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Review

Extracellular matrix as a biological scaffold material: Structure and function

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Abstract

Biological scaffold materials derived from the extracellular matrix (ECM) of intact mammalian tissues have been successfully used in a variety of tissue engineering/regenerative medicine applications both in preclinical studies and in clinical applications. Although it is recognized that the materials have constructive remodeling properties, the mechanisms by which functional tissue restoration is achieved are not well understood. There is evidence to support essential roles for both the structural and functional characteristics of the biological scaffold materials. This paper provides an overview of the composition and structure of selected ECM scaffold materials, the effects of manufacturing methods upon the structural properties and resulting mechanical behavior of the scaffold materials, and the in vivo degradation and remodeling of ECM scaffolds with an emphasis on tissue function.
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Keywords: Extracellular matrix; Scaffold; Remodeling; Degradation; Ultrastructure

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1. Introduction

Biological scaffold materials composed of extracellular matrix (ECM) have been shown to facilitate the constructive remodeling of many different tissues in both preclinical animal studies and in human clinical applications. The ECM from which these scaffold materials are derived from a variety of tissues, including heart valves [1–7], blood vessels [8–11], skin [12], nerves [13,14], skeletal muscle [15], tendons [16], ligaments [17], small intestinal submucosa (SIS) [18–20], urinary bladder [21–23] and liver [24]. The mechanisms by which biological scaffold materials promote site appropriate tissue reconstruction are not well understood and there is legitimate controversy concerning the relevant importance of the composition vs. structure of these materials. The composition of ECM scaffolds consists of a complex mixture of molecules that mediate structural and/or biological properties. These molecules are arranged in unique three-dimensional (3-D) patterns that are ideally suited to the tissue from which the ECM is harvested. Typically, such scaffold materials are biodegradable unless processed in such a manner that irreversible cross-links are created between the resident molecules. The composite structure of these ECM molecules, as well as their in vivo degradability, has marked effects upon the host response and the remodeling events that determine the eventual clinical outcome. A partial list of commercially available products composed of extracellular matrix is provided in Table 1 as a testament to the clinical relevance of these concepts.

Although well-designed and informative studies have been conducted on a variety of ECM scaffold material, the most comprehensive studies regarding mechanical and structural properties, macro- and ultrastructure and biological activity have been reported for urinary bladder matrix (UBM) and SIS.

The objective of this paper is to provide an overview of structure/function relationships within these two biological scaffold materials, and to extend these relationships to other biological scaffold materials when possible. In the context of this overview, the term "function" is used in the broadest sense including biomechanical and physiologic effects.

2. Composition of biological scaffold materials

ECM scaffolds consist of the structural and functional molecules secreted by the resident cells of each tissue and organ from which they are prepared. Therefore, the specific composition and distribution of the ECM constituents will vary depending on the tissue source. The ECM scaffold derived from porcine small intestinal submucosa (SIS–ECM) is the biological scaffold material that has been most extensively characterized, and therefore will be used as a prototypical ECM scaffold. By dry weight, SIS–ECM scaffold is composed of greater than 90% collagen. The large

Table 1
Commercially available biological scaffold materials

Product	Company	Material	Processing	Form
AlloDerm	Lifecell	Human skin	Natural	Dry sheet
AlloPatch®	Musculoskeletal Transplant Foundation	Human fascia lata	Natural	Dry sheet
Axis™ dermis	Mentor	Human dermis	Natural	Dry sheet
Bard® Dermal Allograft	Bard	Cadaveric human dermis	Natural	Dry sheet
CuffPatch™	Arthrotek	Porcine small intestinal submucosa (SIS)	Cross-linked	Hydrated sheet
DurADAPT™	Pegasus Biologicals	Horse pericardium	Cross-linked	Dry sheet
Dura-Guard®	Synovis Surgical	Bovine pericardium	Cross-linked	Hydrated sheet
Durasis®	Cook SIS	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Durepair®	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
FasLata®	Bard	Cadaveric fascia lata	Natural	Dry sheet
Graft Jacket®	Wright Medical Tech	Human skin	Natural	Dry sheet
Oasis®	Healthpoint	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
OrthADAPT™	Pegasus Biologicals	Horse pericardium	Cross-linked	Dry sheet
Pelvicol®	Bard	Porcine dermis	Cross-linked	Hydrated sheet
Peri-Guard®	Synovis Surgical	Bovine pericardium	Cross-linked	Dry sheet
Permacol™	Tissue Science Laboratories	Porcine skin	Cross-linked	Hydrated sheet
PriMatrix™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Restore™	DePuy	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Stratasis®	Cook SIS	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
SurgiMend™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Surgisis®	Cook SIS	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Suspend™	Mentor	Human fascia lata	Natural	Dry sheet
TissueMend®	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Vascu-Guard®	Synovis Surgical	Bovine pericardium	Cross-linked	Dry sheet
Veritas®	Synovis Surgical	Bovine pericardium	Cross-linked	Hydrated sheet
Xelma™	Molnlycke	ECM protein, PGA, water		Gel
Xenform™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Zimmer Collagen Patch®	Tissue Science Laboratories	Porcine dermis	Cross-linked	Hydrated sheet

majority of the collagen is type I, with minor amounts of collagen types (Col) III, IV, V and VI also present [25]. Urinary bladder matrix (UBM–ECM) also contains the same collagen types as SIS–ECM, with greater amounts of Col III being present, as well as Col VII. Col VII is an important component of the epithelial basement membrane that distinguishes this particular ECM scaffold from most other ECM scaffold materials [26]. SIS–ECM contains a variety of glycosaminoglycans (GAGs), including heparin, heparan sulfate, chondroitin sulfate and hyaluronic acid [27]. The amount of GAGs remaining in a tissue after decellularization depends greatly on the method of decellularization. For example, ionic detergents are often used in the decellularization process and such detergents can remove GAGs from the ECM [28]. SIS–ECM has been shown to contain adhesion molecules such as fibronectin and laminin [26,29], the proteoglycan decorin and the glycoproteins biglycan and entactin (unpublished data). Various growth factors are also present in SIS–ECM, including transforming growth factor-β [30,31], basic fibroblast growth factor (b-FGF) [31,32] and vascular endothelial growth factor (VEGF) [33]. Several of these growth factors have been shown to retain their bioactivity even after terminal sterilization and long-term storage [30,32]. In summary, biological scaffolds composed of extracellular matrix have a complex composition with a variety of diverse molecules that are perfectly suited to support the cellular processes necessary for optimal function of the tissue and organ from which they are harvested. The ability of an ECM harvested from one tissue to function as a biological scaffold material for the same or different tissue may vary.

3. Structure of ECM biological scaffold materials

The ultrastructure and 3-D architecture of ECM scaffolds can be largely preserved throughout processing steps required for decellularization of the tissue if care is taken to avoid harsh chaotropic agents [26,34]. There is morphological evidence that scaffolds composed of ECM from specific organs retain defining structures, such as the basement membrane of the urinary bladder in UBM and the stratum compactum of the small intestine [26]. Microscopic and ultrastructural features of the matrix play important roles in modulating the behavior of cells that contact the scaffold by controlling the cells' ability to migrate into the scaffold [26] or by influencing tissue specific cell phenotype [35,36]. For example, an intact basement membrane can largely prevent in vitro cell penetration into the underlying matrix and foster the formation of confluent cell populations that cover the surface [26]. Alternatively, an irregular fibrous surface architecture can facilitate penetration of selected cell types into the midsubstance of the ECM scaffold [26]. The ECM can dramatically affect the differentiation pathway of human embryonic stem cells and selected progenitor cell populations [35,37,38].

The collagen fiber architecture of an ECM scaffold plays a critical role in determining its biomechanical behavior. The alignment and organization of collagen fibers are dependent on the function of the source tissue from which the ECM is derived. For example, the collagen fibers within a ligament or tendon are highly aligned along the long axis of the tissue to provide the greatest resistance to strain in a load-bearing application. Thus, the use of ECM derived from tendons and ligaments is a logical choice for repair of structures, such as the anterior cruciate ligament [17,39,40]. The small intestinal submucosa also has a characteristic collagen fiber organization that is related to its native in vivo function. SIS–ECM has a preferred alignment along the native longitudinal axis of the small intestine, and it appears that this preference is a composite of two populations of collagen fibers with their centroids shifted ~30° from the longitudinal axis of the intestine [34]. This spiral arrangement of collagen fibers with their adjacent smooth muscle cell layer allows the small intestine to constrict in a manner that promotes the efficient transport of a bolus of biomass (i.e. peristalsis). When the SIS–ECM is subjected to biaxial mechanical testing, this preferred fiber orientation leads to an anisotropic biomechanical behavior, with greater strength and tangent modulus along the preferred fiber direction [34].

The degree of alignment of the collagen fibers within an ECM scaffold changes as the scaffold is loaded (Fig. 1). Not only do the collagen fibers straighten from their typical crimp pattern, but populations of fibers can rotate towards the direction of stretch. The collagen fiber rotation is reversible until the tissue reaches a tissue specific maximum strain and this collagen fiber alignment shift can be predicted using a simple affine model [41]. However, with large, asymmetric strains, the rotations are permanent, no longer fit an affine model and can lead to measurable changes in the overall biomechanical behavior of the scaffold. A recent study showed that the collagen fiber alignment of UBM–ECM changed based on the direction in which mechanical force was applied to separate the submucosal and muscular layers from the mucosa during preparation of the material [42]. As a result of the tension required to separate the tissue layers, the material that delaminated in the longitudinal direction showed a highly aligned population of collagen fibers along the longitudinal axis, while the material that separated circumferentially showed no distinct preferred direction. Biaxial mechanical testing showed that these distinct structural differences due to processing methods changed the mechanical behavior of the resulting ECM scaffolds. The longitudinally separated material showed highly anisotropic behavior and the circumferentially separated material showed more isotropic behavior. Therefore, certain predictions and expectations can be made regarding the physical and mechanical properties of an ECM based on an understanding of its collagen fiber architecture. The collagen fiber alignment of an ECM scaffold and methods to alter the collagen fiber alignment during processing may also be used as design considerations to develop scaffolds with desired mechanical proper-

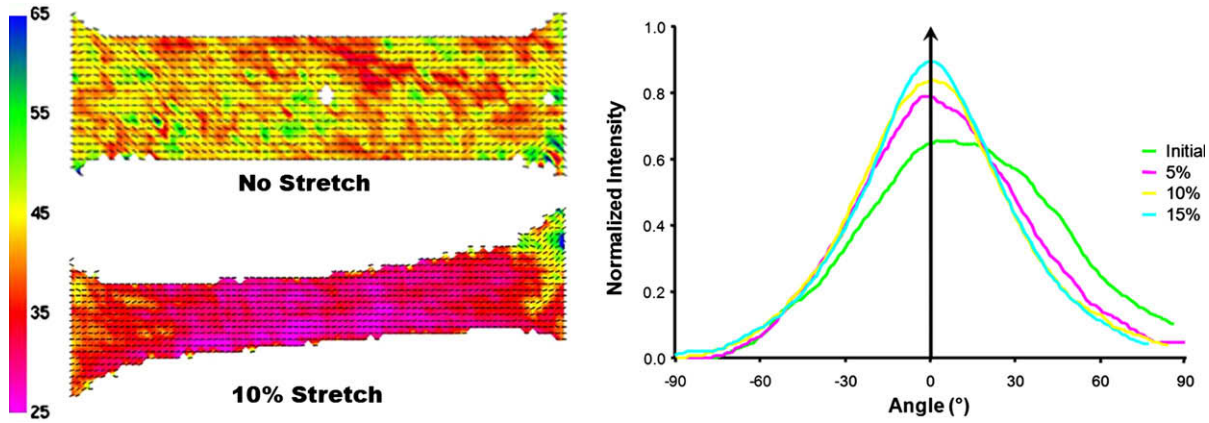

Fig. 1. (Left) Maps of the collagen fiber architecture for SIS–ECM with no stretch and 10% stretch obtained using small angle light scattering. The hash marks represent the preferred fiber direction and the color represents the degree of alignment based on the orientation index with a scale on the left side. A low orientation index indicates a high degree of alignment. (Right) The fiber distribution distributions show that the preferred fiber direction shifts towards the direction of stretch and the fibers become more aligned as the normalized intensity increases.

ties, as will be discussed in greater detail in the section on dehydration which describes methods to manufacture multilaminate devices.

4. Effect of processing upon structure and function of biological scaffold materials

The preparation of an ECM scaffold material from intact mammalian tissue requires several processing steps that can markedly affect both the structure and the type of host response that these materials elicit when utilized as a template for tissue reconstruction. The native tissue from which an ECM scaffold is prepared must be mechanically or physically separated from unwanted tissue structures, decellularized, often disinfected and dehydrated or lyophilized and terminally sterilized. Each of these processing steps can alter the integrity and architecture of the matrix as described above and, in turn, influences the mechanical and material properties of the ECM. A schematic showing the various forms of ECM scaffold is shown in Fig. 2.

4.1. Decellularization

The effective removal of antigenic epitopes associated with cell membranes and intracellular components of tissues and organs is necessary to minimize or avoid an adverse immunologic response by allogeneic and xenogeneic recipients of the ECM scaffold material. The tissues from which the ECM is harvested, the species of origin, the decellularization method and the methods by which the material is sterilized can vary widely. Xenogeneic and allogeneic cellular antigens are recognized as foreign by the host and result in an adverse inflammatory response or overt immune-mediated rejection [43–45]. The molecules which constitute the extracellular matrix are however, conserved across species lines and are tolerated well even by

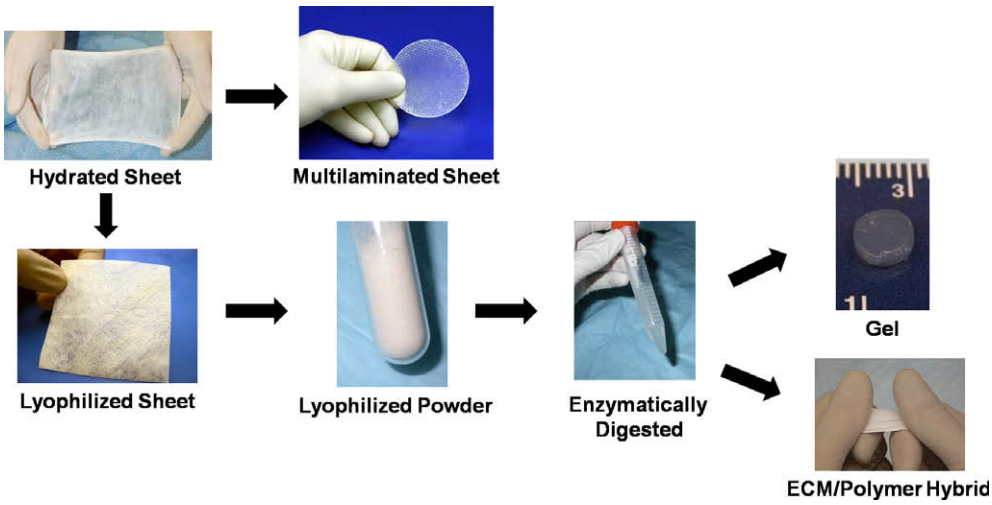

Fig. 2. Layout of processing steps for various forms of extracellular matrix scaffolds. The hydrated sheet can be used as a scaffold material directly. Several hydrated sheets can be vacuum pressed to make a multilaminate scaffold device. The hydrated sheets can also be lyophilized sheets. The lyophilized sheets can be comminuted to make a particulate form of the material. The comminuted material provides can be enzymatically digested to make a liquid form of the material, which can be repolymerized into a gel or mixed with a synthetic polymer to make a hybrid scaffold.

xenogeneic recipients [46–49]. Certain antigens, such as the galactosyl-α-1,3-galactose, have been shown to be present in porcine SIS–ECM but fail to activate complement or bind IgM antibody, presumably because of the small amount and widely scattered distribution of the antigen [50,51]. The ultimate goal of any decellularization protocol is to remove all cellular material without adversely affecting the composition, mechanical integrity and eventual biological activity of the remaining ECM. Commonly used methods of decellularization include a combination of physical and chemical treatments. Sonication, agitation and freezing and thawing are commonly used methods to disrupt cell membranes, release cell contents and facilitate the subsequent rinsing and removal of cell remnants from the ECM. The commonly used decellularization methods appear to be insufficient to achieve complete decellularization, as most, if not all, ECM scaffold materials retain some DNA [52,53]. Although it seems logical that the decellularization process will by definition affect the structure and composition of the extracellular matrix, the intent of the process is the preservation of as much of the native mechanical properties and biological properties of the original ECM as possible. Some detergents used to facilitate decellularization have been shown to disrupt collagen of certain tissues, thereby decreasing the mechanical strength of the tissue, while the same detergent may have no effect on the collagen in another tissue [17,39]. Studies have shown that removal of GAGs from the scaffold can have a negative effect on viscoelastic behavior of the scaffold, which is not surprising since water retention is one of the major functional characteristics of GAGs within a tissue [54]. Therefore, the decellularization method requires optimization for each tissue to remove cellular material without compromising the mechanical properties of the tissue.

4.2. Hydration

Very few biological scaffold materials maintain a hydrated state throughout the decellularization and sterilization process. Avoidance of the loss of water from the ECM can prevent changes in the tissue architecture, such as collapse of the collagen fibers upon each other, and the formation of physical bonds between the ECM molecules. Environmental scanning electron microscopy of the abluminal side of UBM in a hydrated and lyophilized state shows the tissue architecture changes that occur after removal of water (Fig. 3). Biologic scaffolds that retain their hydrated state throughout the decellularization and sterilization process tend to support cellular attachment and cell infiltration in vitro better than scaffolds that are subjected to a dehydration step followed by rehydration [55]. A major disadvantage of the hydrated materials, however, is the continuous leaching of soluble growth factors (such as VEGF and b-FGF) from the material during packaging and shelf life.

4.3. Dehydration

Biological scaffolds are often dehydrated by lyophilization (freeze drying) or by vacuum pressing prior to terminal sterilization. Dehydration tends to make the scaffolds easier to handle and limits the loss of growth factors during storage. Lyophilization is a process by which the water is removed from the material by sublimation at low temperatures and low pressures. Lyophilization is commonly used to preserve biological graft tissues, such as bone [56–58] and tendon [59,60] and commercially available biological materials, such as Bard® Dermal Allografts (Bard, Inc.), Acell Vet™ (Acell, Inc.) and Oasis™ (Cook Biotech, Inc.). Although lyophilization can extend the shelf life of ECM scaffolds without causing significant changes in strength values, it may alter collagen fiber morphology and the in vitro growth of cells upon the material [55]. Lyophilization can significantly decrease the thickness of the scaffolds (typically by ~30%), resulting in a more compacted fiber morphology, and will decrease the ability of the material to re-absorb the same amount of water that was present prior to the lyophilization process [61,62], possibly due to the disruption of GAGs within the tissue [63].

An alternative method used to dehydrate ECM scaffold is vacuum pressing. The vacuum pressing process allows

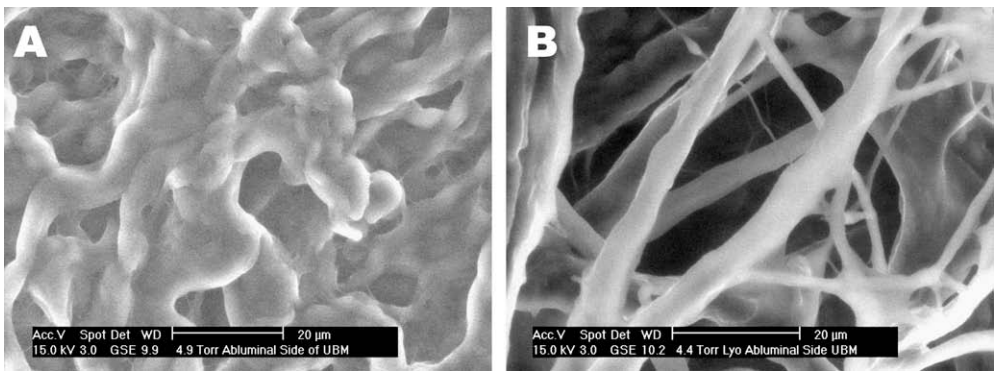

Fig. 3. Environmental scanning electron microscopy images of the abluminal side of (A) hydrated and (B) lyophilized forms of UBM. Hydration has a dramatic effect on the ultrastructure of the UBM, with the collagen in the hydrated UBM showing a swollen appearance. The lyophilized material showed compacted collagen fibers. The impact of hydration on the local arrangement of adhesion molecules or the conformation of glycosaminoglycans is unknown.

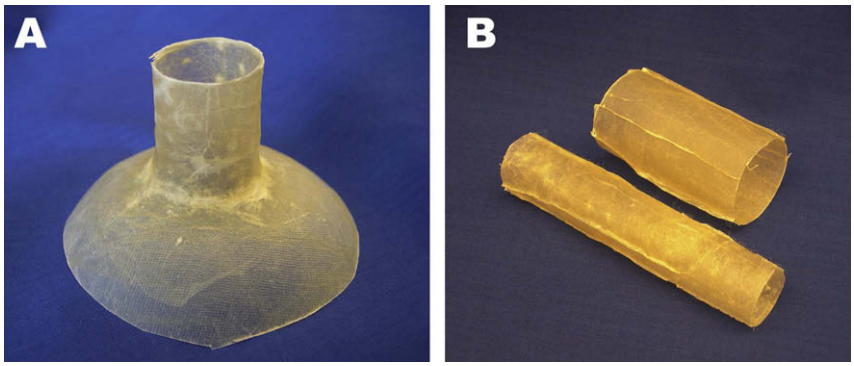

Fig. 4. Multilaminate form of UBM shaped to match the gastroesophageal junction and the esophagus.

for the lamination of multiple sheets of ECM to increase the strength and/or design-in specific mechanical behavior based upon knowledge of the collagen fiber architecture. For example, Restore® (DePuy Orthopaedics) is constructed of 10 layers of SIS–ECM, with two layers oriented every 72°. The resulting sheet provides strength at the time of implantation that exceeds the strength of a native rotator cuff tendon tissue (i.e. the tissue for which it was originally intended to function as a biological reinforcement scaffold) and imparts isotropy to natively anisotropic materials. Lamination via vacuum pressing of ECM scaffolds also reduces the extensibility, and changes the ultrastructural morphology of the resulting construct [22,64]. Vacuum pressing is an effective method for constructing a variety of 3-D shapes of ECM scaffold materials (Fig. 4).

Although constructive in vivo remodeling has been observed with the use of hydrated, lyophilized and multilaminated forms of ECM scaffolds [65–71], ultrastructural changes that occur as a result of dehydration can affect cell attachment, in vivo degradation rate and cellular infiltration [55]. The optimal configuration and method of processing of an ECM scaffold should be determined for each clinical application.

4.4. Powdered ECM scaffolds

Lyophilized sheets of ECM can be comminuted into an ECM powder or particulate form [23]. A particle form allows for the delivery of the ECM as a suspension via minimally invasive techniques (e.g. needle injection) to the site of interest or for the manufacture of 3-D scaffolds by compaction methods. The particles present in comminuted ECM retain the ultrastructural, 3-D surface characteristics of the parent ECM sheet. Particle sizes ranging from 50 to 200 μm in diameter can be reproducibly manufactured. Suspensions made from a particulate (powdered) form of lyophilized UBM have been successfully used as an injectable scaffold for the treatment of urinary incontinence in preclinical studies [72], but the needle size required to accommodate passage of the particles is prohibitive (i.e. too large external diameter) for many clinical applications. Carriers such as glycerin are typically required to increase the viscosity of a suspension of particles intended for clinical use. Acellular human dermal matrix has been investigated as a micronized form for injection into laryngeal tissue, but tissue augmentation is not possible due to its rapid in vivo degradation [73]. Powdered forms of ECM scaffolds may also be used for topical delivery or may be combined with synthetic polymers to create hybrid scaffolds. Since a particulate form of such scaffold materials are not expected to serve load-bearing functions, the physical properties of the ECM, such as particle size, surface area and type of liquid carrier, are the relevant variables that affect the ease and convenience with which the material can be delivered to the intended site.

4.5. Gel form of ECM scaffolds

A liquid or gel form of ECM can further expand the clinical utility of an ECM scaffold by allowing the delivery of the material via minimally invasive methods to sites of interest. Stated differently, a gel form can be delivered to a site of interest in its pre-gel liquid state more readily than a suspension of particles. The solubilized ECM can be delivered by catheter or needle-based surgical techniques to irregularly shaped anatomic sites. A gel form can also serve as a cell delivery vehicle when appropriate. The rheological properties of the gel can be designed to be similar to those of the tissue that is being repaired. Ideally, the gel processing methods would minimize or avoid purification steps that could remove or destroy the active growth factors and low molecular weight peptides present in the native ECM, and the gel form would retain the native bioactivity of the parent ECM scaffold. Previous studies have shown that a gel form of an ECM derived from SIS can be produced that is able to support the growth and differentiation of a variety of cells in vitro, but the preparation of this gel form required an aggressive collagen purification process that likely resulted in the loss of bioactive molecules [74].

Recently, a preparation of a gel derived from UBM has been reported in which no purification steps were necessary. Lyophilized UBM powder was enzymatically (i.e. pepsin) digested at low pH resulting in a viscous solution. A pepsin-digested form of UBM–ECM was able to self-

Table 2
Comparison of the complex viscosity of UBM gels with commercially available injectable materials

Material	k	n	r^2	Frequency (Hz)	Ref.
Collagen type I (3 mg ml^{-1})	6.92	−1.117	0.995	0.01–15	[75]
Urinary bladder matrix (3 mg ml^{-1})	2.35	−1.062	0.988	0.01–15	[75]
Urinary bladder matrix (6 mg ml^{-1})	5.69	−0.955	0.999	0.01–15	[75]
Gelatin (Gelfoam)	149.39	−0.903	0.997	0.01–15	[113]
Zyplast™	99.851	−0.915	0.998	0.01–15	[113]
Zyderm™	66.395	−0.915	0.998	0.01–15	[113]
Zyderm™	12	−0.860	0.977	0.01–100	[114]
Cymetra®	19.9	−0.778	0.972	0.01–100	[114]
Hyaluronic acid–DTPH	3.19	−0.744	0.974	0.01–100	[114]
Human abdominal subcutaneous fat	23.576	−0.951	0.994	0.01–15	[113]

The complex viscosity ($|\eta^*|$) vs. frequency (f) data were fitted to a power law of the form $|\eta^*| = k \cdot f^{-n}$ where k and n are both constants.

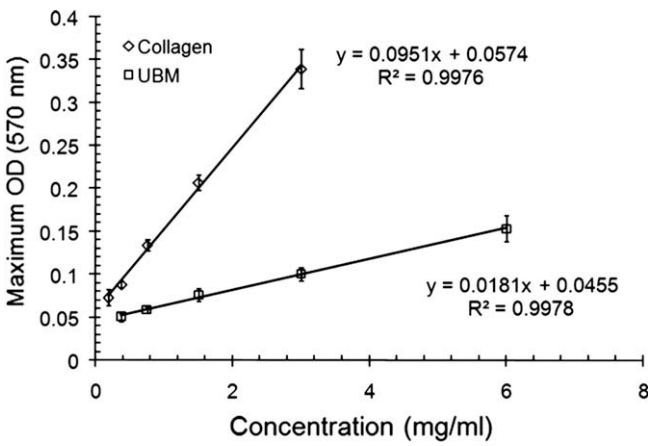

Fig. 5. Steady-state turbidity of collagen and UBM gels as a function of final concentration.

assemble into a gel by raising the ionic strength, temperature and pH to physiological levels [75]. While the buffered pepsin digest is kept at low temperatures (4 °C), the solution behaves as a liquid with low loss modulus, storage modulus and viscosity values. After the temperature is raised to 37 °C the storage modulus and the loss modulus of the material increase as a function of time and reach a steady state value after ~12 min. The storage modulus is greater than the loss modulus, suggesting that the UBM gel behaves like a solid material after self-assembly. The stiffness of a UBM gel varies as a function of protein concentration making it possible to tune the rheologic properties for specific applications. Under oscillatory shear stress, the storage moduli of the gels increase from ~20 Pa at a concentration of 3 mg ml^{-1} to ~50 Pa at a concentration of 6 mg ml^{-1} for a shear deformation of 25%. Table 2 compares the rheological properties of UBM gels with commonly used injectable materials and shows how concentration affects the final rheological properties. Differences in the final turbidity values between Col I gels and UBM gels at similar concentrations suggest that UBM has a different ultrastructure than purified Col I (Fig. 5). UBM gel has been shown to support in vitro cell growth of several cell types, including myoblasts, cardiomyocytes, smooth muscle cells and endothelial cells [75].

5. Hybrid scaffolds composed of ECM

ECM scaffolds are inherently constrained by the material properties of the tissue from which they are derived, including shape, mechanical properties and oxygen diffusivity. The material properties of ECM scaffolds can also be affected by the manufacturing process (i.e. mechanical decellularization vs. chemical decellularization) and by the age and health status of the animal from which the ECM is harvested. There are limited ways in which the material and mechanical properties of ECM scaffolds can be manipulated to tailor the functional properties of the device for specific applications. For example, the maximum strength of thin sheet forms of ECM (e.g. SIS–ECM and UBM–ECM) can be increased by creating multilaminate ECM constructs of varying number of layers [16,17], as previously described. Mechanical strength can also be increased by cross-linking the structural components of the scaffold with chemicals such as glutaraldehyde, carbodiimide and hexamethylene-diisocyanate [76], or by non-chemical methods. However, cross-linking reduces the in vivo degradation rate of the ECM scaffold and changes the host tissue response from an anti-inflammatory, constructive remodeling response to a pro-inflammatory, foreign body response [77–79].

Synthetic scaffold materials are an alternative to naturally derived ECM scaffolds and are typically characterized by uniform and reproducible mechanical and material properties. However, synthetic materials lack the bioactivity and constructive host tissue response characteristic of ECM-derived scaffolds and often result in fibrous encapsulation following in vivo placement. Combining a synthetic material with ECM may capture the advantages of both types of materials, i.e. mechanical and material properties, which can be manipulated with the synthetic component, and bioactivity, which is provided by the ECM. Similarly, the combination of synthetic and naturally occurring materials may manifest the disadvantages of both materials, i.e. the host inflammatory response to many synthetic materials and the biological variability of an ECM material. One example of a synthetic polymer–ECM hybrid scaffold is the combination of powdered SIS with poly(D,L-lactide-

co-glycolide) to create tissue-engineered bone [80]. Another example includes the combination of UBM with a poly(ester-urethane)urea (PEUU) to create an elastomeric hybrid scaffold [81]. The combination of PEUU with UBM results in an elastomeric material with increased maximum stiffness, strength and strain when compared to lyophilized UBM sheets. When implanted in a subcutaneous location, hybrid scaffolds degrade faster than purified PEUU but slower than lyophilized UBM sheets [81].

6. Terminal sterilization

Several studies have shown that terminal sterilization of ECM scaffolds can have a detrimental effect on the mechanical properties of the scaffold. Recently, it was shown that UBM-ECM had decreased uniaxial and biaxial mechanical properties after exposure to ethylene oxide (750 mg h^{-1}), gamma irradiation (20 kGy) and electron beam irradiation (22 kGy) [82]. Several studies have also investigated the effects of a wide range of gamma irradiation exposure on dermis ECM [83,84]. At low dosages of gamma irradiation (<15 kGy), the strength and modulus of the scaffold increased, but the mechanical properties decreased above 15 kGy in a dose-dependent manner [83]. These changes appear to be due to an increase in collagen cross-linking due to the low dose of irradiation that levels off after approximately 5 kGy, along with collagen chain scission that continues to increase with the irradiation dose [84]. The mechanisms for changes in mechanical properties following ethylene oxide and electron beam irradiation have not been studied in detail.

7. Changes in mechanical behavior of ECM scaffolds during in vivo remodeling

The mechanical behavior of ECM scaffolds changes during the process of in vivo remodeling [85], and such changes are dependent on factors such as the local tissue microenvironment, the rate of scaffold degradation, forces present within the mechanical environment, and the rate and extent to which the infiltrating cells deposit new ECM. The most studied ECM scaffold during the in vivo remodeling process is SIS–ECM. In a canine model of Achilles tendon reconstruction, a segmental defect was created and replaced with a tube of SIS–ECM (native geometry, not laminated) [25]. The stifle joint was immobilized for 5 weeks, but weight bearing was allowed. At 1 week after implantation, the strength of the tendon repaired with SIS–ECM was less than 100 N, or 10% of the original strength. As new collagenous connective tissue was formed, the strength of the remodeled SIS–ECM implant tissue gradually increased until the strength exceeded that of the native musculotendinous junction and the insertion into the calcaneus by 12 weeks after surgery [25]. In a goat model of anterior cruciate ligament reconstruction, a remodeled SIS–ECM scaffold showed a decreased strength at 3 months after surgery, followed by an increase to a level comparable to a patellar tendon autograft at 1 year [86]. In a dog model of rotator cuff reconstruction, a 10-layer multilaminate device (Restore™) was used to replace a completely resected infraspinatus tendon, and the animals were allowed unlimited cage activity after surgery [87]. Although no mechanical data was presented for the early phase of remodeling, the failure loads of the tendon repaired with SIS–ECM eventually increased by approximately threefold from the time of repair, and were comparable to failure loads for rotator cuffs subjected to a primary repair, despite having a cross-sectional area that was half the size [87]. In addition, when SIS–ECM was used to repair the gap defect in the medial collateral ligament of a rabbit knee, the strength at failure and modulus of the ligament substance were increased at both 12 and 26 weeks of healing compared to no treatment [88,89]. The improved mechanical properties of the remodeled tissue compared to new host tissue that formed with no treatment is thought to be a result of decreased expression and synthesis of Col V and various proteoglycans, increased collagen fibril diameter and increased collagen fiber alignment in the ligament repaired with SIS–ECM [88,90,91]. Clearly and logically, the structural changes that occur during in vivo remodeling of ECM scaffolds are associated with marked changes in scaffold strength.

The mechanical behavior of remodeled ECM scaffolds has also been evaluated in a number of muscular tissue applications. An eight-layer SIS–ECM device used to repair the abdominal body wall (skeletal muscle) of a dog decreased to 50% of its initial strength by 10 days after implantation, but subsequently increased to twice its initial strength by 24 months [85]. After repair of the canine urinary bladder (smooth muscle) with a three-layer SIS–ECM device [92], it was found that the compliance of the remodeled graft increased substantially compared to the device at the time of reconstruction (30-fold difference), to levels that were not statistically different from the normal urinary bladder. Furthermore, the remodeled SIS–ECM showed contractility responses that were similar to the normal bladder as a result of innervated muscle formation. UBM–ECM has been used to repair canine esophagus (smooth muscle) and myocardium (cardiac muscle) [93,94]. For the esophageal repair, the ECM graft rapidly remodeled to approach the pressure-diameter response and compliance of the adjacent native esophagus within 90 days [93]. The newly formed esophageal tissue also showed peristaltic activity, although the muscle contraction was asynchronous with the adjacent native esophagus [93]. In a study of myocardial repair in a canine model, a single layer of ECM (~100 μm) remodeled and showed evidence of contractility and improved regional function of the heart within 8 weeks [94].

These examples show that in the short term after implantation, ECM scaffolds typically show a decrease in strength that is temporally associated with the rapid in vivo degradation of the scaffold [68]. Quantitative assessment of scaffold degradation has been performed in both

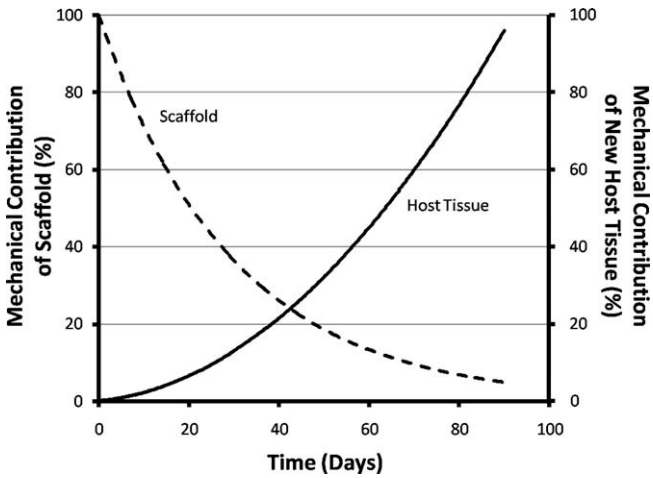

Fig. 6. Schematic of the mechanical contribution of an ECM scaffold over time as it degrades and the mechanical contribution of the new host tissue as it forms during ECM remodeling in the presence of appropriate mechanical loading.

canine urinary bladder and Achilles tendon applications, with similar results in both cases. Degradation of SIS–ECM occurs rapidly, with nearly 50% of the scaffold degraded by 1 month and complete degradation by 3 months [68,95] (Fig. 6). These degradation kinetics provide insight into the rate of new matrix production. In the early phase of remodeling, degradation occurs quite rapidly, before newly deposited ECM has the opportunity to fully organize, thus the initial decrease in scaffold strength. However, once the infiltrating cells have established residence and begin producing site-specific new ECM, rapid scaffold remodeling occurs, with an increase in the strength and site-appropriate mechanical behavior [85,93] (Fig. 6). In the application of esophageal reconstruction, the graft became more compliant, while in the application of tendon or ligament reconstruction, the load to failure increased.

8. Role of mechanical loading on ECM remodeling

In contrast to the fact that most preclinical studies with SIS–ECM successfully utilized early mobilization, some early clinical applications of SIS–ECM used immobilization and non-weight-bearing conditions following surgery to protect the scaffold material during the phase of rapid ECM degradation. To determine the effects of immobilization on the remodeling of an SIS–ECM scaffold, a study was performed in which an SIS–ECM scaffold was used to repair a segmental defect in a rabbit Achilles tendon with different immobilization protocols [96]. Rabbits were separated into five groups. In four groups, a 1.5 cm section of the Achilles tendon was surgically removed and repaired with an SIS–ECM interpositional graft, after which the surgically repaired limb was immobilized for 2 weeks. The first group of animals was sacrificed at the end of 2 weeks as a control. The other three groups had their hind limb braced to allow full range of motion, partial range of motion (60–90° of flexion) or no range of motion for an additional 4 weeks, at which point all animals were sacrificed. In the control group, a sham operation was performed in which the Achilles tendon was exposed, but no defect was created and no SIS–ECM material was implanted. The sham-operated hind limb was immobilized for 2 weeks prior to sacrifice. Histological analysis of the groups with a partial or full range of motion showed dense collagenous connective tissue oriented along the longitudinal axis of the tendon. Spindle-shaped cells were distributed throughout the tendon and oriented along the longitudinal axis of the tendon. The only difference between the partial and full range of motion groups was that fewer cells were found in the center of the remodeled graft with partial motion.

The SIS–ECM remodeling process showed an entirely different morphologic response when no period of remobilization followed immobilization of the limb. In all animals, there was histologic evidence of the SIS–ECM scaffold at the defect site with only limited deposition of disorganized new host connective tissue. The cellularity was limited to the periphery of the graft, with almost no cells found in the middle of the device. In summary, active loading of a remodeling ECM scaffold accelerates the remodeling process and results in the formation of a robust, site-appropriate tissue.

In vitro models are now being developed that will increase our understanding of the role of mechanical loading in the constructive remodeling response observed with ECM scaffolds in vivo. A recent study showed that cyclic uniaxial stretching of fibroblasts seeded on the SIS–ECM scaffold led to increases in the expression of Col I, while the expression of Col III decreased slightly [97]. In vitro studies that have investigated the expression of Col I and Col III by fibroblasts seeded on silicone substrates have shown that expression of both genes is increased in response to mechanical stimuli [98–100]. The SIS–ECM environment in the presence of mechanical loading appears to facilitate a more normal Col III to Col I ratio [101] and a more normal distribution of collagen fibril diameters in the healing tissue [102–104] compared to the collagen fibril diameter that is present in the absence of mechanical loading. In a separate in vitro study that investigated the effects of cyclic uniaxial stretching on fibroblasts seeded on SIS–ECM, the constructs showed increased stiffness, probably due in part to new collagen synthesis and reorganization of existing collagen [106]. The findings of these in vitro studies may partially explain the improved mechanical properties that have been reported in in vivo studies in which physiological mechanical loading was allowed during the remodeling process [88,89]. Future studies may provide additional information on the effects of the mechanical environment of other cell types that have been shown to be important contributors to site-specific remodeling, such as macrophages [78] and bone marrow-derived cells [65,107,108].

9. Functions of solubilized/degraded biological scaffold materials

Separate from the mechanical and structural functions of biological scaffold materials are the biological activities associated with the host tissue response. Biological scaffolds composed of extracellular matrix have been shown to markedly affect angiogenesis, cell proliferation, cell migration and cell differentiation. Such biological activities are typically caused by cell signaling mechanisms that involve soluble molecules. Scaffolds composed of ECM have been shown to be rich in growth factors [31,33], bifunctional molecules such as fibronectin [29] and various types of collagen [25,26], among other structural and functional molecules. More recently, degradation products of the parent ECM molecules have been shown to have significant biological activity themselves [109–112]. Stated differently, there is significant functional activity attributed to the degradation of the native scaffold structure and release of the inherent bioactive constituents. Unlike the mechanical and structural properties that are dependent upon an intact 3-D structure, the biological activities are in large part dependent upon just the opposite; that is, the degradation of the intact 3-D structure.

Processing methods that inhibit degradation of biological scaffolds, such as chemical cross-linking will significantly alter its functional profile and therefore the host tissue response to the biological material. From this perspective, degradable biological scaffolds may be considered as controlled release devices for a variety of functional molecules.

The concept of functionality that is a result of scaffold degradation by necessity implies that mechanical and structural properties will be in a dynamic state. Accurate predictions of the biological functionality will depend upon an understanding of the rate of scaffold degradation, the composition of the materials from which the biological scaffolds are constructed, and the nature of degradation products and their local and systemic distribution following in vivo placement.

10. Summary

The consideration of structural and functional relationships of biological scaffolds includes an understanding of the 3-D architecture of biological materials, the biochemical composition of such materials, the manufacturing processes involved in producing such materials, and, perhaps most importantly, an understanding of the changes that occur with such materials following in vivo placement and host remodeling. Although this work largely describes the structural and functional characteristics of SIS–ECM and UBM–ECM, the principles can apply to other ECM-based scaffold materials with variations depending on the tissue source of the ECM and processing methods. It is now recognized that mammalian extracellular matrix represents an excellent scaffold material suitable for many therapeutic applications. The structural support and biological signaling that allow ECM scaffolds to promote constructive remodeling are likely the same characteristics that have evolved for tissue homeostasis and repair and replacement following injury. The successful utilization of mammalian ECM as a therapeutic device will depend in large part upon our ability to understand and take advantage of the native structure/function relationships of the biological scaffold material.

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