Extracellular Matrix as a Strategy for Treating Chronic Wounds

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Abstract

The dermis normally directs all phases of skin wound healing following tissue trauma or disease. However, in chronic wounds, the dermal matrix is insufficient to stimulate healing and assistance by external factors is needed for wound closure. Although the concept of the extracellular matrix directing wound healing is not new, ideas about how best to provide the extracellular matrix components required to ‘jump-start’ the healing process are still evolving. Historically, these strategies have included use of enzyme-inhibiting dressing materials, which bind matrix metalloproteinases and remove them from the chronic wound environment, or direct application of purified growth factors to stimulate fibroblast activity and deposition of neo-matrix. More recently, the application of a structurally intact, biochemically complex extracellular matrix, designed to provide the critical extracellular components of the dermis in a single application, has allowed for the reconstruction of new, healthy tissue and restoration of tissue integrity in the previously chronic wound. This review focuses on this third mechanism as an emerging tactic in effective wound repair. Intact extracellular matrix can quickly, easily, and effectively provide key extracellular components of the dermis necessary to direct the healing response and allow for the proliferation of new, healthy tissue. Its application may promote the healing of wounds that have been refractory to other, more conventional treatment strategies, and may eventually show utility when used earlier in wound healing treatment with the goal of preventing wounds from reaching a truly chronic, nonresponsive state.

1. Background

Chronic wounds represent a difficult problem for the clinician and are a cause of prolonged suffering for the patient. Typically caused by impaired vascular perfusion due to diabetes mellitus,[1] venous hypertension,[2] or chronic pressure secondary to sustained immobility,[3] many of these wounds fail to heal even after 3 months of standard treatment and require active intervention to promote closure.[4-7] The extent of the problem is tremendous, both in economic terms and in reduced quality of life. For example, of the 14.7 million persons in the US diagnosed with diabetes in 2004,[8] 6% of them may be expected to develop foot ulcers over a 3-year period,[9] at an estimated annual cost of approximately $US6 billion (2001 value).[10] In addition to the economic costs, most patients with chronic venous leg ulcers report pain (81%), itching (69%), and loss of sleep (67%) as a result of their wounds,[16] together with a significantly lower quality of life.[17]

A fundamental problem with chronic wounds is that they lack a functional extracellular matrix (ECM) to stimulate, direct, and coordinate healing. A functional ECM is central to wound healing and its absence in chronic wounds stalls the healing process. By restoring the ECM to its natural state through the addition of exogenous components that specifically address the failing ECM in a chronic wound, it may be possible to stimulate the healing response and bring about successful wound closure. To fully understand how such a strategy may improve the healing of chronic wounds, it is necessary to understand the components of the natural dermal ECM and how they interact to promote healing, how the ECM is deficient in chronic wounds, and how different
strategies that are available to the clinician can be used to facilitate restoration of the ECM.

2. Major Components of the Dermal Extracellular Matrix (ECM)

The ECM is a complex scaffold consisting of structural and functional proteins, proteoglycans, glycoproteins, and glycosaminoglycans arranged in a tissue-specific orientation. Within the ECM, these individual proteins perform a wide range of physiologic functions. For example, fibrillar collagens provide the support and tensile strength that give the ECM its structural integrity. Other proteins, such as fibronectin, provide attachment sites for cells of various types and coordinate remodeling of the ECM.[18,19] Still other components, such as heparin and hyaluronan (hyaluronic acid), aid in retaining matrix hydration, act as signaling molecules that direct all stages of tissue repair and regeneration, and bind growth factors that are essential to the normal functioning of the ECM.[20] Growth factors and matrix metalloproteinases (MMPs) contribute to matrix turnover: growth factors actively direct the local cells to increase matrix production, initiate angiogenesis, and migrate to where they are needed,[21,22] while MMPs degrade the ECM to facilitate cell migration and remodeling of newly synthesized matrix.[23] Taken together, the ECM is a dynamic environment in which cells and matrix constituents interact to maintain homeostasis in the uninjured state, and to restore homeostasis in the case of injury or tissue loss.

Human skin is the largest and one of the most complex organs in the body,[24] forming a functional barrier between the internal and external environments. Its functionality depends upon the establishment and maintenance of the composition and organization of the dermal ECM. The dermal ECM underlies the epidermis and provides structural support for the cutaneous surface. It consists primarily of filamentous type I and III collagens and elastin, with lesser amounts of other ECM components.[25] Lying within the dermis are also the epidermal appendages, nerves, and cutaneous vasculature. In its natural state, the dermis contains occasional tissue-resident inflammatory cells, but the majority of cells are fibroblasts that secrete and maintain the ECM that surrounds them. Collagens comprise approximately 98% of the dermal ECM; they provide structural stability to the skin. Elastic fibers comprise approximately 2% of the dermal ECM and provide elasticity. In addition to these major matrix components, the dermis also contains small amounts of a wide variety of other ECM constituents, such as glycoproteins, proteoglycans, glycosaminoglycans, cytokines, and growth factors, all of which are important to maintaining the anatomy and physiology of the skin and directing tissue repair and wound healing.

The collagens are a large family of proteins that play diverse structural and signaling roles in the matrix. For example, type I, III, and V collagens, the main fibrillar collagens in the dermal matrix,[26] are thought to contribute primarily to matrix strength and structure. Recent findings also suggest that during angiogenesis, endothelial cells use the fibril diameter of type I collagen to regulate their migration patterns, either invading the matrix or forming monolayers upon it in direct response to the fibril size.[27] Similarly, fibroblast behavior appears highly regulated by the collagen density of the matrix.[28]

In addition to the fibril-forming collagens, other members of the collagen family play important roles in matrix integrity and assembly. In skin, type VII and XVII collagens are essential for maintaining the integrity of the dermal-epidermal junction, and type VI collagen is a key organizing macromolecule of the matrix.[25] An extensive array of type VI collagen microfibrils is distributed throughout the dermal matrix, interspersed between the major collagen fibers, and juxtaposed with cellular basement membranes, blood vessels, and nerves.[29-31] Type VI collagen forms highly flexible networks, and is capable of multiple interactions with a wide variety of other matrix molecules and cells.[32-34]

The short triple helix and numerous disulfide bonds found in type VI collagen make it highly resistant to degradation by bacterial collagenase and MMP, although it is highly susceptible to degradation by serine proteases present in inflammatory wound fluid.[35]

In addition to their important role in maintaining the structural integrity of tissue, collagens are involved in a wide variety of other functions. Collagens mediate cell and matrix interactions through specific receptors, such as integrins and specialized proteoglycan receptors.[36] For example, interactions between fibroblasts and collagen are mostly mediated by a subset of β1 integrin receptors, such as α1β1, α2β1, and α11β1 integrins, which are essential for establishing collagen contacts and transducing signals through the matrix.[37] Signaling by these receptors regulates adhesion, differentiation, growth, response to injury, and angiogenesis,[38] and contributes to the overall phenotype of connective tissue fibroblasts. Collagens also contribute to the entrapment, storage, and local delivery of growth factors and cytokines, and therefore play important roles in development, wound healing, and tissue repair.[39]

For example, type I collagen binds decorin, and may therefore indirectly block the action of transforming growth factor-β (TGFβ) within the tissue.[39] Additionally, fragments of type I collagen stimulate cytokine secretion by leukocytes during the initial stages of inflammation and therefore contribute to the chemoattraction of various cell types needed for wound healing.[40]

Elastic fibers comprise the largest non-collagen component of the dermal ECM and function primarily to impart elasticity to the skin. In mature skin, elastin is a complex, insoluble polymer that is...
extrinsically stable with very slow turnover. Regional differences in elastin bundle diameters exist within the dermis, with thinner fibrils present in the papillary dermis and increasingly thicker fibrils present in the deeper layers of the reticular dermis. Despite its very hydrophobic nature, elastin is readily hydrated by water and is therefore thought to also contribute to the retention of skin hydration. Of the additional ECM components found in skin, one of the most important constituents is fibronectin. Fibronectin is essential to matrix remodeling and controlling the deposition of other matrix components such as type I collagen and thrombospondin. Without the ability of cells to secrete fibronectin and polymerize it into effective three-dimensional networks, control of matrix organization, composition, and stability is lost. In the context of wound healing, it is the ability of fibronectin to polymerize that provides cells with a means of precisely controlling cell-ECM signaling events, such as specific localization of the β1 integrin and tension to cell-matrix binding sites, which regulate cell proliferation, migration, and differentiation.

### 3. The ECM in Dermal Wound Healing

Acute wounds normally heal in a very orderly and efficient manner characterized by four distinct, but overlapping, phases: hemostasis, inflammation, proliferation, and remodeling. The normal healing response begins immediately following injury, as platelets from the blood come into direct contact with exposed collagen and other ECM components. This contact triggers the platelets to release clotting factors, growth factors, and cytokines, leading to hemostasis within minutes and the deposition of a fibrobin clot as a provisional matrix. Following hemostasis, neutrophils recruited to the area as a result of platelet degranulation enter the wound site and begin to phagocytose foreign material, bacteria, damaged tissue, and dead cells. Macrophages are recruited later to continue the process of phagocytosis. They also release additional cytokines and growth factors, beginning the proliferative phase of wound healing by signaling tissue fibroblasts to migrate in and deposit new ECM. The new matrix becomes cross-linked and organized during the remodeling phase, which can take months to occur.

Following tissue trauma, type I collagen in the dermis stimulates the respiratory burst, granule exocytosis, and cytotoxic secretion by human leukocytes, thereby stimulating chemotaxis of cell types needed for wound healing. Fibronectin in the provisional matrix attracts and binds cells via integrins, allowing them to repopulate the site of injury. As additional fibronectin is secreted by the recruited cells, fibronectin polymerizes into stable, three-dimensional networks that precisely regulate cell proliferation, migration, and differentiation. Laminin helps direct the formation and stabilization of blood vessels and also provides attachment sites for fibroblasts and endothelial cells. Laminin-5, a key component in the dermal-epidermal junction, serves as a scaffold for cell migration, initiates the formation of hemidesmosomes, and accelerates basement membrane restoration at the dermal-epidermal junction to give the skin resistance against frictional stress. Heparin and heparin sulfate bind growth factors such as fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF), thereby protecting them from rapid degradation and storing them in the provisional matrix for ready release when they are needed. Hyaluronic contributes to water retention by the matrix, and these and other glycosaminoglycans act as cell-signaling molecules that direct endothelial cells and fibroblasts to secrete additional growth factors and cytokines important for the progression of healing. Hyaluronic has also been shown to inhibit the excessive formation of scar tissue by inhibiting platelet aggregation and release of platelet-derived growth factor and other cytokines. Heparan sulfate, as a part of larger proteoglycan molecules, enhances the responsiveness of local connective tissue fibroblasts to the effects of locally secreted growth factors.

Growth factors stored in the dermal ECM act as a ready supply of preformed cytokines, which stimulate the early phases of inflammation and healing, induce the influx of inflammatory and connective tissue cells, and direct local cellular activity. For example, TGFβ and connective tissue growth factor (CTGF) stimulate collagen deposition following injury and inhibit matrix degradation. CTGF also contributes to ECM accumulation in wound healing by enhancing the affinity of fibronectin for fibrin. VEGF, CTGF, and basic FGF-2 all contribute to the re-establishment of the local vascular supply needed to provide nutrients for healing and rid the damaged area of dead cells, tissue debris, and metabolic waste. Platelet-derived growth factor stimulates the deposition of granulation tissue and fibroblast migration, and keratinocyte growth factor facilitates the epithelialization of wounds.

Working and interacting together, the components of the dermal ECM direct the wounded tissue through the processes of acute inflammation, healing, and tissue remodeling to achieve a state of stasis, re-epithelialization, and homeostasis. It is the presence of all these factors and their inhibitors in tightly controlled concentrations and states of activity that leads to successful healing.

### 4. ECM Material for Chronic Wounds

Because of various inherited or acquired pathologies, dermal wounds often do not heal. As acute wounds remain open, their likelihood of healing decreases. The localized environment of
chronic wounds, characterized by high levels of matrix-degrading enzymes, fails to support wound repair because the rate of matrix breakdown exceeds the rate of matrix deposition by the local cells; the ECM is corrupt and cannot support healing. Cytokine expression and distribution are altered, and the presence of senescent fibroblasts is increased. Chronic wound fibroblasts are unable to effectively reorganize the ECM and are unresponsive to growth factors and other signals that are essential for driving the healing response. Fibronectin, a key component of the developing ECM, is rapidly degraded by proteases in chronic wounds, as are growth factors. Additionally, fibroblasts in chronic wounds lack the cell surface receptor integrin α5β1, which is necessary for fibronectin binding and keratinocyte migration. These biochemical features suggest that ECM dysfunction in chronic wounds is substantial and must be addressed in order for healing to proceed.

Strategies to correct ECM dysfunction in chronic wounds may involve addressing any one or any combination of the aforementioned deficiencies. A general principle that must be addressed is that the catabolic nature of the chronic wound, associated with altered growth factor distribution, cellular dysfunction, and increased enzymatic activity, must be overcome and shifted toward an anabolic state, in which the deposition of new matrix tissue exceeds the rate of matrix breakdown. Given the multitude of interactions between cells and ECM needed for successful wound healing, this shift may not be a simple matter of inhibiting enzymes, but rather may require a multifaceted approach, which includes attempts to normalize structure as well as signaling in the wound.

Because type I collagen binds matrix-degrading enzymes, a possible approach to altering the local chronic wound environment is to selectively control MMP activity through the delivery of a collagen-rich, MMP-binding material, thus effectively removing the enzyme from the wound bed and altering the wound microenvironment. Another valuable therapeutic approach may be to deliver growth factors directly to the wound bed where they can readily act to stimulate healing. Yet another approach may be to apply a naturally derived, structurally intact ECM material that can support the healing process to gain wound closure and restore native tissue architecture. In particular, it is thought that materials approximating the complex tissue structure and composition of the dermal ECM can provide the cues needed to direct chemotaxis, adhesion, differentiation, growth, deposition of new dermal matrix, and re-epithelialization and restoration of the normal architecture of the skin.

All three of these clinical approaches have been tried with varying degrees of success. For example, wound care materials derived from biologic sources have been shown to promote granulation and epithelialization of dermal wounds compared with standard care treatments with varying degrees of efficacy. Highly purified collagen products, which have been available for several decades, produce 12-week healing rates of chronic diabetic ulcers in the order of 35%, and have been shown to effectively bind and remove MMPs from chronic wound fluid in vitro. Although their efficacy in removing MMPs from chronic wounds in vivo has been recently questioned, their apparent effectiveness in modulating the ratio of MMPs to the levels of their inhibitors is a plausible mechanism by which these materials may act. Recently, naturally derived, structurally intact ECM materials have been used in a manner similar to these other materials, but unlike other approaches that act via a single mechanism, these materials may affect the status of the chronic wound environment in multiple ways: (i) by providing a collagen-rich material to absorb excess proteases including MMPs; (ii) by providing additional ECM components such as proteoglycans and glycosaminoglycans to bind, protect, and enhance growth factor functions; and (iii) by providing a provisional ECM scaffold into which cells can migrate and remodel. Application of a naturally derived, structurally intact ECM material has been shown to be successful in promoting complete healing in up to 49% of chronic diabetic ulcers and up to 55% of chronic venous ulcers – both statistically significant improvements on standard of care therapies. Because of the potential complications associated with chronic wounds (e.g. infection) and the reduction in quality of life that they cause, it may be logical to try ECM replacement before wounds become truly chronic and nonresponsive. This hypothesis remains to be tested, and may be an important question for future research.

5. Conclusion

The ECM is nature’s template for tissue remodeling. All of the components within the ECM act together to direct all stages of tissue repair and regeneration, and are essential for maintaining homeostasis and directing cellular responses. The dermal ECM supports the epidermis and is responsible for skin regeneration. In instances where the dermal ECM is dysfunctional, appropriate strategies must be employed to provide both structure and signals for healing and permit the multitude of active, ongoing interactions needed to successfully regenerate tissue. Novel approaches that are currently being used include use of purified growth factors to stimulate fibroblast secretion and deposition of a provisional...
matrix, use of purified matrix factors that act as MMP-binding agents to tilt the wound environment toward an anabolic state, and application of a naturally derived, structurally intact substitute ECM material that reproduces the structure and function of the dermis. Use of the appropriate ECM can quickly, easily, and effectively provide the extracellular components of the dermis necessary to direct the healing response and allow for the proliferation of new, healthy tissue, which may promote the healing of chronic wounds. The eventual widespread application of such novel technologies may finally lead to improved healing of wounds that have been refractory to the routine standard of care therapies available today. Perhaps a next step with these therapies is to evaluate their use earlier in wound healing treatment, with the goal of preventing wounds from reaching a truly chronic, nonresponsive state.

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