Minireview

Naturally derived biomaterials for addressing inflammation in tissue regeneration

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Abstract

Tissue regeneration strategies have traditionally relied on designing biomaterials that closely mimic features of the native extracellular matrix (ECM) as a means to potentially promote site-specific cellular behaviors. However, inflammation, while a necessary component of wound healing, can alter processes associated with successful tissue regeneration following an initial injury. These processes can be further magnified by the implantation of a biomaterial within the wound site. In addition to designing biomaterials to satisfy biocompatibility concerns as well as to replicate elements of the composition, structure, and mechanics of native tissue, we propose that ECM analogs should also include features that modulate the inflammatory response. Indeed, strategies that enhance, reduce, or even change the temporal phenotype of inflammatory processes have unique potential as future pro-regenerative analogs. Here, we review derivatives of three natural materials with intrinsic anti-inflammatory properties and discuss their potential to address the challenges of inflammation in tissue engineering and chronic wounds.

Keywords: Chitosan, decellularized matrix, amniotic membrane, inflammation, tissue regeneration, biomaterial

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Introduction

The field of tissue engineering aims to develop strategies to guide the body's response to tissue disruption due to injury or disease. The implantation of scaffold materials, cells, and/or soluble molecules such as growth factors (together referred to as the tissue engineering triad) is used not to replace the damaged tissue but to promote normal deposition of tissue with eventual material degradation for the restoration of native structure and function.[1] The extracellular matrix (ECM), primarily composed of collagen and glycosaminoglycans, is the three-dimensional microenvironment in which cells reside within tissues and organs of the body. The ECM is responsible for providing instructive signals which can modulate cell bioactivity via compositional, structural, and mechanical properties, among others. Following tissue injury, the likelihood of complete restoration of these ECM properties is low as the human body's wound healing mechanism prioritizes wound closure (repair) over tissue regeneration.[2] Therefore, even after months of ECM remodeling, natively repaired tissues exhibit disorganized matrix and decreased mechanical properties.[3] Tissue regeneration strategies have traditionally relied on designing biomaterials that closely mimic native traits of the ECM as a means to potentially promote normal, site-specific cellular function.

Following tissue injury, the body immediately begins to work to clear the injury site of debris, close the wound, and lay down new ECM. In adult wounds this wound healing cascade can be described in three overlapping phases: inflammation, proliferation, and remodeling (Figure 1). During the inflammatory phase, the chemotaxis of inflammatory cells (neutrophils, macrophages) to the wound site is stimulated by platelet release of platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β).[5] Pro-inflammatory, wound macrophages secrete key inflammatory cytokines, tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1) during this phase.[5] The transition to the proliferation phase is characterized by the macrophage shift to an anti-inflammatory, tissue repair phenotype[6] and the presence of fibroblasts along with the upregulation of genes associated with matrix proteins (collagen, proteoglycans, fibronectin).[5] The final phase of wound healing, remodeling, can continue for weeks or months as the tissue matrix is reorganized.

Inflammation, while a necessary component of wound healing, can hinder tissue regeneration in cases such as biomaterial implantation or chronic wounds. Inflammation is

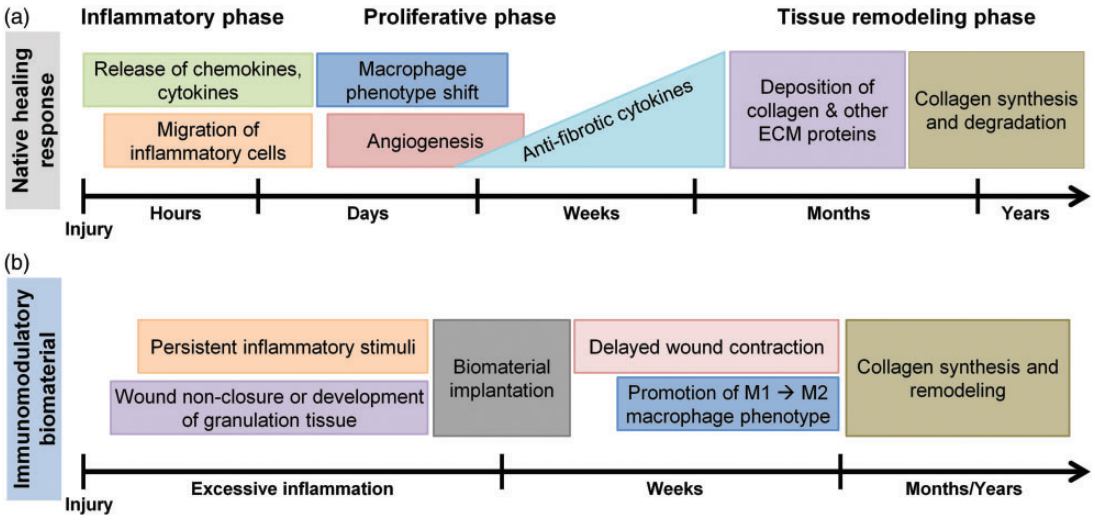

Figure 1 (a) Sequence of events in the body's normal response to injury (adapted from Bentzen[4]). (b) This chain of events can change depending on the tissue type, age of the individual, disease state, and the acute/chronic nature of the defect. In some cases, wounds undergo excessive inflammation leading to chronic wounds or scar formation. Biomaterials with immunomodulatory properties have the potential to enhance healing. (A color version of this figure is available in the online journal.)

not only a result of the original injury; the surgical implantation and biomaterial presence can also stimulate inflammation through the foreign body reaction (Figure 1). Therefore, it should follow that the design of biomaterials for tissue regeneration, normally focused on biocompatibility and recapitulation of the composition, structure, and mechanics of native tissue, should also include features that mitigate the inflammatory response. The goal for scaffold design in the future should be twofold: to provide instructive signals necessary for cell attachment, proliferation, and function in the context of the native tissue (bioactivity) and also to modulate the immune response.

Physical properties of scaffolds, such as composition,[7,8] mechanical properties,[9] and microarchitecture,[10] can all influence cell bioactivity. But these features may also explicitly regulate the immune response. Aligned, electrospun polycaprolactone fibers implanted subcutaneously in a rat model showed increased cell infiltration and a thinner fibrous capsule as compared to fibers with random orientation.[11] Variations in pore size of polytetrafluoroethylene materials result in differential expression of pro-inflammatory cytokines *in vitro* and decreased fibrous capsule thickness *in vivo*.[12]

Traditionally, materials for reducing the inflammatory response of biomaterials have relied on the incorporation of anti-inflammatory therapeutics. One technique is to use plasmids of the anti-inflammatory cytokine IL-10 in the pretreatment of cells or to incorporate them into a collagen biomaterial scaffold.[13,14] This approach reduces inflammation and enhances MSC survival *in vitro*[13] and in an *in vivo* model for myocardial infarction, there was increased wall thickness, increased ratio of collagen III to I, and a shift to a regulatory macrophage phenotype leading to functional recovery.[14] The incorporation of anti-inflammatory drugs such as ibuprofen[15] and tetrandrine[16] into polylactic acid (PLA)-based scaffolds has also shown reduced inflammation and improved tissue regeneration in *in vivo* rat models.

The ability to leverage biologically derived materials with intrinsic anti-inflammatory properties for tissue regeneration has the potential to generate a new class of biomaterials with the capacity to promote regeneration and alter the inflammatory response in the wound site. Coordinated efforts in biomaterial design may offer the possibility to improve regenerative potential because of the ability to alter native inflammatory responses. Pro-inflammatory signals are not inherently detrimental to healing; in fact, they are necessary for repair as long as they subside in a timely fashion.[17] It is hypothesized that biomaterials that first promote the M1 macrophage phenotype and then M2 would enhance ultimate healing.[17] These observations reinforce the idea that biomaterial design should not simply reduce or enhance inflammatory response, but that the kinetics of the inflammatory response offer intriguing targets for biomaterial design. To date, the study of a wide range of naturally derived materials for their potential immunomodulatory/anti-inflammatory capability and their ability to support tissue regeneration has begun. From this wide variety of materials, this review focuses on three particular classes of biomaterials—chitin, decellularized ECM, and amniotic membrane (AM)—that show particularly intriguing properties in the context of biomaterial design. While many current observations described in the following sections and seen in Table 1 focus on solely reducing the inflammatory response, future generation tissue engineering products are likely to exhibit more nuanced control over the inflammatory cascade.

Immunomodulatory activities of chitin-derived materials

Chitin is one of the most abundant polysaccharides in nature, second only to cellulose.[37] It is an inexpensive and readily available material that is found in the exoskeletons of invertebrates, such as crabs and shrimp, as well as the cell walls of fungi and yeast.[37,38] Chitin is a linear polymer composed of *N*-acetyl-D-glucosamine subunits.[37] Since chitin is not readily

Table 1 Materials studied for the modulation of inflammation during wound healing

	Material form	Immunomodulatory function	Reference
Chitin derived	Low molecular weight chitosan oligosaccharides	Significantly inhibited expression of asthma related cytokines *in vitro* and *in vivo*	Chung *et al.*[18]
	Chitosan oligosaccharides	Suppressed NF-κB activation, reduced proinflammatory cytokine production, inhibited TNF-α and oxidative stress-induced apoptosis *in vitro* and relieved intestinal inflammation *in vivo*	Yousef *et al.*[19]
	Nanoparticles of chitosan and alginate	Inhibited bacterial-induced pro-inflammatory cytokine production in macrophage and keratinocyte *in vitro* culture	Friedman *et al.*[20]
	Chitosan films	*In vitro* monocyte culture showed variation in pro- and anti-inflammatory cytokine production over time (corresponding to monocyte to macrophage differentiation)	Oliveira *et al.*[21]
	Chitosan fibers	Promoted migration of inflammatory cells to the wound site and collagen matrix deposition in open skin wounds	Ueno *et al.*[22]
	Chitosan hydrogels	In treatment of third-degree burns, this chitosan material favors inflammatory cell migration and angiogenesis	Boucard *et al.*[23]
Decellularized matrix	Bovine pericardium	Monocyte-derived macrophages seeded on the decellularized matrix *in vitro* exhibited decreased spreading and lower levels of pro-inflammatory interleukins	Ariganello *et al.*[24,25]
	Skeletal muscle	Decreased levels of pro-inflammatory cytokines and increased levels of anti-inflammatory cytokines (*in vitro*); polarized macrophages towards M2 phenotype (*in vivo*); decreased T-cell proliferation	Fishman *et al.*[26]
	Porcine colon	Extensive cellular infiltration and degradation *in vivo* as compared to colonic ECM that had been chemically cross-linked	Keane *et al.*[27]
	Porcine ureter	Carbodiimide cross-linking corresponds to M2 macrophage phenotype shift in subcutaneous rat model; cross-linking with glutaraldehyde or genipin leads to M1	Koch *et al.*[28]
Amniotic membrane based	Amniotic membrane sheet	In treatment of adhesions between digital flexor tendons and their sheathes, AM sheets exhibited no inflammation and restored function to the fingers	Pinkerton[29]
		Chronic ulcers treated with AM were healed within 10 weeks	Troensegaard-Hansen[30]
		In ocular surface applications, AM leads to rapid epithelialization and reduced inflammation	Azuara-Blanco *et al.*,[31] Tseng *et al.*,[32] Chen *et al.*[33]
	Collagen-amnion dermal substitute	Results in few inflammatory cells, rapid healing, and good neovascularization in porcine full thickness skin defect	Kim *et al.*[34]
	Micronized, dry amnion–chorion membrane	Injected, reduces cartilage degeneration in osteoarthritic rat model	Willett *et al.*[35]
	Collagen–amnion porous scaffolds	Decreased expression of TNF-α and MMP-3 genes, increased expression of COLI under pro-inflammatory condition *in vitro*	Hortensius *et al.*[36]

dissolvable, its deacetylated derivative, chitosan, is frequently studied and used in biological applications.[38,39] Chitosan can be further degraded and deacetylated to form chitooligosaccharides (COS).[18,19,40–42]

Chitin, chitosan, and their derivatives have been extensively studied for their anti-inflammatory, anti-microbial, antioxidant, and anticancer effects.[37,38,43] The range of anti-inflammatory applications for chitin-derived materials encompasses the treatment of chronic inflammatory conditions (asthma,[18] inflammatory bowel disease,[19] sepsis,[42] arthritis[44]), the use of chitosan composite particles for drug delivery,[20] and the modulation of immune responses

of implanted chitin-based biomaterials.[21] Low molecular weight chitosan oligosaccharides significantly inhibit asthma-related cytokines (IL-4, IL-13, TNFα) *in vitro* and *in vivo*.[18] Additionally, oral administration of COSs has been effective in attenuating pro-inflammatory cytokine production and reducing intestinal inflammation in models of induced colitis.[19] Bacterial-stimulated inflammation, such as that seen in acne, can also be treated with chitin-derived materials.[20] Nanoparticles of chitosan and alginate (without antiacne mediation) were able to inhibit bacterial induction of IL-12 in macrophages in a dose-dependent manner. Further, the presence of these nanoparticles was able to also inhibit the induction of IL-6 in keratinocytes.[20]

The anti-inflammatory mechanism of orally administered chitosan, in particular, has been attributed to its release of *N*-acetyl-*β*-D-glucosamine during degradation.[37,45] Glucosamine is a structural unit of hyaluronan, a glycosaminoglycan shown to have roles in the promotion of cell motility and accelerated wound healing. Glucosamine is also a primary component of proteoglycans of the native ECM of cartilage and connected tissue. A decrease in the occurrence of osteoarthritis-related total joint replacements has been linked to treatment with glucosamine sulfate.[44] Further disruption of inflammation via chitin-derived materials (outside the field of tissue regeneration) has been observed through the inhibition of pro-inflammatory MMP-2,[46] the decrease in IL-1β-induced chondrocyte apoptosis,[47] and the attenuation of nitric oxide and inflammatory cytokine (such as TNF-α, IL-6, IL-1β) production in *in vitro* dose-dependent culture[40,41] as well as *in vivo*.[42]

An early study of chitin as a wound healing accelerant was conducted by Prudden *et al.*[48] Additional study has shown that chitosan fibers stimulate early cell migration and production of granulation tissue in canine open wounds.[22] More recent work has focused on the response of specific wound healing cells to chitosan-based implants. Immune cells, particularly macrophages, have roles in inflammation and tissue regeneration. Through the production of cytokines and chemokines, macrophages attract or decrease the inflammatory cells within the injury site.[21] By modulating the macrophage response to an implant, the rate and outcome of healing can be altered and chitosan has been found to promote anti-inflammatory macrophage polarization *in vitro* without the use of exogenous growth factors/cytokines.[21]

Chitosan scaffolds for use in skin, bone, cartilage, liver, nerve, and blood vessel wounds have been well summarized.[49,50] However, considering the range of anti-inflammatory uses for chitin derivatives, the study of chitosan scaffold-based therapies for immunomodulation in tissue regeneration is limited. Chitosan fibers[22] and hydrogels[23] have been evaluated for skin regeneration. In both forms, chitosan promotes migration of inflammatory cells to the wound site and collagen matrix deposition. In the hydrogels, chitosan also promoted angiogenesis resulting in vascularization of the new tissue.[23] These findings suggest that chitin-based materials may have potential in future tissue engineering products. However, significant new efforts to link current observations regarding immune response with functional metrics of tissue regeneration are required.

Decellularized matrix as scaffold for tissue regeneration

Scaffolds derived from decellularized matrix (from both allogeneic and xenogeneic sources) have been investigated as materials for regeneration in a range of tissues: heart valve,[24,25,51,52] nasal cartilage,[53] skeletal muscle,[26] gastrointestinal tract,[27,54] ureters,[28] liver,[55] and flexor tendons.[56] Both segmented and whole tissues can be decellularized.[54,55] The prevailing advantage to using decellularized matrix scaffolds is the maintenance of important properties of the native ECM. The ability to use site-specific tissue, in particular, is advantageous for tissue regeneration applications. This ensures that the distinct matrix architecture and composition are appropriate for the functional cells specific to that tissue, allowing for the enhancement of tissue-specific differentiation[57] and promoting chemotaxis and proliferation of progenitor cells.[58] Additionally, decellularized matrices have been shown to have immunomodulatory properties and the ability to influence the host response *in vivo.* Removing the cellular component of the transplanted tissue is key to this result as the cellular presence contributes to a predominantly M1 macrophage phenotype.[53,59]

Decellularized ECM scaffolds intrinsically have the chemical composition of the natural tissue; they contain ECM proteins, particularly collagen and glycosaminoglycans, that are highly conserved across species.[24,26,59] This conservation of biomolecules lends these materials to be biocompatible *in vivo*, exhibiting a low immune response.[26,59] Also maintained within the scaffolds are the complex three-dimensional microstructure (important for directing cellular phenotype via geometric cues)[53] and growth factors that have roles in cell attachment, proliferation, migration, and differentiation.[53] Further, the matrix retains its mechanical properties following decellularization allowing for mimicry of the native tissue and long-term function.[25] This property, in particular, negates the need for exogenous chemical cross-linking, presenting a material that is accessible to naturally occurring degradation and remodeling.[27] In contrast, manufactured collagen-based materials rely on cross-linking to increase their mechanical properties which significantly reduces the cellular degradation of the material.[60] The ability of cells to remodel their surrounding matrix is critical for regenerative success. Through the process of constructive remodeling, the degradable matrix template is gradually replaced by native tissue with the appropriate function, matrix organization, and cellular phenotype.[61] This remodeling outcome *in vivo* is dependent on a favorable response from the innate inflammation associated with both the original injury and/or the biomaterial implantation.[17,61]

While chemical cross-linking is commonly used in collagen-based ECM matrix analogs to stabilize the scaffold, it has been proposed that the inhibition of

decellularized scaffold degradation, and therefore, the release of degradation products through chemical cross-linking holds great influence over not only the regenerative capacity (noted earlier) but also the cellular immune response. Indeed, the products of enzymatically degraded porcine urinary bladder influence cell migration *in vitro*.[62] However, reports on the effects of chemically cross-linking decellularized matrices differ based on the tissue type and cross-linking agent. Porcine colonic decellularized ECM (coECM) exhibited extensive cellular infiltration and degradation *in vivo* as compared to coECM that had been chemically cross-linked with *N*(3-dimethylaminpropyl)-*N*′-ethylcarbodiimide hydrochloride.[27] Additionally, the cross-linked matrices exhibited evidence of encapsulation and disorganized connective tissue, results that are not desired in tissue regeneration applications.[27] Conversely, carbodiimide cross-linked porcine ureteral scaffolds implanted in a subcutaneous rat model showed a macrophage phenotypic switch to the anti-inflammatory M2 phenotype.[28] Cross-linking with glutaraldehyde or genipin stimulated a pro-inflammatory M1 macrophage switch *in vivo*.[28] These data are consistent with glutaraldehyde cross-linking of decellularized bovine pericardium which resulted in limited attachment and survival of macrophage-like cells (U937) and increased release of pro-inflammatory cytokines (MMP-1).[52] Future work should carefully consider the balance between scaffold degradation and the inflammatory wound healing response in the use of decellularized matrix for tissue regeneration applications.

Beyond the role chemical cross-linking can play in the cellular response to decellularized matrices, researchers have extensively studied how these biomaterials influence the inflammatory response *in vitro*[17,24,25–28,52] and *in vivo*.[17,26–28,56] The overwhelming observation, detailed in the following paragraphs, is that decellularized matrix can modulate the immune response by exhibiting anti-inflammatory effects and promoting a shift in macrophage phenotype from M1 to M2.

In studies of monocyte-derived macrophages cultured on decellularized bovine pericardium (DBP), researchers have observed decreased cell spreading and decreased formation of multinucleated cells on DBP (compared to polydimethylsiloxane (PDMS) and tissue culture polystyrene (TCPS)), indicating a low inflammatory response.[24,25] An *ex vivo* culture model was used to monitor the cellular events at the interface between a fresh tissue (porcine bladder) and decellularized tissue (porcine acellular bladder matrix).[63] There, macrophages were the first cells to move from the tissue into the decellularized matrix, where they matured to $CD163^+$ (M2 phenotype) cells.[63]

Autologous tissue grafts have the advantage of not requiring immunosuppression but their use is limited to static organs (i.e. skin) or those that move passively (i.e. blood vessels, bladder, trachea, heart valves)[26] and by supply (like in cases of significant injury to the flexor tendons of the hand).[56] Decellularized tissue provides a potential solution in each of these cases. Skeletal muscle was decellularized and either cultured with rat $CD3^+$ splenocytes or implanted into the tibialis anterior muscle of an immunocompetent rat.[26] Results showed decreased T-cell proliferation in both experiments. Decellularized scaffolds had slower degradation times and polarized macrophages toward an M2 phenotype *in vivo*.[26] *In vitro*, the decellularized skeletal muscle led to decreased levels of pro-inflammatory TH1 cytokines (IL-2, TFN-γ) and increased levels of anti-inflammatory TH2 cytokines (IL-10) compared to fresh tissue, effectively modulating the immune response.[26] Emerging work combining decellularized bone matrices with the sequential release of growth factors/cytokines that can stimulate M1 and then M2 macrophage phenotypes has shown the ability to enhance healing and vascularization.[17]

The mechanism of immunomodulatory behavior by decellularized matrices is not completely understood. It is hypothesized that the removal of MHC classes I and II during the decellularization process contributes to the anti-inflammatory effects of the matrix *in vivo*, the decreased T-cell proliferation *in vitro*, and reductions seen in IL-1 and IFN-γ expression.[26] The theory that the decellularization process exposes critical surface molecules for immunomodulation could be another explanation.[26]

Like any biomaterial, there are disadvantages associated with the use of decellularized matrices. These are a result of the variations between properties on the basis of tissue source and processing (method of decellularization, cross-linking, sterilization methods)[27] along with the potential for pathogen transfer to the host.[64] Cell-derived extracellular matrices present an exciting alternative for future study.[64]

Fetal wound-inspired materials for regeneration and scarless healing

The wound healing process and outcome is fundamentally different in fetal as compared to adult wounds. While adult wounds exhibit inflammation and result in disorganized matrix and scar formation, healing in fetal wounds proceeds in a regenerative fashion, with neither the typical inflammatory response nor the formation of scar tissue.[65–67]

The most prevalent example of fetal wound healing in literature is that of skin which has been well reviewed elsewhere.[66–69] Fetal skin has the potential to heal via complete regeneration (scarless), with normal tissue architecture and a minimal inflammatory response, but this response is dependent on gestational age and the size of the wound.[70–73] This scarless healing phenomenon does not only occur in skin tissue. Similar outcomes have been seen in tendon injury as well, where the restoration of function in adults can be hindered by the development of adhesions.[74] It should be noted that fetal scarless wound healing is not universal to all tissues. It has also been seen in heart and lung tissue but fetal wounds in the stomach, intestine, and diaphragm heal with a scar.[75]

Two leading differences between adult and fetal wound healing are the cytokines/growth factors expressed following injury and the composition of the native ECM. The decreased inflammatory response in fetal wounds can be attributed to the minimal infiltration of immune cells due to lower levels of PDGF, TGF-β1 and TGF-β2, and pro-inflammatory cytokines such as IL-6 and IL-8.[66,76–80] Additionally, the anti-inflammatory cytokine IL-10 is significantly expressed in fetal wounds.[81] Studies have

shown that treating adult wounds with IL-10 results in decreased inflammation and scarless wound repair.[82,83] Growth factors TGF-β1 and TGF-β2 are highly expressed in adult wounds as compared to uninjured tissue but their baseline levels are maintained in fetal wounds.[84] Fetal wounds contain the third TGF-β isoform, TGF-β3, which decreases scar formation through the regulation of collagen deposition as demonstrated in fetal rat skin.[85,86] Collagen type I is the primary ECM protein in both adult and fetal skin (normal and wounded) but fetal skin exhibits a higher ratio of collagen III to collagen I.[87] Hyaluronic acid (HA), a non-sulfated glycosaminoglycan, is also prevalent in the ECM and promotes cell proliferation and motility. Although HA is present in both adult and fetal wound fluid, it has been detected later in the healing process in fetal wound environments.[88]

The results of fetal wound healing cannot be solely attributed to the sterile, intrauterine environment and the interface with the surrounding amniotic fluid. Marsupial pouch young, who develop outside of the uterus, have the ability to heal cutaneous wounds in a scarless fashion.[89] Even without the uterine environment, the wounds created on the backs of grey short-tailed opossums were able to quickly undergo re-epithelialization and dermal repair.[89] The rate and degree of regeneration is dependent on the pouch age of the animal. It has also been shown that scar formation occurs when adult tissue is placed in the fetal environment.[90] Being perfused with fetal blood and bathed in amniotic fluid is not sufficient to induce fetal-like, scarless wound healing.[90] Fetal skin can also heal without scar in an adult subcutaneous environment.[91] These observations have led researchers to focus on the role the fetal ECM has on inflammation and resultant healing.

The AM (amnion) has been investigated as a valuable biomaterial for promoting healing with low immune response and decreased scar formation.[92] The AM, found in the fetal environment, is the innermost layer of the placenta, surrounding the fetus *in utero*. It is composed of a single epithelial layer and a collagen-rich (collagen I as well as types III, IV, V, and VI) ECM that also contains laminin, fibronectin, elastin, and proteoglycans.[92–95] Following birth, it can be easily removed from the placenta and separated from the interfacing maternal membrane (chorion). From there it can be utilized in a range of forms: (1) living tissue containing cells, (2) decellularized matrix, or (3) in combination with other scaffold materials. Isolated AM matrix contains and elutes a range of biological factors (growth factors and cytokines) that are important components of the wound healing cascade.[96] The presence of anti-inflammatory cytokines such as IL-4 and IL-10 as well as inhibitors of matrix degradation (TIMPs 1, 2, 4) is of particular interest.[96]

The AM has long been recognized for its anti-inflammatory properties and wound healing capacity. The use of amnion sheets for treatment of adhesions (scar formation) between digital flexor tendons and their sheathes was reported in 1942.[29] A 1950 report details the use of boiled AM to treat chronic skin ulcers.[30] In it the authors describe six case studies in which patients with longstanding, unhealed leg ulcers were treated with a dressing of amnion. Each of the wounds was healed within 10 weeks of treatment and the resulting skin was thick, healthy, and elastic.[30]

More recently, it has been shown that the intact amnion membrane sheet is effective in the treatment of corneal surfaces,[31–33] skin wounds,[34,96] oral cavity reconstruction,[97] and many other reconstruction applications summarized elsewhere.[92,98] AMs applied to the ocular surface in cases of surface abnormalities,[31] limbal stem cell deficiency,[32] and corneal ulcers[33] have led to rapid epithelialization, reduced inflammation, and surfaces that are smooth and wettable. AMs were implanted subcutaneously in mice and shown to enhance progenitor cell recruitment.[96] In a porcine full thickness skin defect, cross-linked collagen-amnion dermal substitutes exhibited few inflammatory cells, rapid healing, and good neovascularization.[34] Micronized, dehydrated human amnion membrane has been shown to reduce cartilage degeneration in an osteoarthritic rat model.[35]

While the use of intact and injectable AM matrix has been investigated for two-dimensional tissue regeneration applications, the potential of the AM as a bioactive component in 3D biomaterials has not been extensively studied. Three-dimensional matrices made entirely of decellularized AM have been fabricated via lyophilization.[99] The resulting scaffold has been shown to support fetal dermal fibroblast cells *in vitro*. Collagen–glycosaminoglycan scaffolds have been applied in skin, peripheral nerve, and cartilage tissue engineering and as *in vitro* 3D culture models.[100–106] Collagen-based scaffolds, by hindering myoblast recruitment[107] and resultant contraction/scar formation,[108] have induced regeneration and decreased scar formation in skin and nerve defects.[2] Recent work in our lab has investigated scaffolds that combine amniotic matrix particles within lyophilized collagen materials.[36] Here, equine tenocytes were cultured in each scaffold variant and exposed to a range of pro-inflammatory IL-1β-containing media concentrations. The cells cultured in the collagen–amnion scaffold showed increased metabolic activity over the seven-day experiment, even in the pro-inflammatory media conditions. Further, scaffolds containing AM led to decreased expression of TNFα and matrix metalloproteinase-3 genes and increased expression of collagen I. In this we have shown that incorporating fetal wound inspired matrix components in a collagen scaffold for tissue regeneration supports cellular health and tempers pro-inflammatory gene expression *in vitro*.

Conclusions

In this review, we have highlighted emerging efforts in the field of tissue engineering that seek to develop biomaterials that not only promote cell bioactivity using conventional, *tissue-specific* metrics (e.g. proliferation, differentiation, matrix synthesis), but that also begin to see the biomaterial as a vehicle that can modulate the host immune response after injury. Wound healing cascades are complex and vary tissue to tissue, but also in the context of age, disease, and the acute or chronic nature of the defect. Typical design parameters for tissue regeneration biomaterials are focused on providing the appropriate ECM analog to mimic the

native tissue compositionally, structurally, and mechanically. These signals aid local cells in building new tissue as part of the wound healing cascade. However, the process of wound healing can be hindered by the presence of an extensive inflammatory response. Recent results in the literature suggest the intriguing possibility that biomaterial design might also be considered as a tool for modulating the immune response following injury. We have summarized here three naturally derived materials with potential to address the challenge of inflammation in tissue engineering and chronic wounds via their intrinsic anti-inflammatory properties. Chitin-derived materials degrade into monosaccharide molecules that are present in native ECM and have roles in accelerated wound healing. Decellularized extracellular matrices provide the advantage of tissue-specific composition and architecture as well as promoting M2 macrophage polarization. Decades of use as a two-dimensional material in the treatment of a range of wounds have shown that the AM has great potential as an anti-inflammatory material. While not specific or nuanced to date, the idea of instructive signals changing the kinetics of the wound healing cascade offers an exciting glimpse into the future of tissue engineering. Anti-inflammatory properties have been observed in each of the materials described here but the mechanism behind these properties within the context of tissue regeneration has yet to be defined and should be a point of focus in the future. The careful design of tissue regeneration biomaterials to both avoid the stimulation of an immune response and promote the resolution of any existing inflammation will be critical for efficient healing and functional outcomes, suggesting the development of immunomodulatory biomaterials as an emergent subfield of tissue engineering with significant potential for clinical success.

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