electrical stimulation (ES) [2]. *In vitro* and *in vivo*, ES has been shown to increase dermal fibroblast proliferation, migration, adhesion, differentiation, and alignment [1]. ES also modulates the secretion of growth factors and cytokines relevant to wound healing, making electroactive collagen constructs promising platforms for wound closure and skin rejuvenation where the goal is not merely wound coverage but to support active regeneration with controlled remodelling [2].

Physiologically, skin injuries are associated with transient injury currents and transepithelial potentials that provide a directional electrical cue for cell migration [1]. Around acute wounds, fields in the range of ~40–200 mV/mm have been reported and are thought to promote re-epithelialisation [3]. Harnessing or mimicking these cues in engineered systems offers a means to bias fibroblast motility and matrix synthesis toward functional wound closure, and to counteract disordered remodelling that leads to hypertrophic or keloid scarring [1]. While the exact transduction cascade varies by cell type and stimulus parameters, the empirical picture for dermal fibroblasts is consistent: appropriate field strengths and waveforms can increase proliferation and motility [3], enhance matrix protein production [1], and stimulate secretion of factors such as FGF-1 and FGF-2 [2]—all processes central to timely wound resolution.

From a materials standpoint, collagen's intrinsic electromechanical behaviour provides a particularly relevant substrate for "electroactive" devices that aim to deliver cues while remaining biologically native [4]. Although the molecular origin of collagen's piezoelectricity remains debated, cross-linking can substantially enhance shear piezoelectric coefficients [5]. Collagen's charged backbone also allows additional electroresponsive strategies that couple surface charge, ion mobility, and hydration to macroscopic deformation [6]. Polyelectrolyte functionalisation is one of these strategies that has been shown to enhance or modify the electromechanical behaviour of the base biopolymer. When combined with fabrication techniques such as the electrospinning technique to create scaffolds, constructs with increased structural stability and good mechanical properties can be produced alongside the modified chemistry [7]. These features are increasingly leveraged in scaffold and film designs intended for cutaneous repair [2]. Such material attributes become clinically meaningful when translated into improvements in re-epithelialisation rates [4], granulation tissue quality [5], and scar outcomes [6]—three axes that frame the applications below.

2.1.2 Open Wounds

Open cutaneous wounds require rapid haemostasis and inflammation control followed by fibroblast-driven matrix deposition, angiogenesis, and contraction [1]. Exogenous ES can accelerate several of these phases. One study demonstrated that ES increased dermal fibroblast activity and promoted myofibroblast transdifferentiation [2], both of which are associated with faster wound contraction and robust granulation tissue formation. Importantly, these effects were achieved without detectable cytotoxicity on LDH assays across the stimulation regimen used [1]. The same body of work reported enhanced secretion of FGF-1 and FGF-2 under ES [2]—growth factors long implicated in fibroblast proliferation and angiogenic support, both hallmarks of progressing wounds rather than stalled, inflammatory lesions.

The responsiveness of fibroblasts to waveform and frequency provides a handle for tuning constructs to specific wound contexts [8]. For example, degenerate wave (DW) stimulation down-regulated collagen I expression in keloid fibroblasts while preserving viability [8], suggesting waveform-dependent control over profibrotic outputs. In non-keloid dermal fibroblasts, alternating current (AC) fields at 10–60 Hz showed frequency-dependent changes in collagen I and PAI-1 gene expression [6], with 150 mV/mm at 10 Hz often producing the highest overall collagen gene expression among conditions tested [8]. These findings highlight two practical levers—field strength and frequency—to balance matrix synthesis against the risk of excessive deposition as healing progresses.

Electric-field–guided migration further supports closure of open wounds, particularly where cellular density is initially low at the wound edge [1]. Multiple studies document increased fibroblast motility and traction generation under direct current (DC) fields on the order of ~200 mV/mm [1], with concurrent increases in contractility and growth factor secretion [2]. Such magnitudes are squarely within the physiological range near wound sites, aligning with the rationale to reproduce endogenous cues exogenously. In addition, ES can alter alignment: fibroblasts stimulated with pulsatile fields tend to orient perpendicular to field lines [9], which can be exploited to direct collagen fibre deposition and tensile axes of the nascent matrix in ways that reduce anisotropic strain concentrations during later remodelling [6].

A practical constraint in open wounds is avoiding cytotoxicity while still delivering a potent cue [8]. Collating results across studies, the lowest stimulation frequency reported in this area was 1 Hz, with many investigations clustering between 5–60 Hz and field strengths from ~20 to 460 mV/mm [10], [11]. Within these windows, a general trend emerges increasing magnitude tends to increase growth, migration, and matrix protein production—up to cell-type–specific limits [8]. That said, DC stimulation appears more prone to inducing apoptosis compared with AC or DW stimulation in certain contexts [10], underscoring the need for waveform choice guided by wound status and cell phenotype [2].

ES can also be combined with mechanical stimulation for additive or synergistic benefits [12]. Using stretchable silver-nanowire microelectrodes as an integrated electromechanical stimulator, investigators reported increased fibroblast alignment, procollagen production, and polarisation relative to single-modality stimulation [6]. Intriguingly, the combined effects were not simply additive, suggesting partially overlapping or convergent mechanotransduction pathways [12]. For open wounds, where both strain fields (from motion) and injury currents are present, devices capable of concurrent electromechanical delivery may thus better recapitulate the healing milieu.

2.1.3 Scarring

Scar formation represents a continuum from normotrophic remodelling to hypertrophic and keloid phenotypes [1], driven by persistently elevated fibroblast activity, myofibroblast persistence, and dysregulated ECM turnover [8]. ES offers a route to modulate that balance. In keloid fibroblasts, DW stimulation downregulated collagen I expression while maintaining viability [8], pinpointing waveform control as a means to damp pathological collagen synthesis without broadly suppressing cell function. Complementing this, low-to-moderate AC fields (e.g., ~150 mV/mm at 10 Hz) tend to increase collagen I expression in non-keloid dermal fibroblasts [6] This is useful in early reparative phases, but the same frequencies can be dialled upward (to 60 Hz) to reduce expression as the matrix matures [8], providing a rational, phase-specific tuning strategy.

The ability of ES to influence cell cycle and proliferation kinetics also intersects with scarring risk [10]. Exposure of fibroblasts to AC fields around ~41 mV/mm at 10 Hz has been reported to push cells into the growth phase [10]. This effect could be harnessed transiently during proliferation but tapered once a sufficient cellular density is reached. Moreover, DC-elicited increases in contractility and FGF secretion [2] should be weighed against the same waveform's pro-apoptotic tendencies at higher magnitudes [8]—especially in hypercellular scars where apoptosis may be less of a toxicity concern and more of a corrective mechanism if tightly controlled.

Beyond amplitude and frequency, timing within the healing timeline matters [1]. Early augmentation of fibroblast migration and collagen deposition can speed closure, reducing infection risk and mechanical irritation that themselves drive scarring [8]. Later restraint of collagen I synthesis and myofibroblast persistence is desirable to limit stiff, aligned scar architecture [8]. The keloid-focused finding that DW waveforms downregulate collagen I suggests one template: ES regimens might begin with AC at lower frequencies and transition to DW or higher-frequency AC once re-epithelialisation and early granulation are complete [8]. The same conceptual framework can be integrated into electroactive collagen constructs by switching their delivered waveform through embedded electrode designs [3], [6].

Finally, cell alignment under ES has mechanical consequences for scar anisotropy [9]. Pulsatile fields that orient fibroblasts perpendicularly to field lines could, if applied with controlled spatial patterning, interrupt the formation of uniformly aligned collagen bundles typical of hypertrophic scars [6], distributing strain more evenly and softening the macroscopic scar.

2.1.4 Burns

Thermal injuries pose additional hurdles beyond simple full-thickness defects: extensive protein denaturation, vascular damage, and neurotrophic deficits create a hostile, hypoxic, and often dehydrated bed in which cell migration is impeded [1]. Yet, because endogenous wound fields are generated by ion transport across epithelia and disrupted tight junctions, large burn surfaces may also present an opportunity: amplifying or replacing diminished physiological cues with controlled ES to drive keratinocyte and fibroblast migration from viable margins [2] and to re-establish an ECM suitable for re-innervation and angiogenesis [1].

The dermal fibroblast literature—though largely derived from non-burn models—consistently shows that ES increases migration, contractility, and growth factor secretion (FGF-1/FGF-2) [2], all beneficial for establishing granulation tissue in deep partial-thickness burns once infection and necrosis are controlled [1]. Choosing the waveform for a burn context likely benefits from the same tuning logic as for open wounds and scarring [8], but with stricter guardrails for cytotoxicity given the breadth of the injured area. AC and DW are comparatively less pro-apoptotic than DC at equivalent effective cues [10], and frequency can be used to bias collagen expression up or down depending on whether one is in the early proliferative phase or the later remodelling phase [8], [6].

Because eschar and exudate can alter local field distribution, constructs that integrate electrodes into conformal, flexible substrates—such as thin, stretchable networks—may provide more uniform coverage and reproducible dosing over large curved surfaces [12]. Early work using stretchable microelectrodes demonstrates that integrating mechanical strain with ES further enhances alignment and procollagen output [6], which could be exploited during the transition from granulation to early remodelling in burn care [12].

A particularly relevant consideration in burns is the long-term quality of regenerated tissue and the risk of contracture [1]. Here, the ability to modulate myofibroblast behaviour by ES becomes vital. Reports of ES-induced myofibroblast transdifferentiation in wound models indicate a potential to accelerate early contraction [2]. But to avoid late contractures, later-stage waveform transitions (e.g., toward DW) that damp collagen I synthesis could be deployed [8]. In practice, this suggests a staged protocol embedded in the device itself: initial AC at 10 Hz and ~100–150 mV/mm to encourage proliferation and deposition [2], followed by either frequency elevation or DW once epithelial coverage is secure [8], with careful monitoring of range of motion and pliability.

2.1.5 Skin Rejuvenation

Skin ageing is a multifactorial process involving both intrinsic (chronological) and extrinsic (environmental) factors. Healthy young skin is characterised by abundant and well-organised collagen and elastic fibres, supporting tensile strength, resilience, and repair capacity. With age, however, dermal collagen density decreases, elastic fibres become fragmented, and fibroblast activity diminishes [13]. These structural changes lead to thinning, reduced elasticity, impaired barrier function, and delayed wound healing. The skin's bioelectric environment also shifts as ion gradients across the epidermis weaken, and fibroblast responsiveness to endogenous electrical cues declines. Importantly, the difference between ageing skin and acutely wounded skin lies not in the absence of repair mechanisms but in their reduced sensitivity and slower activation [14].

Given these changes, skin rejuvenation therapies aim to restore extracellular matrix integrity, stimulate fibroblast function, and enhance collagen production. Current approaches include topical retinoids, laser resurfacing, microneedling, and the delivery of growth factors or peptides [15]. More recently, electrostimulation (ES) has gained attention as a non-invasive strategy to promote collagen synthesis, increase fibroblast proliferation, and improve dermal blood flow. Clinical and cosmetic technologies, employ low-intensity electrical currents to enhance skin permeability and drive active ingredients into the dermis *via* electrophoresis and iontophoresis [16]. These modalities not only improve delivery efficiency but also appear to directly activate fibroblasts and enhance collagen deposition, thereby counteracting age-related ECM depletion.

In this context, collagen-based electroactive biomaterials offer an attractive platform for minimally invasive skin rejuvenation. By combining a structural scaffold with the ability to conduct or modulate electrical cues, such materials have the potential to mimic the skin's bioelectric environment while simultaneously reinforcing its extracellular matrix. The parallels between wound healing and rejuvenation are clear: both involve stimulating fibroblast migration, alignment, and matrix synthesis. Electroactive collagen constructs could therefore serve a dual purpose, aiding in wound repair while also supporting the restoration of ageing but otherwise healthy skin.

2.2 Collagen

Collagen is the most abundant protein in the mammalian extracellular matrix, forming the structural and mechanical backbone of skin, tendon, bone, cartilage, and a range of other tissues [3]. Its hierarchical organisation, unique mechanical behaviour, and inherent bioactivity make it an indispensable material for tissue engineering and regenerative medicine [17]. In the context of wound healing and skin rejuvenation, collagen provides the scaffold on which cells migrate and deposit new tissue, while also presenting biochemical motifs that influence cell adhesion, proliferation, and differentiation [3]. Its ubiquity in the dermis and its compatibility with multiple fabrication strategies underpin its role as a preferred substrate for electroactive medical devices designed to modulate fibroblast behaviour [2], [6].

2.2.1 Structure of Collagen

At the molecular level, collagen consists of three polypeptide α-chains wound into a right-handed triple helix [3]. Each α-chain follows a repeating Gly–X–Y sequence, where X and Y are frequently proline and hydroxyproline, respectively [17]. The small glycine residues at every third position permit the tight packing of the chains, while proline and hydroxyproline stabilise the helix *via* steric constraints and hydrogen bonding [17]. This triple-helical structure assembles into higher-order fibrils, which in turn bundle into fibres observable at the tissue scale. In skin, collagen fibres are oriented according to Langer's lines, optimising the dermis to withstand directional mechanical loads [17].

Collagen's structure is highly conserved but can vary depending on the collagen type. Type I collagen, the dominant isoform in skin, tendon, and bone, is heterotrimeric (two α1 chains and one α2 chain) and forms thick, highly tensile fibrils [17]. Type III collagen, often co-localised with type I in the dermis, forms thinner fibrils and is more prevalent during early wound healing, where it is later replaced by type I as the tissue matures [2]. This isoform switching is an important determinant of scar quality: a persistent elevation in the type III/I ratio is associated with immature, mechanically weak tissue [17].

At the supramolecular level, collagen fibrils exhibit a characteristic 67 nm D-periodicity arising from the quarter-staggered packing of triple helices [4]. This periodicity has been suggested to underpin both the tensile mechanics and the piezoelectric properties of collagen, as the non-centrosymmetric arrangement of charged amino acids generates a net polarisation under mechanical deformation [5]. Such intrinsic piezoelectricity provides a natural interface with electroactive wound-healing devices, enabling bidirectional coupling between applied electrical fields and mechanical stress within the scaffold [6].

2.2.2 Properties of Collagen

Collagen's mechanical properties derive from both its molecular structure and its higher-level organisation. Type I collagen fibrils exhibit high tensile strength (up to 1 GPa modulus) while maintaining flexibility due to the triple-helical conformation [5]. In hydrated forms, as in native tissue or hydrated scaffolds, the modulus is significantly lower, but this compliance supports dynamic mechanical loading without brittle failure [17]. The high tensile strength and resilience make collagen ideal for dermal applications where tensile stability and tear resistance are important [5].

From a biological perspective, collagen is intrinsically bioactive. Its degradation products can act as chemotactic signals, attracting fibroblasts and macrophages to the wound site [17]. The native molecule also presents integrin-binding domains, such as the GFOGER motif, that mediate specific cell adhesion *via* α1β1 and α2β1 integrins [17]. These domains influence cell shape, migration, and survival, and can directly modulate gene expression related to extracellular matrix synthesis and remodelling.

Electrically, collagen's piezoelectricity—stemming from its polar triple helix and staggered packing—enables it to generate measurable voltages under mechanical load [4]. *In vivo*, this property may contribute to mechano-electrical transduction, potentially influencing cell behaviour through localised electrical cues [2]. In engineered systems, piezoelectric collagen scaffolds can convert mechanical motion, such as patient movement, into electrical stimulation of resident cells, complementing active ES delivered by an integrated device [6].

Collagen is also chemically versatile. Its numerous lysine and hydroxylysine residues provide sites for covalent cross-linking, either enzymatically (e.g., *via* lysyl oxidase) or artificially (e.g., carbodiimide chemistry, genipin, glutaraldehyde) [18]. Cross-linking alters degradation rate, mechanical stiffness, and thermal stability, allowing tuning for specific wound-healing timelines [17]. In electroactive device applications, cross-linking can also increase piezoelectric coefficients by restricting molecular mobility and enhancing dipole alignment [4], [5].

2.2.3 Methods of Altering Collagen Structure

Collagen's properties can be tailored by modifying its molecular, fibrillar, or macroscopic structure. Physical methods such as plastic compression, freeze-drying, and electrospinning influence fibril density, orientation, and porosity [17]. Freeze-drying produces macroporous scaffolds with pore sizes tunable by the freezing rate, directly affecting fibroblast infiltration and matrix deposition [17]. Directional freezing can align pores, influencing both cell orientation and mechanical anisotropy—parameters important for skin, which must resist strain in specific directions [5].

Chemical modification offers another layer of control. Cross-linking agents such as EDC/NHS increase stiffness and reduce enzymatic degradation [18], whereas glycation can alter charge distribution and piezoelectric response [4]. More advanced chemical strategies include functionalisation with conductive polymers such as polypyrrole or PEDOT:PSS [2]. In addition, ionomers which are polymers containing a minor fraction of covalently attached ionic or ionizable groups are used because of their tunable mechanical properties to create hybrid electroactive scaffolds [6]. Incorporation of sulfonated groups, as in ionomeric extracellular matrices, can enhance ionic conductivity and facilitate electromechanical actuation under low-voltage stimuli [2].

Enzymatic treatments can selectively cleave telopeptides or alter cross-link density, affecting fibrillogenesis and degradation rate [17]. For example, pepsinised collagen lacks telopeptides and is less immunogenic, though it may form weaker fibrils unless re-cross-linked [17]. Enzymatic cross-linking using transglutaminase can improve mechanical stability while maintaining a more "native-like" chemistry than synthetic agents [17].

Top-down approaches can also be combined with bottom-up fabrication methods to produce composite constructs. Embedding aligned collagen fibrils within a hydrogel matrix can produce constructs that mimic the gradient structure of native skin [6], with an electroactive collagen layer integrated into a softer, more hydrated network. This combination allows spatial control over stiffness, degradation, and electrical properties.

Emerging nanofabrication methods allow finer control over structure and function. Electrospun collagen nanofibres can be aligned to guide fibroblast elongation and matrix deposition [3]. Co-spinning with conductive nanomaterials such as carbon nanotubes or graphene oxide can enhance electrical conductivity [6]. In wound healing, these modifications not only provide structural support but also create a platform for controlled electrical stimulation—either harvested from the body's own movement *via* piezoelectricity or delivered through embedded electrodes.

In sum, collagen's native structure offers a unique combination of mechanical, biochemical, and electrical properties ideally suited for cutaneous regeneration [17]. By leveraging physical, chemical, and enzymatic modifications, these properties can be tuned to optimise scaffold performance for specific wound-healing and skin-rejuvenation contexts. Adding electroactive functionalities through ionomeric or conductive-polymer modification represents a promising strategy for integrating structural support with active bioelectrical modulation [2], [6].

2.3 Collagen Constructs

The engineering of collagen into functional constructs is central to its application in wound healing and skin rejuvenation. By controlling its organisation, density, and surface chemistry, collagen can be shaped into structures that guide fibroblast activity, influence extracellular matrix (ECM) deposition, and modulate the mechanical and biochemical environment of healing tissue [17]. These constructs are particularly valuable when integrated into electroactive devices, where collagen's intrinsic bioactivity and piezoelectricity can be enhanced with engineered conductivity or ionic responsiveness to deliver targeted stimulation [2].

Two main classes of collagen constructs dominate dermal applications: porous scaffolds and dense films. Each offers distinct structural, mechanical, and biological characteristics and can be tailored through fabrication methods to optimise performance for specific wound contexts. The choice between scaffold and film formats depends on the desired balance of porosity, mechanical stability, hydration, and—when used in electroactive systems—electrical field distribution.

2.3.1 Scaffolds

Collagen scaffolds are three-dimensional porous structures that act as templates for cell migration, proliferation, and ECM remodelling [17]. Their architecture determines how fibroblasts infiltrate and orient within the construct, as well as the rate and quality of tissue integration. In electroactive applications, scaffolds provide both structural support and a pathway for electrical or ionic current delivery [2].

2.3.1.1 Scaffold properties and composition

The performance of collagen scaffolds is closely linked to pore size and interconnectivity. Larger pores—above approximately 100 μm—can support deeper fibroblast infiltration and neovascularisation [17]. Smaller pores—below 50 μm—tend to result in denser ECM formation but can restrict vascular penetration. Achieving a uniform pore distribution with high interconnectivity is essential for nutrient transport and waste removal, particularly in thicker constructs.

Type I collagen is most often used in dermal scaffolds because of its tensile strength and strong compatibility with dermal fibroblasts [17]. The molecular arrangement of collagen imparts piezoelectric properties, enabling the scaffold to generate small electrical charges when deformed [4]. This property can influence cell behaviour and works synergistically with applied electrical stimulation [6].

Mechanical stability is also critical. Scaffolds must maintain their architecture under physiological strain yet remain compliant enough to move with surrounding tissue. Cross-linking strategies, such as EDC/NHS treatment, can increase stiffness and slow degradation [18]. In electroactive designs, controlled cross-linking can improve piezoelectric output by preserving fibrillar alignment [4].

As previously mentioned, functional modifications can further enhance scaffold performance. Conductive polymers, including polypyrrole and PEDOT:PSS, can be incorporated to create conductive networks within the collagen matrix [6]. Ionomeric modifications, such as protein sulfonation, increase ionic conductivity and hydration, improving electromechanical responsiveness without compromising collagen's bioactivity [2].

2.3.1.2 Scaffold design

Scaffold architecture influences both mechanical behaviour and cellular interactions. Anisotropic designs, achieved through techniques such as directional freezing, can align collagen fibrils and pores along a preferred axis [5]. This alignment guides fibroblast elongation and influences the orientation of newly deposited ECM, promoting organised tissue regeneration.

In electroactive constructs, scaffold design also affects electrical field distribution. Uniform pore alignment can improve ionic conduction across the structure, leading to more consistent stimulation of resident cells [2]. Embedding fine conductive meshes or patterned electrodes within the scaffold can focus stimulation to specific regions, such as the wound interface where fibroblast activation is most beneficial.

The elastic modulus must be optimised for the wound type. More compliant scaffolds may better match the low stiffness of delicate regenerating tissue, while stiffer scaffolds are suited for load-bearing or high-strain environments. In piezoelectric scaffolds, modulus also affects voltage generation during movement, requiring a balance between mechanical support and electromechanical sensitivity [4].

2.3.1.3 Scaffold fabrication techniques

Freeze-drying is one of the most common methods for producing collagen scaffolds [17]. By freezing a collagen suspension and sublimating the ice under vacuum, a porous structure is formed. Slower freezing rates produce larger pores, while directional freezing can align pores for anisotropic properties. These features influence tissue integration and, in electroactive designs, ionic conduction [2].

Electrospinning produces fibrous scaffolds that mimic the nanoscale architecture of native ECM [5]. Collagen can be electrospun alone or blended with synthetic polymers to improve fibre formation. Fibre diameter, alignment, and surface texture can be tuned to influence both mechanical and electrical performance. Adding conductive nanofillers during electrospinning enables hybrid fibres that directly transmit electrical stimuli to attached fibroblasts [6].

Three-dimensional bioprinting allows precise spatial control over scaffold geometry and composition. Collagen-based bioinks can be reinforced with other biopolymers and co-printed with conductive inks to integrate electrode patterns directly into the scaffold [2]. It is of note that while 3D bioprinting offers unparalleled resolution, it is a slow process, limiting its applicability for the manufacture of large scalable structures.

Other methods include plastic compression, which produces dense, robust constructs by expelling water from hydrated gels, and gas foaming, which generates interconnected pores without organic solvents [17]. Each fabrication method offers distinct trade-offs in porosity, mechanical integrity, and scalability.

2.3.2 Films

Collagen films are thin, dense layers produced by casting collagen solutions and drying under controlled conditions [17]. Their continuous surface makes them well suited as wound contact layers, barrier membranes, or encapsulating interfaces for electrodes in electroactive dressings. While less porous than scaffolds, films can be engineered to support cell adhesion and influence fibroblast behaviour through surface topography and biochemical cues.

2.3.2.1 Film properties and composition

Tensile properties of collagen films can be adjusted by varying collagen concentration, cross-linking density, and hydration [18]. Highly cross-linked films are stiffer and more durable, whereas lightly cross-linked films retain flexibility. Surface microgrooving can align fibroblasts and influence ECM organisation [5].

For electroactive uses, films can be coated with conductive polymers such as PEDOT:PSS to provide surface conductivity suitable for low-voltage stimulation [6]. Alternatively, ionomeric modifications can increase ionic conductivity and water retention, enhancing their performance as ionic actuators or conductive interfaces [2].

Films can also be functionalised with bioactive peptides to improve cell adhesion or growth factor binding, allowing them to actively participate in tissue regeneration [17].

2.3.2.2 Film design

Collagen film design balances protective and regenerative roles. Dense films protect against bacterial invasion but may require perforations or blends to improve oxygen and moisture permeability [17].

In electroactive applications, films can encapsulate electrodes, preventing direct metal–tissue contact while permitting field penetration. Patterned electrode arrays embedded within films allow spatially controlled stimulation to guide fibroblast migration or ECM deposition [2].

Multilayer designs can integrate multiple functions—for example, an electroactive collagen base layer delivering stimulation beneath a hydrogel layer that maintains moisture and cushioning [6]. Surface treatments such as plasma modification or chemical grafting can adjust wettability, charge density, and protein adsorption, influencing cell attachment and proliferation [5].

2.4 Fibroblast Response to Collagen Constructs

Fibroblasts are the primary cell type responsible for producing and remodelling the dermal extracellular matrix during wound healing and skin regeneration [17]. Their behaviour—migration, proliferation, and secretion of structural proteins—is highly sensitive to both the biochemical composition and physical organisation of their substrate. Collagen constructs, whether in scaffold or film form, provide a biologically relevant environment that can modulate fibroblast activity through ligand presentation, mechanical cues, and, in some cases, electrical stimulation [2].

2.4.1 Without Electrical Stimulation

In the absence of applied electrical fields, fibroblast response to collagen constructs is governed by surface chemistry, topography, stiffness, and porosity [17]. Type I collagen, the dominant structural protein in skin, presents integrin-binding sites such as the GFOGER motif, which promote stable cell attachment *via* focal adhesion complexes. Engagement of these receptors triggers intracellular signalling pathways, including FAK and MAPK cascades, which regulate proliferation and ECM production.

Fibroblast infiltration is significantly affected by pore size and geometry. Scaffolds with pore diameters exceeding 100 μm facilitate deeper cell penetration and enhanced proliferation, whereas smaller pores are associated with denser extracellular matrix (ECM) accumulation near the scaffold surface [17]. Additionally, high pore interconnectivity promotes efficient nutrient diffusion and waste elimination, thereby supporting cell viability in thicker tissue constructs.

Fibre orientation affects fibroblast morphology and function. Anisotropic collagen arrangements, created through directional freezing or electrospinning, can align fibroblasts along a preferred axis [5]. This promotes organised ECM deposition and helps restore mechanical anisotropy in regenerated skin. Aligned fibrils also influence cytoskeletal organisation, affecting migration patterns and rates.

Mechanical stiffness of the substrate is another determinant of fibroblast behaviour. Compliant scaffolds, with moduli similar to native dermis, promote higher motility but can yield a less organised collagen network in new tissue [5]. Stiffer constructs encourage stress fibre formation and can increase collagen type I synthesis, although excessive stiffness may drive a fibrotic phenotype. Cross-linking methods, such as EDC/NHS treatment, allow tuning of stiffness and degradation rate [18].

Surface modifications can enhance fibroblast activity. Coating collagen films with fibronectin or laminin increases adhesion and spreading, while incorporating peptide sequences such as RGD stimulates integrin-mediated signalling [17]. Controlled degradation of the construct can release bioactive collagen fragments, which act as chemotactic signals to recruit fibroblasts to the wound site.

Hydration state also plays a role. Fully hydrated scaffolds maintain a moist environment that supports fibroblast viability and proliferation, whereas drying can limit migration and reduce ECM synthesis [17]. Scaffold swelling alters mechanical properties over time, dynamically influencing cell–matrix interactions.

2.4.2 With Electrical Stimulation

When collagen constructs are combined with electrical stimulation—through embedded electrodes, conductive coatings, or piezoelectric activity—fibroblast responses can be significantly altered [3]. Electrical fields influence orientation, migration speed, and gene expression, particularly for ECM-related proteins.

One key mechanism is galvanotaxis, the directed migration of cells in response to an electrical field. Fibroblasts generally migrate toward the cathode under direct current (DC) stimulation [3]. This migration involves redistribution of membrane receptors and reorganisation of the actin cytoskeleton, processes affected by field strength and exposure time. Collagen scaffolds with conductive elements can deliver such fields locally, aligning migration patterns with scaffold architecture [2].

Electrical stimulation can also enhance proliferation and collagen synthesis. Moderate DC fields have been shown to upregulate type I collagen and fibronectin gene expression in fibroblasts, especially when the substrate provides complementary biochemical cues [3]. Piezoelectric collagen scaffolds generate

small electrical signals under mechanical deformation, potentially mimicking natural electromechanical signals in tissue [4].

The design of the construct determines field distribution and magnitude. Uniform pore orientation and conductive pathways help achieve even field penetration, avoiding hotspots that could cause inconsistent cell behaviour [2]. Conductive coatings, such as PEDOT:PSS, applied to collagen films can deliver stimulation across their surface, promoting cell alignment and increased ECM production [6].

Electrical stimulation can also affect fibroblast differentiation. Under certain conditions, fibroblasts transition toward a myofibroblast phenotype, marked by α-smooth muscle actin (α-SMA) expression and increased contractility [3]. While beneficial for wound contraction, excessive myofibroblast activity risks promoting fibrosis, so stimulation parameters must be carefully controlled.

Electrical cues can indirectly shape the scaffold microenvironment. In ionomeric collagen matrices, electrostimulated ion movement can improve local pH control, growth factor distribution, and nutrient transport [2]. These effects may enhance cell viability and uniform tissue formation.

Piezoelectric and ionomeric modifications provide distinct stimulation modalities. Piezoelectric scaffolds respond to mechanical deformation with voltage generation, making them useful in dynamic wound sites such as joints or facial skin [4]. Ionomeric modifications respond to applied electrical inputs with mechanical deformation and ionic flow, allowing precise temporal control over stimulation [6]. Both approaches combine structural support with active bioelectrical signalling in a single platform.

By integrating biochemical ligands, mechanical compliance, and electrical stimulation, collagen constructs can be tuned to create an optimal fibroblast microenvironment. This multi-modal design approach has the potential to accelerate wound closure, improve ECM quality, and reduce scarring [2].

2.5 Material Characterisation and Production Methods

2.5.1 Films

Collagen films provide a planar, controllable interface for studying and delivering bioelectrical cues while maintaining biochemical compatibility with dermal fibroblasts. Unlike highly porous scaffolds, films prioritise surface presentation and electrode integration over bulk infiltration, which makes them attractive as wound-contact layers, electrode encapsulants, and electroactive laminates in composite dressings. Their performance depends on three tightly coupled aspects: how they are produced, how their electroactive and capacitive properties are quantified at low, biologically relevant voltages, and how their hydrated mechanical response is tuned to withstand handling and skin-level strain without compromising interfacial biology. Taken together, these parameters define whether a collagen film can operate as a stable, low-voltage electroactive component while preserving ligand availability for cell attachment and signalling. [19]

2.5.1.1 Production Method

Collagen films are typically produced by solution casting from acidic suspensions followed by controlled drying and post-processing to stabilise fibrillar networks. Casting onto inert, low-adhesion substrates and evaporating under regulated humidity encourages uniform thickness while limiting

surface skinning that can entrap residual solvent. After drying, films are commonly cross-linked to improve wet stability and adjust degradation and stiffness. Carbodiimide chemistry (EDC/NHS) is widely used because it forms zero-length amide bonds without introducing bulky bridges; importantly, EDC-mediated cross-linking can be tuned to preserve critical binding motifs for integrin-mediated recognition while still raising modulus and lowering solubility. [20]

Alternative stabilisation routes include photochemical methods. Ultraviolet treatment has been optimised to conserve cell-binding sites while increasing resistance to hydrolysis, providing a route where ligand presentation is prioritised alongside mechanical reinforcement—particularly useful for films intended as wound-contact interfaces. [21] Natural cross-linkers (e.g., genipin) offer another lever; genipin reacts with primary amines to create blue-tinted networks, generally increasing toughness and reducing enzymatic degradation with a lower propensity for cytotoxic aldehydes than some synthetic agents. [22]

At the molecular and fibrillar scales, the processing history governs how triple helices assemble into fibrils and how fibrils pack in the plane of the film. Nanoscale testing has shown that native and cross-linked type I collagen fibrils exhibit distinct force–extension behaviours, highlighting why cross-link density and distribution must be matched to the intended strain regime in use. [23] When films are envisaged as barrier-electrode laminates or as the active layer in ionic actuators, the production protocol typically includes mild neutralisation and rinsing to remove residual reactants that could screen charges, followed by hydration to equilibrate the ion content that underpins ionic conductivity during subsequent characterisation. [24]

Finally, production must anticipate downstream biological selectivity. Cross-linking regimes that elevate stiffness can inadvertently mask or modify specific collagen binding sites. Systematic work has shown how carbodiimide parameters alter recognition by collagen-binding receptors, providing a practical map for selecting formulations that retain cell attachment while achieving the targeted wet strength—guidance that is directly applicable to the processing of dense films. [25]

2.5.1.2 Electrical & Capacitive Characterisation

Electroactive films are evaluated under low-voltage stimuli to confirm that the material can respond at amplitudes compatible with cell culture and potential clinical use. A first tier of measurements quantifies bending or in-plane strain in response to direct current (DC) or low-frequency waveforms; these quasi-static tests probe electromechanical coupling arising from ionic transport or dipolar reorientation within the hydrated collagen network. Background principles and test fixtures for such measurements follow the conventions used across electroactive polymers, with emphasis on low-voltage actuation and large-strain sensitivity. [26]

Because hydrated collagen behaves as a weak polyelectrolyte network, electrical characterisation also includes frequency-dependent impedance spectroscopy. This captures capacitive storage from interfacial polarisation and mobile counter-ion accumulation, as well as resistive pathways through the water-rich phase. Methodologically, electroactive polymer practice recommends reporting magnitude/phase over several decades of frequency together with fitted equivalent circuits when the objective is to compare formulations or cross-link densities. [24] Collagen's intrinsic polar structure adds a separate contribution: dipolar mechanisms linked to the non-centrosymmetric arrangement of triple helices can participate in the response, and the relative importance of these intrinsic contributions increases as the network becomes more ordered or is restricted by cross-links. [27]

Shear piezoelectric measurements—often implemented with specialised jigs or scanning probe methods—are used to isolate the collagen-specific contribution to electromechanical coupling. Chemical cross-linking has been shown to increase shear piezoelectric coefficients in collagen by

promoting bundle self-assembly and restricting molecular mobility, which enhances dipole alignment under load. This provides a rationale for formulations that simultaneously target mechanical robustness and stronger signal generation. [28] For biomedical relevance, studies of conductive and electroactive scaffolds demonstrate that adding an electrical cue at modest amplitudes can influence cell behaviour; while those studies frequently use different base polymers, the same low-voltage, frequency-aware characterisation logic translates to collagen films designed for contact-delivery of fields. [29]

In sum, a coherent electrical characterisation workflow for collagen films comprises (i) low-voltage actuation tests to map displacement–voltage–frequency space, (ii) impedance spectroscopy to separate capacitive and resistive channels within the hydrated matrix, and (iii) targeted piezoelectric assays where relevant, interpreted in light of cross-linking-induced changes in collagen packing. This approach aligns with best practice for electroactive polymers and captures the specificities of collagen's hierarchical polar structure. [30]

2.5.1.3 Mechanical Characterisation

Mechanical testing of collagen films proceeds in the hydrated state to reflect use conditions. Uniaxial tension provides the tensile modulus, ultimate tensile strength, and strain-to-failure needed to design around handling and in-service loads. At the fibril level, native versus cross-linked collagen shows distinct stiffness and failure transitions, which scale up to film-level behaviour as cross-link density increases. [23] Photochemical routes that conserve binding sites can maintain better cell recognition while still raising wet stability, an attractive compromise when films serve as the cell-facing layer in a dressing or bioreactor insert. [21]

EDC-mediated coupling typically increases modulus and reduces extensibility by forming additional inter- and intrafibrillar bridges; beyond mechanical effects, this chemistry can alter the presentation of collagen motifs recognised by cell receptors, which has implications for mechanotransduction at the film surface. Detailed analyses of carbodiimide cross-linking reveal how processing parameters map to changes in cellular recognition—results that should be considered alongside tensile data when selecting a formulation for skin-contact devices. [20] Broader overviews of cross-linking strategies emphasise that mechanical outcomes are interwoven with biochemical and transport properties: increasing cross-link density reduces enzymatic degradation and swelling but can impede diffusion and dampen viscoelastic energy dissipation if over-applied. [25]

Because electroactivity and mechanics are coupled in collagen, mechanical characterisation should be interpreted alongside electrical assays. Cross-linking and induced self-assembly can enhance piezoelectric output by ordering fibrillar bundles, offering a route to higher signal levels without external conductive additives. However, any stiffening that improves piezoelectric response must not compromise conformability or create stress concentrations at the skin interface. Studies demonstrating enhanced piezoelectricity after chemical cross-linking underline this trade-off and motivate reporting both modulus and shear piezoelectric coefficients for a given film chemistry. [28] At the device level, practical benchmarks drawn from electroactive polymer actuators remind us to target low-voltage, repeatable strain with materials that tolerate cyclic loading—criteria that collagen films can meet when cross-linking and hydration are tuned appropriately. [26]

Finally, when films are incorporated into multilayer constructs (e.g., as an electroactive laminate coupled to a compliant hydrogel), additional tests such as peel, lap-shear, and cyclic bending become relevant. Although these methods are general to soft-material assemblies, their interpretation in collagen systems must account for the time-dependent swelling and relaxation typical of hydrated biopolymers, phenomena well recognised in the electroactive polymer literature. [24]

2.5.2 Scaffolds

Scaffolds represent one of the most widely used collagen constructs for applications in wound healing and regenerative medicine. Their three-dimensional, porous architecture mimics the extracellular matrix (ECM), providing a physical and biochemical environment that supports cell infiltration, proliferation, and extracellular matrix deposition [12]. The inherent versatility of collagen as a structural and bioactive material makes it particularly well suited for scaffold fabrication. Unlike films, which offer planar and relatively dense interfaces, scaffolds provide interconnected pores that facilitate nutrient exchange, vascularisation, and the establishment of organised tissue structures [28]. In electroactive systems, scaffolds also serve as a unique platform where mechanical, electrical, and ionic cues can be integrated into the regenerative process, leveraging both collagen's intrinsic piezoelectricity and engineered functionalisation strategies [2], [6].

2.5.2.1 Production Method

The production of collagen scaffolds encompasses a variety of physical, chemical, and hybrid techniques, each of which influences pore morphology, fibrillar alignment, and mechanical performance. Freeze-drying remains the most commonly applied method for generating porous scaffolds. In this process, a collagen suspension is frozen and then subjected to sublimation under vacuum, leaving behind a porous matrix. The rate of freezing directly governs pore size: slower freezing results in larger pores, while rapid freezing yields finer pores [12]. Importantly, pore interconnectivity can be tuned by adjusting the cooling profile, a factor that strongly impacts cell migration and vascular infiltration. Directional freezing has emerged as a particularly effective modification, producing anisotropic pore channels that not only mimic native tissue anisotropy but also provide guidance cues for fibroblast alignment [28].

Electrospinning offers an alternative method to create fibrous scaffolds with nanoscale features similar to native ECM. Collagen can be electrospun alone or blended with synthetic polymers, such as polycaprolactone (PCL) or polylactic acid (PLA), to enhance fibre formation and mechanical robustness [20]. Parameters such as solution viscosity, applied voltage, and collector speed control fibre diameter and orientation, enabling precise tailoring of scaffold architecture. Electrospun collagen scaffolds can be engineered to promote anisotropic properties, where aligned fibres support directional cell growth and guide ECM organisation, features that are particularly advantageous in cutaneous regeneration [28].

Plastic compression represents another technique by which hydrated collagen gels are compacted into dense, mechanically stable scaffolds. By expelling excess water under applied pressure, this method produces highly cellularisable scaffolds with improved strength compared to unmodified hydrogels [13]. Although less porous than freeze-dried scaffolds, compressed gels can still support fibroblast infiltration while offering superior mechanical resilience. Gas foaming, by contrast, introduces porosity through the generation of gas bubbles within a collagen suspension, producing interconnected pore networks without the use of organic solvents [12].

More advanced techniques such as three-dimensional (3D) bioprinting have further expanded the design space for collagen scaffolds. Bioinks composed of collagen, gelatine, or alginate can be extruded into patient-specific architectures with spatial control over pore size and geometry [28]. Bioprinting also allows for the co-deposition of conductive or electroactive inks, integrating electrodes or ionic pathways directly into the scaffold [2]. The resulting constructs are not only mechanically and biologically relevant but also capable of providing targeted electrical stimulation to cells during regeneration.

Irrespective of the fabrication method, cross-linking remains a critical step in scaffold production. Both chemical (e.g., EDC/NHS, glutaraldehyde, genipin) and enzymatic (e.g., transglutaminase, lysyl oxidase) cross-linking approaches are employed to stabilise the collagen network [13]. Cross-linking enhances mechanical integrity, slows enzymatic degradation, and increases thermal stability. In electroactive contexts, cross-linking can also improve piezoelectric output by aligning dipoles within fibrillar structures, thereby amplifying the scaffold's capacity to convert mechanical deformation into electrical stimuli [4].

2.5.2.2 Electrical and Capacitive Characterisation

Electrical characterisation of collagen scaffolds focuses on both their intrinsic piezoelectric response and the influence of engineered modifications that enhance conductivity or ionic mobility. Native collagen exhibits piezoelectricity due to its non-centrosymmetric triple-helical structure and quarter-staggered fibril packing [4]. Under mechanical deformation, such as stretching or compression, aligned collagen fibrils generate local electrical potentials. These piezoelectric signals, though modest in magnitude, are believed to contribute to mechano-electrical signalling *in vivo*, where they may influence fibroblast orientation, gene expression, and ECM synthesis [3].

For electroactive wound healing devices, quantifying the piezoelectric coefficient of scaffolds is essential. Measurement techniques typically involve dynamic mechanical testing coupled with charge collection, where strain-induced voltages are recorded across electrodes interfaced with the scaffold. Reported values for collagen piezoelectric constants vary widely depending on hydration state, fibrillar alignment, and cross-link density, but modifications such as directional freezing or electrospinning can enhance the anisotropic response [28].

Beyond intrinsic properties, collagen scaffolds can be functionalised to improve electrical performance. Incorporation of conductive polymers such as polypyrrole or PEDOT:PSS into the scaffold matrix introduces percolating conductive networks that support charge transfer [3]. These composites exhibit reduced impedance and improved current distribution under applied fields, enhancing the uniformity of electrical stimulation delivered to resident fibroblasts [6]. Ionomeric modifications, achieved *via* protein sulfonation, increase ionic conductivity by introducing fixed charge groups that facilitate ion migration [2]. When subjected to electrical input, these ionomeric scaffolds act as ionic actuators, producing coupled electro-chemo-mechanical responses that can dynamically modulate the wound environment.

Capacitive behaviour is another important property, particularly for scaffolds intended to store and release charge during stimulation. Porous collagen constructs with high surface area demonstrate measurable capacitance, which can be enhanced by blending with conductive nanomaterials such as carbon nanotubes or graphene oxide [20]. These hybrid scaffolds combine structural biocompatibility with enhanced electrical responsiveness, enabling both passive piezoelectric signalling and active capacitive stimulation. The ability to store electrical energy within the scaffold may permit pulsed or localised delivery of stimulation, reducing the need for continuous external power.

A key challenge in electrical characterisation lies in balancing conductivity with biocompatibility. Excessive loading of conductive polymers or nanoparticles can reduce porosity and impair cell infiltration, while insufficient functionalisation may lead to weak or inconsistent stimulation. Strategies that integrate nanoscale conductive phases within a primarily collagen matrix appear most promising, allowing fibroblasts to engage with a biologically relevant substrate while still experiencing electrical cues [28].

2.5.2.3 Mechanical Characterisation

Mechanical characterisation of collagen scaffolds is crucial, as mechanical performance determines both their durability under physiological strain and their ability to transmit electrical signals generated by piezoelectric deformation. The tensile strength, compressive modulus, and viscoelastic properties of scaffolds depend on their pore structure, cross-linking density, and hydration state. Type I collagen fibrils provide high tensile strength at the nanoscale, but scaffold-level performance is strongly affected by porosity: larger pores reduce stiffness and strength, while smaller pores increase density but may restrict cell migration [12].

Freeze-dried scaffolds generally exhibit relatively low moduli, often in the range of tens to hundreds of kilopascals, due to their high porosity. Mechanical reinforcement can be achieved by chemical cross-linking, which increases stiffness and reduces creep behaviour [13]. Enzymatic cross-linking offers a more biocompatible alternative, though the extent of mechanical improvement may be limited compared to synthetic agents. Electrospun collagen scaffolds, by contrast, often display higher tensile properties, particularly when fibres are aligned, as alignment permits load transfer along the fibre axis [28]. Plastic compression also produces scaffolds of significantly greater stiffness, making them more suitable for applications where mechanical strength is a priority.

Dynamic mechanical analysis (DMA) is commonly used to evaluate viscoelastic properties, measuring storage and loss moduli across a range of frequencies. These parameters are important in understanding how scaffolds will behave under cyclic loading, such as joint motion or facial expression, which are common in dermal applications. Importantly, viscoelastic behaviour also influences piezoelectric output: scaffolds with appropriate damping properties can convert mechanical energy into sustained electrical signals rather than dissipating it as heat [4].

Scaffold degradation kinetics must also be considered alongside mechanical properties. Collagen constructs are gradually resorbed by enzymatic activity *in vivo*, and this degradation must be matched to the rate of new tissue formation. Cross-linking slows degradation, but excessive stabilisation may impede integration and remodelling [13]. Balancing degradation and mechanical performance are therefore essential in scaffold design.

Mechanical testing under hydrated conditions provides the most physiologically relevant data, as hydration dramatically reduces stiffness compared to dry measurements. Hydrated scaffolds more closely replicate the compliance of native dermis, supporting fibroblast proliferation and ECM deposition [12]. Moreover, hydration influences electroactive behaviour, as ion mobility within the scaffold increases, enhancing electromechanical coupling in ionomeric systems [2].

Composite strategies can further enhance mechanical properties. Incorporating synthetic polymers such as PLA or PCL increases stiffness and durability, while retaining a collagen-rich surface to support bioactivity [20]. Alternatively, blending collagen with glycosaminoglycans (GAGs) introduces additional bio functionality and modifies hydration-dependent mechanics. In electroactive systems, conductive nanofillers not only improve electrical behaviour but can also reinforce scaffold strength, provided they are well dispersed [6].

2.6 Conclusions and guiding questions

Collagen-based constructs remain central to the development of advanced wound healing and skin rejuvenation technologies. Their versatility in composition, architecture, and functionalisation allows fine control over fibroblast behaviour and ECM organisation, whether in the form of porous scaffolds or dense films. Section 2.1 outlined the clinical contexts in which such constructs can be applied, from open wounds to burns and scarring, while Sections 2.2 and 2.3 examined the structural, biochemical, and mechanical properties of collagen and how these can be engineered into functional devices.

Scaffolds offer a highly porous, three-dimensional environment conducive to cell infiltration and vascularisation [17]. Their properties can be tuned through fabrication methods, cross-linking, and incorporation of conductive phases [2], enabling synergy between mechanical support and electrical stimulation. Films, while less porous, provide planar, mechanically robust interfaces that can be patterned, coated, or layered for tailored bioactivity and electroactive performance [6].

Fibroblast response to these constructs depends on a combination of biochemical signals, mechanical cues, and, where applied, electrical stimulation. Without stimulation, responses are driven by factors such as pore size, fibril alignment, stiffness, and surface ligand presentation [17], [5]. With stimulation, additional behaviours such as galvanotactic migration, increased collagen synthesis, and controlled differentiation toward contractile phenotypes can be elicited [3], [4]. Both piezoelectric and ionomeric modifications to collagen provide opportunities for integrating structural and bioelectrical functions into a single regenerative platform [2], [6].

Despite significant advances, several critical questions remain:

1. **Optimising electroactive parameters** — What are the optimal electrical field strengths, frequencies, and durations for stimulating fibroblasts *in vivo* without inducing fibrosis or excessive contraction?
2. **Balancing degradation and function** — How can degradation rates of collagen constructs be tuned to match tissue regeneration timelines while maintaining mechanical integrity and electroactive performance?
3. **Scaffold–film hybrids** — Can hybrid systems combining the porosity of scaffolds with the barrier properties of films deliver superior regenerative outcomes compared with single-format devices?
4. **Personalisation of architecture** — How can patient-specific wound geometry and mechanical requirements be incorporated into construct design using advanced manufacturing methods such as 3D bioprinting?
5. **Long-term bioelectrical integration** — To what extent can piezoelectric or ionomeric collagen constructs sustain stable electrical properties over extended implantation periods in dynamic wound environments?

Addressing these questions will be key to refining collagen-based electroactive devices for clinical use, ensuring that both the biological and electrical aspects of these constructs are tuned for optimal regenerative performance. The next chapter will outline the experimental approach taken to investigate these areas.

3. Methods and Materials

This chapter describes the materials, fabrication procedures, and experimental protocols employed to develop and evaluate collagen–taurine electroactive constructs. The aim was to generate reproducible

films and scaffolds with defined structural, mechanical, and electrical properties, and to test their performance in *ex vivo* skin models.

The first sections outline the preparation of collagen slurries, film and scaffold fabrication, and chemical crosslinking strategies. This is followed by the description of specimen preparation for mechanical testing (compression and tensile assays) and electroactive characterisation (impedance spectroscopy and actuation studies). Finally, the methodology for *ex vivo* wound and burn experiments on human skin off-cuts is presented, including specimen preparation, treatment protocols, and evaluation of closure.

Together, these methods establish the experimental foundation for assessing the suitability of collagen–taurine electroactive materials as potential wound dressings and skin rejuvenation platforms.

3.1 Fabrication

3.1.1 Collagen slurry fabrication

Suspensions of type I Achilles bovine collagen (Sigma-Aldrich, C9879) and taurine (Sigma-Aldrich, T0625) were successfully prepared by hydration in 0.05 M acetic acid (Thermo Fisher Scientific, 13552310) on a roller bed at 4 °C for 12 hours. The hydrated suspensions exhibited a uniform viscous appearance with no evidence of visible phase separation. Subsequent homogenisation using a T 10 basic ULTRA-TURRAX homogeniser for 10 minutes at speed level 6 produced a fine collagen slurry with consistent turbidity and without observable aggregates. The resulting slurries were stable at 4 °C for at least 7 days, maintaining homogeneity and suitable viscosity for downstream processing.

3.1.2 Fabrication of Collagen Films

Collagen films were produced from the homogenised slurries by drop-casting 5 g of the mixture onto 5 cm × 5 cm square anti-static weighing boats. The cast slurries were left to dry under ambient conditions on an orbital shaker for 48 hours. This procedure yielded uniform, thin films with a smooth surface appearance and consistent thickness across replicates. No visible cracking or delamination was observed during the drying process. The dried films were subsequently subjected to crosslinking to enhance mechanical integrity and stability for subsequent testing.

3.1.3 Crosslinking of Collagen Films

To improve structural stability, the dried collagen films were crosslinked using a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) solution. The linking solution was prepared in 75% ethanol at a mass ratio of 1.15 : 0.276 : 1 (EDC:NHS:collagen), corresponding to a molar ratio of 5:2:1 for EDC:NHS:COO^-, assuming 1.2 moles of collagen contain 1 mole of carboxylate groups[25],[31]. This solution was designated the 100% concentration solution.

Films were fully immersed in the EDC–NHS solution for 2 hours at room temperature under constant agitation (240 rpm). Following crosslinking, all samples were washed three times in deionised water (5 min soak followed by a rapid rinse) to remove residual reagents.

For comparative analysis, four film groups were generated:

C: collagen only (control)

CT: unlinked collagen–taurine

CE: EDC–NHS crosslinked collagen

CTE: EDC–NHS crosslinked collagen–taurine

The resulting films displayed improved handling properties post-linking, with CTE films showing greater rigidity relative to CT, while maintaining surface uniformity

3.1.4 Fabrication of Collagen Scaffolds

Collagen scaffolds were fabricated using the same slurry preparation method described in Section 4.1.1. A total of 8 mL of the homogenised slurry was pipetted into each well of a 6-well plate, serving as moulds for scaffold formation.

The samples were subjected to freeze-drying using a VirTis adVantage Plus system. The freeze-dryer shelf was pre-cooled to –20 °C, and the filled moulds were frozen overnight for a minimum of 12 hours. To compare the influence of freezing conditions, additional samples were pre-frozen externally at –70 °C and –20 °C before transfer to the freeze-dryer.

Following the freezing phase, scaffold mould lids were removed to permit vapour escape, and lyophilisation was initiated. The drying step was carried out at a vacuum pressure of 450 mTorr for 26 hours. The resulting scaffolds were observed to retain structural integrity with uniform porous architecture, and no visible collapse or surface cracking was noted.

3.1.5 Crosslinking of Collagen Scaffolds

To enhance structural stability, collagen scaffolds were crosslinked using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). EDC (MW 191.7 $g \cdot mol^{-1}$) and NHS (MW 115.09 $g \cdot mol^{-1}$) were obtained from Thermo Fisher Scientific, while taurine (≥99.5% purity, Bio Ultra grade) was purchased from Sigma-Aldrich.

A 75% ethanol solution was prepared as the solvent system, to which EDC and NHS were added to achieve a final cross-linking concentration of 60%. Taurine was incorporated at a weight ratio of 100% relative to collagen. Each scaffold was fully immersed in 10 mL of the prepared crosslinking solution and left to soak for 2 hours under static conditions. Following immersion, scaffolds were washed thoroughly in deionised water (three sequential rinses) to remove residual reagents.

The washed scaffolds were then subjected to a second freeze-drying cycle (–20 °C, 450 mTorr, 26 hours) to ensure complete removal of solvent and stabilisation of the porous architecture.

Four scaffold groups were generated for subsequent analysis:

C: collagen only (control)

CT: unlinked collagen–taurine

CE: EDC–NHS crosslinked collagen

CTE: EDC–NHS crosslinked collagen–taurine (experimental group)

The resulting scaffolds exhibited well-preserved macroporous structures and improved handling properties post-linking, particularly in the CTE group, which retained integrity during compression testing preparation.

3.2 Compression Testing of Collagen Scaffolds

3.2.1 Preparation of Cylindrical Test Specimens

Following freeze-drying, the scaffolds obtained directly from the 6-well plates exhibited variation in overall size and geometry. To ensure consistency for mechanical testing, cylindrical specimens were extracted using a 5 mm biopsy punch. Each punched scaffold was subsequently trimmed by hand to a target height of 4 mm. Due to manual cutting, slight variation in height was observed, with final samples ranging from 3.8 to 4.2 mm in height. These dimensions were used to normalise stress–strain calculations for the determination of compressive modulus. Prior to testing, the specimens were **hydrated in water** to reproduce physiological conditions and simulate in-use application on skin.

3.2.2 Compression Test Protocol

Compression tests were conducted using an Anton Paar MCR 302e Rheometer. Each scaffold cylinder was positioned centrally on the lower plate, and compression was applied at a constant displacement rate of 100 $\mu m \cdot s^{-1}$. A total of 1000 steps were recorded per run, continuing until 100% compression of the scaffold height was achieved. Force–displacement data were continuously logged and subsequently converted to engineering stress–strain curves. The compressive modulus was determined from the initial linear region of each curve (0–10% strain).

3.3 Tensile Testing of Collagen Films

3.3.1 Preparation of Film Specimens

For tensile testing, collagen-based films (C, CE, CT, and CTE) were cut into a **dog-bone geometry (Figure 1)** to minimise grip-edge effects and encourage failure within the central gauge region. Each specimen measured **40 mm in total length**, with **20 mm wide gripping ends** tapering symmetrically to a **10 mm waist** at the centre. Prior to testing, strips were **hydrated in water** to reproduce physiological conditions and simulate in-use application on skin.

Because the cutting of the strips was performed manually, slight **dimensional variability** occurred between samples, particularly in waist width and taper smoothness. These variations were accounted for during stress–strain analysis by normalising the engineering stress to the measured cross-sectional area of each specimen.

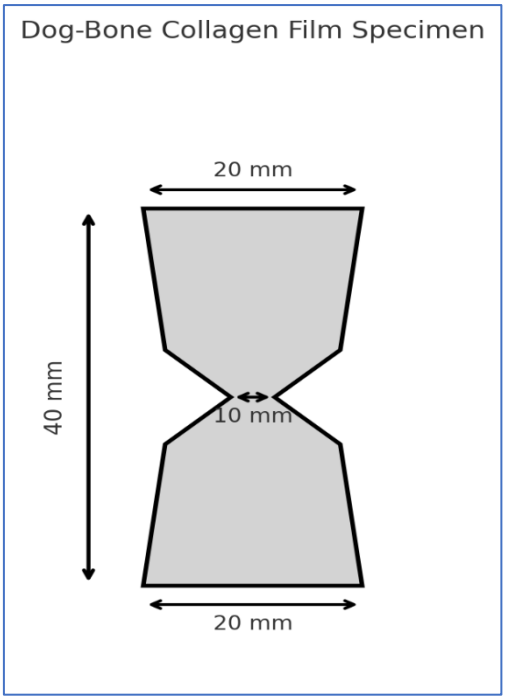

***Figure 1**: Dog-bone collagen film specimen with dimensions (mm).*

3.3.2 Tensile Test Protocol

Tensile testing of collagen-based films was performed using an **Anton Paar MCR 302e Rheometer** equipped with a custom gripping assembly. Each film formulation (C, CE, CT, and CTE) was tested in triplicate. Specimens were mounted vertically between a 3D-printed jig designed to interface with the rheometer and a weighted base, with crocodile clips glued to both fixtures serving as grips (Figure 2).

Each test consisted of **500 steps** applied at a constant extension rate of **–2000 µm·s⁻¹** until specimen failure. Force–displacement data were continuously recorded and converted to engineering stress–strain curves based on the measured cross-sectional area of each specimen. From these curves, the **Young's modulus** was calculated as the slope of the initial linear region (0–5% strain), while the **ultimate tensile strength (UTS)** and **elongation at break** were determined from the maximum stress and corresponding strain at failure.

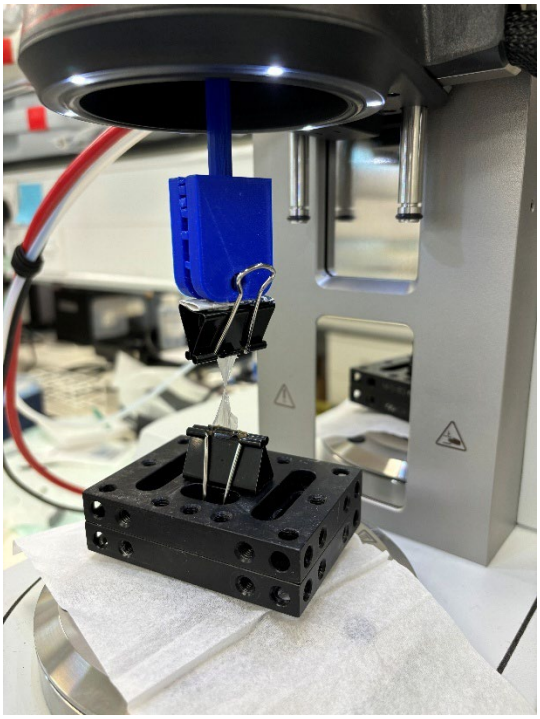

***Figure 2**: Custom tensile test set-up showing collagen film specimen mounted in crocodile-clip grips attached to the Anton Paar MCR 302e Rheometer.*

3.4 Electrical Characterisation of Collagen Films

3.4.1 Preparation of Film Specimens

For electroactive testing, collagen-based films (C, CE, CT, CTE) were first hydrated by immersion in **1× phosphate-buffered saline (PBS, Thermo Fisher Scientific Inc.)** for 5 minutes. Prior to mounting, the films were gently blotted with an absorbent paper towel to remove excess surface water while maintaining internal hydration.

Hydrated films were clamped between copper electrodes, leaving a **15 mm free length** from the clamped end for actuation measurement. A **5 V DC potential** was applied at room temperature between two **2.5 mm × 2.5 mm copper electrodes**.

To improve electrical contact, films measuring **10 mm × 30 mm** were coated on both sides with **100 µL of silver conductive paint (Electrolube, RS Components)** using a porous sponge. Once coated, the films were sectioned into strips of **5 mm × 20 mm**, with edges trimmed to prevent direct electrode–electrode contact.

3.4.2 Electrical Characterisation Test Protocol

Electroactive testing of collagen films (C, CE, CT, CTE) was conducted under hydrated conditions using an impedance spectroscopy approach. A **5 V DC potential** was applied across each specimen, and the frequency was reduced in steps from **4 MHz down to 10 Hz**. At each frequency point, both **impedance magnitude (Ω)** and **phase angle (°)** were measured.

Each film formulation was tested in triplicate ($n = 3$), with a total of **202 frequency points recorded per run**. Data were exported as frequency–impedance and frequency–phase spectra for further analysis.

3.5 *Ex-Vivo* wound experiments

3.5.1 Preparation of *Ex Vivo* Skin Models

Human skin off-cuts were obtained as surgical waste from the through the Oxford Musculoskeletal Biobank (REC09/H0606/11+5). The tissue was sourced from a single patient and was received in irregularly shaped fragments. To standardise sample geometry, an 8 mm biopsy punch was first used to create uniform circular pieces. A second 4 mm biopsy punch was then applied by hand to introduce a full thickness wound to an approximate depth of 1 mm, producing a reproducible defect while accounting for manual variation.

Prepared skin specimens were transferred into a 24-well culture plate and soaked in supplemented culture medium to maintain tissue hydration and viability prior to treatment with collagen films.

3.5.2 Application of Collagen Films

Each collagen film formulation (C, CE, CT, CTE) was tested in triplicate ($n = 3$ per group) using prepared *ex vivo* skin specimens. In addition, three untreated specimens served as controls. For each treatment, a 5 mm × 5 mm section of the respective film was hydrated and carefully placed over the wounded area, ensuring full coverage of the 4 mm biopsy-induced defect.

Films were left in place for a total of 5 days under continuous culture conditions without substitution or external stimulation. Culture medium was replenished as required to maintain specimen hydration. Throughout the experimental period, the collagen films remained adherent to the wound surface, permitting assessment of wound closure and tissue regeneration.

3.6 Ex-Vivo burn experiments.

3.6.1 Preparation of *Ex Vivo* Burn Models

Uniform skin specimens were prepared from human surgical off-cuts using an 8 mm biopsy punch. Thermal burn injuries were generated by applying a soldering iron with a 4 mm tip, pre-heated to 120 °C, and pressed against the tissue surface for 30 seconds. This procedure produced consistent circular burns of approximately 4 mm in diameter.

Following injury induction, the burned specimens were transferred to a 16-well culture plate containing supplemented medium to maintain tissue hydration. Experimental groups were established as follows:

- **Control** (untreated burns, $n = 3$)
- **CE** (crosslinked collagen films, no stimulation, $n = 3$)
- **CTE** (crosslinked collagen–taurine films, no stimulation, $n = 3$)
- **CE + stimulation** (crosslinked collagen films with electrical stimulation, $n = 3$)
- **CTE + stimulation** (crosslinked collagen–taurine films with electrical stimulation, $n = 3$)

Films were hydrated prior to application and placed over the burn site immediately after injury induction. Electrical stimulation protocols are described in Section 4.6.2.

3.6.2 Application of Collagen Films

Hydrated 5 mm × 5 mm collagen films (CE or CTE) were applied directly over the burn site immediately following injury induction. For groups receiving electrical stimulation, two probes were positioned in contact with the film surface and connected to a controlled pulse generator. The stimulation protocol consisted of a 1 Hz train of 100 ms pulses at 5 V DC, followed by 900 ms at 0 V, applied for 2 hours per day on alternate days.

The total duration of the burn healing experiment was 8 days, during which films remained in place without substitution. Burn area was measured macroscopically at Day 0 and Day 8 to assess closure.

4 Results

This study sought to evaluate the mechanical, electrical, and biological performance of collagen-based electroactive films and scaffolds for wound healing and skin rejuvenation applications. Following fabrication and crosslinking of collagen and collagen–taurine films, a series of characterisation experiments were performed. Tensile and actuation tests were used to determine film mechanical properties and electroactivity, while compression testing of collagen scaffolds provided insight into their Young's modulus relative to native human skin. Electrical conductivity and actuation responses were assessed to confirm suitability for electrical stimulation. Finally, *ex vivo* wound and burn healing assays were conducted on human skin off-cuts to investigate the regenerative efficacy of the films.

4.1 Compression Testing of Collagen Scaffolds

The compressive modulus of the scaffolds was determined from the slope of the initial linear region (0–10% strain) of the stress–strain response. The mean values obtained from three replicates per formulation are summarised in Table 4.2, with representative average curves shown in Figure 3.

Formulation	Compressive Modulus (kPa) (mean ± SD)
C (collagen only)	17.3 ± 4.8
CE (crosslinked collagen)	8.5 ± 2.9
CT (collagen–taurine, unlinked)	10.3 ± 2.4
CTE (crosslinked collagen–taurine)	16.0 ± 3.0

All scaffold types displayed the characteristic J-shaped stress–strain behaviour typical of hydrated, porous biomaterials, with a gradual increase in stress at low strain and a rapid rise approaching failure (Figure 1). CE scaffolds exhibited the lowest compressive modulus, reflecting increased compliance after crosslinking. CT scaffolds showed slightly higher stiffness relative to CE, while incorporation of taurine combined with crosslinking (CTE) restored mechanical properties closer to the control collagen scaffolds (C). These findings indicate that taurine plays a role in preserving mechanical integrity when scaffolds are subjected to chemical crosslinking.

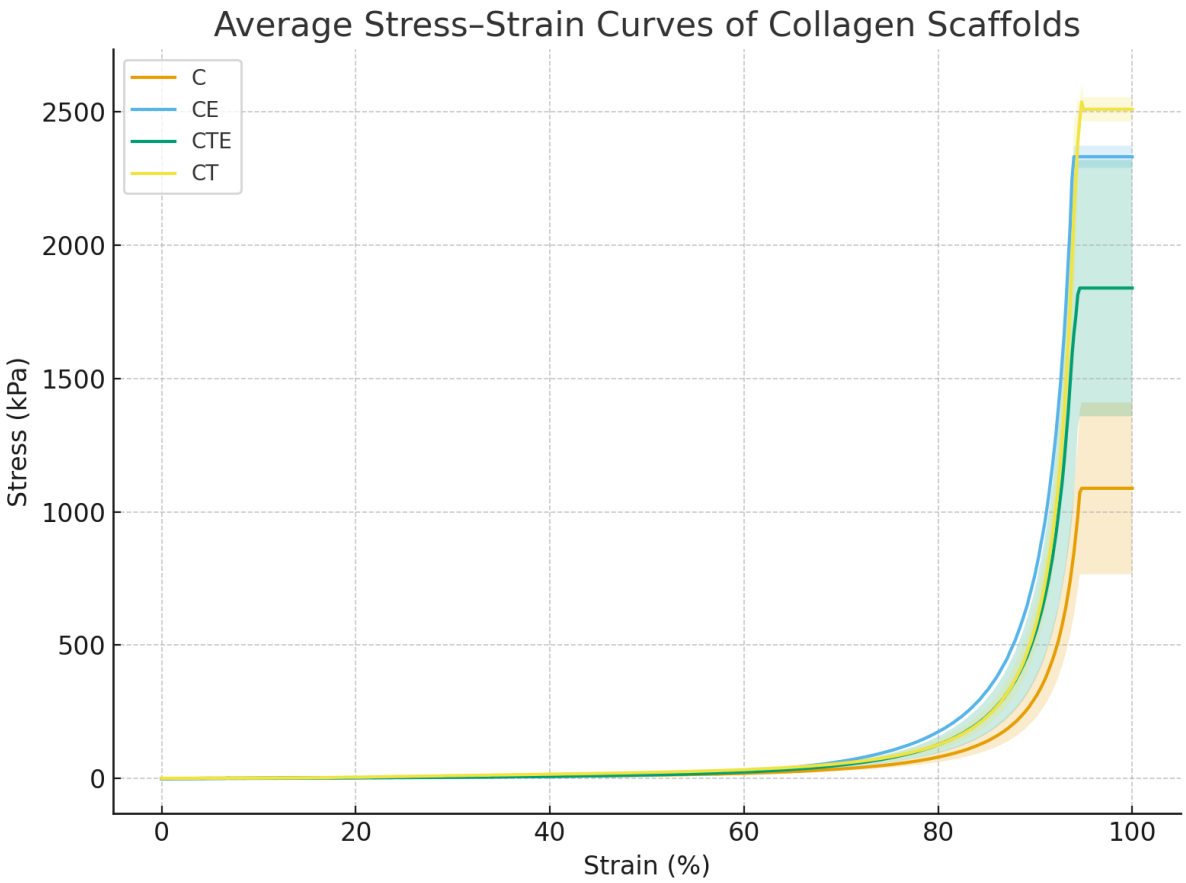

***Figure 3**: Average stress–strain curves of collagen scaffold groups (C, CE, CT, CTE) under uniaxial compression. Shaded regions represent standard deviation across three replicates.*

4.2 Tensile Testing of Collagen Films

The tensile properties of collagen films were evaluated by calculating the **Young's modulus** (slope of the initial linear region of the stress–strain curve) and the **elongation at break**. During preliminary analysis, the very early part of the stress–strain curves (<1% strain) exhibited irregularities due to **instrumental noise at extremely low loads**. This artefact was particularly pronounced because of the thin nature of the films (~10 µm thickness) and the sensitivity of the rheometer at low force levels.

To address this, a **filtering approach** was applied:

- The stress–strain data were smoothed using a moving average to reduce point-to-point noise.
- The modulus was calculated over a broader window (0–10% strain), but with **all data points below 0.01 MPa (10 kPa) stress excluded** to remove the low-load artefacts.
- This ensured that only the mechanically relevant portion of the loading curve contributed to modulus estimation, while elongation at break was calculated directly from the maximum strain attained at specimen failure.

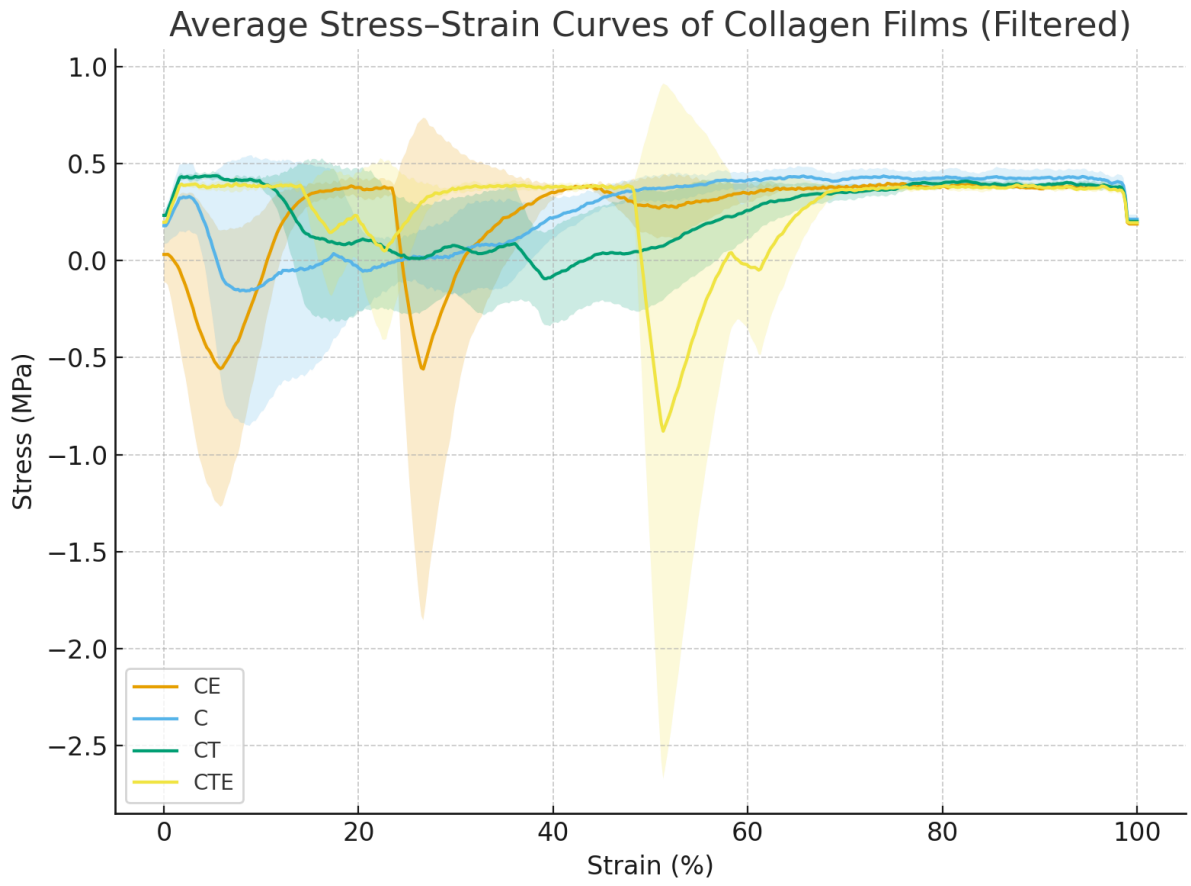

***Figure 4**: Average stress–strain curves of collagen film groups (C, CE, CT, CTE) under uniaxial tensile testing. Shaded regions represent standard deviation across three replicates.*

The results (mean ± SD from $n = 3$ per group) are summarised in the following table:

Formulation	Young's Modulus (MPa, mean ± SD)	Elongation at Break (%)
C (collagen only)	0.07 ± 1.38	99.0 ± 0.0
CE (crosslinked collagen)	0.47 ± 0.00	99.0 ± 0.0
CT (collagen–taurine, unlinked)	0.64 ± 0.04	99.0 ± 0.0
CTE (crosslinked collagen–taurine)	0.81 ± 0.28	99.0 ± 0.0

All film types demonstrated high extensibility, with elongation at break consistently reaching ~99% strain under hydrated conditions (Figure 4). Among the groups, **CTE films** showed the highest stiffness (0.81 MPa), indicating that taurine incorporation combined with EDC–NHS crosslinking improved tensile properties. **CT films** also exhibited greater stiffness than controls, while **CE films** provided only modest reinforcement compared with untreated collagen **C**. The variability observed in C films likely reflects their increased fragility and susceptibility to premature tearing.

4.3 Electrical Characterisation of Collagen Films

Electroactive behaviour of the collagen films was characterised by impedance spectroscopy over the frequency range 4 MHz to 10 Hz. Representative spectra are shown in **Figure 5**. Each formulation (C, CE, CT, CTE) was tested in triplicate, and mean values ± standard deviation were calculated. Outlier capacitance values arising from noise at isolated frequency points were excluded from analysis to ensure consistent comparison.

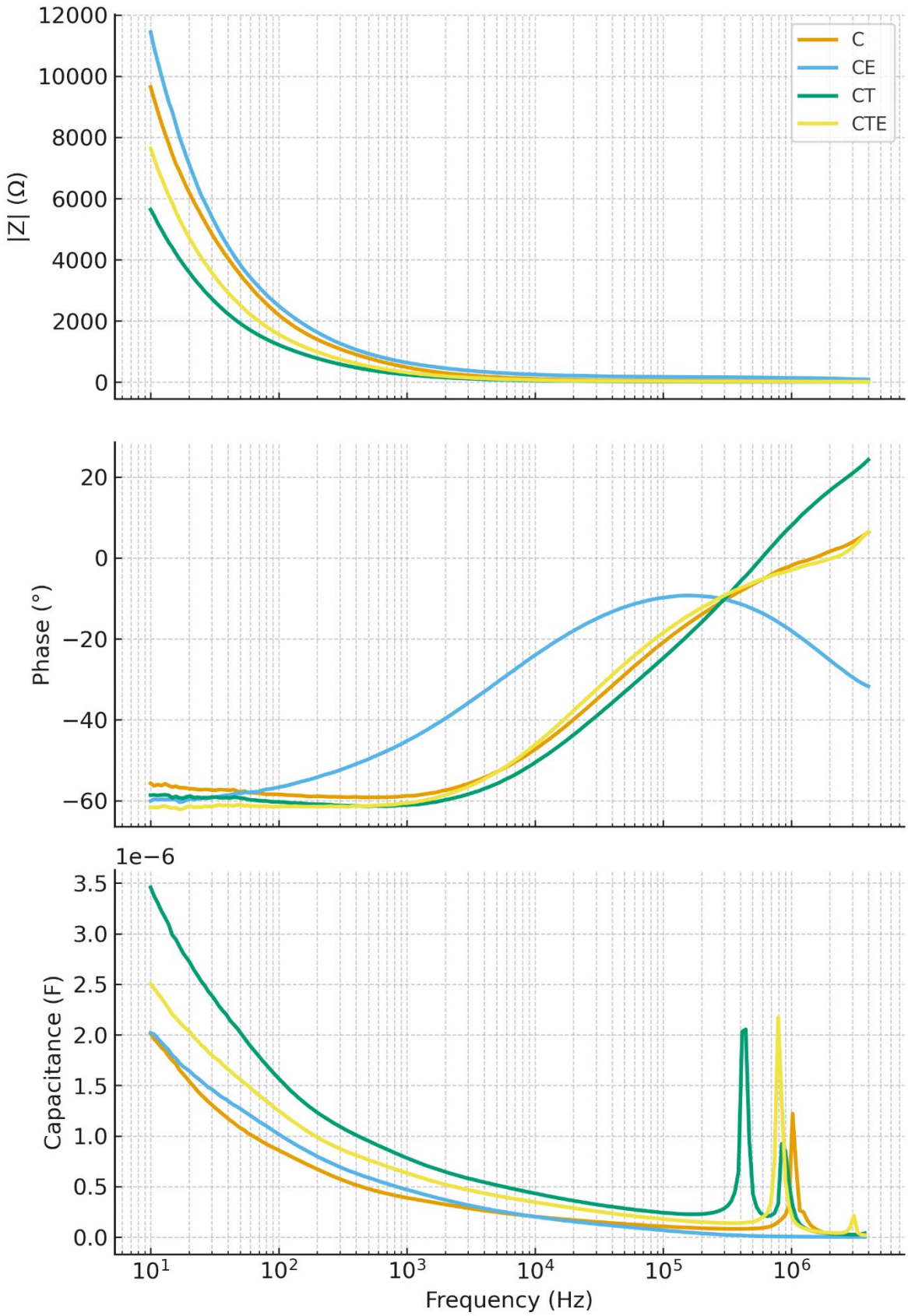

Figure 5. Electrical characterisation of collagen-based films under hydrated conditions by impedance spectroscopy.
(A) Impedance magnitude (|Z|) as a function of frequency,

(B) Phase angle (°) as a function of frequency, and
(C) Capacitance as a function of frequency after removal of outlier points.

Data represent mean responses for each formulation (C, CE, CT, CTE) averaged across three replicates. Error shading omitted for clarity. All formulations exhibited frequency-dependent electrical behaviour, with impedance decreasing at higher frequencies and capacitance peaking in the low-to-mid frequency range. Taurine-containing films (CT, CTE) showed enhanced capacitance relative to C and CE, consistent with improved dielectric storage capacity.

Across all formulations, impedance decreased with increasing frequency, consistent with ionic conduction through hydrated collagen matrices. Phase angle spectra revealed frequency-dependent capacitive behaviour, with characteristic relaxation points evident where the phase approached –45°. Capacitance showed a peak in the low-to-mid frequency range, followed by a gradual decline as frequency increased.

Key electrical properties derived from the spectra are summarised in the following table:

Formulation	|Z| at 10 Hz (Ω, mean ± SD)	|Z| at 4 MHz (Ω, mean ± SD)	Relaxation Freq. (Hz, mean ± SD)	Peak C (F, mean ± SD)	Freq. of peak C (Hz, mean ± SD)
C	9645 ± 824	37.1 ± 16.5	15.2k ± 7.7k	$3.0\times10^{-6} \pm 8.7\times10^{-7}$	346k ± 490k
CE	11,438 ± 6125	90.9 ± 53.2	1068 ± 243	$2.0\times10^{-6} \pm 7.8\times10^{-7}$	10.2 ± 0.3
CT	5639 ± 1164	19.5 ± 6.5	24.5k ± 19.3k	$5.0\times10^{-6} \pm 2.2\times10^{-6}$	427k ± 350k
CTE	7638 ± 1964	21.5 ± 5.4	11.4k ± 3.1k	$4.0\times10^{-6} \pm 2.6\times10^{-6}$	268k ± 379k

4.4 *Ex-Vivo* wound experiments

Macroscopic wound closure was assessed by comparing wound area at Day 0 and Day 5. The photographs below exhibit the progression of the specimen from Day 0 (**Figure 6**) to Day 5 (**Figure 7**).

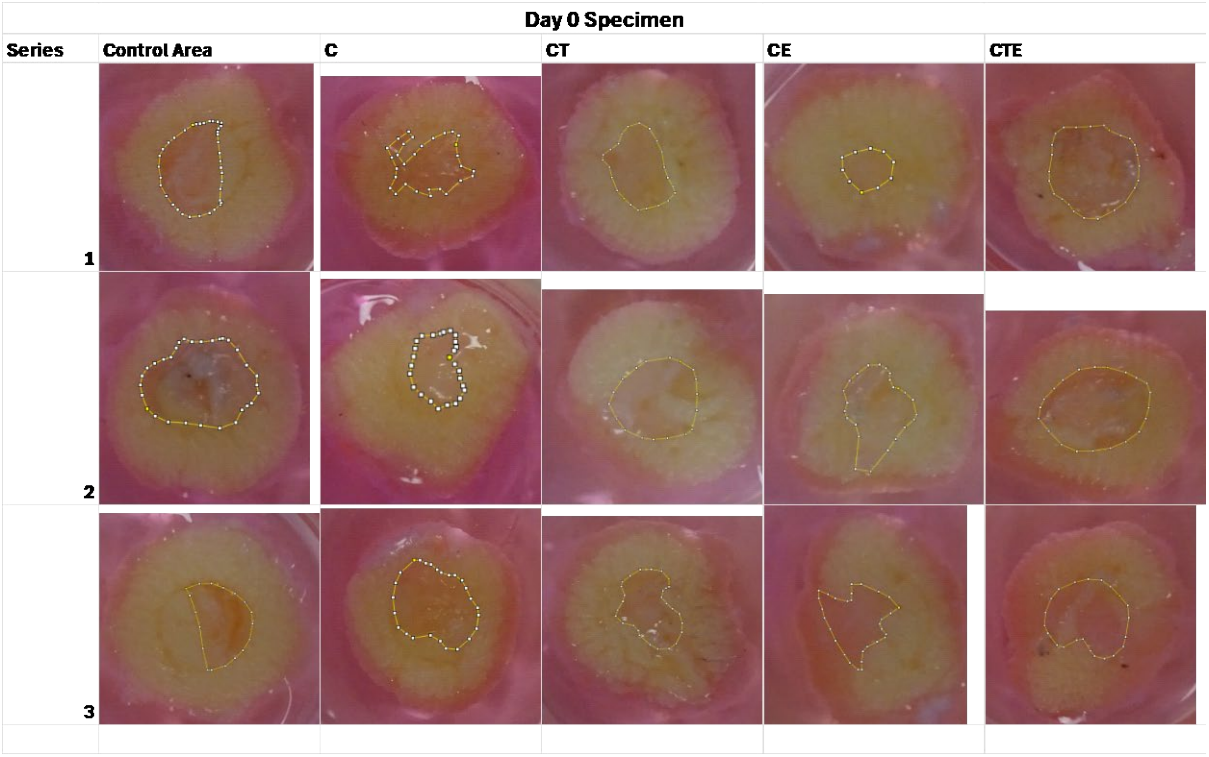

Figure 6: *Photographs of skin off-cuts in well plates across three series of Control, C, CT, CE and CTE. The dotted line indicates the wound area.*

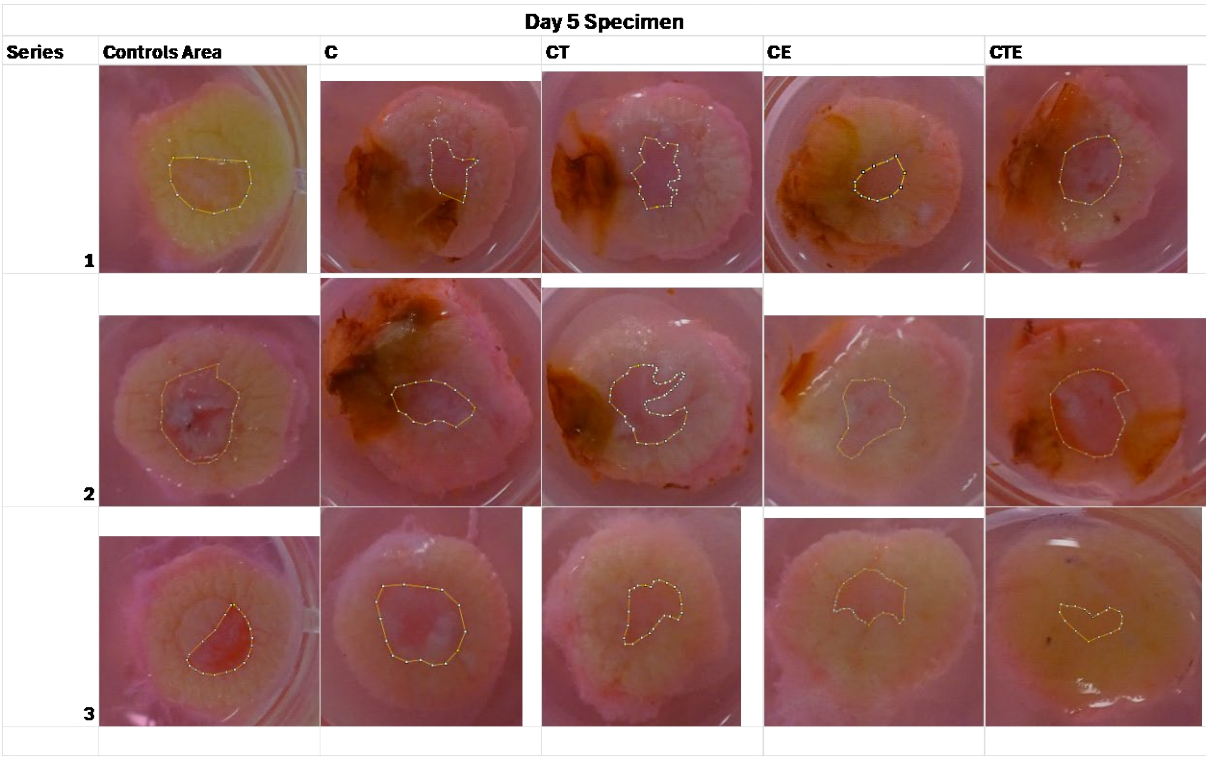

Figure 7: *Photographs of skin off-cuts in well plates across three series of Control, C, CT, CE and CTE. The dotted line indicates the wound area.*

The results are expressed as percentage change in wound area relative to baseline, where negative values indicate wound closure in **Figure 8**.

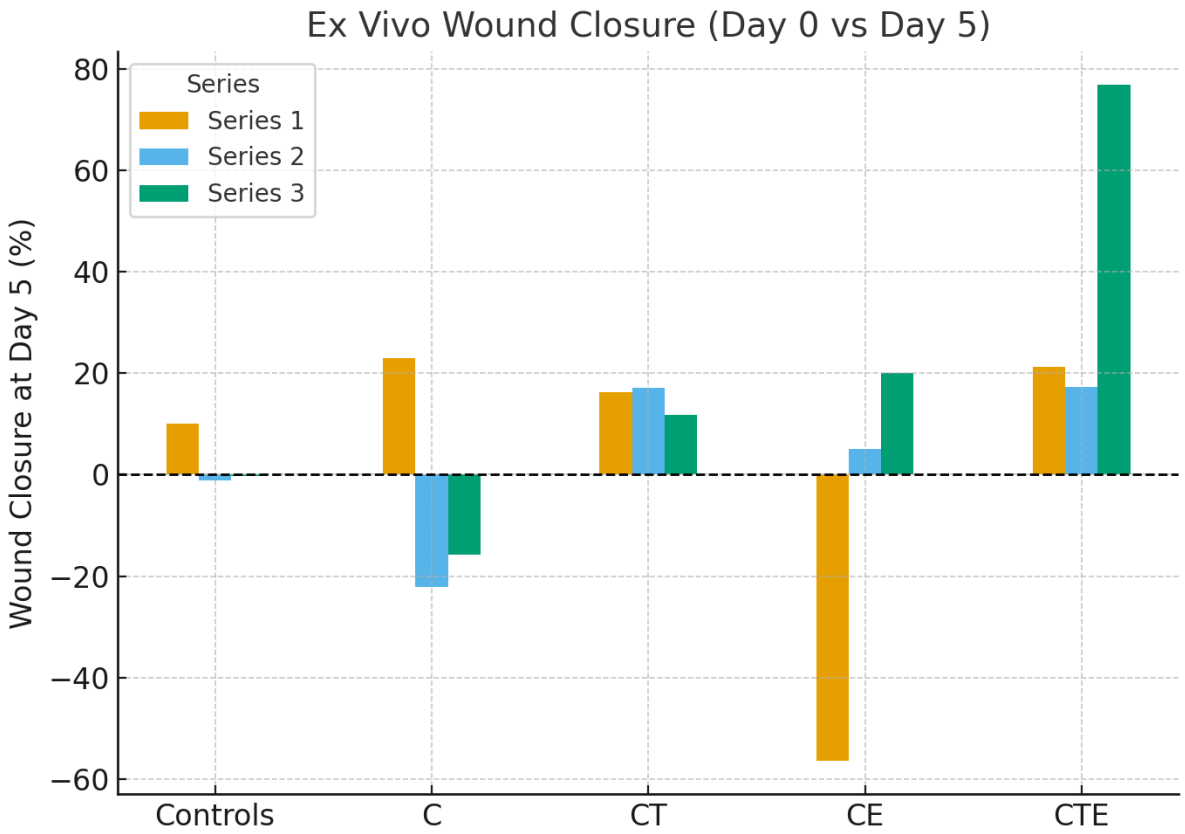

***Figure 8**: Percentage wound closure at Day 5 relative to Day 0 for ex vivo skin specimens treated with collagen films (C, CE, CT, CTE) or untreated controls. Bars represent individual specimens (n = 3 per group).*

The grouped bar chart shows the outcomes for three series of skin samples across all formulations (Controls, C, CE, CT, CTE). Considerable variability was observed both within and between formulations. In some series, wounds enlarged over the 5-day period (positive values), while others demonstrated partial closure (negative values).

Overall, taurine-containing films (CT, CTE) tended to show greater closure compared to controls and collagen-only films (C), although the effect was inconsistent across series. CE films exhibited the highest variability, with some series showing wound enlargement rather than closure. Controls and C films demonstrated negligible closure on average.

The variability in results can be explained by several factors:

1. **Unknown vintage of the surgical off-cuts**, which may have affected tissue viability.
2. **Unknown health status of the donor skin**, as all samples were derived from a single patient.
3. **Manual wound creation**, which introduced differences in wound size and depth despite using biopsy punches.

4.5 Ex-Vivo burn experiments.

Burn area change was assessed macroscopically at Day 0 and Day 8. Results are expressed as percentage change in wound area, where negative values represent burn closure (reduction in wound size), and positive values represent enlargement (**Figure 9**).

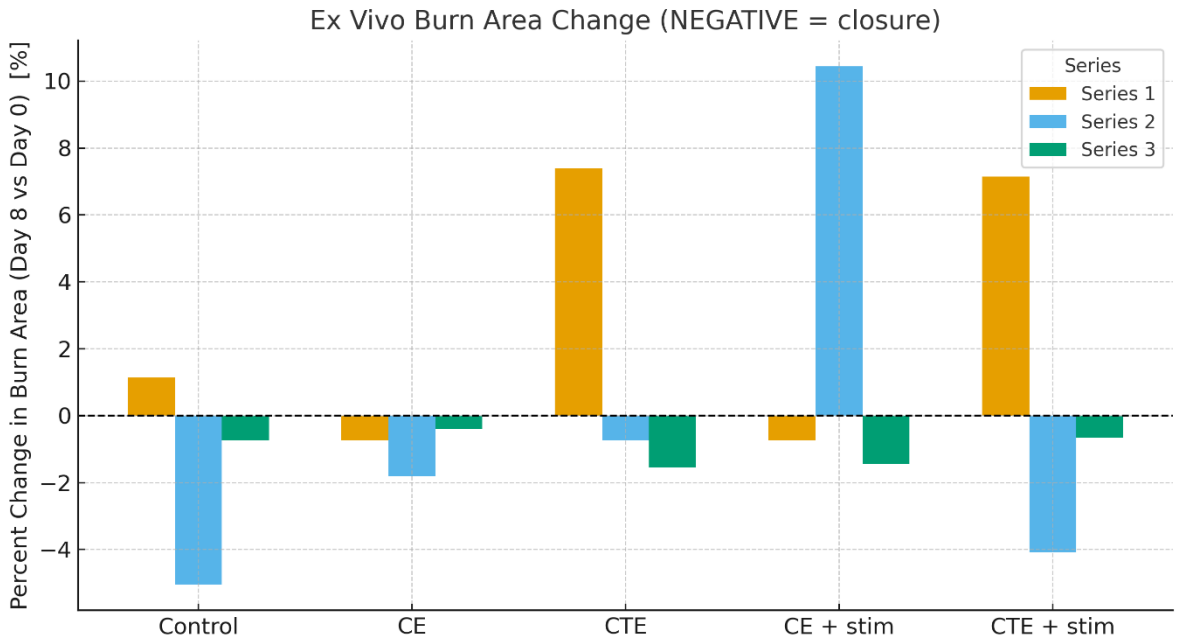

***Figure 9**: Percentage wound closure at Day 8 relative to Day 0 for ex vivo skin specimens treated with collagen films (CE, CTE, CE w/Stimulation, CTE w/stimulations) or untreated controls. Bars represent individual specimens (n = 3 per group).*

Across all groups, **considerable variability** was observed between series.

- **Controls** showed minimal healing capacity, with two series demonstrating slight closure (–5.1% and –0.7%) while one enlarged slightly (+1.1%). This indicates that the *ex vivo* burn model does not spontaneously heal in the absence of treatment.
- **CE films (no stimulation)** produced very small closure across all series (–0.7% to –1.8%), suggesting that crosslinked collagen alone does not strongly influence burn contraction in this model.
- **CTE films (no stimulation)** demonstrated greater closure in two series (–1.6% and –1.6%), but enlargement in the third (+7.4%). This indicates a potential benefit of taurine incorporation for promoting contraction, although the effect is inconsistent.
- **CE with electrical stimulation** exhibited the most variable outcomes, ranging from strong enlargement (+10.4%) to modest closure (–1.4%). This suggests that electrical stimulation may interact unpredictably with CE films in the burn model, possibly due to variability in tissue conductivity or electrode–tissue contact.
- **CTE with electrical stimulation** produced closure in two series (–4.1% and –0.7%) but enlargement in one (+7.1%). These results suggest that taurine-containing films may respond more consistently to stimulation than CE films, but variability still limits reproducibility.

Overall, the results suggest that **taurine-containing films (CTE) tend to support burn closure more effectively than CE**, and that **electrical stimulation can enhance closure in some cases but not consistently**. Variability in outcomes likely reflects:

1. The ***ex vivo* nature of the model**, with limited intrinsic regenerative capacity.
2. **Donor- and series-specific differences**, including unknown tissue viability and health.
3. **Experimental variability** in generating uniform thermal injuries and electrode contact during stimulation.

5 Discussion

This study investigated the fabrication, characterisation, and functional testing of collagen-based electroactive films and scaffolds for potential use in wound healing and skin rejuvenation. Type I bovine collagen was combined with taurine and stabilised through EDC–NHS crosslinking to generate four principal material groups: **C** (collagen only), **CE** (crosslinked collagen), **CT** (collagen–taurine), and **CTE** (crosslinked collagen–taurine). These were assessed for their mechanical, electrical, and biological properties in *ex vivo* skin models.

5.1 Mechanical Properties

Compression testing of scaffolds demonstrated that CTE scaffolds achieved stiffness values comparable to native skin, while CE scaffolds were notably more compliant. Specifically, CTE scaffolds reached an average compressive modulus of ~16 kPa, similar to reported ranges for human skin under compression (20–30 kPa for dermis perpendicular to the surface) [32], [33]. In contrast, CE scaffolds exhibited a mean modulus of ~8.5 kPa, placing them at the lower end of physiological relevance. This indicates that taurine incorporation partially offsets the reduction in stiffness caused by crosslinking.

Tensile testing of collagen films showed a parallel trend. CT and CTE films achieved Young's modulus values of 0.64 ± 0.04 MPa and 0.81 ± 0.28 MPa, respectively, whereas C films (0.07 MPa) and CE films (0.47 MPa) were softer and more variable. These results place CT and CTE films within the lower-to-mid range of reported values for human dermis, which vary from 0.05–0.85 MPa depending on anatomical site, hydration, and measurement technique [33], [32]. Thus, taurine-containing films replicate the stiffness of human skin more closely than collagen-only or CE films, suggesting that taurine provides mechanical reinforcement while maintaining flexibility.

The high elongation at break (~99% strain for all films) also mirrors the extensibility of hydrated skin, which is typically capable of stretching by 30–100% depending on anatomical location [32]. This flexibility is important to prevent tearing and accommodate movement in a clinical setting.

Taken together, these results demonstrate that collagen–taurine films and scaffolds exhibit mechanical behaviour consistent with skin tissue, making them more suitable candidates for wound dressing applications than collagen-only or CE formulations.

5.2 Electroactive Properties

Impedance spectroscopy revealed frequency-dependent behaviour across all collagen film formulations, with **CT and CTE films** exhibiting lower impedance and higher capacitance compared to C and CE. At low frequency (10 Hz), CTE films showed an average impedance of ~7.6 K, while CT films measured ~5.6 kΩ, both lower than controls (~9.6 kΩ) and CE (~11.4 kΩ). At high frequency (4 MHz), impedance dropped to tens of ohms, reflecting ionic conduction through the hydrated collagen

matrix. Capacitance values peaked in the low-to-mid frequency range at ~4–5 μF for CT/CTE, compared to ~2–3 μF for C/CE, indicating that taurine incorporation enhanced dielectric storage.

These results can be contextualised by comparison to **human skin electrical properties**. The impedance of intact human skin has been reported in the **kΩ range at 10 Hz–1 kHz**, with values strongly influenced by hydration, anatomical site, and skin integrity[34]. Capacitance of skin is typically in the **μF range**, reflecting the dielectric contribution of the stratum corneum and hydrated dermis. At higher frequencies (>100 kHz), both skin and the collagen films exhibit decreasing impedance and phase angles approaching zero, consistent with ionic conduction dominating over capacitive behaviour.

The similarity between the impedance and capacitance profiles of **CT/CTE films** and reported values for hydrated human skin indicates that taurine incorporation not only enhances electroactivity but also helps replicate the dielectric properties of skin. This is particularly important for applications in **electrical stimulation therapies**, where the ability of the material to transmit electrical signals into tissue without excessive resistance or signal distortion is critical.

Overall, these findings demonstrate that **CT and CTE films more closely mimic the electroactive behaviour of skin** than C or CE films, reinforcing their potential as electroactive dressings capable of mediating electrical cues in wound healing.

5.3 *Ex Vivo* Wound Healing

In the *ex vivo* wound model, **taurine-containing films (CT, CTE)** demonstrated greater wound closure compared to controls and non-taurine films. **CTE films** achieved the most pronounced closure, though variability across series was evident. The variability likely reflects factors such as the vintage of surgical off-cuts, donor skin health, and challenges in creating consistent wounds by hand. Nevertheless, the trend supports the hypothesis that taurine, through its antioxidant and osmolyte properties, contributes positively to the wound healing microenvironment when delivered in a collagen matrix.

5.4 *Ex Vivo* Burn Healing

In the *ex vivo* burn model, untreated controls and CE films showed negligible closure. **CTE films** produced modest improvements, with some series demonstrating contraction of burn area. The addition of electrical stimulation produced mixed outcomes: while some series exhibited enhanced closure (up to –10%), others showed enlargement. This inconsistency may be attributed to the technical challenges of ensuring reproducible electrode–tissue contact in irregular burn lesions, coupled with variability in tissue viability. Despite these challenges, the observation that taurine-containing films with stimulation could outperform controls in certain cases highlights their potential for electrically assisted burn treatment.

6 Overall Conclusions

The combined results suggest that **collagen–taurine composites, particularly when crosslinked (CTE), offer the most promise** as electroactive biomaterials for skin regeneration. They demonstrate:

- **Mechanical compatibility** with skin tissue, particularly for scaffolds.
- **Enhanced electroactivity**, improving their suitability for electrical stimulation.
- **Improved wound closure** in *ex vivo* wound models compared to collagen-only controls.
- **Potential for burn treatment**, though optimisation of stimulation protocols and delivery systems is required to reduce variability.

While the variability in *ex vivo* experiments limits definitive conclusions, the data support the feasibility of **collagen–taurine electroactive films and scaffolds** as a foundation for next-generation wound dressings. Future work should focus on refining fabrication methods to improve reproducibility, optimising electrical stimulation regimes, and progressing toward *in vivo* validation to better capture the biological complexity of wound and burn healing.

6.1 Future Work

The findings of this dissertation highlight the potential of collagen–taurine electroactive biomaterials, while also underscoring several limitations and opportunities for further research. Future work should focus on the following areas:

1. Optimisation of Fabrication and Crosslinking
Although EDC–NHS crosslinking improved mechanical stability, it also introduced compliance in some formulations (e.g., CE scaffolds). Refining the crosslinking strategy, potentially through combination with enzymatic or physical methods (e.g., dehydrothermal treatment), could enhance reproducibility while maintaining biocompatibility. Additionally, improving control over film thickness and scaffold porosity would reduce variability in mechanical and electrical performance.

2. Mechanistic Studies of Taurine's Role
The beneficial effects of taurine incorporation were evident in both mechanical reinforcement and biological assays, yet the underlying mechanisms remain to be fully elucidated. Future studies should investigate whether taurine acts primarily through antioxidant protection, osmotic balance, or modulation of cell signalling. Controlled release assays, coupled with *in vitro* fibroblast/keratinocyte studies, would provide greater insight into how taurine influences healing pathways.

3. Electrical Stimulation Protocols
The *ex vivo* burn experiments revealed that stimulation outcomes were variable, likely due to electrode contact and tissue conductivity. Optimising stimulation parameters — including pulse duration, amplitude, frequency, and electrode configuration — will be critical for achieving reproducible results. Integration of flexible, conformable electrodes into the collagen films themselves may offer a more clinically translatable solution.

4. *In Vivo* Validation
Ex vivo skin models provide valuable insights but cannot replicate the full complexity of wound healing, including immune responses, angiogenesis, and systemic interactions. Progression to *in vivo* models (e.g., rodent or porcine wound/burn models) will be essential to validate the efficacy of collagen–taurine films and scaffolds under physiological conditions. These studies should include longitudinal assessment of healing rates, scar formation, and histological outcomes.

5. Clinical Translation Pathways
If validated *in vivo*, collagen–taurine electroactive films could be developed into advanced wound dressings for burns, chronic ulcers, and surgical wounds. Future translational research should evaluate manufacturability, sterilisation, storage stability, and regulatory considerations. Early-phase clinical trials could then establish safety, handling properties, and preliminary efficacy in human patients.

6. Integration with Bioelectronics
The electroactive properties demonstrated here open the possibility of pairing collagen–taurine films with wearable or implantable bioelectronic systems for real-time wound monitoring and on-demand stimulation. Developing such integrated devices would represent a significant advance toward personalised regenerative therapies.

In summary, future research should aim to refine fabrication, elucidate taurine's biological role, optimise electrical stimulation, and progress toward *in vivo* validation and clinical translation. These steps will determine whether collagen–taurine electroactive devices can fulfil their potential as multifunctional wound dressings capable of both structural support and bioelectrical modulation.

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