

Xenogeneic extracellular matrix as a scaffold for tissue reconstruction

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Abstract

Bioscaffolds derived from xenogeneic extracellular matrix (ECM) have been used in numerous tissue engineering applications. The safety and efficacy of such scaffolds when used for the repair and reconstruction of numerous body tissues including musculoskeletal, cardiovascular, urogenital and integumentary structures has been shown in both preclinical animal studies and in human clinical studies. More than 200 000 human patients have been implanted with xenogeneic ECM scaffolds. These ECM scaffolds are typically prepared from porcine organs such as small intestine or urinary bladder, which are subjected to decellularization and terminal sterilization without significant loss of the biologic effects of the ECM. The composition of these bioscaffolds includes the structural and functional proteins that are part of native mammalian extracellular matrix. The three-dimensional organization of these molecules distinguishes ECM scaffolds from synthetic scaffold materials and is associated with constructive tissue remodeling instead of scar tissue. The biologic response to these xenogeneic bioscaffolds, including the immune response, is discussed herein.

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1. Introduction

The extracellular matrix (ECM) is a secreted product of cells that populate a given tissue or organ. The composition and ultrastructure of the ECM are determined by factors that influence the phenotype of these cells including mechanical forces, biochemical milieu, oxygen requirements, pH and the inherent gene expression patterns. In turn, the ECM influences the behavior and phenotype of the resident cells. Cell attachment, migration, proliferation and three-dimensional spatial arrangement are strongly affected by matrix composition and structure. Stated differently, the ECM is in a state of ‘dynamic equilibrium’ with the cells, tissues and organs of which it is a part and it serves as an information highway or medium between cells [1–3]. For these reasons, the ECM has been developed as a biologic scaffold for tissue engineering applications in virtually every body system. Individual components of the ECM such as collagen I or fibronectin have been used to modulate cell behavior and augment the in vivo performance of alternative scaffold materials. Methods

of chemically cross-linking the ECM for purposes of increased material strength, slower in vivo degradation or passification of antigenic epitopes have uniformly resulted in a scaffold material that is less biocompatible than ECM that is preserved in its native state.

The ECM plays a central role in mammalian development and physiology and the amino acid sequence and quaternary structure of many components of ECM such as collagen are highly conserved across species lines [4]. This high degree of sequence homology is one reason that xenogeneic ECM can function as a constructive scaffold in mammalian recipients rather than inciting a destructive inflammatory reaction.

The mammalian response to tissue injury involves a complex sequence of events that includes vascular, cellular and humoral components; the outcome of which is either tissue necrosis with scarring or reconstruction of the tissue with return of function. ECM scaffolds can modulate the wound healing response toward tissue reconstruction rather than scar tissue formation. The present review describes the major components of mammalian ECM, the therapeutic use of naturally occurring ECM scaffolds (i.e. not chemically cross-linked) in tissue engineering applications and reconstructive sur-

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gery and the immune response to xenogeneic forms of ECM when it is used in therapeutic applications.

2. Composition of extracellular matrix

The composition of the ECM is a complex mixture of structural and functional proteins, glycosaminoglycans, glycoproteins and small molecules arranged in a unique, tissue specific three-dimensional architecture [5–7]. The logical division of the ECM into structural and functional components is not possible because many of these molecules have both structural and functional roles in health and disease.

2.1. Collagen

Collagen is the most abundant protein within the mammalian ECM. Greater than 90% of the dry weight of the ECM from most tissues and organs is represented by collagen [4]. More than 20 distinct types of collagen have been identified, each with a unique biologic function. Type I collagen is the major structural protein present in tissues and is ubiquitous within both the animal and plant kingdoms. Type I collagen is abundant in tendinous and ligamentous structures and provides the necessary strength to accommodate the uniaxial and multiaxial mechanical loading to which these tissues are commonly subjected. These same tissues provide a convenient source of collagen for many medical device applications. Bovine Type I collagen is harvested from Achilles tendon and is perhaps the most commonly used xenogeneic ECM component intended for therapeutic applications.

Collagen types other than Type I exist in the ECM of most tissues but typically in much lower quantities than Type I. These alternative collagen types provide distinct mechanical and physical properties to the ECM and contribute to the population of ligands that interact with the resident cell populations. For example, Type IV collagen is present within the basement membrane of most vascular structures and within tissues that contain an epithelial cell component [8–10]. The ligand affinity of Type IV collagen for endothelial cells is the reason for its use as a biocompatible coating for medical devices intended to have a blood interface. Type III collagen is found within the submucosal tissue of selected organs such as the urinary bladder; a location in which tissue flexibility and compliance are required for appropriate function as opposed to the more rigid properties required of a tendon or ligament [9]. Type VI collagen is a relatively small molecule that serves as a connecting unit between glycosaminoglycans and larger structural proteins such as Type I collagen, thus providing a gel like consistency to the ECM [10]. Type VII collagen is found within the basement membrane of the epidermis and functions as an anchoring fibril to protect

the overlying keratinocytes from sheer stresses [10]. Each type of collagen is of course the result of specific gene expression patterns as cells differentiate and tissues and organs develop and spatially organize [4,10]. In nature, collagen is intimately associated with glycosylated proteins, growth factors and other structural proteins such as elastin and laminin to provide unique tissue properties [10]. The diversity of collagen types, the association with non-collagenous molecules and the tissue specific ultrastructure of ECM are predictive of the difficulty in recreating such a composite scaffold in vitro (i.e. an artificial ECM).

2.2. Fibronectin

Fibronectin is second only to collagen in quantity within the ECM. Fibronectin is a dimeric molecule of 250 000 MW subunits and exists both in soluble and tissue isoforms and possesses ligands for adhesion of many cell types [11–14]. The ECM of submucosal structures, basement membranes and interstitial tissues all contain abundant fibronectin [13,14]. The cell friendly characteristics of this protein have made it an attractive substrate for in vitro cell culture and for use as a coating for synthetic scaffold materials to promote host biocompatibility. Fibronectin is rich in the Arg-Gly-Asp (RGD) subunit; a tripeptide that is important in cell adhesion via the $\alpha_5\beta_1$ integrin [10]. Fibronectin is found at an early stage within the ECM of developing embryos and is critical for normal biologic development, especially the development of vascular structures. The importance of this molecule and its interactions with other matrix components cannot be overstated with regard to cell–matrix communication.

2.3. Laminin

Laminin is a complex adhesion protein found in the ECM, especially within basement membrane ECMs [13]. This protein plays an important role in early embryonic development and is perhaps the best studied of the ECM proteins found within embryonic bodies [15]. This trimeric cross-linked polypeptide exists in numerous forms dependent upon the particular mixture of peptide chains (e.g. $\alpha 1$, $\beta 1$, $\gamma 1$) [16,17]. 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 reconstruction [18,19]. The crucial role of the beta-1 integrin chain in mediating hematopoietic stem cell interactions with fibronectin and laminin has been firmly established [20,21]. Loss of the beta-1 integrin receptors in mice results in intrapartum mortality. This protein appears to be among the first and most critical ECM factors in the process of cell and tissue differentiation. The specific role of laminin in tissue reconstruction when ECM is

used as a scaffold for tissue and organ reconstruction in adults is unclear but its importance in developmental biology suggests that this molecule is critical for organized tissue development as opposed to scar tissue formation.

2.4. Glycosaminoglycans

The ECM contains various mixtures of glycosaminoglycans (GAGs) depending upon the tissue location of the ECM in the host, the age of the host and the microenvironment. The GAGs bind growth factors and cytokines, promote water retention and contribute to the gel properties of the ECM. The heparin binding properties of numerous cell surface receptors and of many growth factors (e.g. fibroblast growth factor family, vascular endothelial cell growth factor) make the heparin-rich GAGs useful components of naturally occurring substrates for cell growth. The glycosaminoglycans present in ECM include chondroitin sulfates A and B, heparin, heparan sulfate and hyaluronic acid [22,23]. Hyaluronic acid has been most extensively investigated as a scaffold for tissue reconstruction and as a carrier for selected cell populations in therapeutic tissue engineering applications. The concentration of hyaluronic acid within ECM is highest in fetal and newborn tissues and tends therefore to be associated with desirable healing properties. The specific role, if any, of this GAG upon progenitor cell proliferation and differentiation during adult wound healing is unknown.

2.5. Growth factors

An important characteristic of the intact ECM that distinguishes it from other scaffolds for tissue reconstruction is its diversity of structural and functional proteins. The bioactive molecules that reside within the ECM and their unique spatial distribution provide a reservoir of biologic signals. Although cytokines and growth factors are present within ECM in very small quantities, they act as potent modulators of cell behavior. The list of growth factors found within ECM is extensive and includes vascular endothelial cell growth factor (VEGF), the fibroblast growth factor (FGF) family, epithelial cell growth factor (EGF), transforming growth factor beta (TGF-beta), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), platelet derived growth factor (PDGF) and bone morphogenetic protein (BMP) among others [24–26]. 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 methods of encouraging blood vessel formation (e.g. VEGF), inhibiting blood vessel formation (angiostatin), stimulating deposition of granulation tissue (PDGF) and bone (BMP), and encouraging epi-

thelialization of wounds (KGF). However, this therapeutic approach has struggled with determination and delivery of optimal dose, the ability to sustain and localize the growth factor 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 substrate or scaffold for cell growth and differentiation is the presence of all the attendant growth factors (and their inhibitors) in the same relative amounts that exist in nature and perhaps more importantly, in their native three-dimensional ultrastructure. The ECM presents these factors efficiently to resident cell surface receptors, protects the growth factors from degradation, and modulates their synthesis [22,24–26]. If one considers the ECM to be a substrate for *in vitro* and *in vivo* cell growth, it is reasonable to think of the ECM as a temporary (i.e. degradable) controlled release vehicle for naturally derived growth factors. As our understanding of the differences in ECM as a function of tissue type and age increases, our ability to modulate the differentiation (or lack thereof) of host reparative cells, including progenitor cells, within ECM scaffolds at sites of tissue reconstruction will improve.

3. Extracellular matrix as therapeutic scaffolds

The ECM, typically from a xenogeneic source, has been used as a scaffold for the replacement or reconstruction of many different tissues. These studies have utilized ECM that is derived from organs such as the small intestine [27–39], urinary bladder [40–42] or skin [43–45]. An allogeneic or xenogeneic source of ECM has usually been used, thus providing the possibility of an off-the-shelf product for clinical application. There are differing opinions as to whether the ECM should be seeded with site-specific cells prior to utilization as a repair scaffold or should be used in an acellular form. The effect of adding cells to an ECM scaffold upon the biologic remodeling response varies depending upon the intended application and the manner of preparation of the composite scaffold. A cellular component requires an autologous cell source and a controlled environment to assure cell viability during the translocation of the cell-scaffold composite to the recipient tissue site. Both cellular and acellular forms of ECM scaffolds have been used for tissue engineering applications. The examples provided below focus upon the acellular form.

The use of intact (i.e. not chemically modified) ECM as a tissue scaffold provides for a different host tissue response than purified components of the ECM such as collagen I [46]. However, if ECM is chemically cross-linked by such agents as glutaraldehyde, carbodiimide or photo-oxidizing agents, the constructive remodeling that is seen as part of the host response is replaced by a typical biomaterial foreign body reaction: fibrous

encapsulation, slowed or completely inhibited scaffold degradation and chronic inflammation. Stated differently, although the ECM has been viewed favorably as a scaffold for tissue repair, its method of preparation can dramatically alter the host remodeling response.

3.1. Use of ECM scaffolds as an arterial graft

ECM harvested from the porcine small intestinal submucosa (SIS) has been studied extensively for its utility as a vascular graft. SIS has been used as a large diameter vascular graft in dogs with results that show excellent long-term patency and extensive remodeling of the blood vessel wall structures [27,47]. The remodeled scaffolds show complete degradation and resorption of the originally implanted ECM material and replacement by host tissues that form an intimal structure, partially organized bundles of smooth muscle in the vascular wall, and a complete endothelial lining.

In one such study, the infrarenal aorta (5.0 cm length) was replaced in 13 dogs with porcine derived SIS [27]. All 13 dogs maintained patent grafts until the time of euthanasia, which ranged from 4 days to 8 years after surgery. The animals showed no signs of graft infection, aneurysm formation or thrombosis. Histologic examination of the harvested grafts at the time of euthanasia showed confluent endothelial cell growth on the surface of the grafts within 28–42 days, an intimal layer consisting of moderately dense and organized layers of collagen and an external layer of contractile spindle shaped cells that stained positive for smooth muscle actin. These myocyte-like cells were shown to have contractile properties based upon response to topically administered epinephrine and in vitro stimulation with applied electrical current. These findings were among the first to show that an acellular and non-chemically cross-linked ECM, when used as a scaffold for tissue reconstruction, could be populated by organized and differentiated cell types instead of replacement of the scaffold by scar tissue or dense, organized collagen. The addition of cells, such as endothelial cells and/or smooth muscle cells, to tubularized ECM or tubularized Type I collagen has been attempted by many investigators with variable degrees of success [48–55]. Although most would agree that ECM and collagen are excellent substrates for endothelial cell growth and the attachment and proliferation of other cell types, there is no consensus regarding the best way to populate these tubularized scaffolds with muscle cells and endothelial cells as they are developed for clinical use as vascular grafts. The use of ECM scaffolds alone (i.e. without cells) for small diameter vascular graft applications has been less successful than its use for large diameter grafts [28–30]; likely as a result of lower flow rates than observed in large diameter vessels and the absence of an endothelial cell layer at the time of implantation. Although the

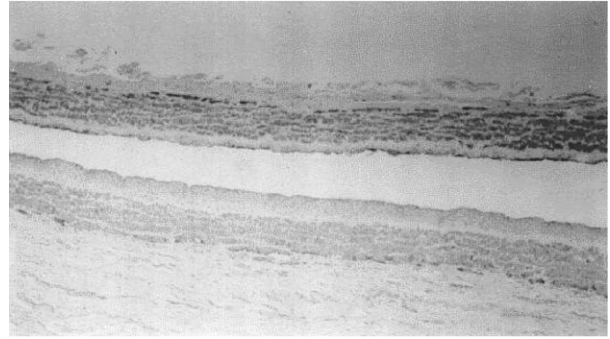


Fig. 1. This photomicrograph shows a longitudinal section SIS one year after placement as a femoral artery graft in a Rhesus monkey. Note the organized bundles of smooth muscle in the wall of the neo-vessel and an intimal layer between the lumenal surface and the smooth muscle.

pattern of scaffold remodeling appears to be the same (i.e. organized smooth muscle and intimal tissue), graft patency beyond a few weeks to months rarely exceeds 50–70%. Of note, it does not seem to matter whether or not the ECM scaffold is of autologous, allogeneous or xenogeneic origin. Porcine derived SIS has been used as a vascular graft scaffold in sub-human primates with identical results to those found in non-primate models (Fig. 1).

The use of ECM scaffolds for venous graft reconstruction [56], and venous valve replacement [57,58] has also shown promising results.

3.2. ECM scaffolds for lower urinary tract reconstruction

The investigation of ECM as a scaffold for tissue reconstruction has been investigated extensively in the lower urinary tract. The source of ECM has included SIS and perhaps more logically, the urinary bladder itself (urinary bladder matrix – UBM). Allogeneic and xenogeneic sources of UBM have been used with equal degrees of success. Pioneering work by Tanagho [59–62] and Atala [63,64] in animal models of urethral, ureteral, bladder wall and vaginal replacement have led to the present use of these materials in human patients with a high success rate. These studies and studies with the use of the SIS-ECM have shown that the matrix itself serves as a temporary, rapidly degraded scaffold that is replaced by organized and functional smooth muscle and site appropriate urothelium [59–79]. The rate of resorption of the scaffold has been surprisingly rapid with as much as 90% of the scaffold replaced within 28 days [65,66,79]. These results indicate that the host cellular infiltrate is rapid, diffuse and different from the typical fibroblastic ingrowth seen with tissue injury or purified collagen scaffold materials. The obvious advantage of no persistent foreign graft material at

the site of functional lower urinary tract tissue suggests that the use of these scaffolds for this therapeutic application will continue to expand. ECM scaffolds are currently used in human patients for stress urinary incontinence, augmentation cystoplasty and urethral and ureteral reconstruction.

3.3. ECM scaffolds for reconstitution of skin

The use of decellularized human dermis (Alloderm®) and porcine SIS (OaSIS™) has been shown to positively affect the healing of chronic ulcers and traumatic skin wounds. These acellular materials serve not only to provide bulk as a dermal substitute but also to promote wound coverage by serving as an excellent substrate for growth of autologous keratinocytes [44,45,80–83]. Depending upon the form of ECM used, there is typically a vascular ingrowth from the wound bed (angiogenesis), dense cellular infiltrate from surrounding dermis and subcutaneous tissue and rapid migration of keratinocytes from the wound edges. Contraction of the wounds can be improved (i.e. minimized) with the use of ECM scaffolds but reconstitution of adnexal structures such as hair follicles and sweat glands has not been shown. Tissue engineered skin substitutes such as Dermagraft™ and ApliGraf® consist of composite grafts including neonatal fibroblasts and secreted ECM, added growth factors and keratinocytes. These composite grafts represent excellent scaffolds for wound repair but the specific advantages offered by the cell component vs. ECM alone are unclear. Without compelling evidence that these composites provide significantly superior results compared to the use of ECM scaffolds alone, the practical limitations such as shelf life, cell sourcing and regulatory approval limit the utility of these devices in human patients.

3.4. ECM scaffolds for orthopaedic applications

Perhaps in no other field have ECM scaffolds been more successfully utilized in human patients than in the field of orthopaedics; especially, soft tissue (i.e. musculotendinous and ligament) orthopaedic surgery. The acceptance of this approach for musculoskeletal repair may be a logical outgrowth of the traditional use of autologous grafts for many orthopaedic surgical procedures. The use of autologous patellar tendon and semitendonosis grafts for anterior cruciate ligament repair, fascia lata for rotator cuff repair and palmaris tendon for ulnar collateral ligament replacement have been accepted approaches for tissue reconstruction in the orthopaedic community for decades with reasonably high success rates. Although these autologous grafts (i.e. transplants) have a cellular component at the time of surgery, the resident cells do not survive the remodeling process. Therefore, an off-the-shelf ECM scaffold (i.e.

free of any cellular component) has many attractive features to this group of surgeons.

The first ECM scaffold material to achieve acceptance by orthopaedic surgeons was the SIS-ECM graft (RESTORE™—porcine small intestinal submucosa) [39,84–88]. Subsequent products to enter the marketplace included Cuff Patch™ (a chemically cross-linked form of the SIS-ECM), Graft Jacket™ (the orthopaedic version of Alloderm), TissueMend™ (a porcine collagen plus ECM component product) and Permacol™ (a porcine dermal collagen implant that is chemically cross-linked and currently only available in Europe). Initial FDA allowances were limited to rotator cuff repair but subsequent studies and use in human patients has shown the ECM scaffold to be effective for the reconstruction of many different tendons and ligaments, including Achilles tendon, biceps tendon, triceps tendon and posterior hip capsule. The remodeled tissues show organized bundles of musculoskeletal tissue instead of the default deposition of scar tissue at the site of injury. In summary, the orthopaedic surgeon community has become fully engaged in the concept of tissue engineering using a xenogeneic ECM scaffold approach.

3.5. ECM scaffolds for replacement of other tissue structures

In addition to studies which have evaluated the utility of the ECM as a scaffold for large diameter and small diameter arterial grafts, venous grafts, lower urinary tract, skin and musculoskeletal structures, there have also been numerous studies investigating its use as a myocardial patch and heart valve substitute [89], dura mater graft following intracranial surgery [90,91], and a hernia repair scaffold [92–95], and for use as a cartilage and bone substitute [96–99]. ECM scaffolds have recently been shown to promote the reconstitution of laryngeal structures including functional vocal cord structures [42]. These preclinical studies have led to the use of SIS-ECM in more than 200 000 human patients.

3.6. Processing of ECM scaffolds for medical use

The host response to an unmodified ECM is distinctly different from the response to synthetic scaffold materials and from the response to either purified components of ECM or chemically cross-linked forms of ECM. The purified components elicit a specific response to the particular molecule: for example, angiogenesis in response to VEGF or bone formation in response to BMP. The response may be desirable for particular medical/surgical needs but typically lacks the complex constructive wound healing response observed when the intact ECM is present. Alternatively, if the intact ECM is chemically cross-linked, thus preventing cell recognition of surface epitopes and preventing degradation by

host tissues, then the scaffold becomes inert and is encapsulated by host fibrous tissue rather than degradable by host cells (i.e. typical of a response to a non-degradable foreign material). Although chemically cross-linked ECM scaffolds typically have greater strength than non-cross-linked ECM scaffolds, these scaffolds may be prone to mineralization and fatigue failure and they lack complete and constructive remodeling by host tissues.

The use of ECM scaffolds alone (without cells) has not been successful for the reconstitution of small diameter tubular structures, especially those that require peristalsis for routine function. For example, the use of SIS-ECM for ureteral repair, common bile duct, vas deferens and small intestine has shown stricture or inappropriate, non-functional remodeling in preclinical studies. The inability to maintain a tubularized form (i.e. collapse during remodeling) and the lack of peristaltic capability during remodeling suggests that a cellular component as a composite approach may be required to achieve a state of clinical utility for these tissue engineering applications.

In all applications, the ECM scaffolds that have been harvested from xenogeneic sources and that have not been chemically cross-linked have shown remarkably consistent tissue remodeling properties. These properties have included extensive and rapid neovascularization, mononuclear cellular infiltration with deposition of new host derived ECM, and tissue remodeling with organization of differentiated cell populations. The tissue remodeling is dependent to a significant degree upon tissue stressors (e.g. mechanical loading, oxygen tension, pH). The biologic response to ECM is reasonably predictable and causes a change from the default healing response of scar tissue formation.

Recent findings have suggested that an important component of the host tissue response is mediated by biologically active degradation products of the ECM itself. In other words, in addition to the biologic effects of the known growth factors and adhesive proteins that are part of the intact ECM (e.g. VEGF, fibronectin, etc.) there are also biologic effects that are spatially and temporally localized to scaffold degradation. Peptides with antibacterial properties [100] and chemoattractant properties for progenitor cells [101] have been identified within the products of enzymatically digested ECM. Studies that have evaluated the ability of ECM scaffolds to resist deliberate bacterial contamination have consistently shown the protective effects of non-cross-linked ECM [47,102]. Such findings are consistent with an innate protective response to injury found within mammalian ECM. Tissue damage resulting in leukocyte mediated ECM degradation would logically release factors (i.e. degradation products) that would resist bacterial infection, stimulate angiogenesis and attract host cells capable of tissue repair and reconstruction. Of

interest, it has been shown that bone marrow-derived cells (not leukocytes) play a significant role in the remodeling of ECM scaffolds [101].

4. Immune response to extracellular matrix scaffolds

The immune response to xenogeneic whole organ transplants has been well studied and has framed our understanding of the present limits of cell, tissue and organ transplantation. However, the mammalian immune response to acellular scaffolds derived from xenogeneic extracellular matrix has received much less attention. The consistency and uniformity of host response to autologous, allogeneic and xenogeneic ECM scaffolds suggests that preservation of the constituent molecules has been significant. The uniformity of response may also suggest that the ECM contains biologic mediators of the immune system that affect the host response to injury.

The preparation of ECM biomaterials for therapeutic use involves decellularization of mammalian tissues or organs. The absence of a cellular component, and thus the absence of all epitopes associated with cells, presents a significantly different type of 'tissue graft' to the host than is typically presented with autogenous, allogeneic or xenogeneic whole organ grafts. The death of cells associated with any tissue graft is accompanied by the release of cell products that elicit inflammation and the associated predictable and well-studied local and systemic responses. An ECM scaffold alone (without cells) contains only the constituent proteins of the ECM and their degradation products as potential antigenic or inflammatory stimuli. The inflammatory and immunologic responses to ECM alone are distinctly different than those observed when cells are embedded within the ECM and cell death and cell debris are components of the host response. In fact, the response observed with ECM alone raises the rhetorical issue of ECM 'viability'. The term viability is generally used in the context of living cells; none of which are present in these scaffold materials. The use of ECM scaffolds for tissue engineering applications generally involves simply translocating the acellular ECM from one tissue or species to another tissue or species.

A typical method of tissue processing would include extensive rinsing in hypotonic saline, treatment with dilute (0.1%) peracetic acid or incubation in Triton 100X and 0.1 ammonium hydroxide and a terminal sterilization step with either ethylene oxide, gamma irradiation or E-beam irradiation (2.0 Mrad) [103,104]. These methods have been shown to eliminate all intact cells (i.e. decellularize) and to degrade any nucleic acid remnants to less than 300 base pairs while leaving the constituent growth factors such as bFGF and VEGF biologically active [105].

4.1. Xenotransplantation and the 'gal epitope'

One of the major barriers to xenotransplantation in humans is the presence of natural antibodies to the terminal galactose alpha 1,3 galactose (alpha-gal) epitope. This epitope is expressed on the cell membranes of all mammals except those of humans and old world primates [106–112]. In humans, these naturally occurring antibodies can be of the IgM, IgG or IgA isotype and can mediate hyperacute or delayed rejection of vascularized grafts through complement fixation [108,110,111,113] and antibody dependent cell mediated cytotoxicity, respectively [106,110,113]. Since ECM scaffolds are avascular at the time of implantation, the problem of endothelial cell lysis and death with attendant thrombosis and ischemia is not an issue. The cells that populate the ECM scaffolds during remodeling are host-derived, and thus, non-immunogenic. Although it has been shown that ECM derived from porcine tissues contains small amounts of the gal epitope it appears that the quantity or distribution of the epitope and/or the subtype of immunoglobulin response to the epitope is such that complement activation does not occur [114]. A study in which gal^{-/-} mice with naturally occurring anti-gal antibodies were implanted with porcine ECM scaffolds failed to show any significant effect upon graft remodeling [115]. Treatment of decellularized ECM scaffolds with alpha galactosidase is a possible solution should alternative methods of ECM preparation be attempted.

The development of increased concentrations of anti-gal antibodies in human patients implanted with SIS-ECM scaffold has been investigated in preliminary studies. To date, there is no evidence for an increase in anti-gal antibody levels in these patients in spite of the presence of low levels of the Gal-epitope in the SIS-ECM scaffold [unpublished data].

4.2. Tissue cytokine and humoral response to ECM scaffolds

In spite of the extensive measures taken to decellularize porcine tissues in the preparation of ECM scaffolds, the complete elimination of all cell membrane and nuclear materials is very difficult and perhaps impossible. It is intuitive that an ECM graft recipient should mount an immune response to the foreign xenograft, either to the cell remnants or to the intact xenogeneic proteins. Several studies have examined the role of Th1 and Th2 lymphocytes in cell mediated immune responses to (cellular) xenografts [116,117]. Activation of the Th1 pathway produces interleukin-(IL)2, interferon (IFN) gamma and tumor necrosis factor-(TNF) beta leading to macrophage activation, stimulation of complement fixing antibody isotypes (IgG2a and IgG2b in mice) and differentiation of CD8+ cells to a cytotoxic

phenotype [118,119]. Activation of this pathway is associated with both allogeneic and xenogeneic transplant rejection [117,118,120,121]. The Th2 lymphocyte response produce IL-4, IL-5, IL-6 and IL-10, cytokines that do not activate macrophages and lead to production of non-complement fixing antibody isotypes (IgG1 in mice). The Th2 pathway is associated with transplant acceptance [122,123].

A preliminary study was conducted to examine the expression of T cell cytokines in antibodies in BALB/c mice implanted with porcine-derived SIS-ECM. Mice were implanted subcutaneously with 1 cm² of the SIS-ECM and local tissue cytokine levels determined. Appropriate positive and negative controls were included in this study [124]. Results showed that all animals implanted with a non-cross-linked ECM scaffold elicited a Th2-type response. Inflammatory cytokines such as TNF- α and IL-1 were decreased in the surrounding tissue while anti-inflammatory cytokines such as IL-5 and IL-10 were increased. ECM implanted mice showed the development of non-complement fixing antibodies (IgG1). Subsequent reimplantation of additional SIS-ECM augmented the Th2-type response instead of sensitizing the mice to the material. The role of the Th2-type response in recipients of porcine-derived ECM scaffolds remains unclear. It is possible that this response is an active part of the remodeling process. Alternatively, the summated effect of the immune response may simply allow the remodeling process to occur without interference from classic inflammatory mediators. These results may also provide a partial explanation for the lack tissue necrosis and subsequent scar tissue formation when the ECM scaffolds are degraded and replaced by site-specific host tissues.

Numerous preclinical studies and biopsies from human patients implanted with the RESTORETM device (porcine SIS-derived product for orthopaedic applications) have shown a vigorous host cellular response in the early (immediate to 3 week) period after scaffold implantation [unpublished data]. These cells are primarily mononuclear after the first 48–72 h but their specific phenotypic characterization is unknown. Plasma cells are not observed as part of the cellular response. Host-derived ECM can first be observed by light microscopy in the graft site as early as 1 week after scaffold placement. Several studies using ¹⁴C-labeled porcine ECM as a scaffold for replacement of urinary bladder, laryngeal tissue and tendinous tissue has shown a very rapid disappearance of the xenogeneic ECM from the graft site. As much as 90% of the implanted ECM scaffold is removed by 28 days post-surgery in selected applications and the scaffold is replaced by host tissues. In applications where multilaminate forms of ECM are used, complete degradation may require 90–120 days. The route of elimination of the graft material appears to be mainly via urinary excretion [65,66,79].

Since non-chemically cross-linked ECM scaffolds are rapidly degraded and removed from the body, it is possible that the stimulus for graft/scaffold rejection is either missing or only transiently present in patients that have received an ECM scaffold. Persistent low levels of the Gal-epitope have been suggested as a cause of chronic graft rejection in whole organ transplants [113]. There have also been suggestions that different tissues (such as porcine heart valves) are inherently less antigenic than other tissues [112,125]. Several studies have evaluated the effects of various methods of chemical cross-linking upon antigenicity. The findings of these studies show that although antigen recognition of xenogeneic implants such as bovine pericardium is diminished with cross-linking procedures, it still persists in spite of very aggressive cross-linking measures [126]. Cross-linking by photo oxidation also elicits low levels of recipient antibody production that can persist for up to 2 years [126].

In summary, the immune response to xenogeneic ECM scaffolds has been partially characterized and suggests a Th2 restricted type of response. The pathophysiology of this response is unknown, but the lack of a cellular component within the grafts likely is an important mediator of the host tissue response.

5. Summary

Scaffolds derived from xenogeneic extracellular matrix have found utility in tissue engineering applications across many body systems. These scaffolds are usually porcine-derived and elicit a constructive remodeling response that is dependent upon many factors. These factors include angiogenesis, support of host parenchymal cell attachment, proliferation and differentiation, and biologic responses that are mediated by degradation products of the ECM scaffold itself. The immune response to xenogeneic ECM scaffolds is consistent with graft/scaffold accommodation rather than graft rejection; the cause of which is not totally clear. Modification of native extracellular matrix by methods such as chemical cross-linking or other methods of cross-linking, convert the biologic response to one which is more consistent with chronic inflammation, tissue destruction, fibrous encapsulation and scarring. Future applications of ECM scaffolds in tissue engineering will likely depend upon the ability to add specific cell types (presumably autogenous) to the scaffolds prior to, or simultaneous to, implantation and the development of strategies for vascularization of three-dimensional ECM constructs.

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