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The extracellular matrix as a scaffold for tissue reconstruction

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The extracellular matrix (ECM) consists of a complex mixture of structural and functional proteins and serves an important role in tissue and organ morphogenesis, maintenance of cell and tissue structure and function, and in the host response to injury. Xenogeneic and allogeneic ECM has been used as a bioscaffold for the reconstruction of many different tissue types in both pre-clinical and human clinical studies. Common features of ECM-associated tissue remodeling include extensive angiogenesis, recruitment of circulating progenitor cells, rapid scaffold degradation and constructive remodeling of damaged or missing tissues. The ECM-induced remodeling response is a distinctly different phenomenon from that of scar tissue formation.

Key words: extracellular matrix / small intestinal submucosa (SIS) / bioscaffolds / tissue engineering / urinary bladder submucosa (UBS)

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Introduction

The extracellular matrix (ECM) is a complex mixture of structural and functional proteins, glycoproteins, and proteoglycans arranged in a unique, tissue specific three-dimensional ultrastructure. These proteins serve many functions including the provision of structural support and tensile strength, attachment sites for cell surface receptors, and as a reservoir for signaling factors that modulate such diverse host processes as angiogenesis and vasculogenesis, cell migration, cell proliferation and orientation, inflammation, immune responsiveness and wound healing. Stated differently, the ECM is a vital, dynamic and indispensable component of all tissues and organs and is nature's natural scaffold for tissue and organ morphogenesis, maintenance, and reconstruction following injury.

Until the mid 1960s the cell and its intracellular contents, rather than the ECM, was the focus of attention for most cell biologists, molecular biologists, developmental biologists and other life scientists. However, with the discovery that the ECM plays a role in the conversion of myoblasts to myotubes[1] and that structural proteins such as collagen and glyocosaminoglycans are important in salivary gland morphogenesis[2] it became obvious that the ECM is much more than a passive bystander in the events of tissue and organ development and in the host response to injury. The discovery of cytokines, growth factors and potent functional proteins that reside within the ECM characterized it as a virtual information highway between cells. The concept of 'dynamic reciprocity' between the ECM and intracellular cytoskeletal and nuclear elements has become widely accepted.[3–5] The translation of this phenomenon to therapeutic use of the ECM as a scaffold for tissue engineering applications has recently been attempted.

The ECM is not static. The composition and structure of the ECM are a function of location within tissues and organs, age of the host, and the physiologic requirements of the particular tissue.[6–8] Organs rich in parenchymal cells, such as the kidney, have relatively little ECM. In contrast, tissues such as tendons and ligaments with primarily structural functions have large amounts of ECM relative to their cellular component. Submucosal and dermal forms of ECM reside subjacent to structures that are rich in epithelial cells such as the mucosa of the small intestine and epidermis of the skin, respectively. These forms of ECM tend to be well vascularized, contain primarily type I collagen and site specific glycosaminoglycans, and a wide variety of growth factors including basic fibroblast growth factor (bFGF), vascular endothelial cell growth factor (VEGF), and epidermal growth factor (EGF).

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1084–9521 / 02 / $– see front matter

RegenX Science Exhibit 1006
Page 1

In contrast, the ECM of the basement membrane that resides immediately beneath epithelial cells such as the urothelial cells of the urinary bladder, the endothelial cells of blood vessels and the hepatocytes of the liver is comprised of distinctly different collections of proteins including laminin, collagen type IV and entactin. All ECMs share the common features of providing structural support and serving as a reservoir of growth factors and cytokines. The ECMs present these factors efficiently to resident cell surface receptors, protect the growth factors from degradation, and modulate their synthesis.[9–12] In this manner, the ECM affects local concentrations and biologic activity of growth factors and cytokines and makes the ECM an ideal scaffold for tissue repair and reconstruction.

Components of the extracellular matrix that support tissue reconstruction

Scaffolds for tissue reconstruction and replacement must have both appropriate structural and functional properties. However, the distinction between structural and functional proteins is becoming increasingly blurred. Domain peptides of proteins originally thought to have purely structural properties have been identified and found to have significant and potent modulating effects upon cell behavior. For example, the RGD peptide that promotes adhesion of numerous cell types was first identified in the fibronectin molecule;[13,14] a molecule originally described for its structural properties. Several other peptides have since been identified in 'dual function' proteins including laminin, entactin, fibrinogen, types I and VI collagen, and vitronectin, among others.[15] If one considers the ECM to be a degradable bioscaffold for implantation, both the structural and the functional components are transient due to the rapid rate of degradation of ECM scaffolds *in vivo*.[16,17] It is reasonable therefore, to consider ECM scaffolds as temporary controlled release vehicles for naturally occurring growth factors.

Collagen is the most abundant protein within the ECM. More that 20 distinct types of collagen have been identified. The primary structural collagen in mammalian tissues is type I collagen. This protein has been well characterized and is ubiquitous across the animal and plant kingdom.[18] Collagen has maintained a highly conserved amino acid sequence through the course of evolution. For this reason allogeneic and xenogeneic sources of type I collagen have been long recognized as a useful scaffold for tissue repair with low antigenic potential. Bovine type I collagen is perhaps the most widely used biologic scaffold for therapeutic applications due to its abundant source and its history of successful use.

Collagen types other than type I exist in naturally occurring ECM, albeit in much lower quantities. These alternative collagen types each provide distinct mechanical and physical properties to the ECM and contribute to the utility of the intact ECM (as opposed to isolated components of the ECM) as a scaffold for tissue repair. By way of example, type IV collagen is present within the basement membrane of all vascular structures and is an important ligand for endothelial cells. Type VII collagen is an important component of the anchoring fibrils of keratinocytes to the underlying basement membrane of the epidermis. Type VI collagen functions as a 'connector' of functional proteins and glycosaminoglycans to larger structural proteins such as type I collagen, helping to provide a gel like consistency to the ECM. Type III collagen exists within selected submucosal ECMs, such as the submucosal ECM of the urinary bladder, where less rigid structure is demanded for appropriate function. This diversity of collagens within a single scaffold material is partially responsible for the distinctive biologic activity of ECM scaffolds and is exemplary of the difficulty in recreating such a composite *in vitro*. In summary, the ECM is a rich source of numerous types of collagen and the relative concentrations and orientation of these collagens to each other provide an ideal environment for cell growth both *in vitro* and *in vivo*.

Fibronectin, one of the 'dual function' proteins mentioned earlier, represents an important component of ECM and is second only to collagen in quantity within the ECM. Fibronectin exists both in soluble and tissue isoforms and possesses many desirable properties of a tissue repair scaffold including ligands for adhesion of many cell types.[19,20] Fibronectin exists within the ECM of both submucosal structures and basement membrane structures.[21,22] The fibronectin component of the ECM scaffold derived from the porcine small intestinal submucosa (SIS) and urinary bladder submucosa (UBS) has been shown to be partially responsible for the adhesion of endothelial cells during *in vivo* constructive remodeling of this xenogeneic bioscaffold.[23] The cell friendly characteristics of this protein have made it an attractive ligand for use as a coating protein upon various synthetic scaffold materials to promote host biocompatibility.

Laminin is a complex adhesion protein found in the ECM; especially within basement membrane ECMs.[21] This trimeric cross-linked polypeptide exists

RegenX Science Exhibit 1006
Page 2

in numerous forms dependent upon the particular mixture of peptide chains (e.g. $\alpha 1$, $\beta 1$, $\gamma 1$).[24,25] The prominent role of laminin in the formation and maintenance of vascular structures is particularly noteworthy when considering the ECM as a scaffold for tissue repair.[26,27] Vascularization of scaffolds for tissue repair is one of the rate limiting steps in the field of tissue engineering and proteins such as laminin are receiving close attention as an important component of endothelial cell friendly scaffold materials.

Glycosaminoglycans (GAGs) are important components of ECM and play important roles in binding of growth factors and cytokines, water retention, and the gel properties of the ECM. The heparin binding properties of numerous cell surface receptors and of many growth factors (e.g. FGF family, VEGF) make the heparin-rich GAGs extremely desirable components of scaffolds for tissue repair. The GAG components of the SIS-ECM scaffold consist of the naturally occurring mixture of chondroitin sulfates A and B, heparin, heparan sulfate, and hyaluronic acid.[28] Hyaluronic acid has been extensively investigated as a scaffold for dermal reconstruction.

The characteristic of the intact ECM that distinguishes it from other scaffold materials is its diversity of structural proteins and associated bioactive molecules and their unique spatial distribution. Although cytokines and growth factors are present within ECM in vanishingly small quantities, they act as potent modulators of cell behavior. The list of growth factors is extensive and includes VEGF, bFGF, EGF, transforming growth factor beta (TGF-beta), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) and platelet derived growth factor (PDGF), among others. These factors tend to exist in multiple isoforms, each with its specific biologic activity. Purified forms of growth factors and biologic peptides have been investigated in recent years as therapeutic means of encouraging blood vessel formation (VEGF), inhibiting blood vessel formation (angiostatin), stimulating deposition of granulation tissue (PDGF), and encouraging epithelialization of wounds (KGF). However, this therapeutic approach has struggled with determination of optimal dose, sustained and localized release at the desired site, and the inability to turn the factor 'on' and 'off' as needed during the course of tissue repair. An advantage of utilizing the ECM in its native state as a scaffold for tissue repair is the presence of all of the attendant growth factors (and their inhibitors) in the relative amounts that exist in nature and perhaps most importantly, in their native three-dimensional ultrastructure.

Sources of extracellular matrix and host response

ECM exists in all tissues and organs but can be harvested for use as a therapeutic scaffold from relatively few sources. The dermis of the skin, submucosa of the small intestine and urinary bladder, pericardium, basement membrane and stroma of the decellularized liver, and the decellularized Achilles tendon are all potential sources of ECM (Figure 1). The host response

Figure 1. ECM harvested from porcine urinary bladder. This thin (60 uM) sheet of ECM is entirely free of any cellular component, has a multidirectional tensile strength of approximately 40 N, and has not been chemically cross linked or modified from its native structure.

RegenX Science Exhibit 1006
Page 3

to ECM scaffolds is largely dependent upon the methods used to process the material.

Chemical and non-chemical means of cross linking ECM proteins have been utilized extensively in an effort to modify the physical, mechanical, or immunogenic properties of naturally derived scaffolds.[29] Chemical cross-linking methods generally involve aldehyde or carbodiimide. Photochemical means of protein cross-linking have also been investigated.[30] Although these cross-linking methods can result in certain desirable mechanical or physical properties, the end result is the modification of a biologically interactive scaffold material into a relatively inert bioscaffold material. The functional tissue engineering result of this scaffold modification is typically a fibrous connective tissue response by the host to be scaffold material, complete inhibition of scaffold degradation, and inhibition of cellular infiltration into the scaffold. Although there may be clinical uses for such modified biomaterials, these properties are counter intuitive to many current approaches in the field of tissue engineering: especially those approaches in which cells are seeded upon scaffolds prior to or at the time of implantation.

In contrast, ECM scaffolds that remain essentially unchanged from native ECM elicit a host response that promotes cell infiltration and rapid scaffold degradation, deposition of host derived neomatrix, and eventually constructive tissue remodeling with a minimum of scar tissue. Therefore, the native ECM represents a fundamentally different scaffold material than ECM that has been chemically or otherwise modified.

Extracellular matrix scaffolds for tissue repair

There is abundant literature on the use of modified ECM scaffolds, especially chemically cross-linked biologic scaffolds, for tissue repair and replacement. Porcine heart valves, decellularized and cross-linked human dermis (Alloderm™), and chemically cross-linked purified bovine type I collagen (Contigen™) are examples of such products currently available for use in humans. Similarly modified ECM scaffolds have been used for the reconstitution of the cornea, skin, cartilage and bones, and nerve regeneration, among others.[30–33]

Porcine derived ECM scaffolds that have not been modified, except for the decellurization process and terminal sterilization, have been successfully used for the repair of numerous body tissues including musculotendinous structures,[34–36] lower urinary tract reconstruction,[37–39] dura mater replacement,[40,41] vascular reconstruction,[42–44] and the repair of full and partial thickness skin wounds.[45] The remodeling process in all of these applications has been remarkably similar. Immediately following implantation *in vivo*, there is an intense cellular infiltrate consisting of equal numbers of polymorphonuclear leukocytes and mononuclear cells. By 72 h post implantation, the infiltrate is almost entirely mononuclear cell in appearance with early evidence for neovascularization. Between day 3 and 14, the number of mononuclear cells increases, vascularization becomes intense, and there is a progressive degradation of the xenogeneic scaffold with associated deposition of host derived neomatrix. Following day 14, the mononuclear cell infiltrate diminishes and there is the appearance of site specific parenchymal cells that orient along lines of stress. These parenchymal cells consist of fibroblasts, smooth muscle cells, skeletal muscle cells, and epithelial cells depending upon the site in which the scaffold has been placed. It has been shown that circulating, marrow derived progenitor cells participate in this remodeling process when ECM scaffolds are used.[46] The role of environmental stressors, such as mechanical loading, have also been shown to be important in the remodeling of ECM scaffolds.[47] Of note, there is an absence of tissue necrosis and scar tissue formation during the remodeling of these xenogeneic ECM scaffolds.

Porcine derived ECM scaffolds derived from the small intestinal submucosa and the urinary bladder submucosa have been used to replace segmental defects in the esophagus of a dog model.[48] The esophagus is noteworthy for its default mechanism of scar tissue formation following injury. Remodeling of the xenogeneic ECM scaffolds showed site specific deposition and organization of skeletal muscle, intact squamous epithelial lining, and normal laminate structure of mucosa, submucosa, and muscular layers (Figures 2 and 3). Although the remodeling of this ECM scaffold did not result in perfectly normal esophageal tissue, the result was a functional structure with multiple organized tissue types. In addition, the absence of scar tissue formation suggested that the default mechanism of esophageal healing had been altered by the use of this ECM scaffold.

ECM scaffolds derived from the urinary bladder submucosa (UBS) have been used for reconstruction of the lower urinary tract with similar constructive remodeling results.[49–61] The UBS scaffolds have been either allogeneic or xenogeneic in origin and have been used both alone or with cultured autologous cells. Sections of urethra, ureter, and urinary bladder

RegenX Science Exhibit 1006
Page 4

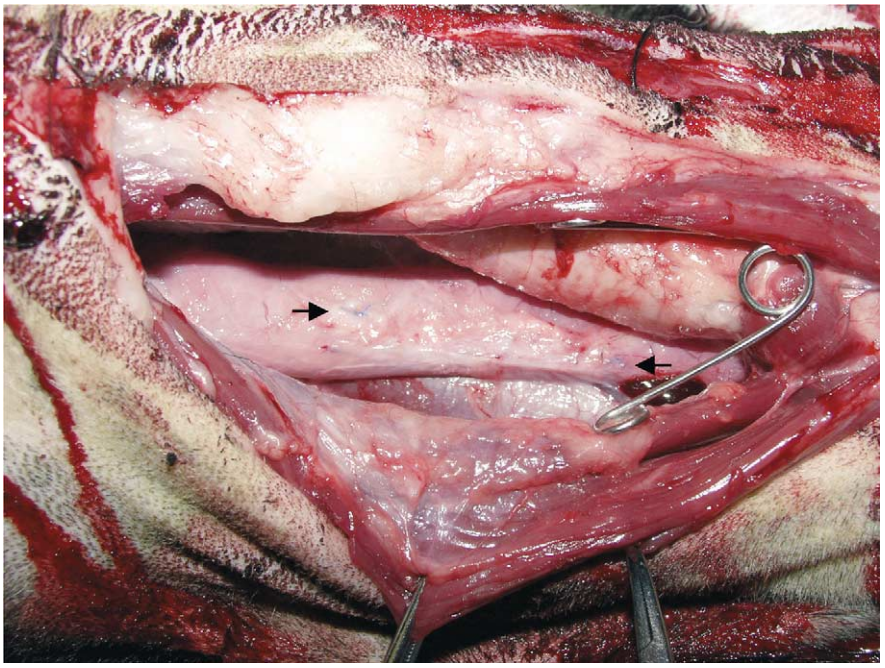

Figure 2. Five centimeter long section of cervical esophagus in a dog that represents the site of placement of a xenogeneic ECM scaffold that has now been remodeled *in vivo*. The scaffold was derived from the porcine urinary bladder. The scaffold has been replaced in 2 months by relatively normal appearing esophageal tissue without stricture, scarring or adhesions to surrounding tissues. The arrows identify sutures that represent the original anastomosis of ECM scaffold to native esophagus.

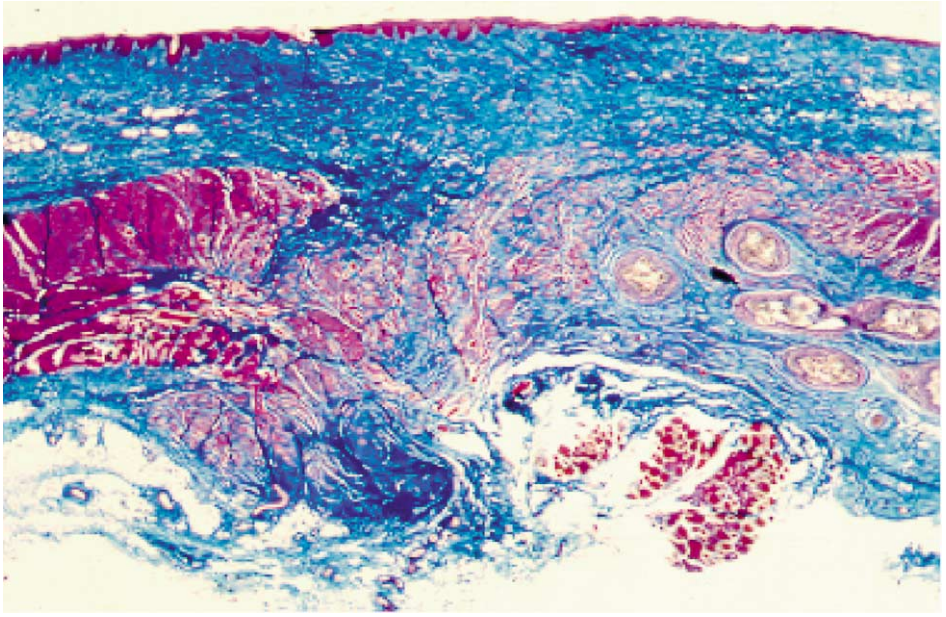

Figure 3. Photomicrograph of tissue shown in Figure 2. There is an intact but not entirely normal appearing squamous epithelium, a lack of normal complement of submucosal glands, partially organized bundles of skeletal muscle and tissue organization that resembles the normal laminar arrangement of tissue types found in the esophagus. Of note, there is a lack of inflammatory cells or scar tissue, and there is no histologic evidence of the originally implanted scaffold.

RegenX Science Exhibit 1006
Page 5

have shown excellent reconstitution with formation or organized and innervated smooth muscle. There is a substantial body of literature developing that supports the use of intact ECM as a scaffold for tissue repair. More than 100,000 human patients have now been implanted with xenogeneic ECM scaffold derived from the porcine small intestinal submucosa for a variety of applications; scaffolds are necessary components for tissue repair and reconstitution.

Conclusions

The ECM represents nature's scaffold for tissue development and tissue repair. The optimal methods for using this scaffold for clinically relevant tissue engineering applications have yet to be determined. Many questions remain to be answered including the optimal source of ECM scaffolds for clinical use, the immunologic response to allogeneic and xenogeneic scaffolds, and the optimal methods for engineering ECM scaffolds with the appropriate mechanical and physical properties. It appears that there is a fundamentally different host response to naturally occurring ECM scaffolds vs. conventional scaffold materials and that ECM has the potential to change the default scar tissue response to injury in adult mammals.

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RegenX Science Exhibit 1006
Page 6

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RegenX Science Exhibit 1006
Page 7