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Extracellular matrix as an inductive scaffold for functional tissue reconstruction

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Abstract

The extracellular matrix (ECM) is a meshwork of both structural and functional proteins assembled in unique tissue-specific architectures. The ECM both provides the mechanical framework for each tissue and organ and is a substrate for cell signaling. The ECM is highly dynamic, and cells both receive signals from the ECM and contribute to its content and organization. This process of "dynamic reciprocity" is key to tissue development and for homeostasis. Based upon these important functions, ECM-based materials have been used in a wide variety of tissue engineering and regenerative medicine approaches to tissue reconstruction. It has been demonstrated that ECM-based materials, when appropriately prepared, can act as inductive templates for constructive remodeling. Specifically, such materials act as templates for the induction of *de novo* functional, site-appropriate, tissue formation. Herein, the diverse structural and functional roles of the ECM are reviewed to provide a rationale for the use of ECM scaffolds in regenerative medicine. Translational examples of ECM scaffolds in regenerative are provided, and the potential mechanisms by which ECM scaffolds elicit constructive remodeling are discussed. A better understanding of the ability of ECM scaffold materials to define the microenvironment of the injury site will lead to improved clinical outcomes associated with their use.

The extracellular matrix (ECM) is a composite of the secreted products of resident cells in every tissue and organ. The matrix molecules represent a diverse mixture of structural and functional proteins, glycoproteins, and glycosaminoglycans among other molecules that are arranged in an ultrastructure that is unique to each anatomic location. The ECM exists in a state of dynamic reciprocity with the resident cells. That is, the matrix composition and organization change as a function of the metabolic adaptations of the cells in response to shifts in the mechanical properties, pH, oxygen concentration, and other variables in the microenvironment.[1] This constantly adapting structure-function relationship, therefore, represents the ideal scaffold for the resident cell population.

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Although the ECM is a known repository for a variety of growth factors, it also represents a source of bioactive cryptic peptides.[2–4] Fragments of parent molecules such as collagen and fibronectin have been shown to have a diverse array of biologic activities including angiogenesis,[5] anti-angiogenesis,[6] antimicrobial effects, and chemotactic effects, among others. These growth factors and bioactive peptides play important roles in defining the microenvironmental niche within which cells function in both normal homeostasis and in response to injury. The matrix has also been shown to be important in fetal development[7] and also plays a critical role in determination of stem/progenitor cell differentiation fate.[8,9]

The tremendous complexity of the composition and ultrastructure of the ECM is only partially understood. Therefore, it is hardly possible to design and engineer a mimic of this complex structure. However, the extracellular matrix can be harvested from parent tissues through decellularization. Attempts to harvest ECM for utilization as a tissue repair scaffold would ideally remove all potentially immunogenic cell products while minimizing damage to the remaining ECM. Many medical device products composed of allogeneic and xenogeneic ECM currently exist (Table I), but the range of performance varies depending upon source of material, methods of preparation, and clinical application. These naturally occurring materials are generally considered as devices by most regulatory authorities. However, depending upon the formulation, these materials may be regulated as a biologic in the future. Regardless of application or regulatory status, optimal clinical outcomes will be obtained if surgeons understand their potential to help define the microenvironment of an injury site.

The purpose of this article is to briefly review the rationale for the selection of ECM as an “inductive” scaffold for regenerative medicine applications and the preparation of ECM scaffolds for such applications. Three recent translational applications of ECM in regenerative medicine are presented and the potential mechanisms by which ECM scaffolds promote “constructive remodeling” outcomes with a particular focus upon the role of ECM degradation products will then be discussed.

ECM AS A SCAFFOLD FOR REGENERATIVE MEDICINE

ECM-based substrates consisting of individual ECM components or of whole decellularized tissues have been used in a wide range of applications in both preclinical and clinical settings.[10–13] These materials, in their many forms, have been used as coatings for tissue culture plastic and as complex as inductive templates for tissue and organ reconstruction in regenerative medicine, a number of which are discussed in detail below. In more complex applications, ECM-based scaffold materials can promote a process termed “constructive remodeling”—the *de novo* formation of site-appropriate, functional tissue.[13] However, as will be discussed in more detail below, the ability to promote constructive remodeling is critically dependent upon the methods used to prepare the scaffold material. Regardless of the application or the outcome, the overall rationale for the use of ECM is similar. Simply stated, the ECM provides a naturally occurring and highly conserved substrate for cell viability and growth. As applications of ECM scaffolds in tissue engineering and regenerative medicine move toward the reconstruction of increasingly complex tissue structures and even whole organs, it is important to understand the mechanisms by which

ECM scaffolds promote constructive remodeling. While the exact mechanisms responsible for such outcomes are not yet fully understood, they clearly extend beyond the role of the ECM as a mechanical substrate and include a number of processes which occur in development and tissue homeostasis.

The ECM as a mechanical substrate

The extracellular matrix provides a 3-dimensional structural support occupying the space between cells, is a substrate for cell migration, and is a transmitter of biomechanical forces. The physical properties of the ECM, such as rigidity, porosity, insolubility, and topography that derive from composition of the matrix largely determine the mechanical behavior of each individual tissue as well the behavior of the cells which reside within.[14,15] For example, the basement membrane is a dense ECM structure which serves as a selective barrier to migrating cells.[16–18] Migration of cells through this structure requires focal remodeling of the matrix. This ultrastructure is in contrast to the more open and porous structure of the underlying connective tissues which allows greater cellular mobility. Numerous studies have demonstrated the potential effects of ECM ultrastructure and mechanics upon cell behavior, migration, and differentiation.[19–21]

ECM components also provide separation between distinct structures within a single tissue. For example, the basement membrane separates the mucosal lining of the intestine from the submucosal tissue. Each tissue compartment serves a particular purpose within the function of the organ as a whole. For example, the basement membrane provides a substrate for growth and maintenance of the intestinal mucosa and acts as a molecular sieve while the adjacent connective tissues primarily provide mechanical support for the organ. The basement membrane is merely 1 example of a specialized form of the ECM, which demarcates the boundary between mesenchymal and parenchymal tissues. There are numerous other examples of boundaries within tissues. In each case, the transition from 1 tissue type to another is accompanied by a shift in the ECM composition and structure.

It is easy to appreciate the potential role of the ECM as a mechanical substrate for tissue engineering and regenerative medicine applications. Many studies have attempted to recreate these structures using synthetic approaches, and electrospinning is the most notable example of the described methods.[22,23] These approaches are capable of producing interconnected networks of randomly distributed fibers on the approximate scale of the fibrillar components of the ECM. However, no approach can account for the varied distribution of fiber diameters nor can they substitute for the biologically active components of the ECM. While these studies have clearly demonstrated the potential role of topography, structure, and mechanics of the ECM in modulating cellular phenotype and migration, each tissue and organ contains a unique ECM composition, which includes hundreds of components—a target which is, practically speaking, beyond the capability of any engineering approach intended to produce an ECM mimic.

ECM composition

The ECM is a combination of both structural and functional components arranged in a 3-dimensional, tissue specific architecture. These components of ECM include collagens,

glycoproteins, proteoglycans, mucins, elastic fibers, and growth factors,[24] many of which are highly conserved across species.[25–29] As additional signaling pathways and mechanisms for ECM-cell interactions are discovered, it is increasingly difficult to separate the mechanical and functional aspects of these components. This multifunctionality is increasingly evident, as will be discussed in additional detail, when one considers the bioactivity of ECM degradation products during tissue remodeling.[30,31] As one would expect based upon varying tissue functions, the composition of the ECM varies greatly from tissue to tissue, and in some cases within a given tissue. For example, articular cartilage contains large amounts of collagen II and glycosaminoglycans, which are specifically tailored to accommodate high water content and allow resistance to and recovery from compressive deformation. In contrast, tissues such as tendon contain much higher amounts of collagen I and an organization designed to resist tensile loading. These tissues, by comparison, are somewhat dissimilar from organs such as the liver and kidneys, which serve few mechanical functions and are primarily physiologic in nature. Therefore, the extracellular matrix composition in these organs is somewhat dissimilar.

Again, the rationale for the use of a decellularized tissue (ie, ECM)-based scaffold becomes clear. Removal of the cellular components will leave an intact mesh-work of ECM components which are both highly conserved across mammalian species, arranged in a tissue specific architecture, and with a composition with functional relevance to the native tissue. As will be discussed in further detail below, ECM can be harvested from individual tissues and organs based upon the application of interest, though this may not be a requirement for constructive remodeling.

Dynamic reciprocity

In addition to its structural role, the pleiotropic effects of ECM upon tissue resident cells are known to include cell adhesion, migration, proliferation, differentiation and death.[15,32] The mechanisms by which the ECM can promote these processes are diverse. The ECM can transmit mechanical cues, and can provide signaling cues through direct cellular binding to ECM components, and through the sequestration and regulation of access to soluble growth factors and cytokines.[14,32] Thus, the ECM can be considered a highly specialized substrate for both spatial patterning and structural support as well as a functional substrate for cell growth and signaling. The ECM, even in fully developed tissues in adult mammals, is by no means static. Rather, the ECM is constantly subject to turnover through a process aptly termed “dynamic reciprocity.”[1,33,34] That is, the ECM exerts effects upon cellular behavior and phenotype and cells, in turn, these cells produce, degrade and remodel the ECM. This dynamic and reciprocal process is important to homeostasis of all tissues and organs. The ability to rapidly and dynamically remodel the ECM is also an essential component of the wound healing process, allowing the host to effectively repair tissue damage and protect itself from further insult.

The ability of the ECM to dynamically modulate cellular activity while simultaneously being remodeled is particularly evident during tissue development and morphogenesis.[15,17] This process is highly regulated and cell signaling and patterning processes must be deployed promptly, transiently, and in a defined temporospatial sequence. The role of ECM

remodeling in multiple developmental processes including epithelial branch morphogenesis and skeletal development and remodeling have been investigated in depth.[15,17,35,36] Both the production, degradation, and remodeling of the ECM are key events in these processes. In branch morphogenesis, both the basement membrane and other ECM components are in a constant state of dynamic remodeling leading to primary bud formation, branch formation, and branch reiteration. Cells participate through the degradation and remodeling the matrix in an exquisitely regulated process that relies heavily upon expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases concurrently with the production of fibronectin, collagen, and laminin. It is important to note that the role of the ECM in this process goes beyond spatial patterning and provision of a physical substrate. The ECM is known to participate in the transmission of mechanical forces, regulation of cell migration, growth factor release and signaling, and tissue polarization.[15,17,32] The mechanisms which underlie each of these processes have been studied in depth,[14,15,17,32] but are beyond the scope of the present review.

It is clear that ECM is intricately involved in the developmental process and is capable of both being remodeled and concurrently directing the cellular response. This feature is unique among the various biomaterials used in tissue engineering and regenerative medicine. Although synthetic materials can be finely tuned to degrade under specific conditions and at specific rates, the degradation process is not accompanied by the release of a variety of bioactive peptides, as will be discussed below, or by concurrent signaling to cells in the process of tissue remodeling. Disruption of the ability to degrade or blocking of cell-ECM interactions through chemical cross-linking in the production of ECM scaffolds can limit the ability to elicit a constructive remodeling response.[37,38]

Bioactive degradation products

It is clear that the ECM represents a highly dynamic and versatile environment. The ability of the ECM to be degraded and remodeled is key to normal tissue homeostasis, the response to injury, and in developmental processes. All of the components of the ECM are degradable and subject to modification. The mechanisms by which ECM is degraded and remodeled have been reviewed in-depth elsewhere.[15] Briefly, the major families of proteinases, which are responsible for degradation of the ECM are the MMP and metalloproteinase with thrombospondin motif families (ADAMTS).[39] There are 23 identified MMP family members[40] and 19 ADAMTS family members.[41] These proteinases target a wide variety of ECM components. It is clear why these proteinases are indispensable for maintenance, remodeling, and developmental processes.

Recent evidence demonstrates that degradation or modification of the ECM by proteinase degradation can result in the exposure of new recognition sites with potent bioactivity. ECM degradation products include cryptic sites, termed matricryptins or matrikines, which have been shown to influence cell behavior through a number of mechanisms including integrin, toll-like receptor and scavenger receptor signaling.[30,31,42] These cellular interactions result in a diverse array of bioprocesses including angiogenesis, anti-angiogenesis, chemotaxis, adhesion, and antimicrobial effects, among others[5,6,30,31,42–46] Additionally exposure of matricryptic sites can play a role in ECM assembly and modification by influencing ECM

multimerization and assembly of ECM-growth factor complexes.[31] Fibronectin, for example, has many functions including self-assembly, multimerization, and interactions with other ECM components and growth factors including VEGF. Many of these processes have been shown to be controlled or affected by the exposure of matricryptic sites within fibronectin.[47–53] The degradation of fibronectin leads to the formation of peptides that can affect cellular behavior. There are now an increasingly large number of ECM fragments with recognized bioactivity (Table II).

One of the best known examples of a matricryptic peptide in the tissue engineering and regenerative medicine field is the Arg-Gly-Asp peptide present within primarily within fibronectin, but also within collagen, vitronectin, and osteopontin.[31,54–58] The Arg-Gly-Asp peptide has been used to promote cell adhesion to synthetic substrates.[59–63] Thus, an additional advantage of the use of an ECM-based biomaterial is that it acts not only as a reservoir of structural and functional proteins, but also as a degradable substrate with an additional reserve of "hidden" bioactive peptides released during context-dependent degradation processes.

ECM as an instructive niche for stem cells

Another important role of the ECM in tissue homeostasis and tissue development is its ability to act as a niche for stem cell differentiation. The niche represents a specialized local microenvironment, which contributes to the establishment and maintenance of stem cell phenotype and stem cell differentiation. Recent studies provide strong evidence that the niche is composed of both soluble factors and ECM macromolecules which direct cell fate.[21,64–66] The ECM composition and the biomechanical properties of the ECM within the niche have shown to play a role in cell fate.

It is now widely accepted that stem cells are present within all tissues of adult mammals and that such cells are associated with a unique niche. However, the anatomic niche has only been defined in a few select tissues. For example, neural stem cells are known to localize along blood vessels of the subventricular zone of the mouse brain.[67] Within this environment, the cells adhere to laminin on the vascular basement membrane, which has been suggested to be essential for the maintenance of stem cell properties within this niche. In another example, hematopoietic stem cells are found within the endosteum and their niche is rich in osteopontin secreted by osteoblasts.[68,69] Osteopontin has been shown to regulate both adherence and quiescence of the cells within the niche. The degradation and remodeling of the ECM within the stem cell niche is thought to mediate the activation and release of cells from the niche. A number of ECM properties including composition, topography, and biomechanics regulate their subsequent migration and differentiation in tissues following their release.[20,21]

The ability to recruit and differentiate stem cell populations is considered a key aspect of regenerative medicine applications. In some applications, cells are delivered within a scaffold-based material into an injury site; in other applications stem cells are recruited from endogenous sources through a number of mechanisms including growth factors.

DECELLULARIZATION AND FABRICATION METHODS

As is outlined above, the ECM can be thought of as a highly complex, tissue-specific reservoir of structural and functional proteins, which is capable both of being remodeled by resident cells and of directing cellular behavior, phenotype, and survival. ECM is known to play a role in development and in the maintenance of stem cell phenotype, and these processes are exquisitely regulated, potentially involving multiple ECM components. Therefore, it is highly desirable to maintain these components to the highest degree possible. The methods by which ECM is isolated from source tissue and is manufactured as a scaffold for tissue reconstruction applications must be specifically and carefully tailored to the tissue of interest.[70,71] However, the desire to maintain the ultrastructure and ligand landscape of the ECM must be balanced against the need to remove as much of the cellular content as possible to avoid a potentially adverse immune response.[72,73]

Generally, decellularization of source tissue involves a combination of physical, ionic, chemical and enzymatic methods.[70,71,74] Each of these decellularization agents and the manner in which they are applied should be tailored to the characteristics of the source tissue of interest. These characteristics may include thickness, density, and intended clinical application of the matrix material. A full review of the methods commonly employed to achieve tissue decellularization is beyond the scope of this review but has been reviewed in-depth elsewhere.[70,71,74] The effects of inefficient decellularization and/or the use of overly harsh methods to achieve decellularization have also been described.[73,75–77] Briefly, excessive cellular constituents within an ECM scaffold or significant disruption of the native architecture and growth factor content by excessive processing have been shown to promote a proinflammatory process which adversely affects tissue remodeling upon implantation. Similarly, the chemical cross-linking used to mask cellular epitope and/or to increase mechanical properties in many commercially available products significantly disrupts the ligand landscape of the material and prevents the release of cryptic peptides from the matrix material. Such cross-linking limits constructive remodeling and promotes a foreign body and encapsulation type response following implantation.

The physical configuration of the ECM scaffold following decellularization is often dependent upon the 3-dimensional shape of the source tissue and the mechanical processing methods used to remove excess or irrelevant tissue prior to decellularization. The majority of the clinically available ECM products are single or multilaminate sheets (Table I). However, ECM materials can be processed into powders, hydrogels, and 3-dimensional constructs depending on the particular application of interest.[78,79] Additionally, though not a focus of the present review, the decellularization of many whole, intact organs has been performed largely via perfusion of the tissues with decellularization agents.[70,80,81] These decellularized organs maintain much of the native matrix including the vascular and lymphatic networks which are key for subsequent re-population of the decellularized organ with host cells.

TRANSLATIONAL APPLICATIONS OF ECM IN REGENERATIVE MEDICINE

There are more than 30 commercially available ECM-based scaffold materials on the market as of the publication of this review. These materials vary in their source tissue and species, method of decellularization and sterilization, and 3-dimensional form. These materials also vary in their indications for clinical use. Largely, however, ECM-based materials are regulated as surgical mesh materials (ie, devices) and used for the reinforcement of soft tissues where weakness exists. However, it should be noted that as more complex forms and applications of ECM technology are developed, some materials may be regulated as biologics. Below, we review 3 emerging applications of ECM-based scaffold materials in challenging anatomic sites where few clinically effective solutions exist. The mechanism by which ECM scaffolds may be capable of promoting the constructive remodeling outcomes observed in these applications is then reviewed.

Esophageal disease

Barrett's esophagus and esophageal adenocarcinoma represent the sixth leading cause of cancer death worldwide and rates of esophageal cancer are increasing yearly.[82,83] Treatment of high grade dysplasia (HGD) and cancer of the esophageal mucosa present significant challenges because of the high propensity of stricture of the esophagus. Stated differently, the default response of the esophagus to injury is fibrotic scar tissue with associated clinical stricture. Therefore, esophagectomy remains the standard of care for patients with HGD or early stage neoplasia.[84,85] Alternative effective methods for treatment of HGD and early stage cancer in the esophagus without the need for esophagectomy are desirable. Endoscopic resection has emerged as a promising treatment for HGD and early adenocarcinoma.[86–89] However, these methods are limited by disease recurrence, the frequent need for concurrent radiofrequency ablation, and ablation of the involved tissue. Endoscopic resection is also limited by the size of the nodule that can be removed, often requiring piecemeal resection and rendering histologic diagnosis difficult. A recent study demonstrated that en bloc resection of a large, full circumference section of the mucosa was possible.[90] However, this method requires the prevention of stricture subsequent to large-scale disruption of the mucosal surface.

Recent preclinical studies utilizing ECM scaffold materials have demonstrated that ECM is an effective material for reconstruction of the esophagus (Fig 1).[91–94] An approach to reconstruction of the esophagus, which included the placement of xenogeneic ECM derived from porcine urinary bladder showed that full thickness defects that included approximately 40%–50% of the circumference and 5 cm of length could facilitate a constructive, nonstenotic healing response with formation of all layers of the esophageal wall in a preclinical dog model.[94] This neotissue was both functional and innervated.[95] Although similar remodeling was not observed when a full circumference, full thickness resection was performed, restoration of a functional mucosa was observed when the subjacent muscularis externa was left intact.[93]

Based upon these preclinical findings, 5 patients with Barrett's esophagus and multifocal HGD were treated using long segment, circumferential resection of the mucosa and submucosa with subsequent placement of a tube-shaped ECM scaffold material derived

from porcine small intestine over the resected surface.[96] The ECM material was held in place by an expandable stent which was removed between 9 and 14 days post-treatment. Results showed that the ECM scaffold material remodeled rapidly resulting in the formation of a new epithelium and submucosal tissue layer. All patients required transient dilation for mild stricture but did not experience recurrence of the disease or long-term stricture.

Volumetric muscle loss

The incidence of volumetric muscle loss is increasing due to increased survival following removal of extremity tumors as well as increased incidence of battlefield injuries. While skeletal muscle has a capacity for regeneration following injury, it is generally accepted that a 20% or greater loss of muscle volume will result in the deposition of scar tissue, chronic weakness, and loss of function rather than regeneration.[97–106] There are no reproducible clinically effective options for reconstruction following large volumetric muscle loss. Current techniques may include autologous tissue transfer of vascularized or free muscle flaps. While these methods may provide some amount of cosmetic improvement and coverage, there is little restoration of function. Further, these methods are often not amenable to the reconstruction of large volumetric defects.

ECM scaffold-based approaches to reconstruction of skeletal muscle have been shown to promote the formation of functional, innervated, and contractile skeletal muscle in preclinical models of volumetric muscle loss.[107–110] In 1 such study, a scaffold material composed of porcine small intestinal submucosa was fabricated into a 3-dimensional shape and implanted into a canine model of musculotendinous junction repair.[109] In this model, the distal third of the gastrocnemius and musculotendinous junction were completely removed and replaced with the ECM implant. The results of this study showed that the implant promoted the formation of vascularized, functionally innervated skeletal muscle that was nearly indistinguishable from native muscle by 6 months post-implantation. These findings have now been translated into a treatment for human patients who have suffered volumetric muscle loss.[111] For example, placement of an ECM scaffold in a large quadriceps muscle injury in a 22-year-old male where all previous treatments had proven unsuccessful resulted in the restoration of new functional skeletal muscle and a significant increase in isokinetic performance and quality of life.

Temporomandibular joint meniscectomy

Temporomandibular joint disorders (TMJD) encompass a wide spectrum of clinical conditions involving the components of the temporomandibular joint (TMJ).[112–115] The exact etiology of TMJD is largely unknown due to the number of suspected causes and their multifactorial nature. Symptoms of TMJD range from mild pain and clicking of the joint to chronic, intractable pain and limited jaw motion. It is estimated that TMJD affects 10–36 million Americans, 90% of which are women between the ages of 18 and 40. For a percentage of these patients, the only treatment, which will relieve pain and restore motion to the jaw, is to remove the firbocartilaginous TMJ disk.[116–123]

Currently, no alloplastic alternatives exist to safely and effectively replace a degenerative, nonrepairable TMJ disk. Previous attempts to use alloplastic materials have resulted in

unsatisfactory outcomes, including increased joint pathology, among other complications.[124–127] Several autogenous tissues, such as temporalis muscle, auricular cartilage, dermis, and abdominal adipose tissue, have been used as replacement materials, but only short-term success has been reported.[128–134] In addition, the use of these tissues has been associated with donor site morbidity, the eventual formation of scar tissue, decreased range of motion of the mandible, and additional joint pathology. Studies have documented a reduction of joint pain after discectomy without a replacement procedure; however, these patients experience varying degrees of subsequent degenerative changes.[117,118,135] Thus, the identification of a suitable off-the-shelf disk replacement material would obviate the associated donor site morbidity and avoid downstream degenerative changes to the condyle. Ideally, such a material would also act as a template for cellular in-growth, integrate with the surrounding host tissues, and eventually restore the native morphology and function of the TMJ disk.

A number of tissue engineering and regenerative medicine approaches to the replacement of the TMJ disk have been suggested.[136–150] However, to date, these studies have focused primarily on the selection of ideal cell sources, growth factors, and scaffold materials for the engineering of tissues that recapitulate the TMJ disk *in vitro*. Many of these approaches involve long culture times and would be considered difficult to implement both from practical standpoint and from a regulatory standpoint. A recent study demonstrated that a device composed of decellularized porcine urinary bladder matrix alone was capable of providing an effective interpositional material while serving as an inductive template for reconstruction of the TMJ disk *in vivo* (Fig 2).[151] In that study, a device consisting of a powdered UBM “pillow” encapsulated within sheets of the same material was placed as an interpositional graft after discectomy in a canine model. The implanted material was observed to progressively remodel from 3 weeks to 6 months after implantation, and the newly formed host tissues resembled the native fibrocartilage of the TMJ disk in both gross and histologic morphology. A follow-up study of 10 dogs demonstrated that the composition and mechanical properties of the remodeled tissue were also similar to that of the native disk.[152] Of note, the placement of the UBM device resulted in formation not only of fibrocartilage within the bulk of the implant, but also muscular and ligamentous attachments resembling those found at the periphery of native menisci.

MECHANISMS OF CONSTRUCTIVE REMODELING

Each of the examples provided above demonstrate the process of ECM scaffold-mediated constructive remodeling. In each case, an initially acellular scaffold is populated by host cells and undergoes a remodeling process resulting replacement of the material with functional, site-appropriate host tissue. These outcomes are distinctly different from the default mammalian response to tissue injury which commonly results in scar tissue formation with a loss of function. In each case, the scaffold used was derived from small intestine or urinary bladder, despite the application in nonintes-tine or bladder applications. The mechanisms by which such scaffold materials are capable of promoting constructive remodeling in diverse applications is not yet fully elucidated but have been shown to include exposure to mechanical forces, modulation of the host immune response, and degradation of the scaffold material with concurrent new tissue development. In the absence of any of these

key factors, constructive remodeling is not observed and outcomes are undesirable. We review each of these below, with a particular focus upon the role of ECM degradation in the constructive remodeling process. An overview of the constructive remodeling process associated with ECM implantation is shown in Fig 3.

Mechanical forces

Several studies have shown that early site-appropriate mechanical loading facilitates the remodeling of ECM scaffold materials into site-specific tissue.[153,154] In a recent preclinical study, partial cystectomies repaired with an ECM scaffold material were exposed to long-term catheterization and prevention of bladder filling, with an associated lack of cyclic distention and decrease in maximal bladder distention, and were compared with bladders that experienced an early return to normal micturition following ECM scaffold implantation.[153] The presence of physiologic amounts of mechanical loading in the early time frame promoted remodeling of the ECM scaffold material into tissue with a highly differentiated transitional urothelium, vasculature, innervation, and islands of smooth muscle cells. Delayed return of normal mechanical loading was insufficient to overcome the lack of early mechanical signals and resulted in degradation of the ECM scaffold material, dense scar tissue deposition, and absence of constructive remodeling. Similar results have also been observed in a study of Achilles tendon repair in rabbits with and without post-surgical immobilization.[154]

The mechanisms by which ECM scaffolds promote site-specific remodeling in the presence of site appropriate mechanical loading have only been partially elucidated. Static and cyclic stretching of cells seeded on an ECM scaffold *in vitro* modulated the collagen expression by the cells, enhanced the cell and collagen alignment, and improved the mechanical behavior of the scaffold.[155–159] In addition, during *in vivo* remodeling, it is thought that the progenitor cells recruited to the site of ECM remodeling will differentiate into site appropriate cells in the presence of local mechanical cues.[160–165] Several *in vitro* studies have shown that mechanical loading can induce progenitor cells to differentiate into fibroblasts, smooth muscle cells, and osteoblasts.[166–168] Lastly, and as described above, mechanical loading of the ECM may lead to the exposure of cryptic sites and thereby initiate ECM multimerization and cell responses that lead to constructive remodeling.

Modulation of the host response

Materials derived from mammaliantissue sources elicit a distinctly different host response than those composed of synthetic materials due to their unique surface topologies and ligand landscapes. Further, naturally derived materials likely experience adsorption of a different repertoire of molecules than do synthetic materials and possess inherent surface functionality.[169] The constructive remodeling response elicited by ECM-based scaffold materials has been shown to be dependent upon the ability to elicit as well as to modulate the host response.[38,72,73,170–173] In general, the host response to a nonchemical cross-linked ECM scaffold material consists of neutrophils at early time points (<48 hours) following implantation changing to a prominent macrophage response by 72 hours postimplantation.[37] The macrophage response to the scaffold material persists through much of the early remodeling process and as long as several months postimplantation depending on the

application. This intense and long-term macrophage response to a biomaterial implant is conventionally associated with negative implications including chronic inflammation and encapsulation or scarring.[174] However, the presence of these cells, and macrophages in particular, are essential for the promotion of a constructive remodeling response.[38,73,172,173] A recent study that investigated the response to ECM scaffolds in animals treated with clodronate to remove circulating phagocytes showed that the ECM scaffold was not degraded or remodeled, suggesting a prominent role for circulating mononuclear cells in the constructive remodeling process.[38]

Subsequent investigations have revealed that ECM scaffolds both promote the host response and modulate the phenotype of the cells which participate.[72,73,170–173,175] Briefly, scaffold materials composed of extracellular matrix (ECM) have been shown to promote a switch from a predominantly M1 macrophage (pro-inflammatory, cytotoxic) population immediately following implantation to a population enriched in M2 macrophages (anti-inflammatory and prohealing) by 7–14 days postimplantation.[73,172,173] In addition to eliciting and modulating the host macrophage response, ECM scaffolds have been shown to consistently evoke a Th2 type immune response,[170,171] which is generally associated with transplant acceptance.[176–178] The mechanisms by which ECM-based scaffold materials promote the M1 to M2 and Th1 to Th2 transition remain unknown. However, the phenotypic profile of the immune cells, which respond to these scaffold materials at early time points, has now been shown to be a strong statistical predictor of the downstream outcome associated with their implantation.[173] For example, modification of such scaffold materials with chemical cross-linking agents which delay or prevent macrophage-mediated degradation inhibits the formation of the beneficial M2 response, promotes the M1 response, and results in downstream scar tissue formation.[172,173] These results suggest that interactions of host cells with intact ligands on the surface of the material, or their degradation products, may be responsible for the observed phenomena.

ECM scaffold degradation

ECM scaffolds are rapidly degraded *in vivo*. A recent study showed that 10 layer ^{14}C-labeled ECM scaffolds were 60% degraded at 30 days postimplantation and 100% at 90 days post-surgery in a model of canine Achilles tendon repair.[179,180] During this period, the scaffold was populated and degraded by host cells and resulted in the formation of site-specific functional host tissue. The major mechanism of excretion of the degraded scaffold was found to be via hematogenous circulation and elimination by the kidneys, urine, and exhaled CO_2. The mechanisms of *in vivo* degradation of ECM scaffolds are complex and include both cellular and enzymatic pathways. The process is mediated by inflammatory cells, such as macrophages, which produce oxidants as well as proteolytic enzymes that aid in the degradation of the matrix. Another study utilizing ^{14}C-labeled ECM scaffolds showed that peripheral blood monocytes are required for the early and rapid degradation of both ECM scaffolds, and that cross-linked ECM scaffolds are resistant to macrophage-mediated degradation.[38]

ECM scaffolds have also been degraded *in vitro* by chemical and physical methods. Recent findings suggest that the degradation products of ECM scaffolds are

bioactive.[43,44,46,160–162,181–184] Studies have shown antimicrobial activity associated with the degradation products of ECM scaffolds[43,185]; however, in the absence of degradation, antimicrobial activity was not seen, suggesting that some of the bioactive properties of the ECM are derived from its degradation products, rather than from whole molecules present in the ECM.[186] Degradation products of ECM scaffolds have also been shown to be chemoattractants for progenitor and non-progenitor cell populations.[44–46,160,161,181] An ECM scaffold that cannot degrade (ie, is chemically cross-linked) may not release bioactive degradation products, including those bioactive molecules that may be responsible for modulating the host response toward constructive remodeling. Furthermore, surface characterization studies have demonstrated that chemical cross-linking alters the ligand landscape of the material potentially altering ligand–receptor interactions important in determining cell–scaffold interactions.[75,76]

One of the biologic effects of ECM scaffold degradation is the recruitment of host stem and progenitor cells to the site of degradation. A study of ECM scaffold remodeling in a mouse Achilles tendon injury model examined the ability of ECM scaffolds and autograft tissue to recruit bone marrow-derived cells.[164] Bone marrow-derived cells were observed in the sites of remodeling associated with both ECM scaffolds and autograft control tissue, among what appeared to be predominantly mononuclear cells at early time points (1 and 2 weeks) postsurgery. Both scaffold types remodeled into tissue resembling the native Achilles tendon; however, by 16 weeks, the presence of bone marrow-derived cells was observed only in the ECM treated group. Another study, also utilizing a model of mouse Achilles tendon injury, examined the ability of ECM scaffold explants to cause the chemotaxis of progenitor cells after 3, 7, and 14 days of *in vivo* remodeling.[162] The results of the study showed greater migration of progenitor cells towards tendons repaired with ECM scaffolds, compared with tendons repaired with autologous tissue and uninjured normal contralateral tendon.

While these studies have demonstrated the effects of ECM scaffold implantation and subsequent degradation *in vivo*, additional studies have investigated the delivery of ECM scaffold degradation products in place of whole ECM scaffolds.[160,181–183] The injection of peptides derived by pepsin degradation of a porcine small intestinal ECM scaffold resulted in the recruitment of multipotent progenitor cells in a model of mouse digit amputation.[160,181] These cells expressed a number of markers of multipotency including Sox2, Sca1, and Rex1. When isolated from the site of injury, these cells were shown to be able to differentiate along mesoderermal and neuroectodermal lineages.[160,181] A proteomics approach to identifying the peptides responsible showed that a single α subunit of the collagen III molecule could promote the chemotaxis of multiple progenitor cells *in vitro* and was able to recruit the Sox2, Sca1 positive progenitor cells *in vivo*.[160] Additional studies demonstrated that this peptide was able to promote the osteogenic differentiation of human perivascular cells *in vitro*.[182]

Undesirable responses to ECM scaffold materials

Although the primary focus of this review is a description of the rationale for the use of extracellular matrix as a bioscaffold and the known mechanisms by which constructive

remodeling is facilitated, it must be noted that variations in outcomes have also been reported.[37,173,186,187] As discussed above, although the mechanisms of constructive remodeling are only partially understood, there are certain aspects of preparation and use of ECM bioscaffolds that may account for variable results.

First, the ability of an ECM scaffold material to act as a highly conserved inductive template for constructive tissue remodeling is dependent upon thorough decdellularization.[71,74] As is logical, a scaffold that has not been fully decellularized will contain both DNA and cellular epitopes such as the α-gal epitope. These molecules are well characterized for their ability to promote an inflammatory or rejection type response.[189–191] ECM scaffold materials containing large amounts of cellular content following decellularization have been demonstrated to promote a more inflammatory, M1 type, response and result in scar tissue deposition rather than constructive remodeling.[73] Although the degree of inflammatory response appears to be linked to the quantity of cellular content, an exact threshold beyond which the constructive remodeling response is affected is unknown.[77]

Second, the use of chemical cross-linking reagents, such as carbodiimide or glutaraldehyde, has been shown to alter the ligand landscape of the material and prevent ECM scaffold degradation.[37,38,75] The conversion to a nondegradable material is clearly associated with a foreign body reaction and downstream encapsulation rather than constructive remodeling.[37,173] Fig 4 demonstrates the host remodeling response to 3 different ECM scaffold materials following implantation into a rodent partial thickness abdominal wall defect model.[173] Similarly, harsh processing methods that disrupt the ECM and remove selected growth factors and other components critical to the account for poor clinical outcomes.[75]

Lastly, it appears to be essential that materials are not only prepared in such a manner as to avoid an undesirable response, but also are used in an application appropriate manner. Each clinical application will pose specific challenges, which must be considered in developing an approach to the use of an ECM scaffold material. Three such examples are provided above. Of particular importance is the exposure of the matrix material to the local tissue microenvironment as well as local mechanical forces. In the absence of the application of mechanical stimuli, remodeling of ECM scaffold materials have largely resulted in degradation of the scaffold material without remodeling.[154,155]

CONCLUSIONS

The ECM is a highly complex and highly dynamic structural and functional environment which is both dependent upon and a critical determinant of cell phenotype and behavior. For these reasons, ECM represents an ideal biomaterial for tissue reconstruction. ECM is commonly used as a simple surgical mesh to bridge and reinforce tissues but also has the potential to act as an inductive template for constructive remodeling. Successful use of ECM scaffolds as inductive templates in increasingly complex and challenging applications requires an understanding of the potential for ECM to define or modulate the injury microenvironment in an application specific manner. Key factors leading to constructive remodeling outcomes include the use of appropriate processing in scaffold preparation,

application of site-appropriate mechanical forces, modulation of the host immune response, and degradation of the scaffold with release of bioactive cryptic peptides. Disruption of any of these processes may affect downstream outcomes. A better understanding of these factors will logically lead to improved clinical outcomes associated with the use of ECM-based scaffold materials.

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Abbreviations

ADAMTS	metalloproteinase with thrombospondin motif families
CO_2	carbon dioxide
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EMR	endomucosal resection
HGD	high grade dysplasia
MMP	matrix metalloproteinase
SIS	small intestinal mucosa
TMJ	temporomandibular joint
TMJD	Temporomandibular joint disorder
UBM	urinary bladder matrix
VEGF	vascular endothelial growth factor

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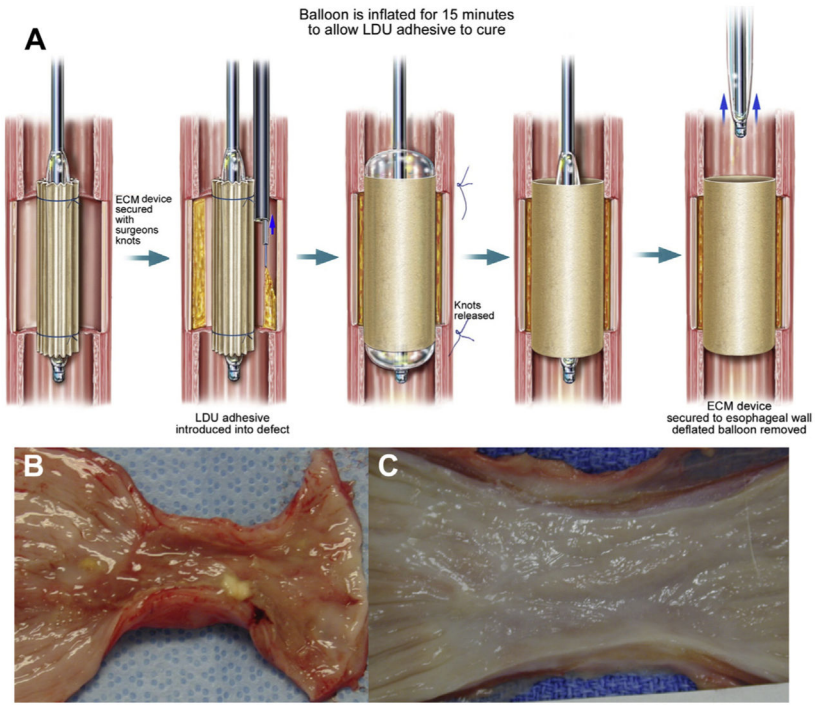

Fig 1.

Use of extracellular matrix (ECM) scaffold material for reconstruction post endomucosal resection (EMR) in a canine model. Schematic of the surgical deployment of ECM device with an achalasia balloon and delivery of the surgical adhesive (**A**). EMR is performed and tubular ECM scaffold is deployed using achalasia balloon. A lysine-derive urethane surgical adhesive (TissuGlu, Cohera Medical) was used to secure ECM scaffold in place. Balloon was inflated and maintained for 15 minutes to allow adherence of the ECM scaffold to the esophagus prior to removal. Gross view of the remodeling EMR areas at 2 months after surgery (**B, C**). The control (**B**) shows pronounced stricture with reductions in the circumference and the length of the injury site. In contrast, the EMR site treated with ECM shows a smooth mucosal surface and limited circumferential and longitudinal reduction (**C**). Reproduced from[91] with permission from Elsevier.

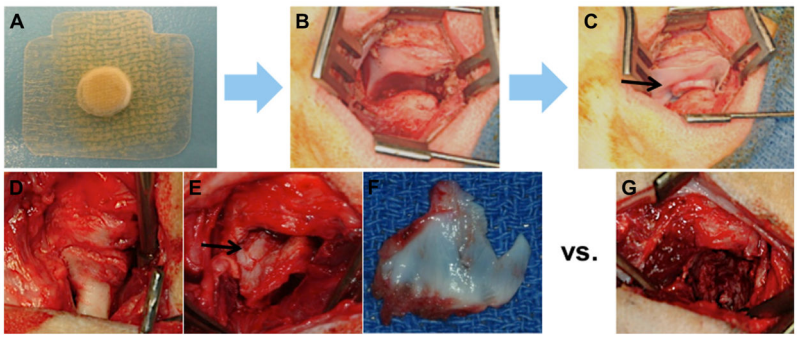

Fig 2.

Use of extracellular matrix (ECM) as a template for reconstruction of the temporomandibular joint (TMJ) disk. An ECM scaffold (**A**) composed of porcine urinary bladder matrix was placed into the TMJ space (**C,** arrow indicates implant) following removal of the TMJ disk (**B**). Results demonstrated that the material was rapidly remodeled, acting as an interpositional material between the condyle and fossa (**D**). At explant the remodeled disk (**E,** arrow indicates explanted material) highly resembled native tissue (**F**). This tissue was also histologically and biomechanically similar to native tissues (not shown) and was shown to include integration with the lateral muscular and ligamentous attachments. This was in direct contrast to the contralateral side (**G**), which was left empty and resulted in the deposition of a small amount of granulation tissue and significant degenerative changes to the joint.

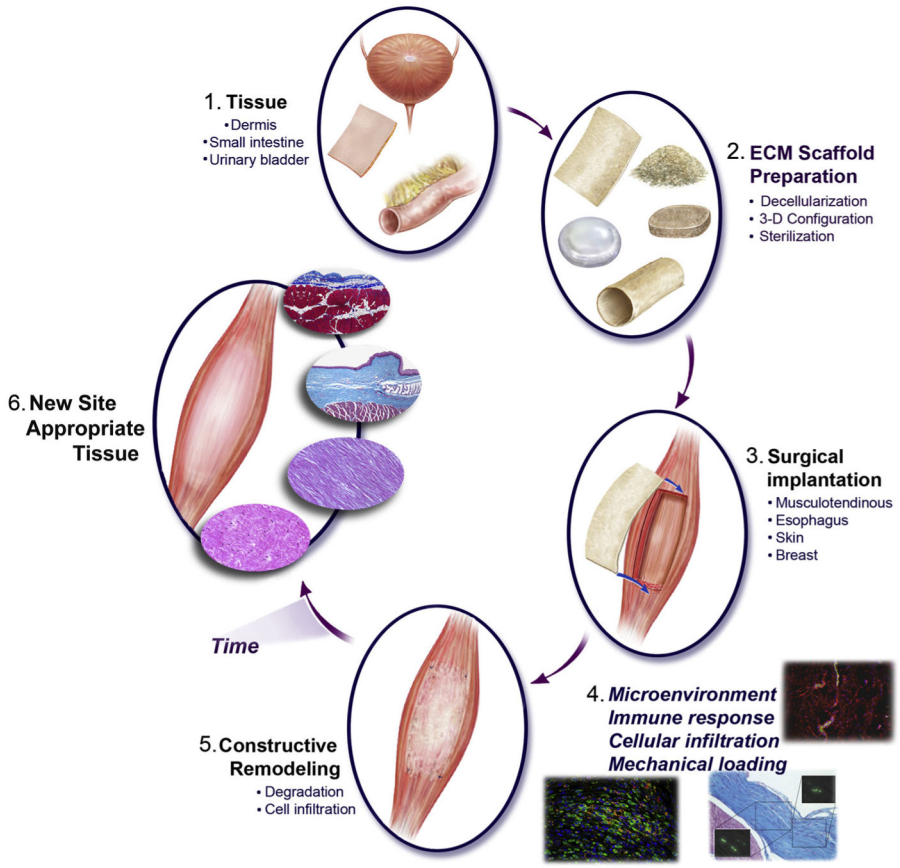

Fig 3.

Overview of the constructive remodeling process associated with extracellular matrix (ECM) scaffold implantation. Scaffold materials obtained from tissue decellularization are processed into application specific formulations. Upon implantation, the material provides a microenvironment for ingrowth of cells and mechanotransduction. The material is degraded rapidly resulting in modulation of the innate and adaptive immune response and recruitment of progenitor cells. Over time, these processes result in constructive remodeling–the formation of new, site appropriate, functional host tissues. Reproduced from[13] with permission from Elsevier.

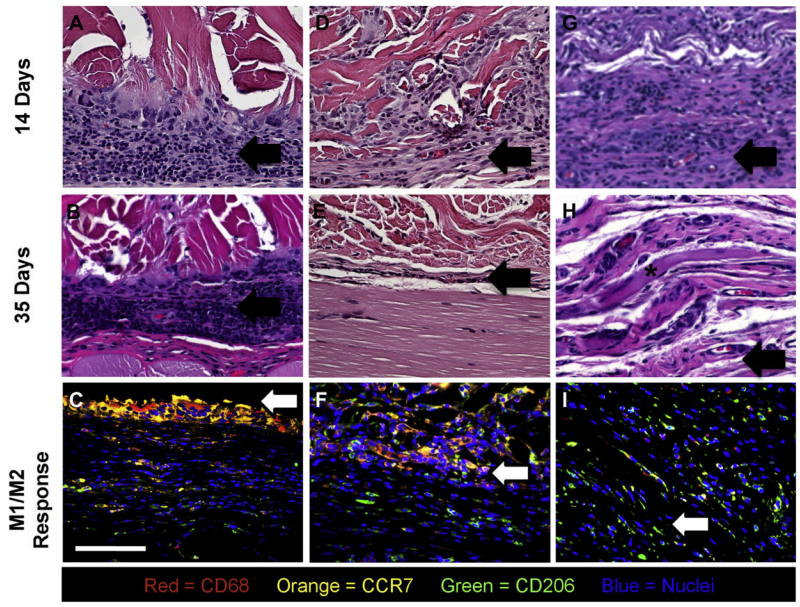

Fig 4.

Outcomes following 14- and 35-day implantation of 3 different extracellular matrix (ECM) based biomaterials in a rodent abdominal wall defect model. Results demonstrated that those scaffold materials, which were cross-linked (Collamend, Bard) were associated with a foreign body type response and an M1 type macrophage response (**A–C**). Scaffold materials, which were not cross-linked but degraded slowly (InteXen, American Medical Systems), were associated with a dense mononuclear cell response early, with reduction of the inflammatory response at later times (**D–E**). These materials were associated with a mixed M1/M2 macrophage phenotype (**F**). Scaffold materials, which were noncross-linked and degraded rapidly (MatriStem, ACell), were associated with a more polarized M2 response and showed signs of early constructive remodeling (**G–I**). Hematoxylin and eosin (**A, B, D, E, G, H**) and immunofluorescent labeling of macrophages (14 days) are shown. Image magnification = ×40. Scale bar = 100 μm. CD68 (pan-macrophage) = red, CCR7 (M1) = orange, CD206 (M2) = green, DRAQ5 (nuclei) = blue. Arrow = interface between scaffold and underlying native tissue. Asterisk = new skeletal muscle bundle formation. Reproduced from[173] with permission from Elsevier.

Table I

Partial list of commercially available scaffold materials composed of ECM

Product	Company	Material		Form	Use
AlloDerm	LifeCell	Human skin	Cross-linked	Dry sheet	Abdominal wall, breast, ENT/head. and neck reconstruction, grafting
AlloPatch	Musculoskeletal Transplant Foundation	Human fascia lata	Cross-linked	Dry sheet	Orthopedic applications
Axis dermis	Mentor	Human dermis	Natural	Dry sheet	Pelvic organ prolapse
CollaMend	Bard	Porcine dermis	Cross-linked	Dry sheet	Soft tissue repair
CuffPatch	Arthrotek	Porcine SIS	Cross-linked	Hydrated sheet	Reinforcement of soft tissues
DurADAPT	Pegasus Biologicals	Horse pericardium	Cross-linked		Repair dura matter after craniotomy
Dura-Guard	Synovis Surgical	Bovine pericardium		Hydrated sheet	Spinal and cranial repair
Durasis	Cook SIS	Porcine SIS	Natural	Dry sheet	Repair dura matter
Durepair	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet	Repair of cranial or spinal dura
FasLata	Bard	Cadaveric fascia lata	Natural	Dry sheet	Soft tissue repair
Graft Jacket	Wright Medical Tech	Human skin	Cross-linked	Dry sheet	Foot ulcers
MatriStem	ACell, Inc	Porcine urinary bladder	Natural	Dry sheet, powder	Soft tissue repair and reinforcement, burns, gynecologic
Oasis	Healthpoint	Porcine SIS	Natural	Dry sheet	Partial and full thickness wounds; superficial and second degree burns
OrthADAPT	Pegasus Biologicals	Horse pericardium	Cross-linked	Dry sheet	Reinforcement, repair and reconstruction of soft tissue in orthopedics
Pelvicol	Bard	Porcine dermis	Cross-linked	Hydrated sheet	Soft tissue repair
Peri-Guard	Synovis Surgical	Bovine pericardium			Pericardial and soft tissue repair
Permacol	Tissue Science Laboratories	Porcine skin	Cross-linked	Hydrated sheet	Soft connective tissue repair
PriMatrix	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet	Wound management
Restore	DePuy	Porcine SIS	Natural	Sheet	Reinforcement of soft tissues
Stratasis	Cook SIS	Porcine SIS	Natural	Dry sheet	Treatment of urinary incontinence
SurgiMend	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet	Surgical repair of damaged or ruptured soft tissue membranes
Surgisis	Cook SIS	Porcine SIS	Natural	Dry sheet	Soft tissue repair and reinforcement
Suspend	Mentor	Human fascia lata	Natural	Dry sheet	Urethral sling
TissueMend	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet	Surgical repair and reinforcement of soft tissue in rotator cuff
Vascu-Guard	Synovis Surgical	Bovine pericardium			Reconstruction of blood vessels in neck, legs, and arms
Veritas	Synovis Surgical	Bovine pericardium		Hydrated sheet	Soft tissue repair
Xelma	Molnlycke	ECM protein, PGA, water		Gel	Venous leg ulcers

Product	Company	Material		Form	Use
Xenform	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet	Repair of colon, rectal, urethral, and vaginal prolapse, pelvic reconstruction, urethral sling
Zimmer Collagen Patch	Tissue Science Laboratories	Procine dermis	Cross-linked	Dry sheet	Orthopedic applications

Abbreviations: ECM, extracellular matrix; ENT, ear nose throat; SIS, small intestinal submucosa.

Table II

Selected examples of “cryptic” peptides within the ECM

Fragment	Parent molecule	Activity
Endostatin	Collagen XVIII	Inhibits angiogenesis
Angiostatin	Plasminogen	Inhibits angiogenesis
Anastellin Fragment III1c	Fibronectin	Inhibits angiogenesis
Canstatin	Collagen IV	Apoptosis, inhibits chemotaxis and proliferation
Restin	Collagen XV	Inhibits migration
Tumstatin	Collagen IV	Inhibits angiogenesis, promotes apoptosis, anti-tumor activity
ABT-510	Thrombospondin-1	Inhibits angiogenesis
RGD	Fibronectin	Promotes adhesion
Hyaluronic acid fragments	Hyaluronic acid	Promotes angiogenesis, increased MMP production
VAVPG sites	Elastin	Promotes chemotaxis, increased MMP expression
C-terminal telopeptide of Collagen III	Collagen III	Promotes chemotaxis, osteogenesis

Abbreviations: ECM, extracellular matrix; MMP, metalloproteinase; RGD, Arg-Gly-Asp.