From Repair to Regeneration: Biomaterials to Reprogram the Meniscus Wound Microenvironment

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Abstract

When the field of tissue engineering first arose, scaffolds were conceived of as inert 3-dimensional structures whose primary function was to support cellularity and tissue growth. Since then, advances in scaffold and biomaterial design have evolved to not only guide tissue formation, but also to interact dynamically with and manipulate the wound environment. At present, these efforts are being directed towards strategies that directly address limitations in endogenous wound repair, with the goal of reprogramming the local wound environment (and the cells within that locality) from a state that culminates in an inferior tissue repair into a state in which functional regeneration is achieved. This review will address this approach with a focus on recent advances in scaffold design towards the resolution of tears of the knee meniscus as a case example. The inherent limitations to endogenous repair will be discussed, as will specific examples of how biomaterials are being designed to overcome these limitations. Examples will include design of fibrous scaffolds that promote colonization by modulating local ECM density and delivering recruitment factors. Furthermore, we will discuss scaffolds that are themselves modulated by the wound environment to alter porosity and modulate therapeutic release through precise coordination of scaffold degradation. Finally, we will close with emerging concepts in local control of cell mechanics to improve interstitial cell migration and so advance repair. Overall, these examples will illustrate how emergent features within a biomaterial can be tuned to manipulate and harness the local tissue microenvironment in order to promote robust regeneration.

Keywords

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Introduction

The soft tissues of the musculoskeletal system (e.g., dense connective tissues such as tendon, ligament, cartilage, and the knee meniscus) are vital for the efficient and pain free execution of the activities of daily living. However, and as a direct consequence of their central role in load bearing, these tissues are commonly injured and present a frustrating scenario in clinical practice. Namely, all of these tissues have a healing response to injury in adults that ranges from 'poor' to 'nonexistent'. For example, torn tendons (which normally transmit forces from muscle to bone) can mount a modest repair response; yet, this process most often culminates in the formation of 'scar' tissue that is disordered, has lower mechanical properties than the original tissue, and is prone to re-injury [1]. Other tissues, such as the articular cartilage lining joint surfaces, mount almost no postnatal repair response [2]. Indeed, a single area of cartilage damage not only fails to heal on its own, but can precipitate more widespread degeneration of the entire joint surface.

Poor healing of dense connective tissues in adults arises in part from the challenging mechanical environment in which healing takes place and is exacerbated by the high density and precise ordering of the extracellular matrix (ECM) as well as the relative paucity of cells in these tissues. Quite interestingly, these same tissues can heal via regeneration (i.e. complete restoration of structure and function) during adolescence [2, 3]. In this earlier developmental state, tissue density is much lower and the number of cells is much higher. As will be described below, our studies in the knee meniscus, for example, have shown that tissue mechanics and matrix density increase with aging, while repair capacity decreases [4]. Defining the critical tissue characteristics that separate these non-healing from healing states may direct new therapies and biomaterial-based interventions.

To address deficits in the repair of dense connective tissues in the adult, we and others have developed a number of tissue engineering strategies with the goal of promoting tissue regeneration. In our case, these approaches are structurally motivated, in that they are based on organized nanofibrous scaffolds composed of ultra-fine biodegradable and biologic fibers [5, 6]. These scaffolds can be fabricated in such a way as to recreate the order of typical dense connective tissues [6], and can also serve as a three dimensional micro-pattern that directs cells at the repair site to produce new matrix with order and directionality comparable to the native tissue [7]. We have used these materials to query basic mechanisms of cell response to scaffold architecture, fiber mechanics, and mechanical deformation [8-10] and have begun to translate these findings towards clinical practice by testing in large animal models of tissue repair. Expanding the potential of these materials, we have also devised novel fabrication strategies to generate scaffolds that are both dynamic and multi-functional [11], and whose bulk mechanical properties can be tailored to match that of the native tissues they are to replace [12]. In the context of fibro-cartilaginous tissues (with a focus on the knee meniscus), we will identify the limitations to endogenous repair, as well as illustrate how such scaffold templates can be engineered to overcome these limitations.

Meniscus Structure, Mechanics, Injury, and Repair

In order to appreciate the inherent challenges to meniscus repair, it is essential to define the function, organization, and macromolecular constituents of the tissue, as each of these factors plays a role in healing response and outcomes. The menisci are semilunar, wedge-shaped structures in the knee that are rich in collagens and proteoglycans (PGs) and function to transfer load from the femur to the tibia [13-15]. Collagen content and directionality (where most fibers are circumferentially oriented) is paramount for meniscus function [16]. When compressive loads impinge on the wedge shaped meniscus, the PG-rich inner zone projects this load outward to engage the collagen fibers [17]. These fibers resist extrusion and enforce joint congruency to promote load transmission [18]. Because the menisci are central to knee function, injury is very common. Meniscus tears may arise acutely (coincident with a traumatic event such as ACL rupture) or with degeneration. Small longitudinal 'bucket handle' tears can progress to involve a significant portion of the meniscus, and loose fragments can displace and interrupt knee function. Tears in the avascular zone (inner 2/3rd) have a poor long-term prognosis, even with suture repair [19]. As a consequence, removal of the damaged portion (partial meniscectomy) is the most common treatment option for meniscal tears. However, the volume of meniscus removal scales with increasing stress on the underlying cartilage and predisposes the joint to osteoarthritic changes [13, 20]. Despite this, partial meniscectomy is performed >750,000 times each year in the US [19], does not provide definitive improvements relative to untreated lesions [21], and predisposes patients to the development of osteoarthritis (OA) [22]. Clearly, there is a need for new therapies that can improve symptoms while preserving meniscus structure and function, particularly with a focus on promoting endogenous repair.

Impediments to Meniscus Repair

In order to develop regenerative approaches to improve meniscus repair, it is important to consider the natural processes of wound repair and impediments to endogenous healing that are present in adult meniscus tissue (Figure 1). It is generally understood that the overall low cellularity (of both endogenous meniscus cells and meniscus progenitors), the dense extracellular matrix, the poor vascularity, and the inflammatory environment present at the wound site, all contribute to failure of meniscus healing and regeneration [23-25]. Based on this understanding, it may be possible to develop and deliver therapeutics to specifically address these limitations through novel biomaterial-based technologies. Of further note, since many dense fibrous connective tissues (including tendon, ligament, and the intervertebral disc) have a limited healing capacity in adults [4, 26, 27], lessons learned in one tissue (the meniscus) might have applicability across many dense connective tissues. In the following sections we discuss the salient features of the meniscus that change with development that may contribute to a failure of repair in the adult.

Tissue Aging and Maturation

From a clinical perspective, meniscal tears are rarely seen in children but are common in adults [28, 29]. Moreover, increasing patient age correlates with worse clinical outcomes after meniscal repair, including higher rates of repair failure [30-33]. Importantly, patients >40 years of age with meniscal tears have significantly fewer meniscus cells at the wound

interface than younger patients [34]. Meniscal fibrochondrocytes (MFCs) are the native cell type within the meniscus and are responsible for maintaining tissue homeostasis [15, 35]. These same cells respond to injury or altered loading by up-regulating production of matrix proteins and/or enzymes to affect repair [36-39]. However, cellularity decreases progressively with age, reaching very low cell densities in adult tissue [4, 28]. Since healing is characterized by cellular migration to the defect, proliferation, synthesis of new matrix to bridge the wound gap, and eventually tissue remodeling and maturation [40], the lack of reparative cells at the wound interface in adults may constitute a significant impediment to repair.

It has also long been thought that deficits in vascularity play a role in the poor healing of the adult meniscus (where nascent vessels serve as a conduit for the delivery of regenerative cells and growth factors to the wound site). Indeed, blood vessels penetrate only the peripheral 1/3rd in the adult [41, 42], while they are present throughout the fetal meniscus. Recent data shows though that fetal meniscal explants cultured in vitro (in the absence of a vascular supply) still exhibit superior integration compared to adult specimens. For example, utilizing an *in vitro* explant model [4], we showed that both fetal and juvenile meniscus repair constructs formed a robust repair over 8 weeks, while a considerable gap remained in the adult meniscus, Figure 2A. Consistent with these findings, others have shown that when adult and fetal ovine tendons are injured, removed, and implanted into the subcutaneous space of adult mice, healing is superior in fetal tissues, and proceeds in a similar manner as in the fetus [3]. Together, these findings suggest that the paucity of repair is not inherent to the adult environment or solely due to the lack of blood supply per se, but rather suggest that impediments to repair originate within the tissue itself as it matures.

Meniscus ECM with Tissue Maturation and Aging

What then changes with tissue maturation that might limit endogenous repair? One obvious alteration with tissue maturation is the density and structure of the extracellular matrix (ECM) [28]. Meniscus ECM density increases markedly with maturation and load bearing use, resulting in higher bulk and local mechanical properties in the tissue [4]. Given the already small pore size within the ECM of this dense connective tissue, increasing matrix density may impede cell migration to the wound site, a requirement for tissue repair [43]. Indeed, unlike migration in 2D, where matrix stiffness directly modulates migration speed [44, 45], cells in 3D interpret not just the adhesive and mechanical features of their microenvironment, but also must migrate through the steric hindrances presented by the ECM itself [46-48]. Interstitial cell migration can occur through either cell-mediated degradation of the matrix (to enable tunneling) or direct migration through the small matrix pores [49, 50], or a combination of the two. As the pores within the matrix become progressively smaller, migration rates decline and cells are eventually rendered immobile [49]. The same is true in very stiff and/or non-degradable artificial matrices, where the matrix cannot be effectively remodeled to allow cell passage [47, 51, 52]. Thus, steric impediments may arise naturally as a consequence of the tissue specialization that enables mechanical function, while these changes may reduce endogenous healing potential, Figure 2B.

Based on this understanding of native meniscus structure, function, and healing capacity, our team has developed novel scaffolds to promote regeneration and repair. Clearly, organization is a critical feature of a functional regenerate meniscus tissue, and this must be one of the first and foremost considerations. However, the cellularity of the healing interface is just as critical, if not more for functional resolution of a defect. Without cellular colonization of the implant and/or wound site, then little if any new tissue can be formed. As will be detailed below, these lessons learned from native tissue healing have informed our scaffold designs, while at the same time we have incorporated lessons learned from the scaffold design and colonization to develop novel strategies to improve native tissue healing.

Engineered Scaffolds to Enhance Meniscus Tissue Formation and Integration: Controlling Porosity

As a starting concept in our scaffold designs, it was clear that the dense and organized nature of the native meniscus was essential for recapitulation, but also that these same network features might pose impediments to repair. This led to the development of a new class of fibrous networks that possessed alignment reminiscent of the native tissue [53-55], along with controllable and emergent porosity. These nanofibrous composites were engineered to include at the time of fabrication 'sacrificial' fiber fractions, where the emergence of porosity and change in mechanics was dictated by the solubility and degradation characteristics of fiber populations within the composite structure [12, 56-58], Figure 3. In in vitro studies, wherein cells were seeded atop these structures, cell colonization and matrix deposition in the scaffold could be tuned by altering the amount and type of sacrificial fiber [59]. Likewise, when these scaffolds were apposed to native meniscus tissue, using an annular/ring integration model [60], scaffold porosity modulated the rate and degree to which cells from the native tissue invested the biomaterial network. When these scaffolds with tunable porosity were coupled to native meniscus, histology and mechanical testing revealed that increasing the sacrificial PEO fiber fraction (and thus pore size) increased the strength of the repair ([61], Figure 3). Taken together, these studies showed that an enabling context for 3D interstitial cell migration could be established by tuning the pore size within the fibrous biomaterial framework, and that this feature could be used to improve both engineered meniscus tissue formation and integration with native tissue.

Reprogramming Local Tissue to Enable Colonization of the Wound Margin: Local Delivery of Degradative Enzymes

The above studies focusing on manipulating scaffold porosity to improve cell colonization and integration led naturally to considerations of what else might be done to further enhance repair. As noted above, the density of adult meniscus ECM likely presents physical impediments to 3D cell migration, dampening endogenous repair capacity by limiting the number of cells that can migrate through the native tissue to the wound interface. Since the sacrificial elements of the scaffold were designed to dissolve and/or degrade soon after apposition with native tissue, these fibers also provide a useful vehicle from which to deliver drugs, growth factors, and other biological agents [62]. As such, we next considered whether fibers within these composites could be harnessed to deliver factors to reprogram the local

tissue environment to a state that would better support interstitial cell migration. In the cartilage literature, it had been shown that partial digestion of the tissue wound edge, via exposure to solutions containing proteolytic enzymes, enhanced tissue-to-tissue integration, with a greater number of cells migrating through the dense matrix to the wound site [63].

To develop this concept in terms of biomaterial-mediated meniscus repair, we first validated that integration was improved in the adult meniscus when repair segments were exposed to soluble collagenase prior to apposition [64]. In that study, using the annular/ring defect model, in vitro culture showed cells and collagen fibrils closing ~92% of the wound gap in adult meniscus repair constructs that were pre-treated with a high dose of collagenase before assembly and culture for 8 weeks. Repair in these samples reached levels were higher than samples treated with a lower collagenase dose (74% closure) and basal media controls (43% closure). To enable biomaterial mediated and targeted enzyme delivery, PEO nanofibers containing active collagenase (PEO-C) were next fabricated. When these collagenase-releasing PEO-C/PCL composite scaffolds (with two fiber fractions) were placed into a juvenile bovine meniscus defect, loss of PG was apparent at the wound edge within 6 hours, with decreased staining intensity persisting through day 7 (Figure 4). This loss of matrix did not extend to the periphery of treated samples, and was not observed in controls, suggesting a local action of the delivered enzyme. Moreover, when these repair constructs (inclusive of two meniscus segments with an interposed bioactive biomaterial) were implanted subcutaneously in nude rats and evaluated over a 4 week period, composite scaffolds delivering collagenase from the PEO fiber fraction (PCL/PEO-C) showed the greatest amount of ECM deposition both within and at the boundaries of the implanted scaffold [64]. DAPI staining of these same constructs also showed marked increases in cellularity in the tissue surrounding the PEO-C scaffolds. Taken together, these studies demonstrated that the storage and rapid release of collagenase from sacrificial PEO nanofibers, when tuned appropriately, could increase cell migration to and matrix deposition at the wound margin as well as cellularity in the adjacent tissue margins. These studies established that by reprogramming the adult tissue ECM towards a more immature state (via partial digestion) one could overcome some of the inherent limitations of endogenous repair.

Recruiting Endogenous Cells to Wound Margin: Homing and Growth Factor Release from Scaffolds

Beyond simply enabling MFC migration through dense ECM via matrix degradation, it may also be essential to provide directional cues, such as chemotactic gradients, to guide endogenous cells to the injury site. Recent studies have shown that simply allowing migration may not in and of itself provide sufficient motivation to drive colonization of the wound site. For example, Greiner et al. showed that a soluble chemical gradient was essential for inducing cell migration through small pores and Mao and colleagues showed in vivo cartilage regeneration only when TGF was released from implanted biomaterials [65, 66]. Others have delivered BMP from alginate hydrogels to increase stem cell recruitment to critically sized bone defects [51]. Interestingly, these studies also highlighted the requirement that the scaffold provide a permissive environment for cell migration along with chemotactic cues to guide cell migration to the wound site.

While a number of molecules have been implicated in meniscus cell migration and biosynthetic activity [67], several recent studies have shown a role for stromal-derived factor-1 alpha (SDF). SDF is a critical regulator of bone marrow progenitor cell (BMC) mobilization and local engraftment at injury sites, is regulated through the SDF/CXCR4 axis [68, 69], and may play a role in meniscus repair. Indeed, in a recent rat meniscus injury model, the SDF/CXCR4 axis was implicated in homing of injected progenitor cells to the site of injury [70]. Due to its short half-life however, SDF is often delivered from biomaterials to enable a sustained response. In recent studies, we used a hydrolytically degradable hyaluronic acid (HA) hydrogel to deliver SDF [71]. In these studies, released SDF, as well as released HA itself, stimulated BMC chemotaxis over a period of 7 days (Figure 5A). In follow on in vitro studies, we queried MFC migration in response to several potential factors, and showed that SDF engendered significantly more meniscus cell migration than either fibroblast growth factor (bFGF) or platelet derived growth factor (PDGF) alone, Figure 5B. Building from these observations, we modified our nanofiber fabrication methods to enable electrospinning of HA fibers [11, 72, 73], Figure 5C. Because of the versatile crosslinking chemistry available with HA, these fibers could be tuned to degrade at different times and so controllably release large molecules at pre-determined rates, Figure 5D, 5E. These novel materials and fabrication methods enable the release of active biologic factors from nanofibrous composites in a controlled fashion, providing yet another means by which endogenous meniscus repair may be improved.

Instruction Upon Arrival at the Wound Margin: Scaffold-Directed Differentiation

In addition to providing a local environment that is amenable to integration and cell migration, as well as chemotactic signals to recruit cells to the defect site, it may be necessary to provide the appropriate biologic and mechanical context for cell differentiation upon arrival at the wound margin. While we and others have released biologic factors from scaffolds to drive differentiation of cells arriving at the wound interface [62, 74], it is now well understood that cells, and particularly stem cells, are also influenced by the local adhesive and biophysical properties [75]. This includes the mechanical properties of the local environment, as well as the amount and distribution of ligands available for integrin binding [76]. To this end, we recently developed tunable fibrous composites in which the fiber mechanics and level of ligand density (i.e., RGD) could be independently altered to investigate the role that these features play in guiding progenitor cell differentiation (e.g., chondrogenesis of human mesenchymal stem cells) [11, 77]. In these studies, fiber mechanics was altered by changing the crosslinking density within each individual fiber, while adhesive ligand presentation was controlled by defining the degree of ligand incorporation in the fiber backbone. As expected, the level of spreading and focal adhesion formation increased as the RGD density on the fibers increased, which in turn influenced the magnitude of cell engagement and pulling on the fibers (Figure 6A). Generally, this increase in ligand density led to a decrease in the level of chondrogenic gene expression and an increase in fibrous gene expression (Figure 6B). Interestingly, for the levels investigated, the mechanical properties of the fibers themselves did not influence chondrogenesis to any appreciable extent, though stiffer fibers increased expression of the fibrous tissue marker

type I collagen. This data demonstrates that, upon arrival, the biophysical properties of the material scaffold, and how cells interact with it, can play a role in the phenotypic decisions made by cells that have migrated to the wound margin. These features might be manipulated to promote fibro-cartilaginous differentiation and matrix production by both endogenous meniscus cells and progenitor cells recruited to the wound site.

Looking Forward: Emerging Concepts in Meniscus Repair

In addition the progress described above in addressing some of the inherent limitations to meniscus repair, other key features are present in the wound environment that must be considered. These include the inflammatory state of the wound margin, as well as the physical properties of the cells within the tissue itself. Each of these factors may provide an additional target for modification to improve repair.

Inflammation and Meniscus Repair

While cell migration to the wound site is crucial for new tissue formation, and newly arrived cells must be instructed to adopt the appropriate phenotype, the inflammatory environment also plays a significant role in meniscus repair by regulating new tissue formation and integration of the wound margins. It is well established that joint injury and degeneration are accompanied by increased synovial inflammation, thus increasing the proteolytic enzyme burden in the synovial fluid [78-80], with matrix metalloproteinase-1 (MMP-1) specifically increased with joint injury [81]. Studies have shown that MMP levels in OA joints are a predictor of future joint space narrowing [79], and that synovial inflammation is a predictor of poor knee function post-meniscectomy [82]. Together, these data suggest that the 'joint as an organ' [82-85] cannot be ignored in strategies to promote meniscus repair. This is particularly relevant at the local level, where *in vitro* studies have shown that even picomolar levels of inflammatory cytokines (TNFα and IL-1β) reduce or eliminate integration between apposed meniscus edges [25, 86, 87], while provision of broad spectrum MMP-inhibitors in this inflammatory context can restore integration capacity [88, 89]. Approaches that limit these proteolytic events, especially at the wound interface, may therefore improve meniscus repair.

The field of biomaterials design has matured significantly over the past decade and we are now able to design triggered responsiveness into scaffolds, including response to local proteases, such as MMPs [72, 73]. We have recently used such MMP-degradable materials to control stem cell behavior [90, 91] and to promote repair in a sheep cardiac infarct model [92, 93]. As with meniscus injury, cardiac injury results in a local increase in MMP activity. Delivery of MMP-cleavable hydrogels to infarcted regions had the effect of decreasing local protease activity, likely through competitive inhibition or quenching of local MMPs, thus promoting repair. Furthermore, inclusion of the MMP inhibitor TIMP-3 as a delivered factor within the MMP-sensitive hydrogel resulted in further recovery of cardiac function [94, 95]. Here, release of the TIMP-3 was governed by material degradation, providing 'on demand' release profiles that could be tuned by the local inflammatory environment. Studies are now underway to explore this new class of materials and mechanism of action in the context of meniscus repair and regeneration.

Cell Mechanics in Interstitial Cell Migration: Applications to Meniscus Repair

As a final point, our recent studies using partial digestion of the meniscus wound interface to loosen the ECM network have shown definitive improvements in wound edge cellularity *in vitro* and *in vivo* [64, 96]. While this finding implicates the dense meniscus ECM as an impediment to repair, an ideal repair strategy would preserve existing ECM (and tissue volume) while promoting integration. Migration in 3D is dependent on the deformability of cells and cell sub-structures [97]. As such, age-related changes in cell mechanics may play a role in meniscus healing. The cell nucleus is considered the rate-limiting organelle in 3D migration, given its large size and stiffness relative to the rest of the cell (2-4 times higher) [98], Figure 7. Nuclear stiffness is determined in part by the amount of heterochromatin (condensed DNA) [99-101], and in part by the filamentous nucleoskeletal network [102]. This filamentous network is comprised of a number of proteins, most prominent of which is the type V intermediate filament protein Lamin A/C, which provides structure and stability to the nuclear envelope [103-110]. Interestingly, while cells with stiff nuclei have limited migratory capacity inside dense collagen matrices [49], cells with compliant nuclei lacking Lamin A/C (such as leukocytes and certain cancer cells) remain highly mobile [49, 97, 111, 112]. Similarly, cells expressing a mutant isoform of Lamin A/C (progerin) that have stiffer nuclei cannot migrate through a constrictive array of micro-posts [113], while reduction of Lamin A/C content enhances migration through small pores [65, 110, 114]. Likewise, modulation of heterochromatin levels influence nuclear deformability [115-117] and cell migration through small pores [118]. Interestingly, both stem cell differentiation and increased tissue micromechanics have been implicated in higher Lamin A/C expression [119], elevated levels of heterochromatin [101, 120-124], and increased nuclear stiffness [110, 119]. Collectively, these data suggest that barriers to interstitial cell migration posed by the dense ECM may be overcome if the endogenous cell nuclei can adopt a 'softer' phenotype, deforming more readily through the small pores within the dense ECM. Materials designed to deliver factors to manipulate the nuclear mechanics of these endogenous cells may therefore promote cell migration to the wound margin, while preserving as much of the existing ECM as is possible.

Conclusions

In current clinical practice, endogenous repair of dense connective tissues culminates in a state in which the regenerate tissue is of inferior quality than the native tissue, and is prone to failure. This failure in repair presents difficulties for the effective treatment of the meniscus, as well as other dense connective tissues such as articular cartilage, tendons, ligaments, and the annulus fibrosus of the intervertebral disc. Over the last decade, and guided by a deeper understanding of the limitations in endogenous repair of these tissues, biomaterials have been developed to reprogram this microenvironment from a state of failed repair towards one that enables true regeneration of tissue structure and function. Based on the predicate of providing an organized template for new tissue formation, and with lessons learned from the population and maturation of such structures in a tissue engineering context, new strategies have been developed to use biomaterials to reprogram the wound microenvironment. These include advances in material design that provide dynamic and multifunctional scaffolds that can lessen local ECM density to improve interstitial cell

migration, delivery of chemotactic cues to draw cells from the native tissue to the wound site, and materials that influence cell fate decisions upon arrival via their biophysical properties and adhesive ligand presentation. In essence, these materials are designed to enable and direct cell colonization of the wound site and instruct cell behavior upon arrival so as to influence the trajectory of repair. Additionally, new concepts are now being developed to further reprogram the repair environment, using the materials themselves to control the inflammatory context of repair and to deliver agents that are tailored to manipulate the underlying cell mechanics to improve repair outcomes. These advanced materials, through their specific attendance to the inherent limitations of dense connective tissue repair, coupled with rehabilitation regimens that dynamically interact and synergize with the state of the repair itself, may one day turn an intractable clinical situation into one that is readily resolved. Improving dense connective tissue repair in the adult will have a profound impact on musculoskeletal health, and restore function to the millions of patients suffering from connective tissue failure.

Acknowledgements

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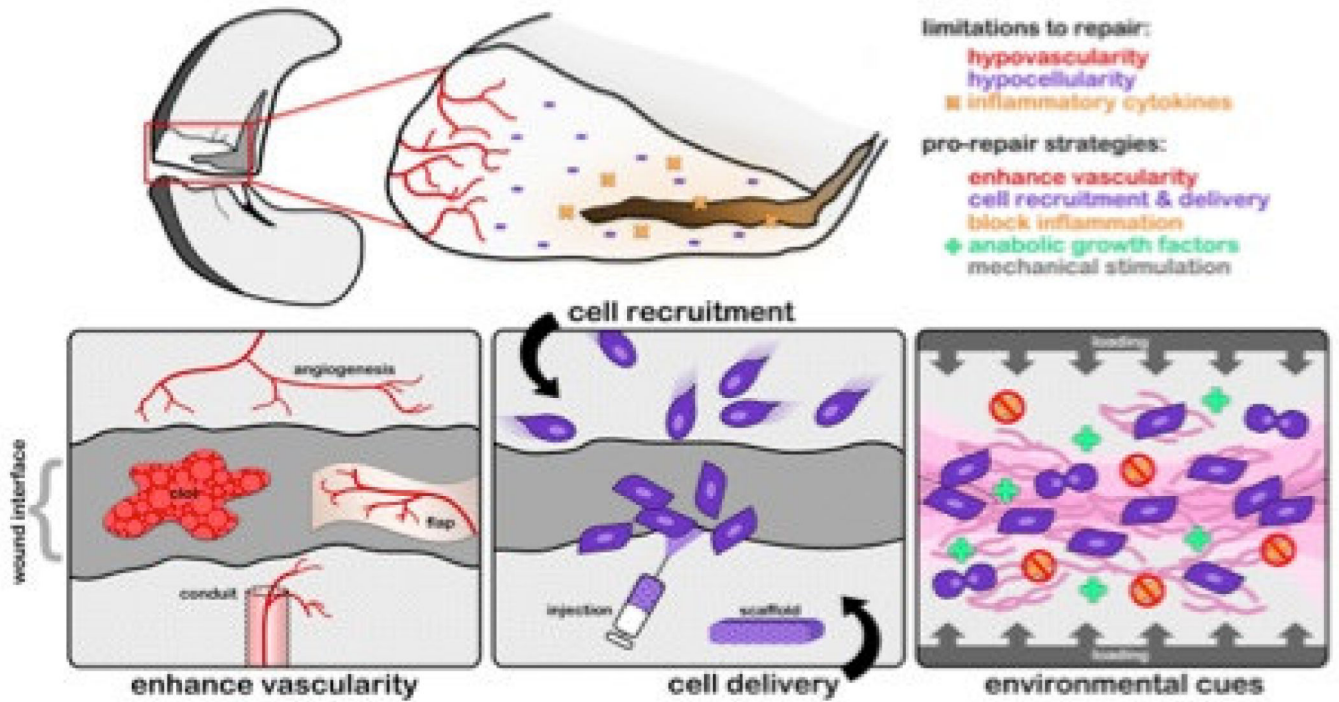
Figure 1. Impediments to meniscus repair

Schematic illustration of a meniscus defect and the underlying impediments to adult meniscus repair, including a lack of vascularity, a loss of cellularity, alterations in ECM density and mechanics, and inflammatory factors in the wound environment. These factors converge to limit endogenous repair and have been incorporated into pro-repair strategies aimed at improving regeneration. Adapted from [96] with permission.
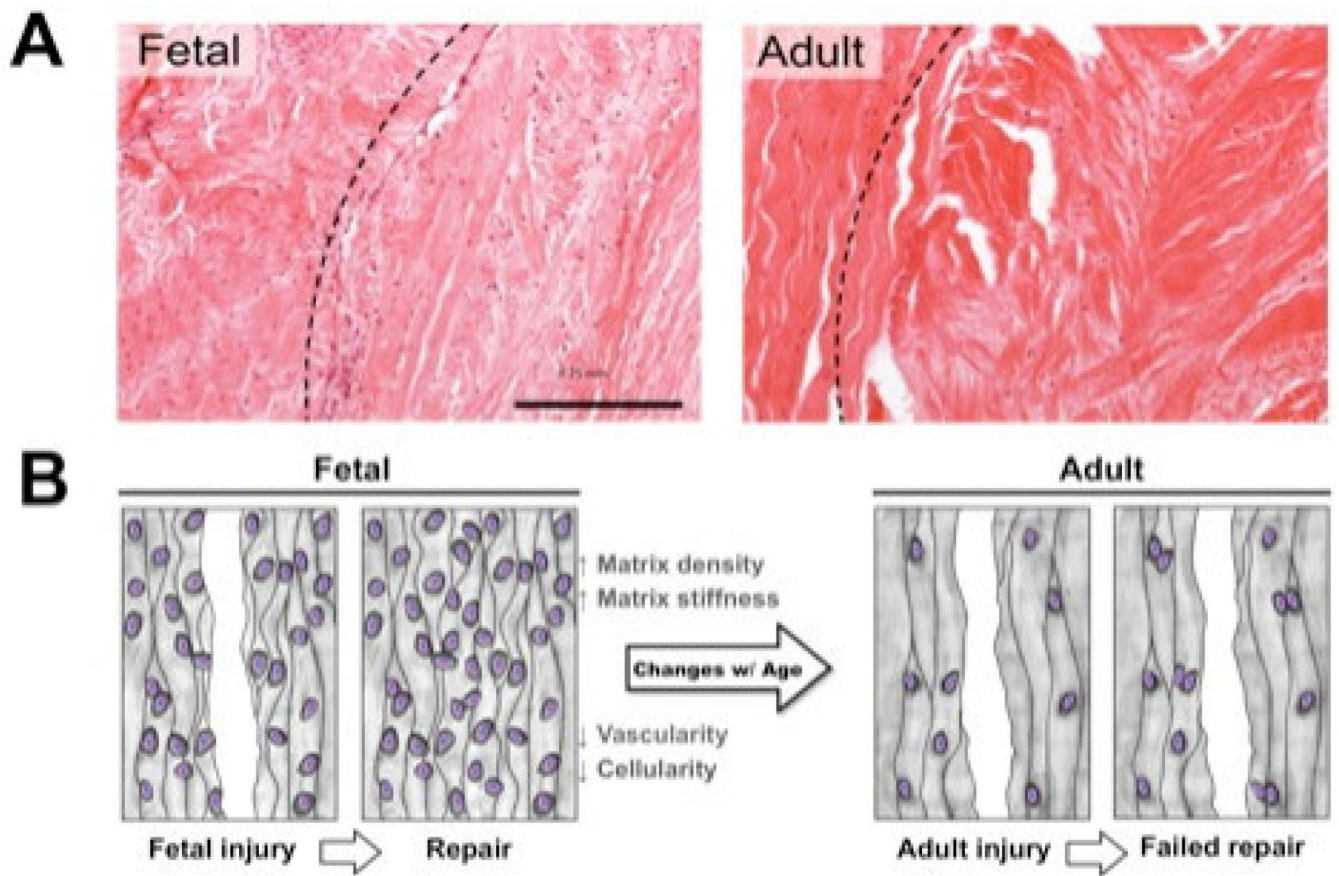
Figure 2. Intrinsic meniscus repair decreases with tissue maturation
A) Histological sections (H&E staining) showing near complete and seamless repair of a
fetal meniscus segment 8 weeks post injury, compared to persistent defects, fissures, and
clefts in an adult tissue repair construct cultured similarly. **B)** Schematic illustration of the
dynamic processes of fetal tissue repair and the intrinsic changes to the tissue that limit
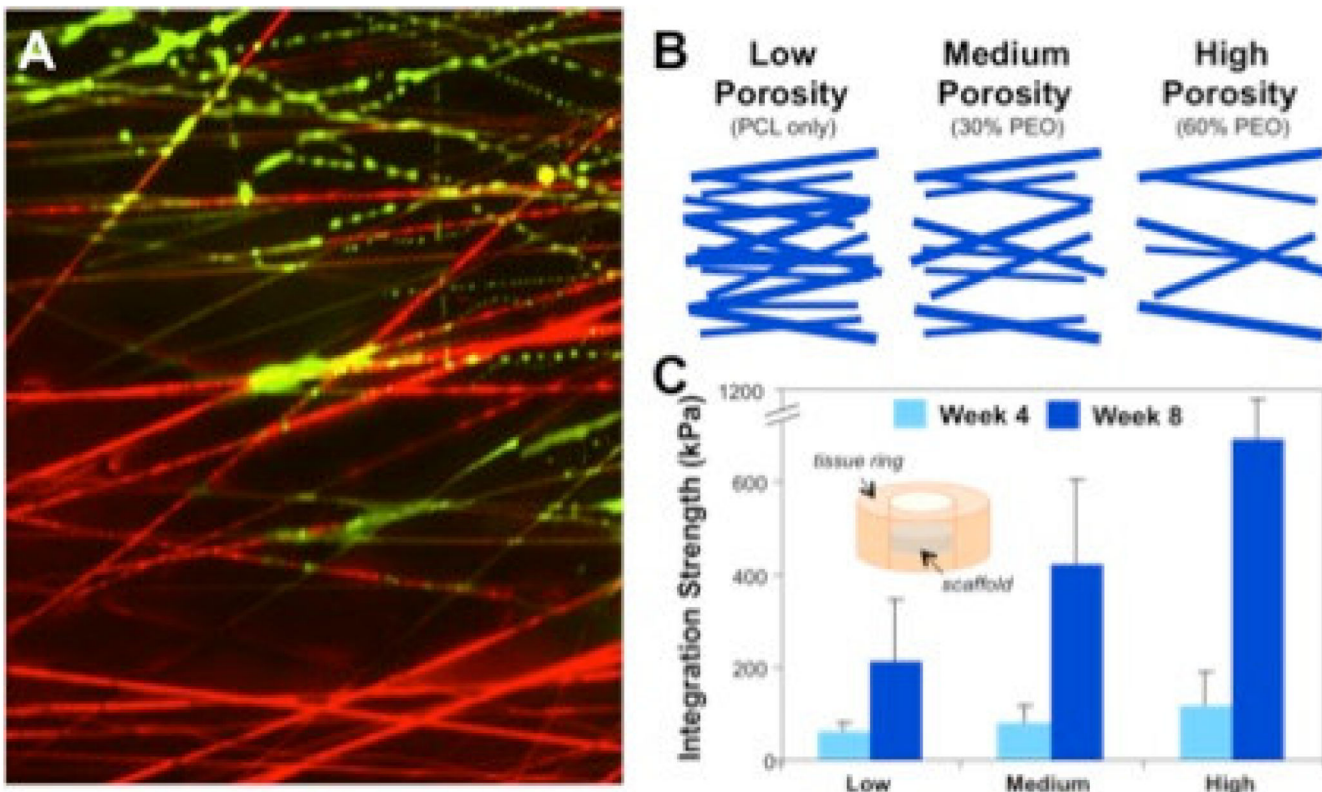repair in the adult. Adapted from [4] and [96] with permission.

Figure 3. Scaffold porosity modulates cell infiltration and integration
A) Dynamic composite fibrous scaffold with a stable poly(ε-caprolactone) (PCL) fiber
fraction (red) and a water-soluble poly(ethylene oxide) (PEO) fiber fraction (green). Here,
the composite is undergoing a transition in porosity as a hydration front advances from the
bottom to the top of the image, with the sacrificial PEO fibers being removed. **B)** Schematic
of fiber composites engineered to present differing levels of porosity based on the fraction of
sacrificial PEO fibers included in the network at the time of fabrication. **C)** Integration
strength as a function of time and scaffold porosity, where scaffolds with higher porosity
integrate with native tissue with higher mechanical strength than those with lower porosity.
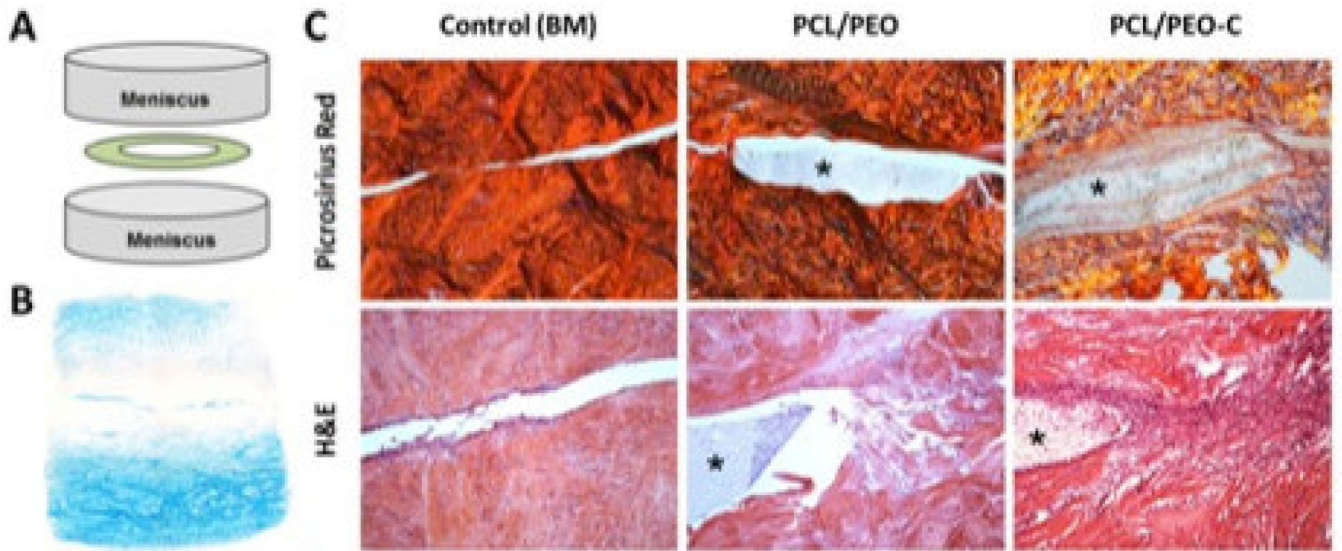Adapted form [59] and [61] with permission.

Figure 4. Bioactive scaffolds modulate local ECM density to improve repair
A) Schematic of meniscus repair construct and **B)** demonstration of matrix degradation (removal of PGs) at the wound interface (Alcian Blue staining, 2X magnification) with local delivery of collagenase from the PEO fiber fraction (PEO-C). **C)** Improved tissue integration as a result of biomaterial-mediated delivery of collagenase (PEO-C) to the wound interface in a subcutaneous model of meniscus repair. Picrosirius red staining of collagen (viewed under polarized light) and H&E staining of the repair interface at 4 weeks (10× magnification). Asterisk indicates scaffold. Adapted form [64] and [96] with permission.
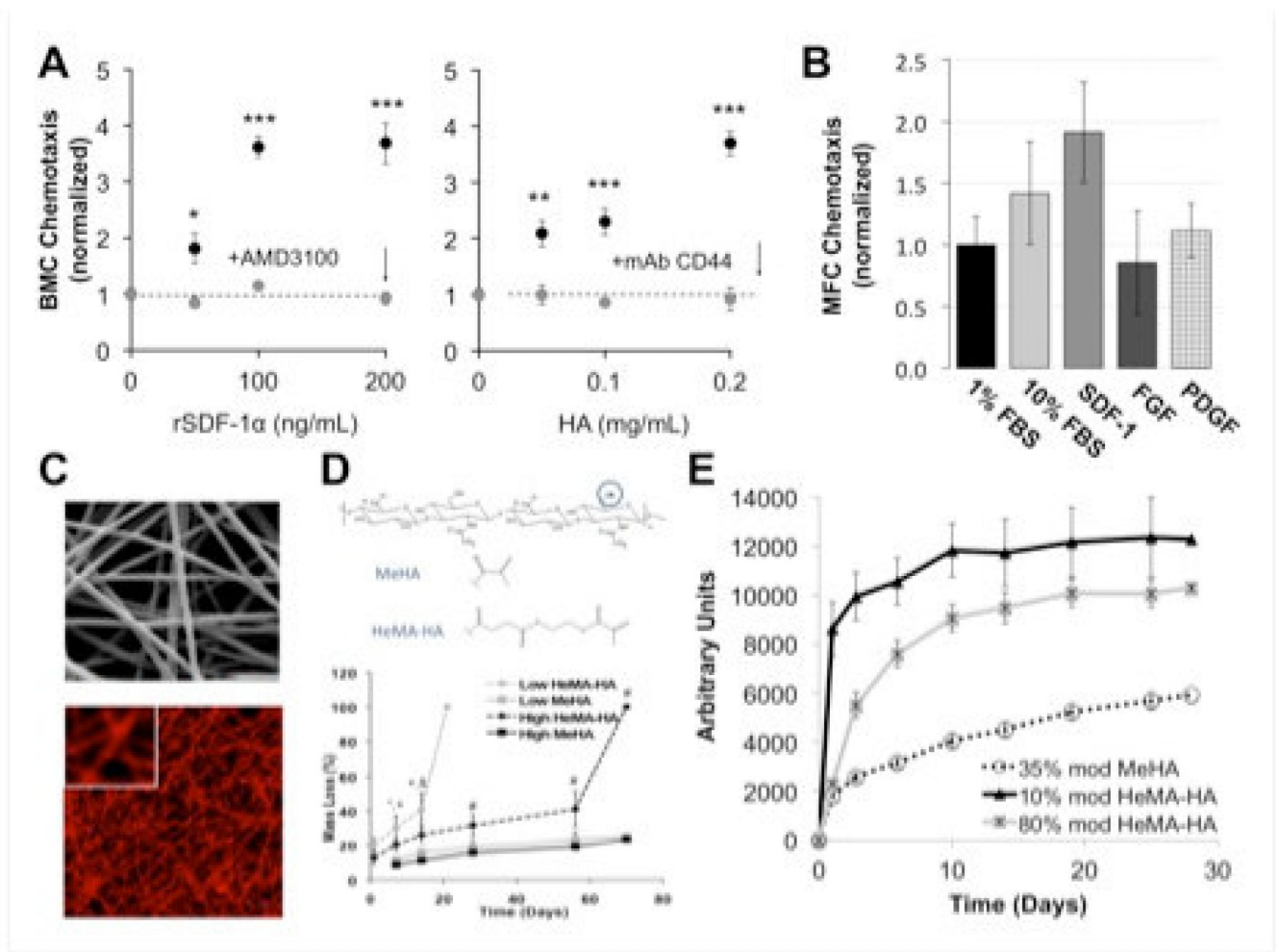
Figure 5. Chemotactic cues and novel delivery methods to improve colonization of the wound site
A) Bone marrow cell (BMC) chemotaxis in response to SDF and HA release from
degradable HA hydrogels. **B)** Meniscus fibrochondrocyte (MFC) chemotaxis in response to
serum (FBS), SDF, FGF, and PDGF (normalized to 1% FBS controls). **C)** SEM and
fluorescent images of HA nanofibers. **D)** Crosslinking chemistries providing stable (MeHA)
and hydrolytically degradable (HeMA-HA) HA-based materials (gels and fiber networks).
E) FITC-conjugated BSA release from HA-based nanofibers as a function of time and
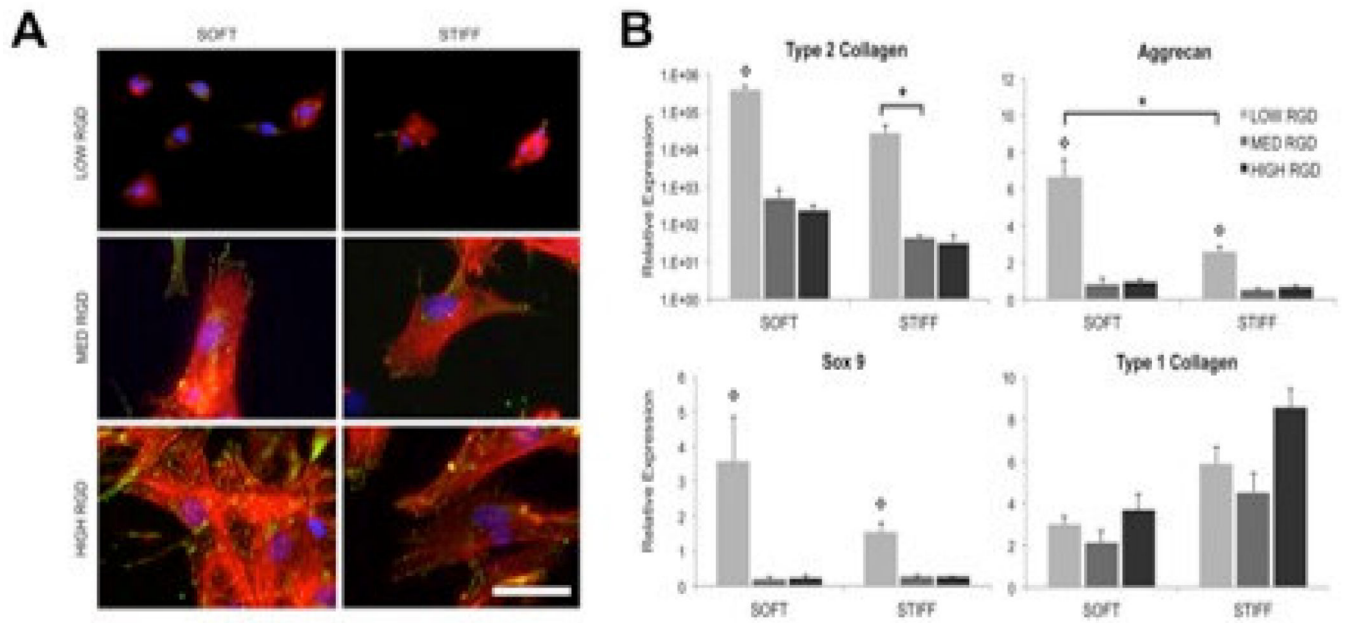degree of modification and type of crosslink. Adapted from [71] and [11, 77] with
permission.

Figure 6. Scaffold mediated instruction upon arrival
A) Vinculin localization (green), actin cytoskeleton (red), and nuclei (blue) staining of human mesenchymal stem cells (MSCs) cultured for 24 hours on fibrous HA scaffolds with varied RGD density and fiber modulus. Scale bar: 50 μm. **B)** MSC expression of chondrogenic markers after 14 days of culture in chondrogenic medium on fibrous HA scaffolds with varied RGD density and fiber modulus. *denotes significance (p<0.05) between groups, and ∻ denotes significance (p<0.05) compared to other RGD densities within the same fiber stiffness condition. Adapted with permission from [11].
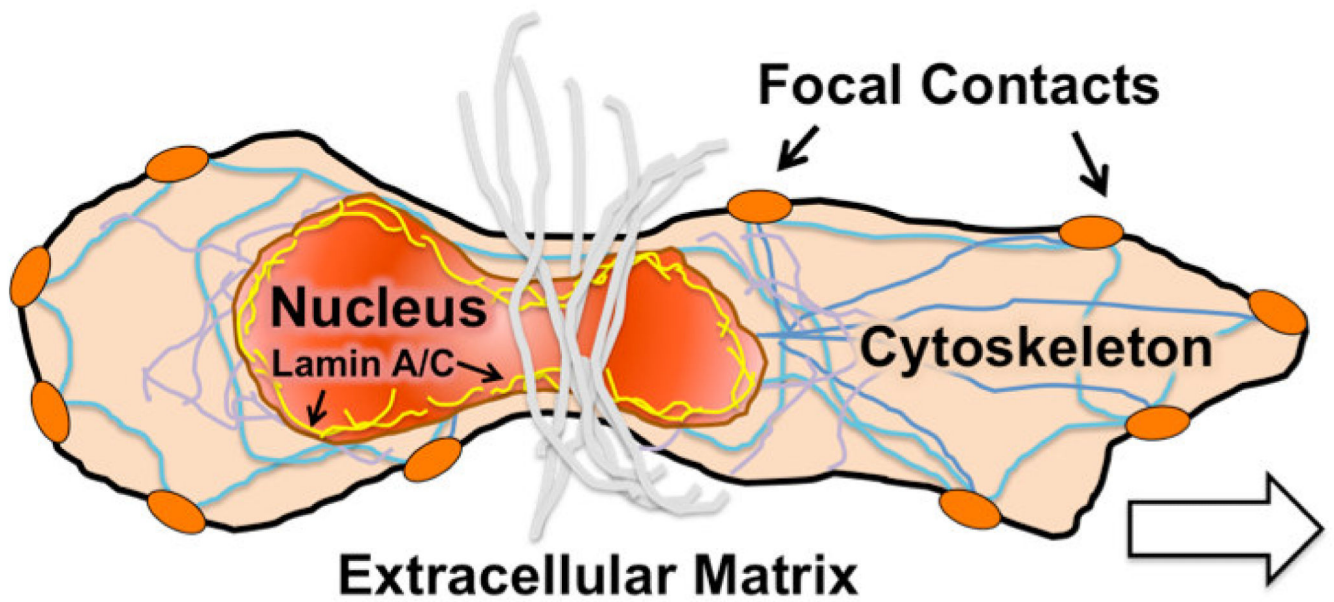
Figure 7. Emerging concepts: manipulating endogenous cell mechanics to improve migration to the wound site

Schematic illustration of interstitial 3D cell migration in dense fibrous networks. Nuclear mechanics, mediated by the amount and distribution of nucleo-structural filamentous components such as Lamin A/C, mediate the ability of cells to squeeze through small pores in dense connective tissues.