Biologic Scaffolds

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Biologic scaffold materials composed of allogeneic or xenogeneic extracellular matrix are commonly used for the repair and functional reconstruction of injured and missing tissues. These naturally occurring bioscaffolds are manufactured by the removal of the cellular content from source tissues while preserving the structural and functional molecular units of the remaining extracellular matrix (ECM). The mechanisms by which these bioscaffolds facilitate constructive remodeling and favorable clinical outcomes include release or creation of effector molecules that recruit endogenous stem/progenitor cells to the site of scaffold placement and modulation of the innate immune response, specifically the activation of an anti-inflammatory macrophage phenotype. The methods by which ECM biologic scaffolds are prepared, the current understanding of in vivo scaffold remodeling, and the associated clinical outcomes are discussed in this article.

Tissue engineering and regenerative medicine strategies for tissue and organ reconstruction/replacement vary widely but typically involve the in vitro and/or in vivo use of a scaffold material to support cell delivery and/or growth. Such scaffolds can be synthetic in nature, such as the polylactic glycolic acid (PLGA) used in the production of the Dermagraft skin substitute (Debels et al. 2015) or the polyglactin 910 used in the production of the Vicryl Mesh for ventral hernia repair (Levasseur et al. 1979). Alternatively, scaffolds can be composed of naturally occurring materials that are components of extracellular matrix (ECM) such as collagen (Glowacki and Mizuno 2008), laminin (Iorio et al. 2015), and chitosan, among others (Rodriguez-Vazquez et al. 2015), or the entire ECM itself.

Cell-laden autologous, allogeneic, and xenogeneic tissues have been used as viable grafts and transplants for heart valves (Angell et al. 1979; Jamieson et al. 1984), coronary arteries and peripheral vessels (Tice et al. 1976; Bortolotti et al. 1981), skin (Tavis et al. 1978; Peters 1980), the anterior cruciate ligament (Indelicato et al. 1992; Olson et al. 1992), and cornea (Tsai and Tseng 1994), among other clinical applications. The obvious limitations of these graft materials include the foreign antigens present on the allogeneic and xenogeneic cell component and the associated adverse immune response, the limited number of allogeneic do-

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nors, the devascularized state of free autografts, and the associated morbidity of the donor site with autologous grafts.

Although the concept of cell removal from various tissues with retention of the ECM had been described as early as 1975 (Meezan et al. 1975), the production of such materials explicitly for the purpose of recellularization, remodeling, and functional tissue reconstruction was not introduced until the early 1990s. Decellularized forms of human dermis (AlloDerm, LifeCell) and porcine small intestinal submucosa (Restore, DePuy, and Surgisis, Cook Biotech) were provided as surgical mesh materials for general surgery, wound care, and orthopedic soft tissue applications. The fundamental difference between these bioscaffolds composed of intact ECM and the above-mentioned scaffolds composed of individual matrix molecules is the retention of both the complex mixture of structural and functional ECM molecules and the native three-dimensional ultrastructure. Recognition of the natural ligand landscape and three-dimensional matrix ultrastructure of the ECM by responding host cells initiates scaffold degradation and the subsequent exposure, release, and/or formation of effector molecules such as embedded growth factor (GF), matrix-bound vesicles (MBVs) (Huleihel et al. 2016), cytokines, and chemokines. These signaling molecules can have profound biologic activity, including the recruitment of endogenous stem/progenitor cells (Beattie et al. 2009; Reing et al. 2009; Crapo et al. 2014) and the modulation of the innate immune response (Brown and Badylak 2014), the sum of which facilitates a constructive and functional remodeling outcome.

In many respects, it is useful to think of an ECM-based bioscaffold as a surgically placed microenvironmental niche rather than simply as a guiding template or a mechanical support device. ECM bioscaffolds have been shown to favorably influence the mitogenesis, chemotaxis (Bornstein and Sage 2002; Vorotnikova et al. 2010), and differentiation fate (Cheng et al. 2009; Ross et al. 2009; Stern et al. 2009; Allen et al. 2010; Barkan et al. 2010; Cortiella et al. 2010; Sellaro et al. 2010) of cells participating in the scaffold-remodeling process, events that are not typically associated with bioscaffolds composed of individual components of the ECM (such as purified collagen).

Presently, there are well more than 80 commercially available products composed of intact ECM. These bioscaffolds are derived from a wide variety of source tissues and organs, and are typically regulated as devices by the FDA and allowed for use in a wide variety of clinical applications. A partial list of these products can be found in Table 1.

The decision of whether to use a synthetic versus biologic material for a given clinical application depends on factors such as required mechanical strength, history of comorbidities and previous surgeries, the risk of bacterial contamination, and cost. The present article will discuss the methods of production, the current understanding of in vivo tissue remodeling of the biologic scaffolds, and an overview of some of the successes and failures in the clinical application of these biomaterials.

PRODUCTION OF ECM BIOSCAFFOLDS

Extracellular Matrix Composition and Structure

Before describing the manufacture of ECM bioscaffolds, it will be helpful to briefly review the composition and structure of ECM as it exists in situ. Indeed, the ultimate goal of ECM bioscaffold production would be the perfect preservation of both composition and structure, an impossible task. Mammalian tissues are composed of cells, a complex mixture of ions and soluble signaling molecules (i.e., growth factors, cytokines, and chemokines), and structural proteins, all of which are arranged in a tissue-specific three-dimensional ultrastructure ideally suited to maintain homeostasis, participate in mechanotransduction events, and respond to injury. ECM composition and structure vary between tissues; however, many functional and structural molecules are common:

1. Collagen represents ~85% of the dry weight of ECM. More than 20 different types of

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Table 1. Partial list of commercially available biologic scaffold materials

Product	Source species	Source tissue	Application focus	Manufacturer
AlloDerm	Human	Dermis	Soft tissue	LifeCell
AlloMax	Human	Dermis	Soft tissue	Bard Davol
AlloPatch HD	Human	Dermis	Tendon, breast	Musculoskeletal Transplant Foundation
NeoForm	Human	Dermis	Breast	Mentor Worldwide
GraftJacket	Human	Dermis	Soft tissue	Kinetic Concepts
Axis	Human	Dermis	Pelvic organ prolapse	Coloplast
Strattice	Porcine	Dermis	Soft tissue	LifeCell
TissueMend	Bovine	Dermis	Soft tissue	Stryker
Avaulta, CollaMend, XenMatrix	Porcine	Dermis	Soft tissue	Bard Davol
Medeor Matrix	Porcine	Dermis	Soft tissue	Koninklijke DSM
DermaPure	Human	Dermis	Chronic wounds	Tissue Regenix Group
ArthroFlex	Human	Dermis	Soft tissue	Arthrex
Suspend	Human	Fascia lata	Pelvic organ prolapse	Coloplast
Tutoplast Fascia Lata	Human	Fascia lata	Opthalmology	IOP
Meso BioMatrix	Porcine	Mesothelium	Soft tissue	Koninklijke DSM
Miroderm, Miromatrix	Porcine	Liver	Soft tissue	Miromatrix Medical
Veritas, Dura-Guard, Peri-Guard, Vascu-Guard	Bovine	Pericardium	Soft tissue	Baxter Healthcare
IOPatch	Human	Pericardium	Opthalmology	IOP
Unite	Equine	Pericardium	Soft tissue, chronic wounds	Synovis Orthopedic and Woundcare
DurAdapt	Equine	Pericardium	Dura mater	Pegasus Biologics
CopiOs	Bovine	Pericardium	Dentistry	Zimmer
Lyoplant	Bovine	Pericardium	Dura mater	B. Braun Melsungen
Perimount	Bovine	Pericardium	Valve replacement	Edwards Lifesciences
Permacol	Porcine	Porcine dermis	Soft tissue	Tissue Science Laboratories
Oasis, Surgisis, BioDesign, Durasis, Stratasis	Porcine	Small intestine	Soft tissue	Cook Biotech
Restore	Porcine	Small intestine	Soft tissue	DePuy Orthopaedics
FortaFlex	Porcine	Small intestine	Soft tissue	Organogenesis
CorMatrix ECM	Porcine	Small intestine	Pericardium, cardiac tissue	CorMatrix Cardiovascular
CuffPatch	Porcine	Small intestine	Rotator cuff	Arthrotek
AxoGuard	Porcine	Small intestine	Nerve	AxoGen
MatriStem	Porcine	Urinary bladder	Soft tissue	ACell

collagen with specific functional attributes have been described. Type I collagen is the major structural protein, providing the strength and load-bearing capability of most tissues. Collagen, with other proteins such as laminin, provides anchoring sites and barrier functions for cells. Type IV and type VII collagen and laminin are major components of the basement membrane[5] of blood vessels and epidermis and ensure the anchorage of endothelial cells (ECs) and keratinocytes, respectively (Akiyama 1996).

[5]Basement membrane is a dense three-dimensional organization of the ECM that functions as a molecular filter and substrate for endothelial and epithelial cells. Some ECM bioscaffolds preserve the basement membrane.

2. Fibronectin is the second most abundant protein of the ECM. It is a dimeric molecule of 250,000 MW subunits containing binding domains for many other ECM proteins, such as collagen. Furthermore, fibronectin is an important cell-adhesion molecule because of the presence of RGD (Arginine–Glycine–Aspartate) domains that interact with the cell membrane integrin α5β1 (Plow et al. 2000).

3. Laminin is a trimeric cross-linked glycoprotein typically present in the basement membrane that facilitates the interaction between cells and other ECM components such as heparin sulfate and collagen (Battaglia et al. 1992; Plow et al. 2000). For example, laminin is required for the normal function of the dystrophin–glycoprotein complex that is responsible for skeletal muscle contraction (Gumerson and Michele 2011).

4. Glycosaminoglycans (GAGs) are negatively charged polysaccharides that bind and retain water and water-soluble molecules, such as GFs (Alberts et al. 2002). GAGs also provide for extraordinary mechanical loading capacity by distributing forces within the tissue, an example of this is hyaline cartilage. Hyaluronic acid (a GAG) in an alternative configuration is widely used for the therapeutic delivery of cells and soluble bioactive molecules, and has been investigated separately as a scaffold for regeneration of cartilage and bone, for stroke repair in central nervous system and for the formation of vessel networks, among others (Collins and Birkinshaw 2013).

5. Growth factors are bioactive molecules responsible for the activation of a plethora of cellular pathways that modulate cell survival, proliferation, and differentiation. The presence of vascular endothelial growth factor (VEGF) (Wang et al. 2013b), fibroblast growth factor (FGF) family (Hoganson et al. 2010), epidermal growth factor (EGF), transforming growth factor β (TGF-β), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) (Soto-Gutierrez et al. 2011; Ren et al. 2013), platelet-derived growth factor (PDGF), and nerve growth factor (NGF), among others, within ECM bioscaffolds has been shown (Meezan et al. 1975; Bissell et al. 1982; Gilbert et al. 2006; Badylak et al. 2008a; Crapo et al. 2012; Wolf et al. 2012).

6. MBVs are extracellular vesicles from 10 nm to 600 nm in size embedded within the ECM. MBVs are highly stable bodies that protect their microRNA (miRNA) cargo from degradation and are resistant to the enzymes and detergents used in tissue decellularization protocols (Huleihel et al. 2016). miRNAs are small noncoding single-stranded RNA molecules of around 22 nucleotides in length that affect multiple biological processes through posttranscriptional gene regulation (Ling et al. 2013). miRNAs are potent yet complex regulators of biological processes because of their nature: one miRNA molecule can regulate multiple genes and one gene can be regulated by multiple miRNAs. MBVs have been shown to mimic/recapitulate some in vitro biologic effects of the ECM such as promoting neurite extensions in neuroblastoma cells and induction of *FIZZ-1* expression in macrophages (Huleihel et al. 2016).

The presence and integrity of these molecules, as well as their three-dimensional ultrastructural organization, greatly affect the downstream clinical outcome when ECM bioscaffolds are used for tissue repair and reconstruction. For example, decellularization of skeletal muscle in ionic detergents such as sodium dodecyl sulfate (SDS) leads to the production of an ECM, which effectively maintains the three-dimensional structure of each myofiber (Perniconi et al. 2011), but markedly disturbs the molecular organization of the basement membrane (Faulk et al. 2014). Porcine urinary bladder matrix (UBM) produced without the use of ionic detergents retains an intact basement membrane that prevents cell penetration and facilitates the formation of a confluent layer of cells on its surface (Gilbert et al. 2006).

The spatial arrangement of structural molecules not only determines cell penetration, attachment, and disposition within the ECM bioscaffold, but is also critical for its mechanical properties. For example, small intestinal submucosa (SIS)-ECM shows a preferred orientation of the collagen fibers along the longitudinal axis of the small intestine, an orientation well suited to the peristaltic action of source tissue. This spatial arrangement of collagen fibers within the ECM results in a polarization of the mechanical properties with greater strength along the preferred fiber direction (Badylak et al. 2009). Thus, the method of production of ECM from source tissues and further processing to obtain a sterile ECM bioscaffold, which is packaged for adequate shelf life, would ideally preserve as much as possible the integrity of the above-mentioned molecules as well as the ultrastructure of the native ECM.

Preparation of ECM Bioscaffolds

The preparation of an ECM bioscaffold requires removal of cells (i.e., decellularization) from source tissue. Because there is no standard by which to determine whether a bioscaffold is decellularized, the term has been used indiscriminately. Relatively stringent criteria for decellularization have been suggested (Crapo et al. 2011), although these criteria may be too conservative for some tissues and applications. It is logical to assume that any form of cell remnant is likely to stimulate an adverse (proinflammatory) tissue response by the recipient. Three criteria have been suggested as metrics of decellularization: (1) lack of visible nuclear material in tissue sections stained with 4′,6-diamino-phenylindole (DAPI)[6] and hematoxylin and eosin staining[7]; (2) less than 50 ng of double-strand DNA per mg of ECM dry weight; and (3) less than 200 base pairs fragment length of remnant DNA (Crapo et al. 2011). A limited number of studies exist that correlate criteria for decellularization (e.g., DNA remnant concentration) with the intensity and characteristics of the host tissue response (Keane et al. 2012, 2015b).

It should be noted that all methods of cell removal from source tissue will adversely affect ECM composition and cause some degree of ultrastructure disruption. Minimization, rather than complete avoidance, of these undesirable effects is the realistic objective of decellularization. Commonly used decellularization methods include physical, chemical, and enzymatic agents. A brief overview of the respective effects of each method on cell and matrix constituents is provided in Table 2. A detailed description of each method can be found in recent articles (Crapo et al. 2011; Keane et al. 2015b).

The most effective agents for decellularization of each source tissue are determined by factors such as the cellularity of the tissue (e.g., liver vs. tendon), density of the matrix (e.g., dermis vs. adipose tissue), lipid content (e.g., brain vs. urinary bladder), and thickness (e.g., dermis vs. pericardium). For thin tissue laminae such as UBM, intestine, pericardium, and amnion, freeze and thaw cycles or mechanical disruption followed by treatment with nonionic or zwitterionic detergents is typically sufficient to achieve an efficient decellularization (Crapo et al. 2011). Thicker and denser laminar tissues, such as dermis, require longer exposure to decellularization agents and use of more harsh reagents such as SDS and trypsin (Crapo et al. 2011). Tissues rich in lipid content (e.g., adipose tissue, brain, and pancreas) require the use of lipid solvents such as alcohols. Isolation of the ECM from tissues typically involves immersion in decellularization agents with agitation for a time that varies with the nature and structure of the tissues. Perfusion decellularization through the vasculature is an attractive alternative to immersion when use of access vessels is possible and practical (Baptista et al. 2009). Porcine liver, kidney, pancreas, and intestine have been decellularized by perfusion techniques (Baptista et al. 2009). Livers from many species (rat, mouse, and pig) have been

[6]4′,6-diamino-phenylindole (DAPI) counterstains chromosome by binding AT regions of DNA and emits blue fluorescence.

[7]Hematoxylin and eosin are the most common histologic stains. Hematoxylin is an acidophilus dye and binds negatively charged DNA, conferring dark purple color to nuclei, whereas eosin is a basophilic dye and binds cytoplasm components that appear pink.

Table 2. Decellularization agents

Technique	Mode of action	Effect on extracellular matrix (ECM)
Chemical Agents		
Acids and bases	Solubilizes cytoplasmic components of cells, disrupts nucleic acids, and often denatures proteins	May damage collagen, glycosaminoglycan (GAG), and growth factors (GFs)
Hypotonic and hypertonic solutions	Cell lysis by osmotic shock, disrupts DNA–protein interactions	Effectively lyses cells, but does not effectively remove cellular residues
Nonionic detergents (e.g., Triton X-100)	Disrupts DNA–protein, lipid–lipid, and lipid–protein interactions, to a lesser degree disrupts protein–protein interactions	Efficacy dependent on tissue; more effective cell removal from thin tissues, some disruption of ultrastructure and removal of GAG, less effective than SDS
Ionic detergents	Solubilizes cell and nucleic membranes, tends to denature proteins	
Sodium dodecyl sulfate (SDS)		Effectively removes nuclear remnants and cytoplasmic proteins from dense tissues, tends to disrupt ultrastructure, removes GAGs and GFs, and damages collagen
Sodium deoxycholate		Mixed results with efficacy dependent on tissue thickness, some disruption of ultrastructure and removal of GAG
Zwitterionic detergents (e.g., CHAPS)	Show properties of nonionic and ionic detergents.	Effectively removes cells with mild disruption of ultrastructure in thin tissues
Solvents		
Alcohols and acetone	Cell lysis by dehydration, solubilizes and removes lipids	Effectively removes cells from dense tissues and inactivates pyrogens, but cross-links and precipitates proteins, including collagen
Tributyl phosphate (TBP)	Forms stable complexes with metals, disrupts protein–protein interactions	Mixed results with efficacy dependent on tissue, dense tissues lost collagen with minimal impact on mechanical properties
Biologic Agents		
Enzymes		
Nucleases	Catalyzes the hydrolysis of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue, potential to invoke an immune response
Trypsin	Cleaves peptide bonds on the C-side of arginine and lysine	Prolonged exposure can disrupt ECM ultrastructure Removes ECM constituents such as collagen, laminin, fibronectin, elastin, and GAGs
Chelating agents (EDTA, EGTA)	Bind metallic ions thereby disrupting cell adhesion to ECM	Typically used with enzymatic methods, ineffective when used alone
Physical and miscellaneous agents		
Temperature (freezing and thawing)	Intracellular ice crystals disrupt cell membrane	Ice crystal formation can disrupt or fracture ECM
Direct application of force	Removal of tissue eliminates cells and force can burst remaining cells	Direct ECM damage

Continued

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Table 2. *Continued*

Technique	Mode of action	Effect on extracellular matrix (ECM)
Pressure	Pressure can burst cells and aid in removal of cellular material	Direct ECM damage
Electroporation	Pulsed electrical fields disrupt cell membranes	Direct ECM damage
Techniques to apply agents		
Agitation	Facilitate chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM
Perfusion	Facilitates chemical exposure and removal of cellular material	Pressure associated with perfusion can disrupt ECM
Pressure gradient across tissue	Facilitates chemical exposure and removal of cellular material	Pressure gradient can disrupt ECM

Data from Crapo et al. (2011) and Keane et al. (2015b).

decellularized by perfusion using various protocols (Faulk et al. 2015). Recently, a study involving the decellularization of 39 human hearts by perfusion of 1% SDS for 4 to 8 days showed that the decellularized organ promotes cardiocyte gene expression in implanted stem cells and allows the organization of cardiomyocytes into nascent muscle with electrical coupling (Sanchez et al. 2015). A sodium deoxycholate-based perfusion protocol led to the decellularization of porcine lung and trachea, which maintained the structural and biomechanical integrity of the native tissue (Weymann et al. 2015).

ECM Bioscaffold Configurations

Biologic scaffold materials are typically configured in shape and size to maximize their use for given clinical applications. Only few bioscaffold materials maintain a hydrated state throughout the decellularization and sterilization process. Maintenance of water content better preserves tissue architecture avoiding collapse of the collagen fibers, and promoting cell infiltration and attachment better than bioscaffolds that have been rehydrated after dehydration (Freytes et al. 2008c). On the other hand, hydrated ECM bioscaffolds are subject to the continuous loss of soluble bioactive molecules, such as growth factors, thus representing a potential disadvantage for clinical applications (Reing et al. 2010).

Sheets and powder forms of ECM bioscaffolds are usually dehydrated by lyophilization before terminal sterilization. The lyophilization process makes ECM bioscaffolds stable and easy to handle, minimizes the loss of soluble molecules, and allows for prolonged storage and preservation. Disadvantages of lyophilization include collagen fiber perturbation that can affect cell growth in vitro (Freytes et al. 2008c), and cause the loss of the ability for full rehydration as a result of a more compacted fiber arrangement (Curtil et al. 1997; Hafeez et al. 2005). An alternative method for dehydration of ECM scaffolds is vacuum pressing. Evaporation of water is favored at low pressure and ice is formed in a thin film (Kasper and Friess 2011). This method allows for the lamination of multiple sheets of ECM, which is used to increase strength and/or design the final bioscaffold for specific mechanical behavior (Reing et al. 2010).

The method of processing and resulting configuration of an ECM bioscaffold should be carefully considered for each clinical application. The following configurations are common and some examples are shown in Figure 1:

1. Single-layer sheet. UBM and SIS are the most studied ECM bioscaffolds in a single-layer configuration. Both single-layer SIS-ECM and UBM-ECM are able to support EC attachment (Badylak 2004), and both have a distinct "sidedness" with respect to surface morphology. Single-layer UBM-ECM has a continuous basement membrane on one side and a more porous irregular surface on the opposite side. SIS-ECM has a

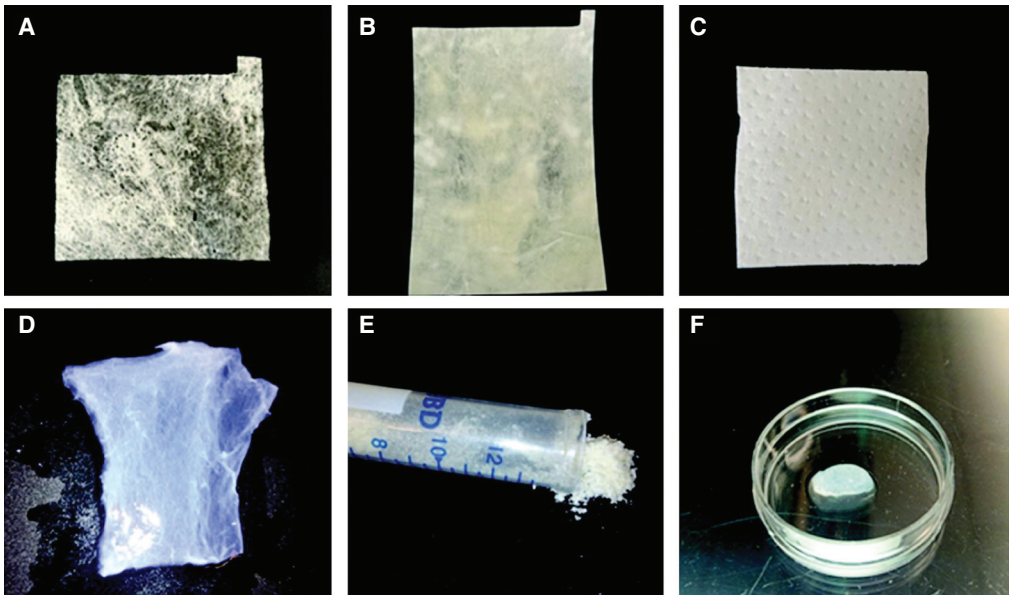

Figure 1. Different configurations of extracellular matrix (ECM) bioscaffolds. Single-layer lyophilized sheets of (*A*) liver-ECM, (*B*) urinary bladder matrix (UBM)-ECM, and (*C*) dermal-ECM. (*D*) Decellularized hydrated UBM-ECM, (*E*) UBM-ECM powder, and (*F*) hydrogel.

compact relatively dense smooth surface on one side and a more open, irregular opposing surface (Brown et al. 2010).

2. Multilayer sheet. Single-layer sheets of ECM represent a good in vitro model to study cellular response. However, when ECM bioscaffolds are used for load-bearing applications, such as tendon reconstruction and body wall repair, single-layer sheets are insufficient to accommodate physiologic loads (Valentin et al. 2006; Gilbert et al. 2007b, 2008; Turner et al. 2012). Multilayered ECM devices can be tailored to increase the mechanical strength (Freytes et al. 2004, 2005). For example, the bioscaffold configured for the repair of the rotator cuff[8] could support a uniaxial ultimate tensile strength of ~1000 N, as in the Restore ECM bioscaffold, which consists of 10 layers of SIS-ECM (Badylak et al. 2008a). Mechanical strength can also be increased by cross-linking the structural components of the scaffold, that is, collagen, with chemicals such as glutaraldehyde, carbodiimide, and hexamethylene-diisocyanate (Lolmede et al. 2009) or by nonchemical methods. Cross-linking has the deleterious effect of reducing the in vivo degradation rate of the ECM bioscaffold, inducing a proinflammatory host tissue response and mitigating a constructive and functional remodeling response (Valentin et al. 2006; Badylak and Gilbert 2008; Badylak et al. 2008b).
3. Powder. ECM powder is obtained by comminuting lyophilized sheets of ECM (Gilbert et al. 2005). Each particle retains the ultrastructural characteristics of the parent ECM (Gilbert et al. 2005) and this process greatly increases the surface area of the ECM bioscaffold enhancing interaction with host cells. In addition, the powder configuration allows for the delivery of ECM as a suspension/emulsion by minimally invasive injection methods.
4. Hydrogel. Typically, hydrogels are derived by the gelation (i.e., a process of polymerization induced by physical–chemical agents) at 37°C of a soluble form of the ECM, called

[8]Rotator cuff is the group of four tendons and muscles that connect the humerus to the scapula.

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pregel (Freytes et al. 2008a). Preparation of a gel derived from UBM, colon (Keane et al. 2015a), cardiac, and skeletal muscle ECMs (Ungerleider et al. 2015), and demineralized bone (Sawkins et al. 2013) has been described. ECM hydrogels from different source tissues have been shown to support in vitro cell growth of different cell types, including ECs, smooth muscle, myoblasts, cardiomyocytes (Freytes et al. 2008a), cardiovascular progenitor cells (Williams et al. 2015), and primary calvarial cells (Sawkins et al. 2013). ECM hydrogels have also been shown to support myogenesis (DeQuach et al. 2012; Wolf et al. 2012) and to recruit cardiomyocytes in vivo (Singelyn et al. 2012).

5. Hybrid. ECM bioscaffold properties, including strength and mechanical behavior, are dependent on tissue source and processing methods, and are not easy to control. The necessity to manipulate the material characteristics, especially mechanical properties, and keep the biological activity of the biomaterial, has led to the development of hybrid materials: a combination of ECM bioscaffolds and synthetic materials. One example of a synthetic polymer–ECM hybrid scaffold is the combination of powdered SIS-ECM with poly(D,L-lactide-co-glycolide) to create tissue-engineered bone (Lee et al. 2004) and intervertebral disc (Kim et al. 2014). Another example includes the combination of UBM-ECM with a poly(ester-urethane)urea (PEUU) to create a hybrid scaffold with increased stiffness, strength, and strain when compared with lyophilized UBM-ECM sheets (Stankus et al. 2008).

ECM Bioscaffold Sterilization

Clinical application of biomaterials as medical devices requires terminal sterilization. The use of chemical agents as disinfectants (e.g., hydrogen peroxide or peracetic acid) can be detrimental for ECM bioscaffolds as a result of oxidation of ECM proteins, GAGs, and collagen fibers, which develop altered cross-linking patterns (Hodde et al. 2007). Heat-based sterilization methods cannot be applied to ECM bioscaffolds because most ECM proteins are irreversibly denatured at 60°C–65°C. Alternative methods of terminal sterilization include ethylene oxide[9] (ETO) and high-energy radiation (electron beam or γ radiation), both of which can have a negative effect on the ECM bioscaffolds. ETO can react with the free amine groups of collagen, affecting the mechanical properties of ECM bioscaffolds, and high-energy radiation damages protein chains and promotes the formation of free radicals (Proffen et al. 2015). Supercritical CO_2 sterilization[10] has been investigated for sterilization of collagen scaffolds and showed minimal compromise of mechanical properties (Bernhardt et al. 2015).

Sterilization is obviously a critical and necessary step and the method should be carefully chosen depending on the intended clinical application of the ECM bioscaffold.

MECHANISMS OF ECM BIOSCAFFOLD REMODELING

Regardless of the size, shape, physical properties, or mechanical strength of a biologic scaffold, the ultimate determinant of success is the host response to the scaffold following implantation. The term "dynamic reciprocity" (Bissell et al. 1982) perfectly describes the interaction between host (recipient) cells and ECM. In fact, the ECM strongly influences cell behavior and phenotype, and cells, in turn, continuously produce, degrade, and remodel the ECM. This reciprocal process is fundamental to tissue development, homeostasis, and wound

[9]Ethylene oxide is a direct alkylating agent that reacts with cellular constituents of organisms such as nucleic acid and functional proteins, including enzymes, which leads to denaturation (Mendes et al. 2007).

[10]Supercritical CO_2 is the state of CO_2 that reaches the supercritical point of temperature (31.1°C) and pressure (7.39 MPa), showing properties and behavior similar to both a liquid and a gas. It has antimicrobial effects at high pressures while still being otherwise nontoxic, noninflammable, nonhazardous, generally chemically inert, and cost effective (Zhang et al. 2006).

healing (Thomas 2001; Rozario and DeSimone 2010).

The host response following implantation of any biomaterial, including ECM materials, begins with the Vroman effect,[11] followed by activation of the innate immune system, including dendritic cells, neutrophils, and macrophages, among others (Badylak and Gilbert 2008; Christo et al. 2015). Depending on the implanted material, activation of the adaptive immune system, which involves lymphocytes (T and B cells), also occurs (Franz et al. 2011; Mora-Solano and Collier 2014).

Degradation of ECM bioscaffold begins immediately after implantation. Degradation can be caused by the action of proteases that are present in the injured tissue (Thomas 2001) or secreted by responding cells (Valentin et al. 2009). The temporal course of degradation of dermal ECM, UBM-ECM, and SIS-ECM has been determined by the use of a ^{14}C isotope incorporated into the collagen of donor animals (Gilbert et al. 2007a). SIS-ECM and UBM-ECM are degraded within 60–90 days of implantation, whereas the more densely organized dermal ECM degraded more slowly over a period of at least 24 months (Carey et al. 2014; Costa et al. 2016). The rate of degradation is likely dependent, in part, on the anatomic site of placement. The degradation process is necessary to promote a constructive remodeling outcome, rather than formation of a fibrous capsule as simple scar tissue. ECM degradation also appears necessary to drive the inflammatory response toward resolution, avoiding the presence of a chronic inflammatory scenario. ECM degradation products produced during tissue remodeling, called cryptic peptides (Anderson et al. 2008; Agrawal et al. 2011a,b; Daly et al. 2012), together with the release of the GFs retained in the ECM (Hodde et al. 2001; Rieder et al. 2004; Badylak 2014; Cavallo et al. 2015), are believed to be responsible for many aspects of ECM-mediated bioactivity. Cryptic peptides are either created or exposed after the proteolysis of ECM components such as collagen, laminin, and fibronectin and their bioactivity is not present in the parent molecule (Anderson et al. 2008; Daly et al. 2012). An example is Arg-Gly-Asp peptide, which is part of fibronectin and collagen and, when exposed, promotes cell adhesion (Brown and Badylak 2014). Cryptic peptides have been shown to contribute to angiogenesis (Stupack and Cheresh 2002; Li et al. 2004; Vorotnikova et al. 2010; Burns et al. 2011; Sicari et al. 2012a), recruitment of endogenous stem/progenitor cells (Heissig et al. 2002; Veevers-Lowe et al. 2011), and promotion of an anti-inflammatory M2 macrophage phenotype (Badylak et al. 2008b; Brown et al. 2009; Keane et al. 2012). The processes involved in scaffold/tissue remodeling are summarized in Figure 2.

Modulation of the Inflammatory Response

The immune response to ECM bioscaffolds derived from allogeneic or xenogeneic source tissue is only partially understood. It is important to note, however, that no scaffold, either synthetic or biologic (even autologous ECM), is inert. The immune response, especially the innate immune response, is largely determinative of the clinical outcome.

ECM bioscaffolds are devoid of cells and most cell remnants. Because the large majority of antigenic epitopes are cell-associated, thorough decellularization should ideally result in a bioscaffold comprised primarily of ECM constituents. The amino acid sequence homology of matrix constituents across species is extraordinarily high (Constantinou and Jimenez 1991; Exposito et al. 1992), and the immune response in recipients of such scaffolds has not been adverse in either preclinical studies or in clinical practice when the scaffold used has been adequately decellularized and has not been crosslinked by chemical methods. The nonadverse nature of the adaptive response was shown in a nonhuman primate study that evaluated the systemic and local tissue effects following repeated autologous, allogeneic, or xenogeneic

[11]The Vroman effect refers to the competitive binding of protein on surfaces, where adsorbed protein on surfaces can be replaced by other upcoming proteins with a higher affinity. This effect is implied in blood platelet adhesion and clotting, in which the plasma fibrinogen, which first binds to surfaces, is later replaced by proteins with a higher molecular weight (Vroman et al. 1980).

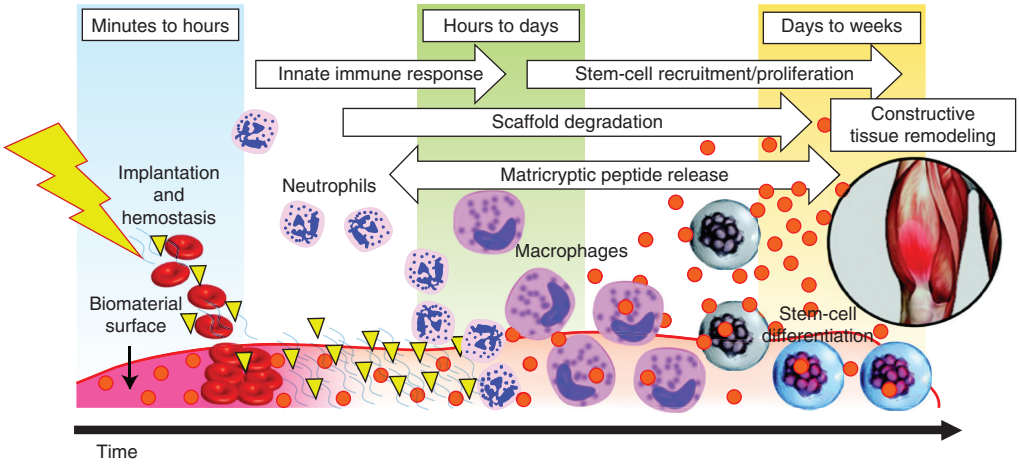

Figure 2. Biomaterial/host interactions timeline.

ECM bioscaffold implantation (Daly et al. 2009). There are well-recognized antigens such as the Gal epitope and *N*-glycolylneuraminic acid (Neu5Gc) both on the cell surface and within the ECM of most species, including porcine (Galili et al. 1988; Yeh et al. 2010; Padler-Karavani and Varki 2011). However, the concentration of these molecules in the ECM is low and, although a serologic response can be detected, attempts to identify any clinically detrimental adaptive immune response to these antigens following implantation have failed (McPherson et al. 2000; Allman et al. 2001, 2002). In fact, preclinical and clinical studies with SIS-ECM have shown a predominant Th2 cytokine and antibody isotype profile following implantation (Allman et al. 2001; Ansaloni et al. 2007). The Th2-like phenotype is associated with an anti-inflammatory, regulatory, and constructive tissue-remodeling response. The absence of documented immune-mediated rejection events in the several million human patients that have been implanted with such materials, including patients with repeated implants, during the past 15 years is testament to the benign nature and safety of ECM bioscaffolds. A recent report of 91 recipients of decellularized allogeneic pulmonic valves failed to show any evidence of a long-term systemic response (Neumann et al. 2014). In summary, the adaptive immune response to allogeneic and xenogeneic ECM bioscaffold is benign and, in fact, may even be constructive.

The innate immune response, which generally precedes the adaptive response, plays a crucial role in the remodeling induced by ECM bioscaffolds. An overview of the default mammalian response to tissue injury can facilitate an understanding of the role of the innate immune response to ECM bioscaffolds.

Following soft tissue injury, the innate immune system is the first to respond with polymorphonuclear cells (PMNs) and monocyte/macrophages. The previous concept of the macrophage as a primary scavenger of cell debris and mediator of chronic inflammation has been replaced by recognition of the remarkable plasticity of this cell type. The macrophage is now recognized as an orchestrator of either a proinflammatory state or a constructive wound-healing state (Novak et al. 2014; Tidball et al. 2014). Functional subsets of monocytes/macrophages are now recognized along a spectrum that ranges from a proinflammatory M1-like phenotype to the M2-like phenotype that has anti-inflammatory, homeostatic, wound healing, and/or regulatory properties (Mantovani et al. 2002, 2004; Mosser 2003; Gordon and Taylor 2005; Mosser and Edwards 2008; Hume 2015). Macrophage polarization toward distinct effector functions is regulated, at least in part, by microenvironmental cues (Mosser and Edwards

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2008). The extent to which ECM bioscaffolds provide or influence these microenvironmental cues determines downstream healing outcomes. A positive correlation between the local tissue ratio of M2-like macrophages to M1-like macrophages (M2:M1) and constructive, functional outcomes has been shown for a large number of commercially available bioscaffold mesh devices (Brown et al. 2009). Normal wound healing in most tissues involves an initial dominance of proinflammatory M1-like macrophages, which then transition into a more prominent M2-like profile (Schwartz 2010; Fraccarollo et al. 2012; Tidball et al. 2014). This transition of macrophage phenotype suppresses and limits the proinflammatory microenvironment and facilitates constructive wound-healing events, such as neomatrix deposition. Absence of this transition from the M1-like to M2-like phenotype results in a persistent and chronic inflammatory state (Tidball and Villalta 2010). Although injured cells following trauma or infection release recognized promoters of inflammation and thus induce an early proinflammatory macrophage phenotype, the in vivo molecular cues that signal the transition to the M2-like phenotype are not well understood. There is an increasing body of evidence that the ECM plays a key role in mediating this transition. It is plausible and logical that ECM bioscaffolds facilitate the same M2-like phenotypic macrophage phenotype.

Macrophages are not only supportive but necessary for in vivo degradation and remodeling of ECM bioscaffolds (Valentin et al. 2009). Preclinical studies have shown a strong positive correlation between ECM bioscaffold degradation, an increased M2:M1 ratio, and constructive remodeling outcomes (Badylak et al. 2008b; Brown et al. 2012; Sicari et al. 2012b). During the process of scaffold degradation, there is generation of bioactive cryptic peptides (Anderson et al. 2008; Agrawal et al. 2011a,b; Daly et al. 2012) and a release of embedded GFs, cytokines, and MBVs from the normal matrix as described earlier. In contrast, chemical crosslinking of scaffolds, as it is performed with some commercially available ECM bioscaffolds (see Table 1), prevents degradation (Badylak et al. 2008b; Brown et al. 2009; Tierney et al. 2009), results in a chronic M1-like proinflammatory response, lack of positive remodeling outcomes, and deposition of scar tissue.

Macrophages are a central component of the innate immune response and are critical determinants of the remodeling outcome. Degradation products of ECM bioscaffolds can promote an increased M2:M1 ratio and are associated with favorable constructive remodeling events and outcomes. Despite identifying the essential role of macrophages for remodeling, the specific signaling molecules and intracellular pathways involved in such events remain to be determined.

Stem/Progenitor Cell Recruitment

In addition to the effects of ECM bioscaffold on the host immune response, there is strong evidence that endogenous stem/progenitor cells are recruited to the site of ECM bioscaffold remodeling and may subsequently participate in anatomic site appropriate differentiation. Within 7–14 days following in vivo implantation, ECM bioscaffolds show robust angiogenesis and the presence of a dense mononuclear cell population including macrophages and stem/progenitor cells (Turner et al. 2010; Agrawal et al. 2011b; Sicari et al. 2014). Within 28–90 days, complete degradation of the ECM bioscaffold typically occurs. Placement of either porcine UBM-ECM or SIS-ECM in the injured rat myocardium has been associated with the presence of cardiomyocyte progenitor cells (Kelly et al. 2009; Zhao et al. 2010). The use of UBM-ECM in patients with volumetric muscle loss[12] (VML) is associated with the recruitment of perivascular stem cells[13] to the site of scaffold

[12]Volumetric muscle loss (VML) is the traumatic or surgical loss of skeletal muscle with resultant functional impairment (Grogan et al. 2011).

[13]Perivascular stem cells surround blood vessels and present differentiation potential similar to the mesenchymal stem cells (i.e., are able to differentiate toward osteogenic, adipogenic, and chondrogenic cell lineages). Perivascular stem cells express adhesion molecules such as CD146 but lack endothelial haematopoietic markers such as CD34 (Crisan et al. 2012).

degradation and the subsequent formation of functional striated skeletal muscle (Sicari et al. 2014). The presence of a population of interstitial stem cell in skeletal muscle has been shown within implanted skeletal muscle ECM for whole muscle reconstruction (Perniconi et al. 2011).

The ECM bioactive molecules able to promote the recruitment of stem/precursor cells at the site of scaffold remodeling as well as the origin of these cells remain largely unknown. Virtually all tissues have a resident stem/progenitor cell population but the endogenous cues that signal these reserve cells to enter a proliferative or differentiation state are only partially understood. In vitro, ECM degradation products are chemoattractant for endogenous stem/progenitor cells (Brown et al. 2009; Brown and Badylak 2014; Crapo et al. 2014). It is plausible that at least some of the cell signaling molecules attracting stem/progenitor cells would reside within tissue ECM in a precursor form. Degradation of the native ECM as occurs following injury or degradation of ECM bioscaffold following surgical implantation would then release such factors (e.g., cryptic peptides, MBVs, and GFs) as part of the wound-healing response. Cryptic peptides of ECM generated by in vitro methods have shown mitogenic, chemotactic, and differentiation properties for a variety of differentiated cells (Agrawal et al. 2011b) and stem cells (Brennan et al. 2008; Crisan et al. 2008; Reing et al. 2009; Crapo et al. 2014). In vivo degradation products have also been shown to have chemotactic properties for stem/progenitor cells (Beattie et al. 2009). However, the specific and definitive role of selected ECM ligands and/or cryptic peptides in the context of ECM bioscaffolds and the constructive remodeling process in vivo has yet to be determined.

Mechanisms by which ECM bioscaffolds recruit stem/progenitor cells are not understood. Cell migration in response to concentration gradient of soluble molecules is called chemotaxis. A cryptic peptide derived from collagen IIIα showed chemotactic activity for many cells, including cortical neural stem cell and myoblasts in vitro and for cells showing stem-cell markers in vivo (Agrawal et al. 2011b). Induction of both proliferation and differentiation of neural stem cells by UBM-ECM has been shown (Wang et al. 2013a), and neural cell differentiation was later reproduced in neuroblastoma cells with MBVs isolated from UBM-ECM (Huleihel et al. 2016). Primitive precursor cells seeded in decellularized kidney proliferated and expressed markers for site-appropriate differentiation (Ross et al. 2009). Murine embryonic stem cells differentiated into epithelial and endothelial lineages within lung ECM (Cortiella et al. 2010). Induction of differentiation of committed precursors has also been shown. For example, skeletal muscle ECM promoted proliferation and differentiation of myoblasts (Stern et al. 2009). It is important to note that ECM bioscaffolds derived from different source tissues can affect the same cell type differently. For example, central nervous system ECM promotes proliferation, migration, and differentiation of glial cells, but UBM-ECM inhibits migration of the same cells while still promoting proliferation and differentiation (Crapo et al. 2012). Similarly, the same ECM bioscaffold can have different effects on different cell types; for example, UBM-ECM promotes migration and proliferation of blastemal cells but does not have the same bioactivity on ECs (Reing et al. 2009).

Other Biologic Activities

Vascularization and innervation are important components of a constructive and functional clinical outcome. During vessel formation in adult and embryos, direct cell–ECM interaction is necessary to promote EC proliferation and survival via integrin-mediated signals. EC migration and vessel morphogenesis (i.e., lumen formation) occurs in part by distributing the required tension through collagen fibers (Senger and Davis 2011). GFs and cytokines that stimulate angiogenesis are embedded in the ECM in the appropriate naturally occurring concentration to guide blood vessel sprouting (Senger and Davis 2011). The angiogenic potential of SIS-ECM has been shown in vitro for human dermal microvascular EC, partially mediated by the presence of VEGF (Hodde et al.

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2001). SIS and dermal ECM implanted subcutaneously showed greater vascularization during remodeling than a scaffold composed of collagen–chondroitin sulfate–hyaluronic acid (Liu et al. 2011). Formation of new blood vessels at the site of the graft 6 months after the implantation of ECM bioscaffold was observed during skeletal muscle regeneration in both mice and human patients treated for VML (Sicari et al. 2014). In the same study, the presence of functionally innervated myofibers within the defect area was shown. Agrawal et al. (2009) showed mature and immature nerve within the area of ECM scaffold remodeling to be a common feature between 1 and 3 months after surgery.

Mechanical Properties of ECM Bioscaffolds

Cell migration, differentiation, and function are guided by both biochemical and mechanical signals. Focal adhesion complexes and focal adherens junctions represent the sensory apparatus for mechanical signals mediated by the ECM (Moeendarbary and Harris 2014). A paradigmatic tissue for the importance of mechanical signals on cell–matrix homeostasis is the hyaline cartilage of the joints. Chondrocytes of the hyaline cartilage subject to normal mechanical stress activate anabolic pathways, secreting ECM components such as GAGs and proteoglycans and promoting the correct collagen fiber orientation. In contrast, absent or traumatic loading causes the activation of catabolic pathways, including the secretion of enzymes that degrade the ECM and collagen. Integrins, transmembrane proteins that regulates cell–ECM interaction, together with soluble molecules (i.e., cytokines and GFs), are mediators of the mechanical signals from the environment to the cells (Ramage et al. 2009). Thus, ECM can mediate mechanical signals to affect cell behavior and cells can respond to mechanical stimuli by modifying the ECM (another example of dynamic reciprocity).

The mechanical behavior of ECM bioscaffolds during the process of remodeling is affected by many factors such as the degradation rate, the forces acting on the tissue, and the extent to which cells infiltrate and deposit new ECMs. Soon after implantation, degradation of ECM bioscaffold begins and, as a result, there is an initial decrease in the strength of the ECM bioscaffold (Gilbert et al. 2007b; Costa et al. 2016). However, once infiltrating cells begin to secrete and organize the appropriate new ECM and tissue remodeling occurs, there is an associated increased strength and site-specific mechanical behavior of the graft (Badylak et al. 2001, 2005; Costa et al. 2016).

CLINICAL OUTCOMES AND FACTORS THAT AFFECT ECM BIOSCAFFOLD REMODELING

The process of ECM bioscaffold-mediated tissue repair depends on multiple factors, including the source tissue and species from which the biomaterials are derived, the efficacy of the decellularization process (Brown et al. 2009; Keane et al. 2012), the extent of postprocessing modifications such as solubilization (Young et al. 2011; Seif-Naraghi et al. 2012), chemical cross-linking (Valentin et al. 2009), and method of terminal sterilization (Freytes et al. 2008b).

Commercially available biologic scaffolds are derived from various sources and manufactured by several established processing methods (Tables 1 and 2). As a result, the extent of decellularization, cross-linking, degradation profile, density, surface topography, and other parameters among these materials varies extensively. In addition, host factors including age, comorbidities, and surgical technique can affect the clinical outcome (Badylak 2014). Considering the large number of variables, clinical results associated with the use of biomaterials have ranged from unacceptable to excellent.

Favorable clinical outcomes are consistently achieved when: (1) scaffolds are thoroughly decellularized, (2) cross-linking via chemical agents is avoided, (3) the scaffolds are free of endotoxin/bacterial contamination, and (4) the scaffolds are placed in an appropriate anatomic location (i.e., in contact with healthy, vascularized tissue and subjected to appropriate physiologic mechanical loads) (Londono and Badylak 2015).

Table 3. ECM bioscaffold clinical applications

Clincal Application	Study	Extracellular matrix (ECM) bioscaffold	Details
Breast reconstruction	Butterfield 2013	Human dermis versus fetal bovine dermis	440 patients: No significant differences in complication rates between the two groups
Breast reconstruction	Tran Cao et al. 2010	Human dermis	14 patients: Evaluation of the postoperative images was not affected by the scaffold
Breast reconstruction	Spear et al. 2013	Porcine dermis	43 patients: Complication rate for scaffolds was 10% of the silicone prostheses clinical data
Trapeziectomy	Belcher and Zic 2001	Porcine dermis	26 patients: Study was terminated early
Rotator cuff tears	Iannotti et al. 2006	Small intestinal submucosa	30 shoulders: Augmentation of repair with small intestinal submucosa (SIS) did not improve rate of tendon healing
Cardiovascular	Woo et al. 2016	Small intestinal submucosa	532 patients: Only 12 patients required explantation
Venous leg ulcers	Mostow et al. 2005	Small intestinal submucosa	120 patients: SIS-treated group had a higher percentage of healing and lower recurrence
Volumetric muscle loss	Sicari et al. 2014	Porcine urinary bladder	5 patients: Three presented functional recovery, all showed signs of constructive remodeling
Colorectal applications	Cintron et al. 2013	Small intestinal submucosa	73 patients: Use of anal fistula plug (AFP) for treatment of fistula-in-ano is safe and modestly effective in reasonable long-term (15-month) follow-up
Dental repair	Gholami et al. 2013	Human dermis	16 patients: Human dermis can be considered a substitute for palatal donor tissue in root coverage
Diabetic ulcers	Lecheminant and Field 2012	Urinary bladder	34 patients: All patients treated with urinary bladder matrix (UBM) progressed to complete healing
Esophageal repair	Agrawal et al. 2011b	Small intestinal submucosa	5 patients: Restoration of normal, mature, esophageal epithelium and return to normal diet without significant dysphagia reported by all patients
Ventral hernia repair	Kissane and Itani 2012	Various	635 patients: Use of ECM bioscaffolds in contaminated surgical fields allows for one-stage repair with little subsequent removal
Vascular applications	Ladowski and Ladowski 2011	Bovine pericardium	845 patients: The use of bovine pericadium for patch closure in carotid endartectomy yields excellent freedom for residual or recurrent postoperative stenosis

Data modified from Londono and Badylak (2015).

The use of ECM bioscaffolds has been well established in various clinical studies (Table 3). The advantages provided by bioscaffolds have been shown in clinical reports for ventral hernia repair (especially class II-III hernias) (Cevasco and Itani 2012; Garvey et al. 2014; Yang et al. 2015). ECM bioscaffolds have also been shown to be a viable alternative to radical esophagectomy in the setting of esophageal adenocarcinoma (Badylak et al. 2011). When these scaffolds are placed in situ following mucosal resection, restoration of normal, mature, squamous epithelium and normal postoperative esophageal function is achieved. The use of an

ECM bioscaffold as a reconstructive patch for the augmentation of the esophageal diameter during primary repair has also been shown. Four patients requiring esophageal reconstruction received patch esophagoplasty with a UBM-ECM bioscaffold replacing the full thickness of the esophagus. All patients had a favorable clinical recovery and resumed normal oral intake after 7 days (Nieponice et al. 2014).

In patients with VML caused by trauma, bioscaffold implantation resulted in neovascularization, innervation, myogenesis, and improved function (Sicari et al. 2014). The implantation of ECM bioscaffolds and physical therapy was associated with mobilization of perivascular stem cells, de novo formation of islands of skeletal muscle within the site of implantation, and increased postoperative performance as measured by increased force production and improved functional task performance (Dziki et al. 2016).

Decellularized human dermis (AlloDerm, LifeCell) is widely used for breast reconstruction. The ECM scaffold is placed as a hammock or sling that provides partial coverage of implant or tissue expander when the pectoralis muscle is lifted from the body wall. The bioscaffold offers benefits such as the option for reconstruction without the need for prior tissue expansion, shorter operative time, improved inframammary fold definition, and reduced postoperative pain. Decellularized human dermis also appears to reduce the time a tissue requires to accommodate an expanded geometry before placement of the permanent implant and appears to reduce the incidence of capsular contracture, an undesired complication of the use of implants (Jansen and Macadam 2011). AlloDerm is also being used as a filler after conservative breast surgery. The sheet is folded onto itself to provide the volume required to fill the space left after a lumpectomy defect. A primary concern when using any material following cancer excision is that the radiologic appearance might alter the natural appearance of the tissue, making it difficult to identify cancer recurrence. A study showed that, on radiographic evaluation, AlloDerm was not discernible from background densities and postsurgical changes, and it did not obscure calcifications, which are important criteria when screening for breast cancer. DC-B MRI identified the device and it was not enhanced on fat-saturated postcontrast T1 sequences making it distinguishable from recurrent disease. Therefore, the use of this ECM scaffold device will not mask recurrence on either mode of screening (Tran Cao et al. 2010).

New indications for the use of bioscaffolds continue to be explored. For example, these scaffold materials are being investigated for cosmetic breast surgery. Porcine dermal matrix (Strattice, LifeCell) has been used for primary and secondary cosmetic breast surgery (Spear et al. 2013). The bioscaffold was used for soft tissue support in areas of inadequate tissue, for soft tissue overlay where there was not enough parenchyma, reinforcing fold repairs in instances of fold malposition, and for capsule replacement after capsulectomy. The use of bioscaffolds is relevant when patients have failed a prior revision surgery and the procedure is being repeated in hopes of better results. In comparison with clinical data from the use of silicone prostheses (Mentor and Allergan), patients treated with biologic scaffolds appear to have 10% of the rate of complications, suggesting that this approach is a safe alternative for specific types of cosmetic breast surgery (Spear et al. 2013)

CorMatrix (CorMatrix Cardiovascular) is a bioscaffold composed of small intestinal submucosa and is used for pediatric heart reconstruction. In a study that included 532 patients in which CorMatrix had been used, 12 patients required explantation. Of the 12 cases, six showed signs of graft failure before the explantation procedure and 11 showed chronic inflammation. Some cases showed active inflammation, calcification, or necrosis and all showed fibrosis of the surrounding native tissue. The cause of these graft failures is unknown. None of the explanted tissues showed signs of constructive remodeling such as integration of mesenchymal cells or myocytes. The investigators concluded that CorMatrix facilitates a favorable clinical outcome for patients undergoing cardiac reconstructive surgery, because only 12 of the

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532 required explantation; however, the lack of availability for biopsy and histologic examination prevented any conclusion regarding site-specific constructive remodeling (Woo et al. 2016). Use of SIS-ECM for repair of congenital heart defects in a different study did show regeneration with favorable outcomes (Scholl et al. 2010).

The clinical success of the use of bioscaffolds for the applications mentioned above does not mean bioscaffolds will always yield a satisfactory outcome. Acellular porcine dermal skin scaffold (Permacol, Medtronic) was used for trapeziectomy. Surgeons replaced the removed trapezius bone with the bioscaffold to maintain the height of the thumb. The study had to be terminated early because of some patients experiencing pain and erythema at the surgical site. Histopathologic examination showed the explanted scaffolds surrounded by a foreign body response. As a result, both control and implant groups had significant shortening of the thumb leading to the conclusion that the xenograft did not provide an advantage to the procedure (Belcher and Zic 2001). It should be noted that Permacol is bovine dermis ECM that has been chemically cross-linked with carbodiimide, thus inhibiting degradation and remodeling. Another example of biologic scaffolds showing less than a desirable outcome is the use of SIS-ECM for augmentation of the surgical repair of chronic severe and massive rotator cuff tears (Iannotti et al. 2006). The SIS-ECM device was used to bridge defects in these cases, although it was only labeled for use in cuff tears without a tissue gap; consequently, the clinical outcome was unsatisfactory. These two examples show the importance of tissue processing (e.g., chemical cross-linking) and use in the appropriate microenvironment in facilitating appropriate remodeling events and downstream clinical outcomes.

Chemical cross-linking is commonly used to increase the mechanical strength of ECM bioscaffolds. However, because cross-linked ECM bioscaffolds are unable to degrade, their persistence in situ typically leads to a foreign body reaction (Anderson et al. 2008) with the formation of connective tissue encapsulation (Reing et al. 2010; Cavallo et al. 2015). Cross-linked ECM biomaterials have also been shown to elicit a proinflammatory macrophage phenotype, rather than the anti-inflammatory and pro-remodeling phenotype induced by the native ECM bioscaffold (Reing et al. 2010; Keane et al. 2012). In the clinical setting, the persistence of the scaffold may affect performance. For example, comparison of cross-linked (Permacol) and non-cross-linked (Strattice) for ventral hernia repair showed that the infection rate was lower with Strattice (5% vs. 21%) with a hernia recurrence rate that was similar in both groups (Cheng et al. 2014).

Not all applications for bioscaffolds are surgical. Biologic scaffolds are also used for topical wound care. The SIS-ECM device, Oasis, has proven effective for the treatment of venous leg ulcers in a 120-patient randomized clinical trial. Oasis and compression therapy was compared with compression therapy alone; the gold standard in treatment for venous leg ulcers. The use of the SIS-ECM scaffold showed superior results with 55% of the wounds healed in comparison with 34% in the control group after 12 weeks of treatment. After 6 months, there was no recurrence of ulcers in the SIS-treated group; in contrast, the control group had a recurrence rate of 30%. This recurrence rate matched the expected recurrence for venous leg ulcers treated with conventional therapies, showing a distinct advantage for use of the biologic scaffold (Mostow et al. 2005).

Overall, biological scaffolds are a legitimate alternative to conventional therapies and have the potential to enhance outcomes or provide solutions that may not be available with synthetic scaffolds. The clinical applications will likely continue to expand as new indications are studied and a more complete understanding of the underlying mechanisms of in vivo remodeling is developed.

CONCLUDING REMARKS

Bioscaffolds composed of ECM are obtained by the removal of cells from source tissues. Effective decellularization and maintenance of the integrity of the native ECM components and

ultrastructure are important factors for a functional clinical outcome. The mechanisms by which ECM bioscaffolds promote constructive functional remodeling of injured or missing tissues include the release of signaling molecules that modulate the innate immune response and recruit stem/progenitor cells to the site of bioscaffold remodeling. Although not optimal, or even preferred for all clinical conditions, the inductive acellular approach to functional tissue repair using ECM bioscaffolds provides an effective alternative.

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