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Prospects for Improving Neovascularisation of the Ischaemic Heart: Lessons from Development

Nicola Smart

Dr Nicola Smart

BHF Ian Fleming Senior Basic Science Research Fellow

Department of Physiology, Anatomy & Genetics

University of Oxford

Sherrington Building

South Parks Road

Oxford

OX1 3PT

Tel: +44 (0)1865 282365

nicola.smart@dpag.ox.ac.uk

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ABSTRACT

Neovascularisation of the ischaemic myocardium post-infarction is necessary to restore blood flow to vulnerable cardiomyocytes and will be indispensable for prospective regenerative strategies, to perfuse newly formed myocardium. Therapeutic attempts to enhance new vessel formation have, to date, yielded modest clinical benefits and innovative approaches are now needed. Intrinsic mechanisms are initiated by the heart in an attempt to rebuild injured vessels but these are poorly understood. Insight into the underlying mechanisms may reveal targets for therapeutically augmenting this low-level neovascular response. Starting from a limited number of descriptive studies, this review summarises what is known of coronary neovascularisation and explores putative mechanisms and cellular sources which may endogenously contribute, or that may be pharmacologically triggered, to support vasculo- or angiogenesis. As injury responses in the adult frequently recapitulate embryological processes, a particular focus is placed on the developmental mechanisms of coronary vessel formation. An understanding of the cellular sources and the regulatory pathways used by the embryo may reveal novel targets for reactivating coronary vessel and myocardial regeneration.

KEYWORDS

Coronary vasculature; Neovascularisation; Epicardium; Sinus venosus; Endocardium

INTRODUCTION

Revascularisation therapies: an urgent unmet need

While cardiovascular diseases remain a major global cause of mortality and morbidity, therapeutic strategies to prevent myocardial infarction and to mitigate the consequences, in terms of cardiac regeneration and repair, are urgently required. Timely reperfusion therapy, by primary percutaneous coronary angioplasty or pharmacological thrombolysis, is the mainstay of ST-segment elevation myocardial infarction (MI) treatment, to limit myocardial loss, adverse remodelling and progression to heart failure. However, a hindrance to reperfusion therapy is the so-called "no–reflow" phenomenon. Now widely demonstrated in human patients and in animal models, "no-reflow" refers to the incomplete reperfusion of the microvascular bed despite adequate restoration of blood flow to the epicardial vessels (reviewed in [102]). Compromised microvascular integrity and patency result from ischaemic endothelial injury, leukocyte plugging and platelet activation upon interaction with damaged endothelium[33,56].

The limited success of pro-angiogenic growth factor therapy in clinical trials[41,116,158] has dictated the need for viable alternative strategies. As a starting point for such therapies, a fundamental understanding of the intrinsic responses of the coronary vasculature, as well as an appreciation of mechanisms that underlie the development (establishment) of the coronary vasculature in the developing embryo, may provide insight. This review summarises the literature, scant though it is, on the endogenous neovascularisation that occurs in the heart in response to ischaemic injury; the potential

underlying mechanisms will be explored, particularly to consider whether, and how, these may recapitulate embryonic coronary vessel development. Although the degree of regeneration achieved intrinsically is insufficient in the mammalian heart, endogenous "DIY repair" mechanisms may be amenable to therapeutic augmentation; developmental mechanisms may be pharmacologically redeployed to re-enact the formative mechanisms for purposes of regeneration in an injury setting.

The Coronary Vasculature

The efficiently functioning coronary vasculature comprises a hierarchical network of arteries, arterioles, capillaries, venules and veins[114], alongside a parallel network of lymphatic vessels[84,99]. Formation of the network requires a precisely orchestrated series of morphogenetic and molecular events which can be divided into three distinct, yet overlapping, processes: vasculogenesis, angiogenesis and arteriogenesis (reviewed in [13]). Vasculogenesis is the initial formation of primitive vascular structures from endothelial precursor cells; angiogenesis is responsible for network expansion by proliferation and migration of microvessel endothelial cells (ECs), either by intussusception, to divide the vessel in two or, by sprouting, to form new branches. Arteriogenesis describes the remodelling required to form mature arteries; the increased diameter of existing arterial vessels in response to enhanced flow and the resultant wall shear stress sensed by ECs is accompanied by acquisition of supporting pericytes and smooth muscle cells. Collateral growth, a specialized type of arteriogenesis, usually refers to the formation of mature arteries from pre-existing interconnecting anastomoses, following coronary artery occlusion. The hierarchical organisation of the coronary vasculature, and the discrete mechanisms that underlie its formation, should be considered in the design of therapeutic strategies. Simply

enhancing the number of small distal vessels cannot compensate for decreased flow in a proximal artery; conversely, restoring patency of epicardial vessels will remain ineffective if defects in the coronary microvasculature are not addressed. An optimal neovascular treatment, used in conjunction with reperfusion therapy, will likely require enhancement of collateralization as well as vasculogenesis/angiogenesis.

Attempts to Regenerate the Coronary Vasculature

Despite the promise of preclinical animal studies, angiogenic growth factor therapy demonstrated minimal efficacy for treatment of coronary artery disease in clinical trials. Notable examples include trials for the chief angiogenic actors, , vascular endothelial growth factor ($VEGF_{165}$; the VIVA trial)[41] and fibroblast growth factor (FGF)-2(the FIRST trial)[116], While numerous other angiogenic factors, including, by way of recent examples, neuropeptide-Y[112] and hepatocyte growth factor[34], have yielded significant new vessel growth in animal models of MI, success will not be assured if translated to aged human patients, with their associated comorbidities. Even if successful in stimulating sufficient coronary angiogenesis, expansion of the capillary network without concomitant arteriogenesis would likely yield immature, unstable vessels which may later regress. Arteriogenesis is not stimulated by angiogenic growth factors or by hypoxia, but by haemodynamic forces, chiefly shear stress [160] and, for this reason, has proven particularly intractable to therapy.

Numerous stem/progenitor cell types have been explored for their ability to induce neovascularisation as a component of myocardial regeneration (reviewed in[118]). Not only are these approaches hampered by limited engraftment and rejection, any regenerative benefits conferred have largely resulted from paracrine stimulation of local vasculature,

recruitment of endogenous stem cells and remodelling of extracellular matrix, rather than incorporation and trans-differentiation of transplanted cells. Although, arguably, stem cells serve as an ideal vehicle to deliver growth factors to the site of injury, an equally effective or superior outcome may one day be achieved by cell free delivery of crucial stimulating factors, perhaps encapsulated for controlled release from a suitable biomaterial.

INTRINSIC NEOVASCULARISATION: A LIMITED, BUT IMPORTANT, ATTEMPT TO MITIGATE ISCHAEMIC INJURY

Despite possessing limited regenerative capacity, the mammalian myocardium deploys a number of adaptive mechanisms in response to stress, both to limit cellular injury and to repair, as much as possible, the damaged tissue. Ischaemia, both acute and chronic, has been shown to stimulate angiogenesis, with hypoxia as the major factor driving neovascularisation. Mediated by hypoxia inducible factor (HIF-1α), a cohort of pro-angiogenic growth factors are up-regulated, including VEGF isoforms and their receptors, flk-1 and flt-1[64] [63], FGF-1 and -2 and Transforming Growth Factor (TGF)-β [1,2]. Unfortunately, such adaptive mechanisms are clearly inadequate to prevent myocardial infarction and angina. The insufficiency of the intrinsic vascular response is confounded by both decreased cytokine production and EC viability in aged[107], diabetic[31,108,159] and hypercholesterolaemic [22] patients and animal models.

Neovascularisation has been reported to proceed primarily via angiogenesis and arteriogenesis, or collateral growth. Intuitively, it may appear that the adult heart should have little or no requirement for vasculogenesis, given the prior existence of a mature coronary vasculature. However, in the absence of experimental tools to unequivocally deduce the source of *de novo* vessels, vasculogenesis cannot be excluded or categorically distinguished

from angiogenesis. Human and large animal studies are unavoidably descriptive in nature and, even in rodent models, tools are limited for fate mapping, due to the paucity of selective molecular markers that distinguish arterial, venous, endocardial, capillary and lymphatic ECs. Single cell transcriptomic profiling may provide the knowledge base from which to engineer lines for selective lineage tracing. Meanwhile, we are limited, essentially, to observational studies in animal models which enable a series of snapshots to temporally track the development of neovessels in the ischaemic heart; careful observation may yield some insight which may, in turn, facilitate the development of appropriate reagents to definitively implicate the underlying mechanisms.

Remodelling of the Microvasculature

At the microvascular level, the most complete description of intrinsic neovascularisation was provided by Ren and colleagues, who reported on the morphological remodelling of the vasculature in the healing canine heart post-MI[103], along with some insight into the inflammatory response which may both drive and, in turn, be modulated by the vascular adaptations. After 7 days' reperfusion, following coronary artery ligation, the infarcted territory was found to be rich in capillaries, with large pericyte-poor "mother vessels" and endothelial bridges. During scar maturation, arteriolar density in the infarct increased and a proportion of microvessels acquired a supportive pericyte coat. Intense endothelial CD31 staining was observed in and around the infarct region, in comparison with remote uninjured regions but, at this stage, vessels were shown to weakly bind biotinylated *Griffonia simplicifolia* lectin I (GS-I). The lectin-binding sugar groups of ECs matured with further remodelling such that, by d28, most vessels displayed intense GS-I binding. This coincided with a decline in angiogenic activity and transition towards the maturation phase, with

pericyte recruitment and formation of a muscular coat. Ren et al drew comparisons between the angiogenic microvessels formed post-MI with the enlarged, dilated "mother vessels" in tumor-related angiogenesis, reported by[87], speculating that the generation of dilated enlarged vessels may be a characteristic feature of VEGF isoform-mediated angiogenesis[28,91]). The delay in pericyte acquisition appeared to be permissive for the angiogenic process, to provide the microvasculature of the infarct with a plasticity window[8], necessary in this dynamic phase of healing to accommodate the rapidly changing cell populations and metabolic needs. Thus, although not formally interrogated, these histological observations are at least consistent with an underlying mechanism of angiogenesis; in further support of this, Virag and Murry quantified the extent of EC proliferation in the murine heart post-MI[145]; 2.9 ± 0.1% of CD31-expressing ECs were BrdU-positive after 4 days, declining to 0.7 ± 0.5% after 1 week and essentially ceased thereafter. Consistent with the canine response, vascular density was found to peak at the granulation tissue stage between 4 and 7 days and decrease with formation of the mature scar at 4 weeks post-MI. Further studies are clearly required to provide a mechanistic underpinning of post-MI microvascular remodelling, an understanding of which will be imperative for the development of therapies for optimal cardiac repair.

Collateral Growth: Functionally Bypassing Occluded Arteries

The extent of collateral growth post-MI varies considerably among species but has been best described in human patients, in whom the coronary circulation is very well developed and these larger vessels are easily visualised by simple angiography[53]. Coronary collaterals effectively provide an alternative source of blood supply to myocardium jeopardized by ischaemia. A third of patients with coronary artery disease (CAD) possess collateral arteries

that suffice to enable 20-25% of the normal flow and prevent myocardial ischaemia. Well-developed coronary collateral arteries in patients with CAD mitigate myocardial infarcts and improve survival[113,114]. Preformed collaterals are found in fewer (approximately one fifth) of individuals without CAD, implying that collateral vessel formation is an adaptive mechanism that accompanies the development of CAD.

Although the propensity for collateral vessel formation varies between species, animal models may provide mechanistic insight. Indeed, utilising an inducible Apln-CreER line to label capillary, but not arterial, ECs, He and colleagues recently proposed that collateral vessels were predominantly formed by the enlarging of pre-existing arteries (arteriogenesis), rather than by angiogenesis into the ischaemic region and recruitment of new vascular smooth muscle cells (VSMCs; arterialization)[40]. Whether or not the mechanisms differ in humans, to any significant degree, is difficult to ascertain and this may only be possible to deduce by inference if therapies based around targeting the murine mechanism prove effective in the clinic. Since ~20% of CAD patients cannot be revascularized by percutaneous coronary intervention or coronary artery bypass grafting, the therapeutic augmentation of collateral growth may be a key treatment strategy in those individuals.

NEOVASCULARISATION: PUTATIVE MECHANISMS

Vascular progenitor cells

Under conditions of homeostasis, cell turnover in healthy blood vessels is low but, under pathological conditions, progenitor cells have been shown to replace injured or dead cells. Although ambiguity still surrounds the molecular identity and extent of contribution of such

cells, they have been proposed either to reside within the vascular wall and/or to be mobilised, via the circulation, possibly from a bone marrow source.

Circulating progenitor cells

Endothelial Progenitor Cells (EPCs) of the peripheral blood and bone marrow have been the most widely studied of the vascular progenitor cell subtypes, with reported contributions to neovascularisation in tumour angiogenesis[83], wound healing[4] and ischemia[58] and to intimal re-endothelialization after vessel wall injury[39]. Controversies surrounding the molecular definition and differing isolation methods have hampered the study of EPCs, as discussed in[104]. The evidence for EPC contribution to neovascularisation in the ischaemic heart is by no means extensive but Kocher and colleagues demonstrated that human adult bone marrow contains endothelial precursors, with phenotypic and functional characteristics of embryonic hemangioblasts, that can direct vasculogenesis and angiogenesis after experimental MI and contribute to improved functional recovery[58]. Cytokines, including Granulocyte-colony stimulating factor (G-CSF), stromal-derived factor (SDF-1), VEGF-A isoforms, erythropoietin (EPO), endothelial nitric oxide synthase (eNOS), Angiopoietin-1 (Ang-1) and platelet-derived growth factor (PDGF) have since been used to mobilize EPCs to the heart for revascularisation of the infarcted myocardium (reviewed in [122], but the extent of direct contribution, as opposed to angiogenic paracrine benefits, remain to be precisely determined.

Vascular Wall progenitor cells

A range of multipotent and lineage-restricted progenitor cells have been documented to reside within the mural layers of postnatal blood vessels (reviewed in[97]; these include multipotent

progenitors such as mesenchymal stem cells (MSCs)[55,88], multipotent vascular stem cells (MVSCs)[131], adventitial macrophage progenitor cells (AMPCs)[96], as well as lineage-restricted populations such as endothelial progenitor cells (EPCs)[47], microvascular pericytes[128] and smooth muscle progenitor cells (SPCs) [46]. Once triggered, these cells proliferate and generate EC, VSMCs, hematopoietic or mesenchymal cell progeny. The tunica adventitia has emerged as a compartment particularly rich in progenitor cells, as its matrix properties resemble a stem cell niche and sustains progenitor cell signalling[72,73]. Vascular wall progenitor cells have been shown to perform a homeostatic function during postnatal growth, aging and in disease states, to maintain structural integrity and function of vessels[95], although contribution to disease progression has also been proposed, for example in the context of neointima formation[46] and[131].

Pericytes have been described as multipotent progenitor cells that reside within the vessel wall, although they are considerably more abundant than other stem cell populations and regarded as mural, not perivascular cells, with well-defined roles in vessel formation and function[3]. They regulate vascular patterning, EC growth and differentiation during angiogenesis as well as vessel tone, permeability and stability in established vessels[35]. Pericytes are phenotypically heterogeneous and they share properties with VSMCs, fibroblasts and mescenchymal stem cells[97]. Like the other vascular wall progenitors and EPCs, difficulties in defining pericytes, due to the lack of a distinguishing molecular marker, has confounded full characterisation and deduction of their reparative potential. Pericytes are essential for angiogenesis and vessel maturation and, intriguingly are capable of both promoting sprouting and vessel stability. Whether such opposing roles are played by distinct pericyte sub-populations or whether pericyte phenotype adapts to perform distinct functions is an important unanswered question[97]. Pericyte contribution to neovascularisation has been more widely explored in other injury or disease settings (reviewed in[52]), however, a

small number of studies exploring roles in coronary microvascular remodelling have also been published. Human saphenous vein-derived pericyte progenitor cells (SVPs)[49] and human pericytes from skeletal muscle[15] have been delivered to infarcted murine hearts. In each case, cell transplantation led to attenuated ventricular dilatation and improved cardiac function, associated with diminished fibrosis and a moderated inflammatory response. A significant increase in neovascularisation was reported, including a small degree of direct incorporation of transplanted pericytes[15], the predominant effect seemingly resulting from paracrine stimulation. SVPs were shown to secrete VEGF-A, Ang-1, and a raft of chemokines along with miRNAs, which, in part, accounted for the pro-angiogenic properties[49].

A role for Macrophages?

Immune cells, notably macrophages but also mast cells, dendritic cells and subsets of T cells, contribute to vasculogenesis and angiogenesis in a number of ways; they secrete an array of angiogenic growth factors and coordinate a range of cell-cell interactions (reviewed in[69]). Recent work has helped to clarify the role of macrophages in coronary vessel development[60]. At least 2 distinct populations of macrophages were found to co-exist in the embryonic heart, identified on the basis of C-C chemokine receptor type 2 (CCR2) surface expression, the receptor for monocyte chemoattractant protein-1 (MCP-1). CCR2+ macrophages, derived from foetal lymphomyeloid progenitors, were located predominantly within the trabecular projections of the ventricular endocardium, whereas $CCR2^{-}$ macrophages, derived from yolk sac progenitors, were found almost exclusively within the myocardial wall. While CCR2+ macrophages were found to be dispensable for embryonic coronary vasculogenesis, $CCR2^{-}$ macrophages were shown to be essential for patterning and remodelling of the microvasculature[60]. A similarly careful analysis would be informative,

if performed in the adult MI setting, particularly as CCR2+ bone marrow cells were shown to be involved in *de novo* collateral formation post-MI (collaterals were reduced in both CCR2-/- and MCP1-/- mice)[163]. Whether neovascularisation post-MI recapitulates the embryonic process or whether there is a distinct requirement for an alternative macrophage population at postnatal stages would be well worth knowing. Of particular interest would be how the embryonic roles map onto the populations described in the ischaemic heart, the M1 classically activated, pro-inflammatory macrophages versus the M2 alternatively activated macrophages, which are pro-angiogenic and anti-inflammatory[14], and whether either population participates in neovascularisation post-MI.

The Potential for Redeployment of Developmental Mechanisms for Neovascularisation

A relatively recent paradigm in regenerative medicine is that embryonic mechanisms used in the generation of tissues during development may be reactivated, either as a component of the suboptimal endogenous reparative response or following exogenous stimulation, to induce regeneration via the same pathways and processes. Thus, a detailed understanding of how coronary vessels are formed during development may provide the necessary insight to identify the appropriate target cells and stimuli for their redeployment.

In contrast to the paucity of literature on the extent and mechanisms of intrinsic neovascularisation in the ischaemic heart, the origins and formation of the coronary vasculature have been well documented after more than 30 years of study. That is not to say that the process is entirely understood. Although we appear to be nearing a consensus, following recent advances, some inconsistencies and outstanding questions remain which require further fine-tuning The focus of much of the controversy has been the origin of the EC precursors that constitute the coronary vasculature (previously discussed at length in[106,134,135], although several key publications have emerged in the intervening years). In

contrast, views on the origin of coronary smooth muscle and adventitial fibroblasts remain largely unaltered since their original identification: those of the ventricular walls derive from the (pro)epicardium while the neural crest and second heart field contribute to the proximal arteries at the base of the heart and the great vessels[43]. The origins of the cardiac lymphatics was even more elusive[99] until recently, when Klotz and colleagues revealed a heterogeneous composition of multiple origins, which include both extra-cardiac venous endothelium and a non-venous source which may arise from the yolk sac haemogenic endothelium [57] (as discussed in [84]).

Establishing the Capillary Plexus

Initially forming as a collection of discontinuous endothelial patches, the primitive capillary plexus expands over the surface of the heart, largely in a dorsal to ventral direction, to envelope the embryonic heart from embryonic day (E)9.5. The source of the contributed ECs was long held to be the proepicardial organ (PEO), a transient extracardiac mesothelial cell population, which migrates to give rise to the epicardium, the outer layer of the heart. A subset of epicardium-derived cells (EPDCs) delaminate and undergo epithelial-mesenchymal transition (EMT) to generate mesenchymal derivatives which have been reported to include VSMCs, pericytes, fibroblasts, cardiomyocytes and endothelial cells ([12,27,36,51,77,79,147,148,172]. More recently, the sinus venosus (SV) and the ventricular endocardium were alternatively proposed to account for the origin of coronary ECs. A number of factors may underlie the protracted ambiguity. Species-specific differences may, in part, account for some of the apparent inconsistencies between the early avian[77,89,90] and more recent mouse data [12,164,172]. The close proximity of PEO, SV, liver sinusoids (which were also considered[65,92]), and the endocardium, at least at its atrioventricular junction, being co-located at the inflow region of the embryonic heart, may also be a

confounder. Undoubtedly, this had the potential to undermine early studies which relied on localised retroviral labelling[77] and vital dye (DiI) injection, as well as the persuasive quail–chick chimera studies, in which a quail proepicardial explant was transplanted into in a chick host[78,90,92]. Over and above complications of physical separation, the proximity to inductive paracrine secretion and some degree of common functional roles, means that it has been difficult to identify truly lineage-specific markers with which to label selected populations. There have been examples of gene or reporter expression in cell types other than those intended to be labelled by the genetic reporter; although fate mapping studies in which these lines have been used have, in general, been carefully controlled, they need to be cautiously interpreted. Examples include *Nfatc1*(to label endocardium*[153]*, but also expressed in sinus venosus[81] and epicardium[21]); *W-t1* (to label epicardium[21], but experession reported in ECs[29,149]); *Tbx18* (to label epicardium[12], expressed in cardiomyocytes [20]); *Gata5* (to label epicardium[76], but expression detected in cardiomyocytes[109]) The heterogeneity of progenitor populations, such as those of the PEO[51,105], and the lack of understanding on the extent of marker overlap, or how this affects cell fate, further compounds difficulties in interpretation. Thus, while cre-lineage tracing has undeniably advanced our knowledge of coronary vessel development, due diligence is warranted, particularly given the acknowledged pitfalls of Cre-based fate mapping, discussed in detail in [106,134].

These caveats aside, lineage-tracing studies in mouse have allowed a seemingly accepted consensus for coronary vessel development. Those using the established PEO markers, *Tbx18*[12] and *Wilm's Tumour-1* (*Wt-1)*[172] confirmed PEO-derived coronary VSMCs, fibroblasts and pericytes, but demonstrated a minimal contribution, if at all, to coronary ECs. This prompted a search for alternative sources of coronary ECs and focus turned to the sinus venosus (SV), the endothelial-lined cavity that returns venous blood to the embryonic heart.

Using a combination of the apelin-lacZ knock-in mouse line, an inducible VE-cadherin Cre line and an ephrinB2-LacZ reporter for fate mapping and clonal analyses, Red-Horse and colleagues[101] proposed that VE-cadherin+ve ECs from the SV migrate into the heart to contribute the bulk of the coronary plexus. Coronary sprouts were continuous with the sinus venosus and did not appear to arise from other vasculogenic sources. The authors proposed that coronary artery progenitors arise from differentiated veins, demonstrated by a switch from a venous (EphB4+) to arterial (ephrinb2+) molecular signature in the emerging coronary sprouts. Of note, the processes of sprouting, dedifferentiation and redifferentiation were dependent on elusive inductive signals from the ventricle and epicardium. VEGF-C was later identified as one of the key epicardial signals required for angiogenesis of SV-derived ECs to underlie expansion of the coronary plexus[16].

While the Red-Horse study concluded that SV contributes the majority of ECs to the embryonic heart, they also reported a modest contribution from the endocardium. In contrast, using fate mapping based on the *Nfatc1* marker, along with live imaging and tissue transplantation, Wu and colleagues concluded a more substantial contribution from the ventricular endocardium [153]. The endocardium is the inner layer of the heart, a smooth membrane composed of endothelial cells. In the mouse, endocardial cells are first detected at E7.5, deriving from mesodermal precursors of both the first and second heart fields[98]. Far from being a quiescent, terminally differentiated population, endocardial cells give rise to the atrioventricular valves[98] and were shown by Wu *et al[153]* to be active angiogenic cells that form coronary endothelial networks, in response to myocardial VEGF-A; coronary angiogenesis, and arterial formation specifically, was inhibited by deletion of either myocardial *Vegf-a* or endocardial *Vegfr-2*. In contrast, endocardium-derived venous ECs were few and veins formed independent of myocardial VEGF signalling[153]. Thus, in a departure from the previous philosophy of a common source for the coronary vessels, Wu

and colleagues proposed distinct origins and mechanisms for the formation of coronary arteries and veins. However, a subsequent publication[133] proposed that subepicardial endothelial precursors (labelled by Apln-CreER, and likely SV-derived) contributed most of the intramyocardial coronary arteries and that a subset of subepicardial vessels remained in the subepicardial space and formed coronary veins. With this lineage trace, only the interventricular septal ECs were found to derive from the endocardium. Using an additional reporter, Npr3-CreER, to label endocardial descendants and support the group's prior findings with the Nfatc1 reporters[133], Zhang et al[164] concluded that endocardium minimally contributes coronary ECs to the ventricular free wall during embryonic stages.

By way of reconciliation of a PEO contribution, as advocated by the prior studies, Katz and colleagues identified additional PEO sub-populations, defined by expression of *Semaphorin3D* (*Sema3d*) and *Scleraxis* (*Scx*), that give rise to coronary ECs by contributing to the SV and endocardium[51], respectively. By and large, *Sema3D* and *Scx* were found not to overlap with *Tbx18* and *Wt-1* expression within the heterogeneous epicardial populations, but revealed the existence of other important PEO domains. Whether *Sema3d*- and *Apelin*-expressing SV cells represent a common, or distinct, EC progenitor population will require further investigation. Given that *Sema3D*-derived cells populated the heart with an epicardial-to-endocardial gradient and *Scx* delineated an endocardial contributing population, the expectation may have been that these SV and endocardial populations would contribute venous and arterial ECs, respectively; an interpretation which may also be supported by the previous SV clonal analyses, in which only 1-3% of arterial EC clones were found to arise from venous ECs[101]. However, both the *Sema3d* and *Scx* lineages were found to contribute equally to arterial and venous ECs, preferentially to medium-sized arterioles and venules[51].

Are we Nearing a Consensus?

Chen and colleagues sought to evaluate the relative contributions of the putative lineages to the coronary circulation. Using *ApjCreER* (SV), *Nfatc1Cre* (endocardium) and *Sema3dCre* (proepicardium) lineage-tracing tools, they mapped onto the whole intact heart compartmentalized contributions traced from each source[16]. The SV contributed to a large number of arteries, capillaries and veins on the dorsal and lateral sides of the heart (their angiogenesis stimulated by epicardium-derived VEGF-C). Vessels in the midline of the ventral aspect and ventricular septum were primarily derived from the endocardium (in response to myocardial VEGF-A[153]). The proepicardium gave rise to a smaller fraction of vessels and these were spaced relatively uniformly throughout the ventricular walls[16].

Although, by overall consensus, the epicardial EC contribution is more modest than originally thought, and seemingly contributed by a specific sub-population, a concept that remains somewhat difficult to reconcile is the striking and consistent phenotype of impaired coronary vessel development when any one of a plethora of diverse genes is deleted from epicardial cells, using one of the reliable and canonical epicardial Cre drivers, such as Wt-1CreERT2. Many examples have been previously catalogued e.g. in[121] and, by way of a recently published example, Singh and colleagues defined the requirement of the Hippo signalling mediators, Yap and Taz[117]. Their deletion leads to impaired epicardial EMT, proliferation and differentiation into coronary ECs. When deleted using a Sema3d-Cre, a modest reduction in coronary ECs may be anticipated, but that deletion driven by Wt-1CreERT2 should manifest as a reduced number of epicardial-derived PECAM-1 +ve ECs[117], suggests that the precise roles of the epicardial sub-populations in coronary vessel development is still far from understood.

Postnatal Expansion and Maturation of the Coronary Vasculature

Maturation of the coronary vasculature after birth is associated with a 2.8-fold increase in capillary growth within the first 5 days of postnatal life, to accommodate the 80% increase in cardiac mass[137]. While this was previously considered to occur predominantly by intussusception, or splitting, of pre-existing capillaries[142], recent studies convincingly demonstrate a *de novo* vessel contribution from an endocardial origin[132]. By pulse-labelling endocardial ECs (with a tamoxifen inducible Nfatc1-CreER) alongside thelabelling of SV derivatives (with Apln-CreER or Fabp4-creER), at a range of developmental stages, Tian and colleagues found that a substantial proportion of coronary VECs arise *de novo* perinatally, not simply by angiogenesis of pre-existing (SV-derived) vessels but by endocardial to vascular EC conversion [132]. A profoundly different mechanism was proposed; coincident with trabecular compaction, endocardial cells on the trabeculated surfaces became trapped within the compacting myocardium and subsequently coalesced, leading to rapid vessel formation and perfusion of the newly compacted myocardium[132]. This appears to be a distinct mechanism from that proposed for embryonic endocardium-derived ECs, that form by conventional angiogenesis in response to myocardial VEGF-A[153],

Although by no means unequivocal, the current consensus for EC contribution to the embryonic coronary vasculature can be summed up as follows (see **Figure 1**): during embryonic stages, the majority of ECs contributed to the free ventricular wall derive from the SV, with a more modest contribution from PEO subpopulation(s), and an endocardial contribution to interventricular septal ECs. The coronary plexus grows toward the aorta and penetrates the wall of the aorta via distinct openings, known as ostia[30]. Upon perfusion, the plexus remodels[50], with arteriogenesis and recruitment of mural cell support, as discussed below. A second wave of new vessel formation ensues shortly after birth, with additional

endocardially-derived ECs contributed, coincident with trabecular compaction, to become incorporated into the expanding vascular network.

Coronary Smooth Muscle Cells, Pericytes and Adventitial Fibroblasts

The embryonic origin of coronary VSMCs has, at least thus far, proven much less divisive, and shown to arise from the epicardium in several studies, with multiple methodologies[12,79,172] (**Figure 1**); adventitial fibroblasts also derive from an epicardial origin[93]. EMT for inward migration and differentiation to form these cell types was shown to be dependent upon PDGF Receptor β (PDGFRβ) signalling[75,126]. Volz and colleagues characterised an intermediate cell type, resembling vascular pericytes in marker profile ($PDGFR\beta^{+}$ $Notch3^{+}$ $NG2^{+}$ $SM\text{-}MHC^{-}$ $SM\alpha^{-}$ $PDGFR\alpha^{-}$)[147]. The authors demonstrated that these cells derived from the epicardium and populated the entire coronary vasculature, wrapping around microvessels. Those that surrounded coronary arteries differentiated into VSMCs by EC to pericyte Jagged-1 – Notch3 signalling. A recent study revealed that the majority of epicardial VSMCs are derived from Wt-1(+), Gata5-Cre(+) cells that migrate before E12.5, and only a minority derive from a later-emigrating, *Wt-1*(+), Gata5-Cre(-) population[151]. That being so, a further matter to resolve would be the origin of the VSMCs required to support the endocardial ECs contributed postnatally. By temporally labelling the inducible Wt1CreER traced epicardial cells, Liu and colleagues posited that a proportion of early labelled EPDCs remain undifferentiated in the outer myocardial wall until the neonatal endocardial vessels form; at such time, the early specified cells re-migrate deeper into the inner myocardial wall and undergo VSMC differentiation to support the endocardial derived vessels[68].

ARE EMBRYONIC MECHANISMS INTRINSICALLY REDEPLOYED OR CAN THEY BE REACTIVATED POST-MI FOR CARDIAC REGENERATION?

As outlined above, detailed descriptions of neovascularisation in the ischaemic heart are scant and significantly more research is required to investigate the underlying mechanisms by which the reported neovascularisation is brought about. **Figure 2** illustrates some of the putative mechanisms that may contribute. Whether neovascularisation occurs primarily by angiogenesis or, additionally, with a contribution from reactivated embryonic mechanisms has not been systematically assessed. Beyond any intrinsic self-repair, by low-level reactivation of vasculogenic precursor populations, the potential to do so pharmacologically will depend upon i) understanding the competency of the progenitors, and how they are altered in the embryonic versus adult heart; ii) identifying the key signalling factors required to restore embryonic potential and the ability to proliferate, migrate and differentiate. Insight into the latter will likely, though perhaps not exclusively, derive from understanding the equivalent mechanisms in development, as discussed below.

Potential for Vasculogenesis from Embryonic Sources

From the standpoint of a healthy adult heart, the quiescent nature of the few residual embryonic progenitor populations, does not imply great promise for contribution at a time of need. The sinus venosus is a transient developmental structure and doesn't exist, as such, in the adult; however, coronary veins may represent an equivalent source. The endocardium, we now recognise, is not merely a dormant lining of the myocardium, but a dynamic source for contribution of *de novo* vessels to the postnatal heart[132]. However, the trabecular compaction that facilitates this contribution concludes shortly after birth and this may limit scope for further involvement in later life. The epicardium, similarly, loses inherent capacity

to migrate, differentiate and convey potent paracrine factors to the myocardium shortly after birth[17], coincident with loss of epicardial marker expression[119].

Fortunately, a small number of studies have revealed that, in the context of myocardial injury, both the epicardium[119,170,173] and the endocardium[80] respond to endogenous injury cues and, moreover, others have identified signalling factors that can recapitulate embryonic roles and therapeutically stimulate reactivation and enhance regenerative potential. Any SV-equivalent structures have not been explored and considerably more research is required to understand the mechanisms of activation and reparative roles of the epicardium and endocardium.

Epicardium

The epicardium has been a prime focus of attention as a potential source of cells for repair following myocardial injury. The Zebrafish possesses an innate capacity to regenerate its myocardium and replace damaged vasculature following injury[94] and the associated re-expression of epicardial tbx18, wt-1, and aldh1a2 appears to be an integral component of repair[61]. Indeed, blocking epicardial EMT with a dominant-negative FGF receptor resulted in failure of neovascularisation and regeneration[61]. Lineage tracing experiments demonstrated that EPDCs gave rise to perivascular cells and myofibroblasts, but not cardiomyocytes[54]. Reactivation of embryonic epicardial gene expression, including Wt-1, Tbx18, Raldh2, and Tcf21, occurs even in the non-regenerating mouse heart, following MI[37,119,173]. After activation, EPDCs invade the underlying myocardium; to an extent, the fate of EPDCs in the infarcted heart is similar to that in development. Lineage traced EPDCs expressed fibroblast markers and a minority expressed *bona fide* VSMC markers. Direct contribution to revascularization was not reported but the authors demonstrated an

important paracrine role to support angiogenesis[171]. This finding is consistent with the demonstration that it is predominantly the early migrating Wt-1+ epicardial progenitors that contribute to the VSMC lineage, whereas later migrating populations contribute to non-VSMC lineages or remain in the epicardium[151]. Pericyte contribution appears not have been explored in this or any other epicardial fate mapping post-MI but is warranted in light of the identification of pericytes as the intermediaries between EPDCs and VSMCs in the embryo[147]. While direct cellular contribution from the epicardium may be sub-optimal in the MI setting, there is potential to enhance activation and shift cell fate, as was shown with Thymosin β4 (Tβ4[119], discussed below).

Endocardium

If endocardial contribution to the postnatal (neonatal) heart depends upon trabecular compaction[132], it may be intuitive to assume that limited endocardial potential remains in the compacted adult heart, not discounting the possibility of an angiogenic mechanism, as proposed, albeit contentiously[164], for embryonic stage endocardium[153]. However, the plasticity of the adult endocardium was revealed in a study by Miquerol and colleagues[80], which described the formation of elegant vascular structures, resembling 'flowers', that derive from the endocardium post-MI. Foci of ECs, labelled by Cx40-GFP and VEGFR2 but negative for endoglin, were identified within the endocardium overlying the infarct zone and distinguishable from surrounding Cx40-/VEGFR2-/Endoglin+ endocardium. 'Flowers' associated with subendocardial VSMCs and were connected, via stalk-like structures, to the underlying coronary vessels. The mechanism of endocardial flower formation appears to consist of angiogenic and arteriogenic steps, with early VEGFR2 expression and active proliferation of adjacent endocardial and smooth muscle cells[80]. The extent to which 'flowers' facilitate the perfusion of the ischaemic myocardium remains to be determined and the molecular mechanisms that underlie the conversion of endocardial cells to coronary ECs

should be explored, with the hope of uncovering putativte targets for enhancing neovascularisation.

MOLECULAR MECHANISMS OF CORONARY VESSEL DEVELOPMENT AND PATHWAY REACTIVATION POST-MI

After MI, the injured and border zone myocardium and infiltrating immune cells secrete potent paracrine factors, many of which have been shown, at least in development, to regulate coronary vessel formation from the SV, endocardium and epicardium. Activation of these pathways may underlie the intrinsic reactivation that occurs following injury or may represent key targets to reinitiate regenerative mechanisms post-MI. Moreover, activation and up-regulation of chemokines, such as stromal cell-derived factor 1 (SDF-1), stimulates the mobilization of bone marrow progenitor cells and their homing to the myocardium[122]. While a mechanistic understanding of neovascularisation, in terms of inductive signals and responsive cell types, is far from complete, some insight from developmental studies may shed light on vasculogenic and angiogenic processes, even if the progenitor cell types available for stimulation differ in the adult heart. Some of the main pathways are précised below, with a brief discussion of their developmental roles and expression in the infarcted heart to highlight possible recapitulation of mechanisms in the context of MI. For more detailed overviews, please refer to [86] and [123], and to citations in each for discussion of individual pathways.

Growth factors of the archetypal angiogenic VEGF family play multiple roles in the development of the coronary vasculature[130]. VEGF-A isoforms, VEGF-B, and VEGF-D are expressed in cardiomyocytes, while VEGF-C is expressed in epicardium[16] and pericytes[59] and their receptors are expressed in the epicardium[136], endocardium[165,166] and sinus venosus[16]. During development, myocardial VEGF-A

signalling induces epicardial EMT, endocardial contribution of coronary artery ECs[153], as well as EC proliferation, migration and tube formation[138], whereas epicardial VEGF-C expression regulates the formation and growth of coronary sprouts from the sinus venosus[16]. MI leads, via HIF-1α, to the up-regulation of VEGF-A isoforms in the epicardium[170], endocardium[168] and border zone myocardium[168], as well as their secretion, at high levels, from EPCs[157] and BMSCs[129]. VEGF (unspecified isoforms) and HIF-1α expression correlated with increased microvessel density in the infarct area[18]. As well as potently inducing coronary angiogenesis by stimulating ECs, VEGF signalling post-MI may stimulate vasculogenesis via the epicardium or endocardium.

During development, Angiopoietin1 (Ang1) is strongly expressed in the myocardium and signals to Tie2 expressed on both the epicardium and endocardium [16], to regulate coronary vasculogenesis and angiogenesis[150]. Cardiomyocyte-secreted Ang1 also promoted sprouting of SV ECs and was shown to be selectively required for formation of subepicardial coronary veins, not intramyocardial arteries[16]. The other ligand, Ang2, also influences coronary development, synergistically activating Tie2, along with VEGF-A, to increase capillary density[146]. Expression of cardiac Ang2 and Tie2, but not Ang1 or Tie2, increases within the first 48 hours of reperfusion following MI in the rat[115], however, the specific cell types that up-regulate the receptor and ligand need to be determined. Of note, Ang-2 was identified as a reliable biomarker of incident acute MI in patients, independent of traditional risk factors[48].

Although myocardial FGF-epicardial FGFR1 signalling appears not to be necessary for epicardial EMT[109], it is required for EPDC invasion of the myocardium, for differentiation and remodelling of the subepicardial mesenchyme and angiogenesis[143]. FGF levels increase in the post-MI heart. FGF-1 is expressed strongly in inflammatory cells within the border zone and FGF-2 is secreted by ECs[45]; this is complemented by increased

expression of the receptors in ECs and in the epicardium [167].Deletion of EC FGFR1 and FGFR2 impaired cardiac functional recovery post-MI, with a failure of vascular remodelling[44]. In adult zebrafish, FGF signalling critically regulates epicardial EMT and regeneration following injury, as evidenced by expression of a dominant negative mutant FGFR1[61]

TGFβ signalling regulates EMT[25] and endothelial-to-mesenchymal transition[74] in multiple tissues. TGFβ-TGFBRI activation induces the transition in epicardial cells via p38 MAP kinase, p160 rho kinase[38] and by production of hyaluronic acid[23] in the extracellular matrix, to promote angiogenesis and VSMC differentiation. Myocardial TGFβ additionally contributes by stimulating phosphorylation of the BMP receptor, ALK2, leading to smad-mediated transcriptional changes[85]. TGFβ isoforms are up-regulated following MI to exert pro-fibrotic[11,144] and pro-inflammatory effects but may also stimulate neovascularisation as part of this wide-ranging cardiac repair programme.

During development, PDGF signalling is required for epicardial EMT and invasion[109], via mechanisms which appear to include PDGFRα-mediated WT-1 repression[7], and PDGFRβ-mediated activation of Rho kinase and phosphatidyinositol-3 kinase pathways [70]. Additional roles in cell fate determination are also likely since activation of PDGFRα has been implicated in fibroblast differentiation[109], whereas PDGF-BB/PDGFRβ potently induces VSMC fate[70]. Following MI, PDGFRβ signalling is activated in perivascular cells within the infarct[174] and found to be important for maturation of infarct vasculature as PDGFRβ blockade enhanced capillary density but produced vessels which were dilated, lacking mural cell support with increased permeability[174].

Erythropoietin (Epo) signals in the embryo via its epicardially expressed receptor (EpoR) in the embryo, to regulate both vasculogenesis and angiogenesis[154]. In the adult,

Epo was shown to protect the heart after MI by inducing angiogenesis as well as preventing cardiomyocyte apoptosis[140]. In rats with heart failure, exogenous EPO increased myocardial VEGF expression (isoforms not specified) to induce a 37% increase in capillary density and improved cardiac performance[152] but the mechanism underlying neovascularisation remains to be explained. The suggestion that endogenous EPO may function in vascular repair is supported by the demonstration of a positive correlation between EPO levels and coronary collateral development in patients with coronary chronic total occlusion[156,161].

Notch signalling plays a key role in angiogenic sprouting, in establishing arterio-venous identity and in regulating endothelial-mural cell interactions[111]. VEGF gradients establish highly specialized, filopodia-rich endothelial tip cells, which lead sprouting angiogenesis, with less motile, yet highly proliferative, stalk cells further down the sprout[42]. VEGF signalling to VEGF receptors 2 and 3 on tip cells activates expression of Notch ligand Delta like ligand 4 (DLL4), which activates Notch transmembrane receptors in adjacent tip cells. Notch signalling in the stalk cell inhibits the tip cell phenotype, therefore maintaining the newly formed sprout (reviewed in [42]). EC Notch activation induces expression of arterial markers and suppresses venous markers[111] and drives vessel wall maturation by regulating EPDC-VSMC differentiation[26], in part by inducing Pdgfrb expression and cooperating with TGFβ signalling to induce VSMC genes. Notch signalling underpins heart regeneration in zebrafish[100] and is up-regulated in the injured mammalian heart[24].“Notch-activated" epicardial progenitors expanded following MI and secreted potent mitogenic and cardiotrophic growth factors including Tβ4, PDGF-A, TGF-β, BMP-1 and -4 and FGF-7. They contributed fibroblasts to the remodelling heart and revealed a modest epicardial-cardiomyocyte differentiation potential[110].

Exogenous Therapies to Enhance Neovascularisation

Given the complex multi-level spatiotemporal control required to orchestrate and fine tune the processes of coronary vasculogenesis, angiogenesis and vasculogenesis in the developing embryo, it is unlikely that neovascularisation post-MI could be achieved by single growth factor therapy. Successful strategies will probably require combination therapies or reactivation of orchestrated processes, as achieved in the embryo, by the identification of key inductive stimuli or master regulators, which may be a chemokine, non-coding RNA or signalling peptide. Sequential release of $VEGF_{165}$ and PDGF, from a fibrin gel applied to the rat heart post-MI, provides just one proof-of-concept example of the efficacy of a combination strategy[5], which led to improved angiogenesis, survival, and cardiac function, with diminished inflammation and fibrosis, compared to the application of free growth factors. In a further example, modulating metabolism with the thyroid hormone analogue, 3,5-diiodothyropropionic acid (DITPA) enhanced levels of VEGF (notably 165 and 188 isoforms of VEGF-A), FGF-2, Ang-1 and Tie-2 in the rat heart post-MI, leading to increased arteriolar density and improved left ventricular function[169].

Following the recent proliferation of studies on the involvement of non-coding RNAs, both microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), an understanding into the higher level control that they exert on vasculogenic mechanisms will likely bolster our attempts to therapeutically enhance neovascularisation. A detailed discussion is beyond the scope of this review but can be found elsewhere e.g.[6,32,62,127,139]. By way of a case in point, miR-126 has been described as a master regulator of physiological angiogenesis[19]. Expressed at high levels in ECs and EPCs, miR-126 initiates angiogenic signalling, supports EC differentiation and maturation in embryonic vasculogenesis, yet contributes to maintenance of quiescent phenotype in ECs during homeostasis. In response to vessel injury or hypoxia, miR-126 activates EPCs and ECs for vascular healing and neovessel

formation[19]. Elucidation of regulatory networks that control vessel development and regeneration is key to enable optimal neovascularisation and cardiac repair.

Thymosin β4 (Tβ4) was identified as a paracrine regulator of the EMT of embryonic EPDCs, that stimulated their inward migration and differentiation into vascular progenitor cells to form the coronary vasculature[125]. The developmental role of Tβ4 could be extrapolated into the adult setting, as treatment of adult murine cardiac explants with the small peptide restored activation, migration and cardiovascular differentiation potential. Following migration away from the explant, epicardin-positive EPDCs differentiated into fibroblasts, smooth muscle and endothelial lineages *in vitro*[125]. The epicardium, particularly following MI, is a rich source of fibroblasts; pre-treatment with Tβ4 appears to shift the balance of EPDC contribution towards cardiomyocytes and coronary vascular progenitors[119,120,124] and away from fibroblasts, which, by and large, appear to be the default fate[171]. Molecular phenotyping of murine adult EPDCs, following MI and Tβ4 treatment revealed them to be a highly heterogeneous population, chiefly defined by expression of cardiac progenitor and mesenchymal stem cell markers, including Wt-1, Sca-1, CD90, CD44 and PDGFRβ, thus significantly different from their embryonic counterparts at E12.5, despite the common expression of embryonic epicardial markers[9]. A comparison of populations expressing a *Wt-1*-GFP reporter and/or Sca1 revealed that greatest vasculogenic potential resided within the *Wt-1*-GFP negative population. Although, compared with a Sca1 negative epicardial subpopulation, the GFP+ Sca-1+ fraction expressed significantly higher levels of Isl1 and Flk1, embryonic markers of multipotent cardiac progenitors and vascular specification[82], non-GFP+ epicardial cells, activated by Tβ4 and injury, expressed considerably higher levels of *Pecam1* and *Sm22α* than the WT-1-expressing population. Not only is this in keeping with the limited vasculogenic potential of the *Wt-1* fate mapping

studies, it raises the intriguing possibility that vasculogenic potential may reside within an adult epicardial subpopulation that does not express *Wt-1* [9]; therefore, a direct, if limited, contribution to neovascularisation post-MI cannot be ruled out.

Prokineticins (PKs) have also been shown to reactivate adult epicardial cells[141]. Acting via its G protein-coupled receptor, PKR-2, the secreted peptide, PK2, was shown to promote coronary vessel formation and cardiomyocyte protection post-MI. PK2 and its receptors, (PKR)-1 and -2, are elevated for 7 days following MI in mouse and overexpression of PKR-1 promoted vascular regeneration via epicardial activation[10].

Synthetic modified RNA encoding human vascular endothelial growth factor (VEGF-A mod RNA) was shown to induce cardioregenerative effects by activating epicardial cells, inducing their proliferation, inward migration and biasing their differentiation potential in favour of endothelial cells (58%) and a contribution (5%) of cardiomyocytes[71,162]. These findings will serve to reignite the debate surrounding epicardial differentiation potential, EC contribution being an elusive epicardial fate and cardiomyocyte contribution viewed with much scepticism. Given the significant implications for repair, key questions to be addressed are whether, and how, Tβ4 and/or VEGF-A fundamentally alter epicardial progenitor cell fate, either directly or via cooperation with injury or inflammatory signalling, as opposed to the greater prevalence of ECs and cardiomyocytes simply reflecting enhanced epicardial activation and size of progenitor pool.

More recently, cardiac-specific overexpression of human stem cell factor (hSCF) was shown to effect enhanced activation and migration of WT-1–expressing epicardial cells, 3 days post-MI and increased arteriolar density 5 days post-MI[155]. In vitro, hSCF treatment promoted EPDC proliferation and growth factor expression, amongst them VEGF-A and FGF-2, effects which were abrogated with an antibody against c-kit, the cell surface receptor

for SCF. Although Wt-1 lineage traced αSMA+ were detected, these were not associated with vessels in the peri-infarct region, as previously reported[124,170], and may identify myofibroblasts. These findings are also intriguing because a number of studies, notably[9], have suggested that *Wt-1* and *c-kit* label epicardial sub-populations that are largely non-overlapping[66,67]. Further research is required to understand epicardial heterogeneity, the overlap and interplay between the various populations.

FUTURE PROSPECTS FOR CORONARY NEOVASCULARISATION

The intrinsic repair mechanisms which attempt to restore blood flow to infarcted myocardium are poorly understood and, surprisingly, under-investigated, given the urgent need for vascular therapies. Improved insights into the embryonic programme and cellular origins of coronary vessel development may uncover new knowledge and potential for augmenting vessel regeneration following infarction. Notwithstanding the limited availability of Cre-loxP based tools to selectively label adult endothelial populations in order to delineate their contribution to the *de novo* capillary network, key questions remain regarding the plasticity and potential of these cells to contribute in the adult. Productive starting points may be the piecing together of the regulatory networks that control, at the signalling/transcriptional levels, sinus venosus sprouting, endocardium-coronary EC coalescence and arterial/venous EC specification and assessment of their recapitulation in the heart post-MI. If epicardial activity and endocardial plasticity are greatest during embryonic stages, what are the injury-induced signals that lead to partial restoration and what are the limiting factors that prevent a complete reversion to the foetal state? A more complete picture of the embryonic mechanisms, combined with an appreciation of the vascular remodelling that occurs endogenously in the injured heart may reveal opportunities to therapeutically enhance cardiac neovascularisation and regeneration.

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FIGURE LEGENDS

Figure 1. Developmental Mechanisms of Coronary Vessel Development. In the embryo, endothelial progenitors derive primarily from the sinus venosus (SV), but with likely contributions from proepicardium (Sema3d+/Scx+ populations) and endocardium. These migrate via the subepicardial space into the myocardium and assemble to form an endothelial (EC) capillary plexus, which expands via angiogenesis. Vascular smooth muscle cells (VSMCs) derive from the proepicardium to support the maturing vessels. During the perinatal period, the coronary network markedly expands with addition of new vessels from the endocardium.

Figure 2. Putative Mechanisms that may Contribute to Neovascularisation of the Myocardium Post-MI. Although poorly understood, insight into the mechanisms that underlie the formation of new coronary vessels in the ischaemic heart is urgently needed. Based on known roles in development and tumour vasculogenesis, and evidence of signal pathway reactivation in the heart post-MI, a number of processes and progenitor cell sources may be expected to contribute. Angiogenesis and collateral growth (not shown) are likely the predominant processes for restoring blood flow. Other putative mechanisms may include reactivation of embryonic progenitor populations, namely the epicardium and endocardium, the latter having been shown to contribute to an extent [78]. Infiltrating stem cells from the bone marrow or activated progenitors from within the vascular walls may also contribute. EPDCs: Epicardium-derived cells; EPCs: Endothelial Progenitor Cells; BMSCs: Bone marrow-derived (mesenchymal) stem/stromal cells; VWPCs: Vascular Wall Progenitor Cells.