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Scarring vs. Functional repair: Matrix-based strategies to regulate tissue healing

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Abstract

All vertebrates possess mechanisms to restore damaged tissues with outcomes ranging from regeneration to scarring. Unfortunately, the mammalian response to tissue injury most often culminates in scar formation. Accounting for nearly 45% of deaths in the developed world, fibrosis is a process that stands diametrically opposed to functional tissue repair and regeneration. Strategies to improve wound healing outcomes therefore require methods to limit fibrosis. Wound healing is guided by precise spatiotemporal deposition and remodeling of the extracellular matrix (ECM). The ECM, comprising the non-cellular component of tissues, is a signaling depot that is differentially regulated in scarring and regenerative healing. This Review focuses on the role of key matrix components during mammalian wound healing alongside a comparison to scar-free repair and regeneration followed by an overview of matrix-based strategies that attempt to exploit the role of the ECM to facilitate functional tissue remodeling.

Keywords

extracellular matrix (ECM); wound healing; fibrosis; tissue engineering

2 Introduction

The extracellular matrix (ECM) is the complex three-dimensional acellular environment that is present in all tissues and is essential for life. Controlled remodelling of the ECM is required for development, wound healing, and homeostasis; however, uncontrolled remodelling contributes to a variety of disease states. The ECM is crucial for tissue repair, acting as a dynamic scaffold to protect and support healing wounds and as an instructive platform for reciprocal signaling with cells. Precisely regulated spatiotemporal biochemical and biomechanical cell-matrix interactions are key to the efficacy and quality of repair

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processes. Understanding matrix signaling events during wound healing provides insights for the rational design of matrix mimics to control the outcome of tissue repair.

All vertebrates have evolved mechanisms to replace or restore damaged tissue. The spectrum of wound healing outcomes ranges from complete regeneration in zebrafish and salamanders to scar formation in adult mammals. The mammalian wound healing response is classically characterized by three stages (i.e., inflammation, proliferation and remodeling) with each stage inextricably linked to the ECM. Tissue repair commences immediately following tissue injury and, in post-natal mammals, most often culminates in the replacement of the damaged tissue with an acellular fibrotic matrix (i.e., scar tissue). Fibrosis is a major impediment to functional tissue repair. While scarring of minor skin wounds will have little or no functional effect on the organism, fibrosis in other tissues (e.g., heart, esophagus, spinal cord) contributes to the manifestation and progression of a myriad of diseases. The present review focuses on some of the common regulatory features of ECM during wound healing and tissue regeneration. An overview of the deposited and remodeled matrix components during mammalian wound healing is provided alongside a comparison to natural examples of scar-free repair and regeneration. Finally, we review pre-clinical and clinical matrix-based strategies that attempt to exploit the role of the ECM to facilitate functional tissue remodeling.

3 Matrix deposition and dynamics in the wound healing cascade

Faithful wound healing requires finely tuned ECM deposition and remodeling. Disruption of matrix remodeling events and excessive matrix deposition can lead to impaired wound healing (i.e., fibrosis). The deposition of ECM proteins and matrix-associated proteins during the wound healing cascade is well-orchestrated, and key to successful wound healing (Table 1). Fibrin and fibronectin are released from vasculature immediately after capillary damage to prevent extensive blood loss. Platelets release chemotactic growth factors to re-establish the epithelium, while neutrophils and macrophages begin clearing excess matrix and cell debris. Fibroblasts activate to myofibroblasts and deposit collagen, glycosaminoglycans (GAGs) and fibronectin (i.e., granulation tissue). Myofibroblasts, originating from epithelial-to-mesenchymal transition of various cells, produce collagen I enriched scar tissue to provide strength and protection to the healing wound. Myofibroblasts contract along matrix fibers to support wound closure and subsequently undergo apoptosis. Epithelial cells deposit a basement membrane to establish tissue integrity and cell polarity. Angiogenesis along the newly deposited basement membrane leads to the formation of blood vessels to restore circulation. Collagen fibrils are then arranged and cross-linked over a period of months, and excess collagen is cleared (Figure 1).

3.1 Major ECM components in mammalian tissue repair

3.1.1 Collagens—Collagens are the most abundant and diverse ECM proteins with 28 members (type I - XXVIII) of the collagen family identified to date [1]. Collagens are trimers assembled from three polypeptide chains (α-chains) that form a triple helix with repeating amino acid motif Gly-X-Y and are classified as fibrillar, fibril-associated collagens with interrupted triple-helices (FACIT), basement membrane network-forming, hexagonal

network-forming, and filamentous. The collagens contribute to tissue repair via cell interaction sites for adhesion and migration, by connecting the basement membrane and the interstitial matrix, and providing mechanical strength to the tissue.

Fibrillar collagens III and I are sequentially deposited during mammalian tissue repair. Collagen III regulates fibroblast phenotype in early stages of tissue repair, and is replaced by collagen I after 2-3 weeks [2]. Individuals with collagen III mutations have impaired wound healing. Collagen III deficiency in murine models triggers increased myofibroblast differentiation and accelerated wound closure alongside increased scar formation. Collagen I, the most abundant collagen type, is expressed by fibroblasts. Collagen I fibers offer strength to protect the healing wound, and are involved in mechano-signaling events guiding tissue repair. The scar matrix is predominantly composed of collagen I and is effectively a barrier to tissue regeneration once formed.

Collagen IV provides strength and integrity to the basement membrane – the sheet-like network underlying all epithelium and endothelium. Collagen IV interacts with cells via integrins, and collagen IV fragments exert a variety of biological activity. During tissue remodeling, the basement membrane, and therefore collagen IV, aids in establishing tissue integrity, epithelial cell polarity, and neo-vascularisation. Basement membrane remodeling by matrix metalloproteinases (MMPs) during angiogenesis releases growth factors that are tethered to the dense membrane network, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) [3].

The role of FACIT collagens in tissue repair is not yet well understood. Type XIV collagen appears to regulate early stages of collagen fibrillogenesis, based on studies on Col14a1(-/-) mice [4]. Collagens XII, XIV and VII connect the basement membrane to the interstitial matrix and are crucial to matrix integrity [5, 6]. Collagen VI is expressed by fibroblasts and endothelial cells of newly formed blood vessels in clinical specimens and regulates fibroblast apoptosis *in vitro* [7]. Collagen VI deficiency weakens the basement membrane and alters collagen fibril architecture in Col6a1(-/-) mice [8], and similar defects are present in the dermis of individuals with collagen VI myopathy [9]. Collagen VI deficient matrices are thinner and contain less total collagen than matrices derived from healthy individuals. Collagen VI also affects cell spreading and migration [10]. Interestingly, macrophages secrete collagen type VI to modify cell-matrix interactions during wound healing [11]. FACIT collagen type VII ensures wound closure through the organisation of laminin in the basement membrane and by supporting fibroblast migration [12].

3.1.2 Fibronectin—Fibronectin is a multidomain glycoprotein comprising 12 FN type I repeats (FN1), two FN type II repeats (FNII), fifteen FN type III repeats (FNIII) and one variable (V) region. Fibronectin exists in circulating plasma form and the structurally unique cellular form, which is expressed and assembled during the wound healing cascade. A variety of cell- and tissue-specific isoforms arise from alternative splicing of FNIII domains. This domain structure allows for flexibility in the protein backbone and mechanical dynamics to modulate fibronectin-cell and fibronectin-matrix interactions [13].

Fibronectin acts as a scaffolding protein during tissue repair and a regulator of cell function. Plasma fibronectin, synthesized by hepatocytes, stabilizes the fibrin clot and is a substrate for platelet adhesion, spreading, and aggregation in early stages of wound healing. Cellular fibronectin, secreted by endothelial cells and fibroblasts, is assembled into a dense fibrillar network that enables fibroblast polarization and provides migration highways for cells within the wound bed. The domain architecture and mechanical flexibility of fibronectin confers cell signaling and matrix interactions as different domains comprise different cell-binding and matrix protein-binding sites. Fibronectin a critical regulator of ECM organization and stability and a pre-formed fibronectin matrix is requisite for collagen network formation [14]. The fibronectin matrix is a signaling depot where growth factors are sequestered and activated upon matrix alterations (e.g., transforming growth factor beta 1 (TGFβ1) and VEGF, amongst others). Interestingly, wound gap closure is primarily dependent on fibronectin deposition rather than the availability of a collagen matrix [15].

3.1.3 Laminin—Laminins are glycosylated, 400 kDa multidomain proteins assembled from an α-, β-, and γ- chain to a cross-like tertiary structure. Twelve different mammalian laminin chains have been identified that can form 16 different isoforms with varying chain length and domain architecture (39). Genetic deletion of laminin causes early lethality [16] in contrast to collagen IV deletion that diminishes the stability of the basement membrane and is lethal at a later developmental stage [17]. Remodeling of the basement membrane during wound healing is critical to re-epithelialisation and the formation of blood vessels.

The formation of a basement membrane network is initiated by laminin. Basement membranes are sheet-like matrices, present in all epithelial and endothelial tissues, providing tissue integrity, instructing cell polarity, and acting as a selective barrier for cell transmigration (37, 38). Vascular basement membrane components are required for the initiation of vascularization, and blood vessel growth and branching requires continuous remodeling of the basement membrane. Endothelial cells are quiescent while bound to the capillary basement membrane, but proteolytic degradation of the basement membrane by MMPs leads to the release of pro-angiogenic growth factors (i.e., VEGF and FGF, and cryptic basement membrane fragments) cell migration and blood vessel formation activity [18]. Growth factor tethering within the basement membrane is maintained by agrin, perlecan, and type XVIII collagen. Basement membrane fragments have been suggested as a tool to assess impaired tissue repair and fibrosis [19]. Isoforms of laminin are involved in endothelial and pericyte cell signalling [20], indicating a regulatory role of basement membrane remodeling in angiogenesis. In mice lacking nidogen-1, a protein that connects laminin chains and stabilizes the basement membrane, epidermis remodeling and resolution of granulation tissue resolution is impaired following skin injury [21].

3.1.4 Tenascin—The tenascin family of matrix proteins comprises four different isoforms arising from alternative splicing and protein modifications. Tenascin-C, a well-studied isoform, is a hexameric, multidomain protein around 200kDa comprising FNIII and EGF-like domains which provide a variety of binding sites for cross-talk with cells and the matrix [22]. Tenascin-C is a highly flexible isoform due to its tandem FN-III modules that can be revealed by force-induced unfolding [23]. Tenascin-C is a developmentally associated

protein that is effectively absent in the adult organism except during wound repair. Interestingly, *de novo* tenascin-C synthesis is characteristic at the wound's edge during tissue repair and tenascin-C deficiency elicits abnormalities in wound healing [24]. Tenascin-C depletion reduces the deposition of fibronectin and collagen in the wound healing process where fibronectin-tenascin-C interactions alter cell migration and focal adhesions pivotal to wound closure [25]. A recent study found that supplementation of tenascin-C (in conjunction with another developmentally associated protein Fibrillin-2) on acellular lung scaffolds enhances epithelial proliferation, decreases senescence, aids cell attachment and migration, and improves the morphology and structure of regenerated lung tissue in-vitro and ex-vivo [26]. Tenascin-C accumulation is also detected in fibrotic tissue, suggesting spatiotemporal control of tenascin-C is important to tissue repair [27].

3.1.5 Vitronectin—Vitronectin is a 75kDa blood plasma protein that represents 0.2–0.5% of total plasma proteins with binding domains for integrins and matrix proteins (e.g, collagen and heparin) and non-matrix proteins (e.g., plasminogen activator inhibitor-1) [28]. Vitronectin regulates plasminogen activation during wound healing [29]. Plasminogen activation is an extracellular proteolytic cascade generating plasmin—a protease that cleaves matrix, mediates clearance of fibrin clots, and activates MMPs critical for matrix remodeling and angiogenesis [30]. Vitronectin also binds activated platelets and initiates platelet aggregation [31] and vitronectin depletion results in delayed coagulation [32]. The addition of exogenous vitronectin enhances the wound closure rate of corneal epithelial cells *in vitro* [33]. Vitronectin regulates growth factor activity and has been suggested as a growth facto delivery vehicle for growth factors [34] which has shown promise in early clinical study for treatment of chronic wounds [35].

3.1.6 Glycosaminoglycans and proteoglycans—Glycosaminoglycans (GAGs) such as hyaluronic acid (HA), chondroitin sulfate (CS), dermatan sulfate (DS), and heparan sulfate (HS) are synthesized by fibroblasts and contribute to the hydrophilic granulation matrix [36, 37]. Proteoglycans consist of core proteins or glycoproteins with glycosamino side-chains that interact with granulation tissue, altering matrix biomechanical properties and hydrophobicity, and modifying cell migration and fate. Proteoglycans are a reservoir for growth factors (e.g., TGFβ, VEGF and FGF) critical to tissue repair. For example, the activity of FGF-2 (which stimulates fibroblast growth) is mediated by HS-proteoglycans that facilitates binding and stabilization of FGF-receptors [38].

HA interacting with HA-binding proteoglycans (e.g., aggrecan, versican, neurocan, and brevican) is ubiquitous in the human body and involved in many different tissue repair events, including the regulation of fibroblast phenotypes [39]. Syndecans are transmembrane HS-proteoglycans that control cell migration and wound closure [40], and act as a regulator of heart fibrosis [41]. HA-binding proteoglycans bind growth factors and have been exploited as growth factor delivery vehicles for diabetic wound healing [42]. The HS-proteoglycan perlecan is part of the basement membrane and involved in epithelial cell plasticity and angiogenesis. Recent work has demonstrated the potential to induce angiogenesis and healing of cutaneous wounds with scaffolds functionalised with perlecan and VEGF [43].

3.1.7 SPARC—Secreted protein acidic and rich in cysteine (SPARC) is a 32kDa glycoprotein expressed at sites of injury and matrix remodeling [44]. SPARC is a modulator of matrix organization and cell-matrix interactions through binding of matrix proteins and modulating integrin-linked kinase activity [45]. SPARC-deficient mice exhibit tissue repair defects manifesting as delayed formation of granulation tissue and diminished ECM production [46]. SPARC is also involved in idiopathic pulmonary fibrosis through activation by TGFβ [47]. Inhibition of SPARC attenuates the pro-fibrotic effect of TGFβ [48].

3.1.8 CCN proteins—CCNs are a family of six secreted proteins with key roles in tissue repair and matrix remodeling. CCNs interact with cells and granulation matrix via binding sites for integrins and HS-proteoglycans [49]. Platelets release CCN2, leading to expression of other CCN isoforms at the site of tissue injury. CCN2, alongside TGFβ, promotes expression of matrix proteins and is overexpressed in fibrosis, and is suggested as a potential diagnostic marker of fibrosis [50]. CCN5 has an opposing role to CCN2 in the progression of fibrosis, where CCN5 inhibits cardiac hypertrophy and fibrosis [51]. Interaction with TGFβ-signalling is a common functional motif of CCN proteins. Similarly, CCN4 is up-regulated in idiopathic pulmonary fibrosis. CCN1 is also considered anti-fibrotic as it induces myofibroblasts senescence [52]. CCN1 structurally bridging macrophages to neutrophils through binding of phosphatidylserine on neutrophils and integrins on macrophages and plays a key role in neutrophil clearance, and CCN1 has been suggested as a possible treatment for non-healing chronic wounds [53]. CCN3 promotes fibroblast adhesion and wound healing in skin wounds via integrin-binding and has been demonstrated to be pro-angiogenic [54].

3.1.9 Thrombospondin—Thrombospondins (TSPs) are multidomain oligomeric glycoproteins with distinct domain structure and binding sites for cells, growth factors, GAGs and other matrix proteins [55]. A multivalent structure allows TSPs to link matrix proteins and facilitate matrix organisation. TSP1 is expressed by macrophages and facilitates re-epithelialisation through organising matrix structure and fibrils [56], while also acting as a regulator of latent TGFβ [57]. Depletion of TSP and TSP1/TSP2 results in a prolonged inflammatory response and delayed healing [58]. TSP1 is a mediator of renal fibrosis in the pathophysiology of ischemic kidney failure [59]. TSP2 is necessary for collagen fibrillogenesis [60] and TSP2 is required for platelet aggregation in the early response to tissue injury [61]. TSP2 also acts as an angiogenesis inhibitor and is regulated by protein kinase B (Akt) in fibroblasts, which mediates cell migration, adhesion and differentiation [62].

3.1.10 Osteopontin—Osteopontin is a 44kDa phosphoprotein that has been extensively studied in the context of bone mineralisation and repair. Osteopontin is also a participant in skin and cornea wound healing. Fibroblast phenotype, matrix organisation and collagen fibrillogenesis are all altered when osteopontin is depleted [63, 64].

3.1.11 Fibulin—Fibulins are multidomain proteins with binding sites for several matrix proteins, with strong affinity for basement membrane proteins laminin and nidogen, and consensus sequences for calcium binding [65]. Fibulin is deposited by fibroblasts as

extracellular fibers or secreted into the blood, and five different isoforms have been identified. Fibulin fibers regulate TGFβ and fibroblast migration in cardiac tissue [66, 67]. Fibulin-5 accumulation is associated with abnormal tissue repair in chronic obstructive pulmonary disease [68] while the addition of exogenous fibulin-5 promotes skin wound healing [69]. These elastic fibers of fibulin-5 may not directly impact short-term wound healing, but rather are important for long-term function and integrity of the tissue [70]. While the structure-function relationship of fibulin fibers in repair processes is not well understood, fibulin has a role in long-term tissue repair.

3.2 Matrix mechanics – how stiffness and force regulate tissue repair

Mechano-chemical feedback mechanisms between cells and the matrix regulate the wound healing cascade. The dynamic composition and architecture of the matrix during tissue repair alter the viscoelasticity of the wound bed, and in turn growth factor activation, cell adhesion, migration, and differentiation. Further, cell-generated traction leads to mechanical unfolding of proteins that reveals neo-interaction sites as an additional regulatory feature of ECM-mediated wound healing.

Fibronectin and collagens are the first matrix proteins deposited after tissue injury. Fibronectin fibrils can be mechanically unfolded by cells to regulate protein structure/function. Cytoskeletal tension leads to the reversible extension of fibronectin, exposing FNIII motifs and altering integrin-binding affinities and cell signaling [71]. The elasticity of fibronectin fibers assists collagen assembly, as collagen I fibers preferentially co-localize with more-relaxed fibronectin fibrils [13]. Similarly, tenascin-C and tenascin-X, which differ only in number of FNIII domains, can be mechanically unfolded to generate functional diversity and the ability to sustain cell-ECM or ECM-ECM interactions over long extensions [23].

Collagen morphology determines the physicochemical properties of the collagen matrix and affects collagen degradation, fibrogenesis, and integrin-mediated mechanocoupling. Cell motility and the ability of cells to translocate collagen fibers is modulated by collagen stiffness and porosity [72], which together can induce a change in mode of migration [73]. Collagen I, the major constituent of scar tissue, has a fractal microarchitecture that confers stiff matrix properties that alter cellular responses, such as inducing epithelial-to-mesenchymal transition (EMT) and mesenchymal transcription responses through integrin-mediated mechanosensing [74]. Accordingly, in fibrotic processes, excess collagen I deposition triggers an increase in myofibroblasts (originating via EMT) that in turn deposit matrix and propagate tissue fibrosis. The mechanical properties of collagen are also associated with TGFβ activation via myofibroblast's integrin-mediated growth factor activation [75].

3.3 Proteolytic matrix remodelling

During tissue repair, spatiotemporal expression of enzymes leads to cleavage of matrix proteins that modulates matrix morphology (i.e., mechanical properties) and biochemical signaling by releasing or unravelling bioactive matrix fragments known as cryptic fragments or matricryptins. Among the matrix degrading enzymes important for tissue repair are

MMPs, ADAMs (a disintegrin and metalloproteinase), ADAMTSs (a disintegrin and metalloproteinase with thrombospondin-like motifs), and neutrophil elastase. Proteolysis guides cell migration by local matrix degradation and modulate cell signaling. Moreover, matrix-degrading enzymes release (and activate) growth factors from the ECM.

3.3.1 Matrix metalloproteinases (MMPs)—MMPs belong to the zinc proteinase superfamily and are expressed by cells during tissue injury and repair. MMP activity is regulated by the activation of pro-enzyme or inhibition through endogenous tissue inhibitors of metalloproteinases (TIMPs). Twenty-two different human isoforms of MMP with distinct substrates and tissue distribution have been identified. MMPs are either secreted or membrane-bound (MT-MMPs) and are generally classified by their substrate and mechanism of action. However, redundancy of substrates and identification of new substrates may soon render the historical classification of MMPs obsolete. The MMPs involved in tissue repair processes cleave matrix proteins including collagens, gelatin, laminin, fibronectin, proteoglycans, tenascin, vitronectin, and osteopontin in local matrix remodeling events.

MMPs process matrix proteins during tissue injury and repair and also act on cellular junction and adhesion proteins. MMPs have been shown to cleave E-cadherin, an adherens junction protein responsible for epithelial cell sheet formation, to regulate epithelial cell extrusion and cell migration events [76]. MMPs interact with integrins to modulate cell adhesion and migration [77]. MMPs also regulate vascular remodelling by liberating sequestered angiogenic factors and by generating cryptic anti- and pro-angiogenic molecules [78]. Given the key roles of MMPs in tissue repair, increased MMP expression has been correlated with impaired wound healing and fibrosis [79]. Elevated levels of certain MMPs have been suggested as diagnostic markers of disease-state and therefore MMP-inhibition represents a potential therapeutic strategy for improving tissue repair outcomes [80, 81]. However, pharmaceutical targeting of MMPs is problematic, as poor selectivity and off-target effects of exogenous MMP inhibitors has led to multiple failed clinical trials [82], highlighting a need for unique approaches to MMP targeting.

3.3.2 ADAMs and ADAMTSs metalloproteinases—ADAMs and ADAMTSs are members of the zinc proteinase family and are involved in tissue repair, vascular remodeling and activation of growth factors. ADAMs have been found to be important in skin and human lens epithelium wound healing [83–85]. ADAMTSs degrade proteoglycans [86], process pro-collagen to collagen [87], regulate blood coagulation, and facilitate vascularization [88–90].

3.3.3 Neutrophil Elastase—Neutrophil elastase is secreted by neutrophils and macrophages during the early wound healing response. Neutrophil elastase is responsible for extracellular killing of microorganisms [91], and has been shown to process laminin in regulating neutrophil extravasation [92–95].

4 Scar-free healing: Unique matrix environments in the embryo and regenerating organisms

The ECM provides a pro-regenerative microenvironment in fetal tissue repair and in regenerating vertebrates that may provide therapeutic opportunities for improving wound healing in humans (Table 2). In stark contrast to adult wounds, fetal tissue repairs rapidly and scar-free. Differences exist between the ECM of adult and embryonic tissue, highlighting the role of matrix composition and architecture in scar-free healing [96]. The ratio of type I to type III collagen is higher in adult tissue repair processes compared to embryonic repair [97]. Murine models of collagen III deficiency show increased scar formation, indicating an important role of collagen III in the regulation of scar tissue formation. Collagen I provides strength to the closing wound, but is the major constituent of the scar-forming matrix and a physical barrier to regeneration. Tenascin expression occurs earlier in the fetal wound compared to the adult [98]. Elevated levels of HA, a granulation tissue component and regulator of fibroblast phenotype and cell motility, is elevated in the fetal wound matrix which likely provides a more permissive matrix for cell movement and proliferation [99]. It has been further shown that there are differences in MMP and TIMP ratios between fetal and adult wounds, favoring matrix turnover and cell migration [100]. Changes in matrix composition, architecture and MMP expression further impact the release and activation of growth factors tethered to the matrix, such as TGFβ, to accelerate scar-free tissue repair in fetal tissue.

Insights into scar-free tissue repair can be gained by understanding how some non-mammalian vertebrates undergo tissue regeneration. Not surprisingly, dynamic breakdown and remodeling of the ECM is pivotal to tissue regeneration. Zebrafish, salamanders, and lizards are known for their regenerative capacity. Biphasic expression of MMPs is critical for faithful limb and tail regeneration in lizards and salamanders. The first wave of MMP expression is thought to promote epithelial cell migration while inhibiting basement membrane formation and the second phase of MMP expression is responsible for ECM remodeling and turnover [101]. The delayed basement membrane formation is an important distinction in regeneration vs. mammalian tissue repair. In most mammals, the basement membrane is restored during early stages of wound healing and is associated with a switch from collagen III to collagen I which is progressively cross-linked to an acellular fibrous scar. In salamanders and fish however, expression of basement membrane constituents (e.g., collagen IV and laminins) is delayed until late stages of tissue repair/regeneration [102, 103]. Synthesis and cross-linking of collagen I is also delayed until final stages of scar free repair [103]. Increased expression of hyaluronic acid, tenascin, collagen III, fibronectin, and developmentally-associated collagens (e.g., collagen XII) may also be important to the regenerative ECM landscape [101].

5 Strategies to reduce scarring

Fibrosis is the major contributor to the manifestation and progression of a myriad diseases and represents one of the most compelling health issues in modern medicine. Scarring causes substantial morbidity; for example there is high incidence of fibrotic adhesions of

internal organs in response to trauma, infection, radiation, or surgery that often leads to pain, bowel obstruction, and infertility. In diseases such as idiopathic pulmonary fibrosis, liver cirrhosis, cardiac fibrosis, systemic sclerosis and nephritis, fibrosis can lead to organ failure and death. Despite the fact that fibrotic diseases account for nearly 45% of deaths in the developed world, there is currently no accepted therapy to target fibrosis. However, insights gained over the past 2 decades into the molecular pathways driving fibrosis has informed the development of potential therapies.

Dozens of fibrosis therapies have been developed over the past decade and can be classified as pharmaceuticals, biomaterials, and cell therapies (Table 3). Common therapeutic targets include TGFβ signaling, inflammation, and fibroblasts (i.e., honing, activation, and proliferation). Strategies to target fibrotic disease have had limited clinical success to date, and is reviewed in detail elsewhere [104–106]. Given that fibrosis is often characterized by several simultaneous pro-fibrotic pathways, a multifaceted approach is likely key to success. In the following section, we focus on matrix inspired approaches to limit fibrosis and achieve functional tissue repair.

6 Matrix-based materials for modulating tissue repair

The intimate role of the ECM in post-inflammatory tissue repair, wound healing, and fibrosis dispels the historic view of ECM as simply an inert support structure for cells. The ECM microenvironment in which cells reside is comprised of structural fibers, functional molecules, and a signalling repository which is highly heterogeneous and varies from tissue to tissue. ECM-cell interactions activate cellular responses (e.g., proliferation, differentiation, and migration) and tissue-specific cells are constantly secreting, assembling, and remodelling the ECM. Accordingly, the design and fabrication of ECM mimicking materials has been pursued for decades and represents a core strategy in tissue engineering. Due to the complexity of composition, structural organization, biomechanics, and dynamic roles of the ECM, synthesis of a high fidelity ECM mimic is not yet feasible. For this reason, typical biomaterial approaches focus on a few of the mechanisms by which ECM influences cells and attempts to effectively present these cues for a given tissue. Thus, essentially every material studied in tissue engineering can be classified as an ECM-like material but this review will focus on biologically derived ECM-like materials.

6.1 Components and cryptic fragments of the ECM

In mammals, the ECM consists of over 300 proteins including collagens, proteoglycans, and glycoproteins. Individual ECM components have been isolated from tissues to study constituent effects on cell behaviour and to manufacture scaffolds for various applications. Matrix materials have been generated from purified collagens, fibronectin, fibrin, laminin, HA, and CS, among others. Purified Type I collagen derived scaffolds are among the most common ECM-derived protein and are FDA allowed for several clinical applications, mostly as hemostatics and soft tissue fillers. Enzymatic cleavage of many ECM proteins can release or reveal bioactive fragments that exert biological activity which is distinct from the parent molecule. ECM proteins and their subdomains bind integrins and other cell surface receptors to regulate gene expression, cell differentiation, mitogenesis, and migration. Through the

role of ECM fragments in directing cell activity and cell fate, matrix components regulate inflammation, angiogenesis, and scarring outcomes during wound healing. Given the interaction of the ECM with the wound healing process, there has been considerable interest in the cryptic fragments and degradation products of ECM for tuning wound healing.

Cryptic fragments have been described for a variety of matrix proteins and contribute to tissue repair events ranging from cell migration and epithelial-to-mesenchymal transition to the formation of blood vessels. Selective ECM degradation generates microenvironments with unique biochemical and morphological ECM properties to instruct cell behaviour [107]. Past studies have shown relative success in use of matrikines as inhibitors of angiogenesis and inflammation, and angiogenesis inhibitors have been proposed in clinical studies for cancer therapy [108, 109]. Individual ECM fragments can also alter wound healing outcomes. A peptide fragment of lumican, a small leucine rich proteoglycan, has been shown to promote corneal wound healing following epithelial debridement, in part by mitigating TGFβ signalling [110]. Collagen IV contains hidden signaling molecules, such as arresten and canstatin that become active through proteolytic processing by MMPs and regulate angiogenesis after tissue injury by coordinating basement membrane remodelling and vascular sprouting [111–113]. Similarly, cryptic laminin fragments impact epithelial and endothelial cell migration and adhesion, angiogenesis, MMP activation, and EMT-related events involved in fibroblast differentiation and wound closure [18, 114, 115]. The laminin β1-chain-derived fragment, liberated by MMP processing, interacts with cells via α3β1-integrins and triggers down-regulation of active MMP2 in mouse and human ESCs [116], and was recently shown in a pre-clinical study to prevent peritoneal fibrosis [117].

Individual fragments and cryptic sites of ECM provide interesting insights to the complex process of ECM remodelling during wound repair. Bioactive ECM fragments are potential drugs and have been tested in experimental models of cancer, wound healing, fibrosis and infectious diseases either as full-length fragments, modified fragments or peptides derived from their sequences, but their translation into the clinics is challenging. Clinical trials with recombinant collagen XVIII fragment (i.e., endostatin) as a cancer therapy have been largely unsuccessful in the US despite approved clinical use of endostatin in China [118]. However, there is still potential for endostatin as an anti-fibrotic [119]. Translation to date has been limited largely due to low potency of individual ECM fragments relative to the cognate ligands for the receptors known to trigger cell activation; for example, IL-8 binds and activates neutrophils at nanomolar concentrations [120], while micromolar concentrations of chemotactic matrix-derived peptides are needed to activate neutrophils [121].

The combination of several ECM bioactive fragments as an alternative approach to increase their biological effect has been studied by examining the products released from ECM materials as they degrade. The degradation products of decellularized tissues releases numerous protein fragments and peptides with reported biologic function. Early study in this area showed that peptides generated during solubilisation of SIS are chemoattractants for endothelial cells [122]. The fragments generated by enzymatic degradation of a decellularized urinary bladder matrix (UBM) have shown an increased proliferative and migration response in perivascular stem cells [123], especially in hypoxic conditions similar to oxygen levels after injury [124]. Host-mediated degradation of UBM has also been shown

to be chemotactic for progenitor cells, which established a link between chemotactic properties that result from in vitro methods of ECM scaffold degradation and the in vivo process of scaffold remodeling [125]. Degradation products of UBM injected at the site of digit amputation caused an accumulation of multipotent progenitors expressing Sox2, Sca1, and Rex1 [126] and these effects were later attributed in part to a cryptic peptide from the C-terminal region of collagen III [127]. Bioactive components of degraded fetal cardiac ECM [128] and partially degraded adult cardiac ECM [129] have also been shown to promote the expansion of cardiomyocytes. Most frequently, these studies were conducted using a complex mix of degraded ECM materials which makes it difficult identifying contributions and interpreting mechanism of action. However, it's unquestionable that degradation products of ECM have measureable bioactivity that may be a therapeutic strategy to enhance tissue repair. One approach to deliver several bioactive fragments as a therapeutic that has gained attention over the past decade uses scaffolds and hydrogels derived from decellularized tissue.

6.2 Decellularized matrices

The concept of tissue decellularization is not new. The earliest reports of tissue decellularization date back nearly 7 decades, where in 1948 pulverization and freeze-thaw cycles were used to render muscle tissue acellular [130]. In 1991, an early tissue engineering study used allogeneic acellular dermal matrices as a biomaterial to facilitate reconstruction of skin with the use of autologous keratinocytes and fibroblasts [131]. The first use of xenogeneic tissue was described in 1989, where small intestinal submucosa (SIS) was used as a scaffold for vascular applications [132]. Since then, methods for preparing ECM scaffolds been developed and refined for nearly every tissue in the body [133]. All tissues are comprised predominantly of the same base biopolymers (i.e., collagens, glycans, laminin, elastin) with collagen I representing the majority of the matrix by weight. This unique properties of different decellularized tissues are therefore attributed to differences in biopolymer compositions and structures attributed to the cellular composition of the tissue. Numerous ECM scaffolds are now FDA-allowed and commercially available from a range of source tissues and species (Table 3).

The objective of decellularization is to remove cell and antigenic components from a tissue while limiting damage to the underlying native ECM. The methods required to extract cells is a tissue-dependent procedure due to differences in cell density, matrix density, tissue thickness and shape amongst different tissues. Disruption of and removal of cells from tissues is typically achieved with physical treatments such as freeze-thawing, agitation, sonicaton, or mechanical pressure combined with detergents, ionic solutions, and/or enzymatic treatment which invariably causes some disruption of matrix. Methods of tissue decellularization has been reviewed in detail elsewhere [133]. Optimal decellularization processes require a balance between adequate cell removal and preservation of the matrix. Decellularization processes that are too harsh or too gentle can elicit adverse outcomes and pro-inflammatory responses arising from ineffective removal of antigens (e.g., α-gal or MHC-I epitopes), residual detergents, endotoxin contamination, or changes in the ECM structure [134, 135]. Complete removal of all cell organelles, lipid membranes, cell-derived nucleic acids is likely not possible and there is measureable cellular debris in every ECM

scaffold including those in clinical use [136, 137], but given that such scaffolds generally not associated with adverse reactions by patients it is suggested that levels of cellular debris below some threshold is acceptable. Despite potentially dire consequences of incomplete decellularization [138], quantitative metrics for decellularization still do not exist.

When prepared properly, ECM scaffolds can act as an inductive template for tissue repair. Numerous materials derived from decellularized tissues are used clinically to repair tissue in diverse applications including plastic and reconstructive, dental, orthopaedics, and cardiovascular procedures among others. The most extensive clinical use of ECM scaffolds is for hernia repair, where xenogeneic and allogeneic decellularized tissues like Surgisis®, Allomax®, and AlloDerm®, are considered to have improved performance characteristics over synthetic mesh materials [139–142]. Decellularized tissues have also aided in the repair of critically sized skin defects. Alloderm® has been shown to increase skin flexibility and improve scar quality in full-thickness wounds [143] while Oasis® has facilitated complete healing in 60% of patients with chronic leg ulcers compared to 35% with the standard treatment [144]. Various decellularized dermis mesh materials are increasingly used in breast reconstructive procedures [145, 146], and Alloderm® has also been used to facilitate frozen-banked ovarian tissue transplantation in the first reported pregnancies and live birth using this technique [147]. Decellularized tissues are commercially available in cardiac applications for the repair of heart valves (e.g., SynerGraft®) and pericardium (e.g., CorMatrix® ECM).

Along with the widespread use of ECM-based products, many preclinical and clinical studies continue to use decellularized matrices to improve repair of tissues including muscle, oesophageal, and cardiac. In a clinical report of 13 patients from an ongoing trial (ClinicalTrials.gov Identifier: NCT01292876) with volumetric muscle loss, decellularized tissue was implanted and combined with aggressive physical therapy improved strength by 37% and range-of-motion tasks by 27% [148]. While more study is necessary, particularly to control for the effect of physical therapy and differences amongst 3 different ECM scaffolds used, this and another study [149] suggest ECM can enhance the repair of volumetric muscle lesions to improve strength and function, albeit increased strength may not be attributable to appreciable de novo muscle formation [150].

Decellularized tissues have also supported the reconstruction of tissues in the gastrointestinal tract [151]. In patients with superficial adenocarcinoma, restoration of the normal epithelial lining of the esophagus was achieved by implantation of a tubular ECM scaffold following en bloc circumferential resection of the diseased mucosa and submucosa [152]. A recent follow-up study showed this same strategy of mucosal resection with ECM scaffold placement also facilitates repair of tissue at the opposite end of the gastrointestinal tract in the colon/rectum [153]. The technique, informed by preclinical studies showing the ECM scaffold was necessary to support regrowth of mucosal tissue and prevent recalcitrant stricture [154, 155] is currently being studied in patients with esophageal high grade dysplasia (ClinicalTrials.gov Identifier: NCT02396745).

While an enhanced tissue healing response has been demonstrated with ECM materials, the ultimate goal to achieve scarless wound healing remains largely unmet. Studies in highly

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regenerative species like the salamander and zebrafish have highlighted the importance of the ECM in regenerative healing [101]. Building upon this concept, a recent study has shown that cardiac injection of particulate ECM derived from a healing zebrafish heart induced a regenerative effect in the murine heart following myocardial infarct. The authors showed proliferation of cardiomyocytes and cardiac precursor cells was induced by the injection of decellularized zebrafish cardiac tissue. The effect was associated with the higher level of neuregulin-1 in ECM derived from regenerating zebrafish myocardium compared to adult mouse cardiac ECM, and ErbB2 signaling was involved in inducing the regenerative outcome [156].

Despite many successes of decellularized tissues in preclinical and clinical settings, the field is still developing. It should be noted that outcomes associated with the use of ECM scaffolds have ranged from success to failure, and the reasons for this disparity are now mostly understood. Stated simply, not all commercially available decellularized tissues are created equal—differences exist amongst the source tissue, sizing, storage, proprietary processing, and sterilization. A recognized drawback on decellularized tissue matrices is the inherent heterogeneity amongst source tissues. For example, the composition of decellularized myocardial matrix from different cadaveric donors has measurable donor-to-donor variation [157]. Quantitative proteomics provides a tool that will likely improve the characterization and quality control of tissue derived scaffolds. A recent study described the use of proteomic methods to enable refinement of processing methods to attain high fidelity ECM constructs from lung tissue [158]. Still, heterogeneity between donor tissues can result in heterogeneity in decellularization. Implantation of the first decellularized porcine valve introduced in Europe, SynerGraft®, resulted in the death of 3 of 4 of the pediatric patients undergoing valve repair. Analysis of the SynerGraft® valves showed that decellularization was incomplete and resulted in severe inflammation and valve failure [138]. This sobering outcome together with limitations due to tissue heterogeneity highlight the need for robust quality control measures for decellularized tissues.

6.3 Tissue-derived injectable hydrogels

The generation of tissue derived hydrogels from SIS in 1998 prompted the expansion in use of decellularized materials beyond the sheet or mesh materials described previously [159]. Tissue-derived hydrogels, or ECM hydrogels, represent a growing subset of injectable biomaterials in tissue engineering. Hydrogels are defined as highly hydrated polymeric materials with polymer chains and crosslinks to provide structural integrity. Natural ECM mimicking hydrogels contain natural polymers (e.g., collagen, aliginate, chitosan, or gelatin) and have been extensively studied and reviewed in detail elsewhere. Such hydrogels are composed of one or more purified ECM proteins and have promising applications. Single protein or collection of purified ECM components, although well-defined and relatively simple materials, is not able to recapitulate the complexity of the ECM microenvironment. Matrix-derived hydrogels with increasing complexity, although less defined, can be produced from decellularized tissues and have shown promise in mediating tissue repair outcomes.

Tissue derived hydrogels are formed through solubilisation of decellularized tissues. These gels are typically digested at low pH in a pepsin solution. Once the decellularized tissue is soluble, salt concentration and pH are balanced to physiologic levels and a gel forms spontaneously when warmed to 37°C. The soluble mixture is a viscous liquid below 22°C, and displays intermediate solid-like and fluid-like properties at 37°C [160]. Gel formation occurs through collagen self-assembly and is augmented by the presence glycosaminoglycans and other ECM proteins [161]. The source tissue from which the hydrogel is derived will impact the biochemical makeup and in turn the mechanical properties of the gel. Hydrogels have been formed and characterized from a range of decellularized tissues including adipose, gastrointestinal, heart, lung, and skin among others. A recent review nicely summarizes the structural and functional characteristics of such gels [162]. Tissue derived hydrogels have mechanical properties that are tunable by varying the concentration of matrix, providing some application-specific control of material properties. In some instances the hydrogels retain cues from the source tissue that can enhance cellular responses of tissue-specific progenitors. ECM hydrogels are cell-friendly and commonly used as a cell culture substrate. Several studies have shown improved or equivalent responses of cells grown on/in ECM hydrogels when compared to reconstituted basement membrane extract from Englebreth-Holm-Swarm tumor (i.e., Matrigel®) or purified collagen. Promising results from in-vitro studies with ECM hydrogels has spurred increased study of such hydrogels in pre-clinical in-vivo studies. The delivery of solubilized ECM via catheter or syringe to irregularly-shaped anatomic sites provides increased utility of ECM-derived materials.

ECM hydrogels are injectable materials that allow for minimally invasive delivery to target tissues. The viscous soluble pre-gel ECM material is shear thinning, exhibiting a decrease in viscosity under shear, which enables injection of the ECM. Low viscosity of the pre-gel solution and gelation kinetics can be optimised to achieve minimally invasive injection with sufficient time required for delivery of the pre-gel solution to the anatomic site before gelation begins. The injectability through catheters or 18-27 gauge syringes has been demonstrated demonstrated for ECM hydrogels derived from adipose, cartilage, dermis, intestine, liver, lung, muscle, and urinary bladder. The in-vivo application of ECM hydrogels has shown promise in a number of applications, particularly where there is an existing unmet need for minimally invasive therapies.

Tissue derived hydrogels have been tested pre-clinically as a therapeutic for skeletal muscle injury, defects in the central nervous system (CNS), myocardial infarction, and ulcerative colitis. In-situ polymerizing hydrogels composed of ECM enhance skeletal muscle remodelling in injury models. Hydrogels derived from both UBM and dermal ECM skeletal promote muscle remodelling in partial thickness abdominal wall defects with UBM treatment resulting in relatively higher myogenesis [163]. ECM hydrogel has also shown therapeutic benefits in treating skeletal muscle ischemia reperfusion injuries. In a rat hindlimb ischemia model, treatment with injectable ECM derived from skeletal muscle and umbilical cord increased tissue perfusion with an enhanced regenerative response elicited by skeletal muscle derived ECM hydrogel [164]. A number of studies have investigated the therapeutic potential of ECM hydrogels in treating tissue defects in the CNS. A recently described technique was used to deliver ECM hydrogels intracerebrally to a stroke cavity

[165], where UBM hydrogel at 8 mg/mL was shown to induce a cell infiltrate consisting of 60/30 mix of brain-derived/peripheral macrophage phenotypes that may potentially be exploited for treating stroke lesions [166]. ECM hydrogels have also been applied to traumatic injuries of the brain and spinal cord. Traumatic brain injuries treated with brain-derived ECM had reduced lesions volumes and improved neurobehavioral function [167], while UBM hydrogels reduced lesion size and improved motor function despite no measureable improvement in cognitive recovery [168]. Decellularized spinal cord and UBM hydrogels were used as a substrate to bridge lesions following spinal cord injury [169]. Catheter delivery of porcine myocardial matrix hydrogel has also been demonstrated as safe and effective in improving cardiac function following myocardial infarction in preclinical studies [170] and is currently the subject of clinical study (ClinicalTrials.gov Identifier:NCT02305602). A recent preclinical study in in rats with ulcerative colitis showed functional improvement in epithelial barrier function following treatment with SIS hydrogel [171]. The ECM was delivered as an enema and following in-situ gelation adhered to the colon wall more than 24 hours, potentially expanding the applications for tissue derived hydrogels beyond treating only compartments or lesions of tissues.

6.4 Method of repair: modulating the inflammatory wound healing process

While many studies have demonstrated the therapeutic effect of decellularized tissues, either as a scaffold or hydrogel, the exact mechanism of repair remains to be fully understood. The implantation or injection ECM materials is inextricably linked to acute tissue injury at the site of application, and it's not surprising that the success of ECM materials has largely been attributed to the ability of ECM to modulate the host response that commences upon implantation or injection. ECM materials are rapidly, within hours of implantation or injection, infiltrated by mononuclear cells. The ECM material then undergoes host-mediated biodegradation which is followed by an orchestrated spatiotemporal cellular response characterized by immune modulation and stem/progenitor cell mobilization, proliferation, and differentiation.

ECM degradation is a requirement for constructive tissue remodelling. The ECM material is infiltrated by host cells, especially macrophages, and the release of matrix metalloproteinases (MMPs) and other proteases contribute to the degradation of ECM. As a depot of biological signals, the degradable ECM materials act as vehicles for delivery of a variety of functional molecules. Decellularized tissues are rich in growth factors, bifunctional molecules such as fibronectin and various types of collagen, among other structural and functional molecules. Bioactive fragments of parent ECM molecules such as collagen, fibronectin, and laminin, are also generated by proteolytic degradation of the ECM [116, 117, 122, 172, 173]. The degradation rate of ECM scaffolds varies with the source tissue and the site of application. Besides a few studies with SIS ECM scaffolds, there is limited data on the degradation rate of ECM scaffolds. When used as a scaffold for Achilles tendon repair 60% of the SIS ECM mass was lost by one month after surgery, and the graft was completely resorbed by three months [174]. When placed in the urinary bladder, less than 10% of the original SIS ECM remained after 3 months [175]. In a model of abdominal wall repair, only 20% of the original the SIS-ECM remained after 4 weeks and the SIS ECM was resorbed by 6 months after implantation [176]. The degradation of ECM hydrogels is

relatively fast compared to ECM scaffolds and, again, the rate of degradation/ resorption is dependent on the application. For instance, ECM hydrogels injected into a tissue compartment degrade over weeks [170], while enema preparations of ECM hydrogels delivered to the colon are cleared over days [171]. In summary, depending on the application, ECM degradation is necessary for constructive outcomes and ECM scaffolds are degraded over weeks to months while ECM hydrogels are degraded over days to weeks. Processing methods that affect the degradation of ECM materials, such as chemical cross-linking, will alter the release profile of degradation products and impair tissue remodelling.

Immune modulation is a necessary feature of ECM mediated tissue remodelling. Despite being an underlying contributor to many degenerative and chronic diseases, inflammation plays an essential role in mitigating infection and mediating tissue repair. An intact immune system is necessary for faithful regeneration of the salamander limb [177], zebrafish fin [178, 179] and zebrafish heart [180]. The regenerative capacity of mammalian tissues also relies upon an intact and tuned immune response [181]. While tissue repair and regeneration are initiated by inflammation, excessive or prolonged inflammation often promotes fibrosis. Macrophages, which have garnered the majority of attention in relation to tissue regeneration, act not only through their immune functions, but also control cell function and ECM remodelling. Macrophages are phenotypically diverse, and are key to both initiation/ maintenance of inflammation as well as the inflammation resolution/regeneration processes. Research over the past 10 years with ECM-derived materials has correlated specific macrophage phenotypes with regenerative outcomes [182]. Decellularized tissues skew macrophages towards a predominantly M2 phenotype, which is regulatory and anti-inflammatory, and away from the pro-inflammatory M1 phenotype. This M2 majority seems to arise from a reduction in M1-like macrophages rather than an increase in M2-like macrophages. For example, ECM glycoproteins derived from various matrices diminished M1 function [183], and delivering a porcine SIS gel to the colon in chemically-induced colitis abated the inflammatory response resulting in increased proportions of M2 macrophages [171]. Relatively less studied in the context of regeneration is the adaptive immune response but recent work has begun to elucidate the role of T cells in the immune microenvironment and, similar to macrophages, Th-2 cells have been shown to accelerate bone healing [184] and regeneration of injured muscle [185, 186]. There is still much to be learned about the mechanisms of matrix-based materials in generating a therapeutic immune response, but it’s clear that a regulated scaffold immune microenvironment is a critical component of the regenerative niche [106, 187, 188].

The formation of functional tissue requires that stem/progenitor cells are recruited to the site, proliferate, and site-appropriately differentiate. Chemotactic properties of matrix components for a variety of cells (e.g., skeletal muscle cells, esophageal progenitors, perivascular stem cells, among others) have been demonstrated in-vitro. Matrix degradation products have also been shown to affect differentiation and function of stem/progenitor cells. These effects have also been shown in pre-clinical and clinical application of matrix-based materials, where a variety of endogenous stem/progenitors are recruited and accumulate at the site of tissue remodelling.

7 Conclusions

The essential role of the matrix influencing cell function and guiding wound healing provides a model for therapies to improve tissue repair outcomes. Scar-free healing in the embryo and regenerative organisms is guided by the ECM, and these examples provide opportunities for enhancing mammalian tissue reconstruction and regeneration. Matrix based scaffolds derived from decellularized tissue have been used clinically in reconstructive surgeries for decades. The success of these scaffolds in facilitating functional tissue repair is dependent largely upon a scaffold immune microenvironment and scaffold degradation, which has led to increased study of matrix degradation products. Components and cryptic fragments of ECM are revealed during matrix remodelling and elicit a wide range of signalling events and individual components have been used to tune wound healing outcomes. Hydrogels derived from decellularized tissues, which represents a complex mixture of ECM bioactive fragments, has emerged in recent years as a strategy to modulate the repair of various tissues. Improved understanding of the dynamic matrix properties during regeneration, scar-free healing, and fibrotic outcomes will provide intervention checkpoints that may be exploited to guide clinical methodologies for improved tissue repair.

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Abbreviations

ECM	extracellular matrix
ADAM	a disintegrin and metalloproteinase
ADAMTS	a disintegrin and metalloproteinase with thrombospondin-like motifs
CS	chondroitin sulfate
DS	dermatan sulfate
EMT	epithelial-to-mesenchymal transition
FACIT	fibril-associated collagens with interrupted triple-helices
FGF	fibroblast growth factor
GAGs	glycosaminoglycans
HA	hylaluronic acid
HS	heparin sulfate
MMPs	matrix metalloproteinases

SIS	small intestinal submucosa
SPARC	Secreted protein acidic and rich in cysteine
TGFβ	transforming growth factor beta
TIMPs	tissue inhibitors of metalloproteinases
TSP	Thrombospondin
UBM	urinary bladder matrix
VEGF	vascular endothelial growth factor

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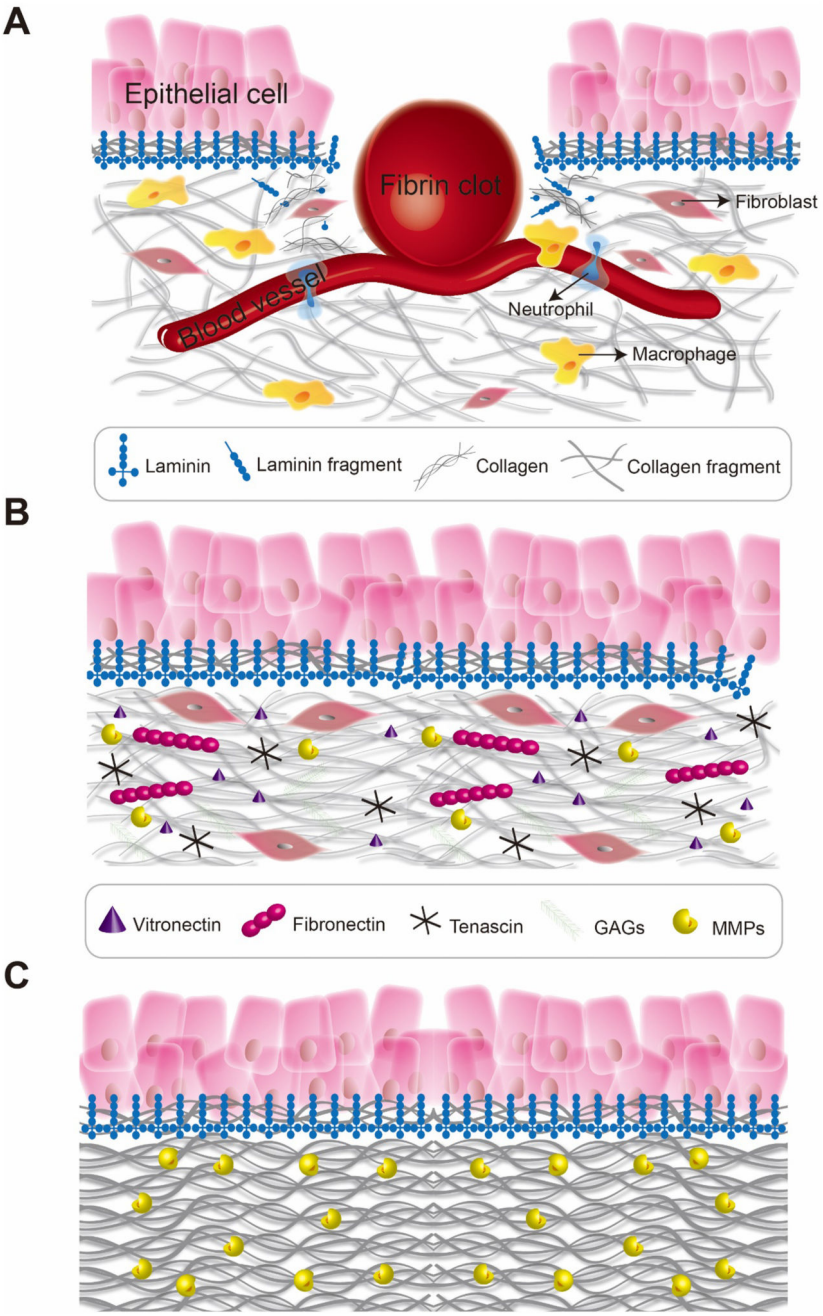

Figure 1. Extracellular matrix deposition and remodelling during tissue repair.

(A) Tissue injury triggers the accumulation of fibrin and plasma fibronectin that are released from the vasculature to immediately close the wound. Resident and recruited immune cells release growth factors and matrix proteins to initiate the wound healing cascade. Fibroblasts are activated and deposit collagen and fibronectin. **(B)** The well-orchestrated release and dynamic remodelling of a variety of different matrix proteins enables cell migration, cell differentiation, matrix remodelling and wound closure. **(C)** Finally, collagen fibers are

remodelled by proteolytic enzymes to protect the healing tissue and to provide strength and support.

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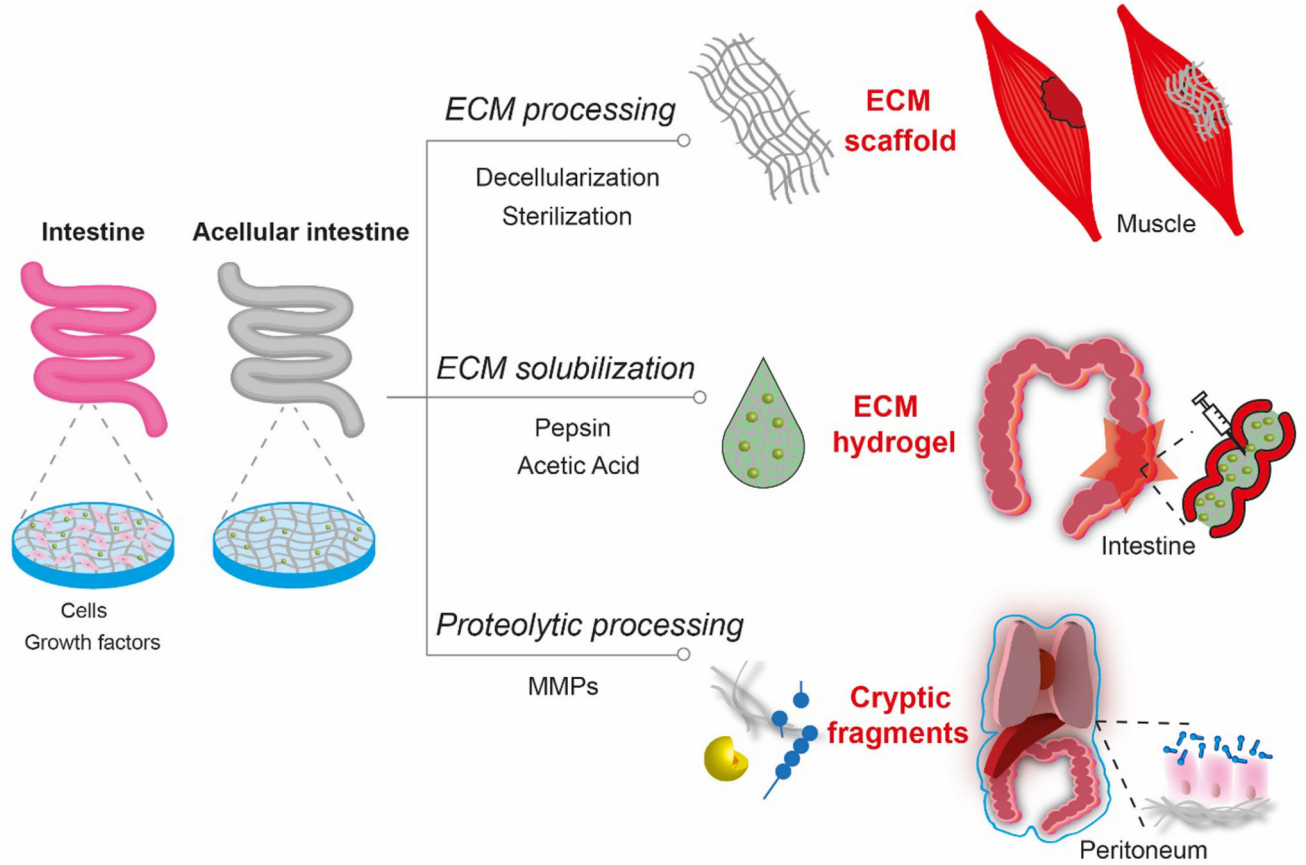

Figure 2. Overview of matrix-based strategies to regulate tissue repair outcomes.

The extracellular matrix (ECM) represents the non-cellular component of every tissue and organ. The matrix can be utilized in various ways based on application of interest. Appropriate tissue decellularization and sterilization processes can be used to prepare a mesh material suitable for filling/bridging tissue defects. Acellular tissue can also be solubilized (e.g., with pepsin or acetic acid) to produce an ECM hydrogel that is suitable for injection or catheter delivery in minimally invasive procedures. Alternatively, specific bioactivity of defined components or cryptic fragments of ECM components can be exploited to mediate tissue repair processes.

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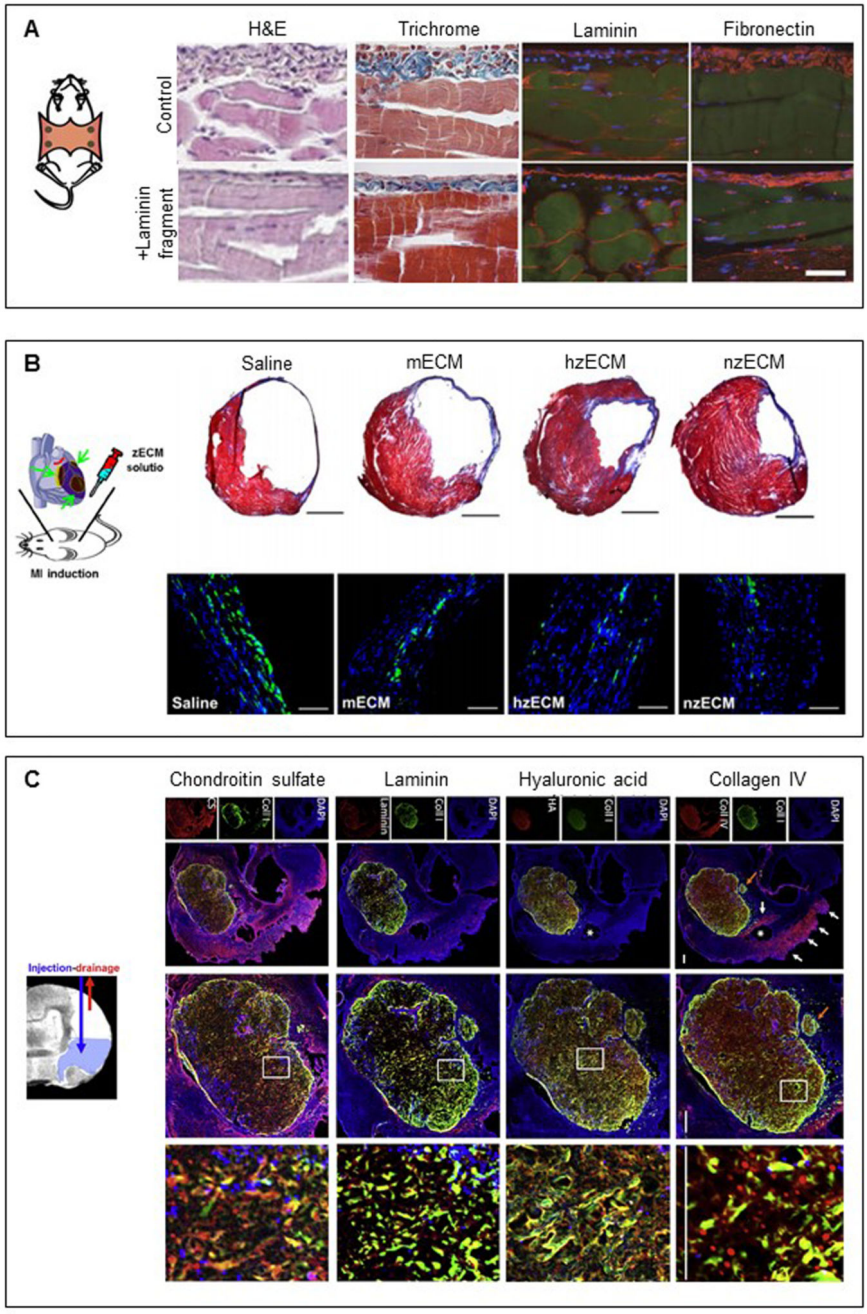

Figure 3. Matrix-based therapies modulate tissue repair.

(A) A cryptic laminin fragment functionalized biomaterial was prevents fibrosis in an established model of peritoneal fibrosis. Compared to controls, mice treated with the cryptic fragment showed a reduced fibrotic response demonstrated by H&E, Trichrome, and fibronectin stains alongside minimal disruption to the basement membrane evidenced by laminin staining (scale bars = 40 μm, Adapted from [117]). **(B)** Zebrafish extracellular matrix induces mammalian heart regeneration. Extracellular matrix from normal (nzECM) and healing (hzECM) cardiac tissue was micronized and injected in an adult mouse acute

myocardial infarct model. Compared to adult mouse cardiac ECM (mECM), the zebrafish ECM, especially the hzECM mitigated scarring as seen in Masson's trichrome stained sections (collagen in blue, cardiac muscle in red, scale bars = 1mm, Adapted from [156]). Heart sections labelled with CD68 showed a reduction in chronic inflammation at 6 weeks post-infarct when mice were treated with hzECM (CD68 in green, scale bars = 50 μm). **(C)** Extracellular matrix hydrogels implanted in a stroke cavity using a newly developed injection-drainage strategy promotes an acute endogenous repair response that could potentially be exploited to treat stroke. The hydrogel is infiltrated by host cells, typically of microglia, macrophage, or neural and oligodendrocyte progenitor phenotype, forms to the shape of the lesion, and the ECM components including GAGs (chondroitin sulphate and hyaluronic acid) and basement membrane proteins (laminin and collagen IV) can be detected over time (scale bars = 500 μm, Adapted from [165]).

Table 1
Role and consequence of dysregulation of key matrix proteins during tissue repair

Matrix protein	Role during tissue repair	Consequences of dysregulation
Collagen type I	• protects healing wound • forms scar matrix • mechano-signalling	• deficiency results in impaired wound healing, defective fibroblast proliferation, slow wound closure • accumulated in fibrosis
Collagen type III	• regulates early stages of repair and fibroblast phenotype	• deletion leads to increased myofbroblast differentiation, accelerated wound closure, and increased scar formation
Collagen type IV	• basement membrane integrity, • regulates neo-vascularization and growth factor release	• deletion results in a weaker basement membrane
Collagen type VI	• regulates fibroblast apoptosis • affects cell spreading and migration • involved in collagen fibril organisation	• deletion leads to weak basement membrane, altered collagen fibril architecture, less total collagen, and a thinner matrix • deficiency affects organization of fibronectin
Collagen type VII	• supports fibroblast migration • connects basement membrane to interstitial matrix • involved in laminin organization	• deletion leads to absence of anchoring fibrils and therefore constant wound burden, and perturbed laminin organisation
Collagen type XIV	• connects basement membrane to interstitial matrix • regulates fibrillogenesis	• deficiency results in altered matrix biomechanics (tissue-dependent)
Plasma fibronectin	• stabilises fibrin clot • substrate for platelet adhesion, spreading and aggregation • essential for post-traumatic opsonization	• cellular fibronectin can compensate deleted plasma fibronectin where applicable
Cellular fibronectin	• enables wound gap closure via fibroblast polarisation and migration • required for collagen network formation • growth factor activation and mechanosignalling	• splicing errors lead to abnormal wound healing • accumulated in fibrosis
Laminin	• critical for basement membrane assembly, re-epithelialization and • angiogenesis • involved in regulation of MMPs	• deletion results in the lack of a basement membrane
Tenascin	• interacts with fibronectin • critical for cell-matrix crosstalk	• depletion leads to reduced collagen and fibronectin deposition and impaired wound closure due to alterations in fibroblast focal adhesions

Matrix protein	Role during tissue repair	Consequences of dysregulation
Vitronectin	• regulates plasminogen activation • initiates platelet aggregation • regulates growth factor activity	• depletion results in delayed coagulation
Glycosaminoglycans	• growth factor reservoir • regulate fibroblast phenotypes • involved in angiogenesis • modulate matrix biomechanics and hydophobicity	• deletion of syndecan leads to delayed wound healing and impaired angiogenesis • loss of heparan sulfate results in impaired wound healing
SPARC	• modulates matrix organisation • activates TGFβ	• deficiency leads to delayed granulation tissue formation and diminished matrix production
CCN proteins	• promote matrix protein expression • interact with TGFβ signalling • involved in neutrophil clearance • promote fibroblast adhesion • modulate myofibroblast senescence	• CCN2 and CCN4 overexpressed in fibrosis
Thrombospondin	• facilitates matrix organisation and re-epithelialisation • regulate latent TGFβ • critical for collagen fibrillogenesis • critical for platelet aggregation • regulates angiogenesis	• depletion results in prolonged inflammatory response and delayed wound healing
Osteopontin	• regulates fibroblast phenotype • important for collagen organisation	• deletion leads to altered collagen fibrillogenesis and matrix organisation
Fibulin	• regulates TGFβ and fibroblast migration • important for long-term tissue repair	• accumulation associated with abnormal tissue repair

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Table 2
Key differences in ECM content in scarring vs. regenerative healing

	Scarring	Regeneration	Function in regeneration
Collagen I	High expression, disorganized, and high crosslinking	Low expression, organized, and low crosslinking	Col I maintains tissue structural integrity but excessive levels and high crosslinking are physical barriers to regeneration
Collagen III	Decreased expression	Increased expression	Increased levels of col III offers a looser matrix network to allow cell migration, proliferation, and differentiation. Col III is required for col I fibrillogenesis.
HA	Low levels	High levels early and sustained	Increased levels of HA in matrix facilitates cell movement, mitigates fibroblast proliferation, and augments inflammation
Tenacin C	Late expression	Early expression	Early expression of tenascins enables cell migration for rapid epithelialization and wound closure
Fibronectin	Later deposition	Early deposition	Early deposition of fibronectin provides cell anchorage points for rapid wound closure
MMP:TIMP ratio	Low	High	High MMP:TIMP ratio favors matrix remodelling and turnover

Table 3
Selected interventions that may limit fibrosis

Category	Name	Class	Potential Target/mechanism	Phase
Pharmaceuticals	Enalpril	Angiotensin converting enzyme inhibitors	Inhibit fibroblast activation	Clinic
	Pirfenidone	Small molecule	TGFβ, inflammation	Clinic
	Nintedanib	Small molecule	Receptor tyrosine kinsases	Clinic
	Anakinra	Monoclonal antibody	Inflammation (IL-1RA)	Clinic
	Fresolimumab	Monoclonal antibody	Inflammation (IL-13)	Phase II
	Endostatin	Recombinant fragment of col XVIII	TGFβ	Pre-clinical
	Lumikine	Peptide fragment of lumican	TGFβ	Pre-clinical
Biomaterials	Integra™	Silicone and bovine col I-chondroitin sulfate mesh	Collagen and GAG mesh provide environment to facilitate repair	Clinic
	Surgisis® Oasis® Biodesign® CorMatrix®	Porcine SIS ECM scaffold	Multi-functional molecules liberated during matrix degradation mediate inflammation and repair	Clinic
	XenMatrix®	Porcine dermis ECM scaffold	See above	Clinic
	Alloderm® Allomax®	Human dermis ECM scaffold	See above	Clinic
	MatriStem®	Porcine UBM ECM scaffold	See above	Clinic
	VentriGel™	Porcine myocardial ECM hydrogel	See above	Phase I
Cell-based therapies	Dermagraft®	Allogeneic neonatal cells on vicryl scaffold	Neonatal cells and scaffold provide environment to facilitate repair	Clinic
	Apligraf®	Allogeneic neonatal cells on bovine col I matrix	Neonatal cells and scaffold provide environment to facilitate repair	Clinic
	Mesenchymal stem cells	Allogeneic stem cells	Paracrine signalling alters inflammation	Phase I