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Antigen removal for the production of biomechanically functional, xenogeneic tissue grafts

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ABSTRACT

Xenogeneic tissues are derived from other animal species and provide a source of material for engineering mechanically functional tissue grafts, such as heart valves, tendons, ligaments, and cartilage. Xenogeneic tissues, however, contain molecules, known as antigens, which invoke an immune reaction following implantation into a patient. Therefore, it is necessary to remove the antigens from a xenogeneic tissue to prevent immune rejection of the graft. Antigen removal can be accomplished by treating a tissue with solutions and/or physical processes that disrupt cells and solubilize, degrade, or mask antigens. However, processes used for cell and antigen removal from tissues often have deleterious effects on the extracellular matrix (ECM) of the tissue, rendering the tissue unsuitable for implantation due to poor mechanical properties. Thus, the goal of an antigen removal process should be to reduce the antigen content of a xenogeneic tissue while preserving its mechanical functionality. To expand the clinical use of antigen-removed xenogeneic tissues as biomechanically functional grafts, it is essential that researchers examine tissue antigen content, ECM composition and architecture, and mechanical properties as new antigen removal processes are developed.

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1. Introduction

Biologic tissues have been explored for their potential to be used as biomaterials in human patients for a variety of applications, particularly those involving replacement or repair of tissues with a mechanical role, from heart valve replacement to ligament and cartilage repair. Biologic tissues are advantageous compared to synthetic materials in that they possess the complex structure, composition, and mechanical properties of the tissue they are intended to replace. Currently, no other material matches the functional properties of native tissues such as cartilage (Huey et al., 2012). Sources of tissue for use as biomaterials may be autologous (obtained from the same patient receiving the repair), allogeneic (obtained from a human tissue donor), or xenogeneic (derived from another animal species). Although autologous tissue transplants are used successfully for some applications, including anterior cruciate ligament (ACL) (Gulotta and Rodeo, 2007) repair and cartilage repair (Revell and Athanasiou, 2009), using tissue from one site in a patient to repair another is limited due to potential donor site morbidity and an inability to fully recapitulate the structure and function of the original tissue. For heart valve replacements, autologous grafts can only be used for specific indications and are associated with potentially life-threatening complications (Henaine et al., 2012). Due to these limitations, allogeneic tissues from human tissue donors have been increasingly used for ACL repair (Reinhardt et al., 2010) and cartilage repair (Ahmed and Hincke, 2010; Revell and Athanasiou, 2009). Allogeneic grafts are also used for some heart valve replacements (Elkins et al., 2001; Ruzmetov et al., 2012). Allogeneic tissues are limited by their relative scarcity, potential for invoking immune reaction, and disease transmission. Xenogeneic tissues are abundant and also typically have composition and properties comparable to their human counterparts (Gilbert et al., 2006). Most notably, chemically fixed xenogeneic pericardium and heart valve tissue are frequently used to replace human heart valves (Courtman et al., 1994; Zhai et al., 2006). Xenogeneic tissues have also been used as scaffolds for tissue engineering of multiple tissue types, including heart valves (Schenke-Layland et al.,2003; Syedain et al., 2013; Wong et al., 2013), tendon (Lohan et al., 2013), trachea (Remlinger et al., 2010; Zang et al., 2012), meniscus (Stapleton et al., 2008, 2011), and articular cartilage (Gong et al., 2011; Kang et al., 2012; Kheir et al., 2011; Yang et al., 2008). Despite their desirable qualities, the major obstacle to using xenogeneic tissues as biomaterials is the presence of antigens that trigger an immune response and, ultimately, graft rejection. To more widely use xenogeneic tissues for clinical applications, it is necessary to remove the antigenic components from the tissues while preserving

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the desirable composition, structure, and function of the original tissue.

2. Decellularization and antigen removal from xenogeneic tissues

Xenogeneic grafts contain antigens, primarily proteins, which are recognized by a patient's immune system as foreign. Such antigenic components of the tissue initiate recipient immune responses that attempt to break down and remove the foreign material. At a minimum, such graft-specific immune responses lead to biomechanical failure of the graft, and at worst it can lead to host death (Trivedi, 2007). A number of strategies have been employed to remove or disguise the antigens in xenogeneic tissues in order to render them safe for implantation into human patients. The most common strategy currently used for clinical applications is the use of a chemical treatment, such as glutaraldehyde, that cross-links the proteins within the xenogeneic tissue. The cross-linking process is intended to make the xenogeneic proteins unrecognizable to a human patient's immune system, but there is evidence that glutaraldehyde treatment does not completely mask important antigens, including the Galalpha1,3-Galbeta1-4GlcNAc-R (α-gal) epitope (Hulsmann et al., 2012). While glutaraldehyde-fixation is successful in overcoming hyperacute or acute rejection response towards xenogeneic heart valve replacements, delayed immune recognition ultimately results in chronic immune degradation and calcification resulting in valve failure (Manji et al., 2006). Removing the antigenic proteins, rather than cross-linking the proteins and leaving them within the tissue, has the potential to improve the longevity of xenogeneic grafts.

Various chemical and physical treatments have been proposed to decellularize xenogeneic tissues in order to reduce their antigen content. Chemical treatments include protocols that apply an exogenous substance to a tissue for the purpose of degrading or solubilizing cell components and other antigens. Chemical treatments, such as acid solutions, hypotonic/hypertonic solutions, detergents, organic solvents, and enzyme solutions, aim to disrupt cells by osmotic pressure (e.g., hypotonic solutions) or by solubilizing the cell membrane (detergents and organic solvents) and to facilitate removal of the cytosolic and membrane-associated proteins. Physical treatments such as freezing and thawing, sonication, and gamma irradiation or photo-oxidation are used to disrupt the cells within a tissue and are typically used in conjunction with a chemical treatment that facilitates removal of the cell components. Gamma irradiation and photo-oxidation both act to cross-link proteins within a tissue and have been examined as sole treatments or in combination with other antigen removal methods (Akens et al., 2001, 2002; Ota et al., 2007; Sarathchandra et al., 2012). The goal of an antigen removal protocol should be to target removal of antigens that trigger an immune response without disrupting the important structural molecules that lend a tissue its mechanical properties.

Failure to sufficiently reduce the antigen content of a xenogeneic tissue or to preserve the composition and functional properties of the tissue will likely lead to graft failure upon implantation (Fig. 1). To produce a successful xenogeneic graft, it is necessary to measure the residual antigen content of the graft material after treatment. In the sections below, it should be noted that the term 'antigen removal' is used in lieu of 'decellularization,' as the goal of decellularization is to reduce the antigen content. Decellularization aims to reduce cell components, such as nuclei, from a tissue and assumes a corresponding reduction of antigens. Antigen removal can be defined as any process that eliminates proteins and other antigen molecules from a tissue. While removal of cell components is necessary, the concept of antigen removal establishes the residual antigen content of a tissue as a crucial endpoint. Historically, cell counts and quantification of the DNA remaining in a xenogeneic tissue have been used as a measure of success in reducing antigen content. There is evidence, however, that cell counts do not correlate with the residual antigen content of a tissue (Goncalves et al., 2005; Wong et al., 2011, 2013). Other means of quantifying or qualifying antigen content in a tissue include immunoblotting (Arai and Orton, 2009; Goncalves et al., 2005; Wong et al., 2011, 2013), ELISA (Galili et al., 1997; Nam et al., 2012), immunohistochemistry (Goncalves et al., 2005; Yoshida et al., 2012), and monocyte activation tests (Rieder et al., 2005; Yoshida et al., 2012). It is important to note that there is no known threshold for sufficient antigen removal and most researchers strive to remove as much antigen content as possible without disrupting the desirable qualities of the xenogeneic tissue. Adversely affecting the composition and mechanical properties of the xenogeneic tissue is just as likely as incomplete antigen removal to result in degradation of a xenogeneic graft if the graft material is incapable of withstanding the stresses it is subjected to.

3. Structure-function relationships of biomechanical tissues

Biomechanical tissues are capable of withstanding repetitive, compressive, tensile, and/or shear stresses. Although many tissues are subject to some mechanical stress, the tissues of the musculoskeletal and cardiovascular systems are subject to the greatest magnitude of stress and number of cycles during normal physiologic activity over the lifetime of an individual. In order to withstand mechanical stress, these tissues have highly specialized extracellular matrix (ECM) composition and architecture. To preserve the functional properties of a xenogeneic tissue for use as a biologic graft material, it is essential to understand the structure–function relationships of the xenogeneic tissue as well as the tissue it is intended to replace.

A hierarchy of ECM components exists that creates the functional properties of biomechanical tissues. The concentration and distribution of the major ECM macromolecules, collagen and proteoglycans, have a key role in determining mechanical properties. Other proteins, such as elastin, contribute to the mechanical properties of some tissues (Scott and Vesely, 1995; Vesely, 1998). The collagen type and alignment within the ECM provide a tissue's tensile stiffness and strength. At the ultrastructural level, collagen fiber diameter, spacing, and crimping all contribute to tensile properties (Silver et al., 2003). Although the collagen network is also an essential prerequisite for tissue compressive stiffness (Williamson et al., 2001), proteoglycans are largely responsible for providing viscoelasticity and resistance to compression, especially in cartilage (Katta et al., 2008). The combination of collagen, proteoglycans, and other ECM molecules determines the biomechanical characteristics of a tissue (Hinton and Yutzey, 2011; Zhu et al., 1993).

Collagen type and alignment have a large influence on the tensile properties of a tissue. The ECM of tendons, ligaments, fibrocartilage, and heart valves consists primarily of coarse bundles of type I collagen. In tendons, the type I collagen fibers are arranged helically, parallel to the axis of force transduction, yielding uniaxial tensile stiffness and strength (Aparecida de Aro et al., 2012). The arrangement of collagen fibers in ligaments depends on the particular function of the ligament. In the anterior cruciate ligament (ACL), fibers are organized in a complex fashion providing a non-linear stress–strain relationship that is characterized by increasing stiffness with increasing loads (Dienst et al., 2002). The fibrocartilaginous meniscus has collagen fibers arranged circumferentially as well as radially allowing the meniscus to resist expansion under compression during loading of the knee (Almarza and Athanasiou, 2004;

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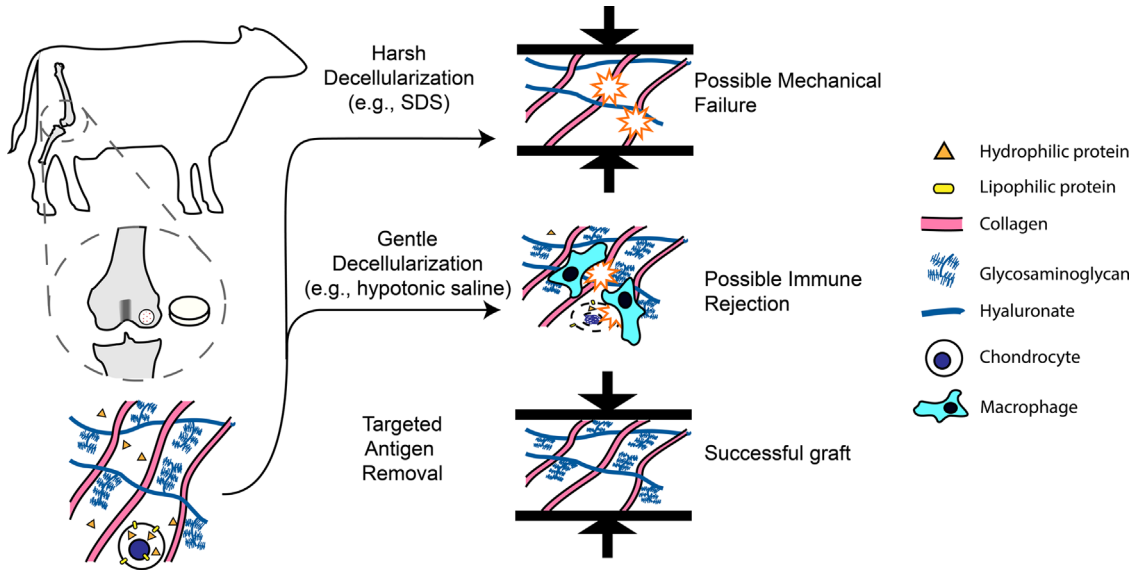

Fig. 1. A bovine articular cartilage explant is obtained from the distal femur. The extracellular matrix (ECM), consisting of collagen, hyaluronate, and glycosaminoglycans (GAG), and a single chondrocyte are depicted. Hydrophilic antigens are present in the cytosol and ECM. Lipophilic antigens are present associated with the cell membranes. Following decellularization with a harsh chemical treatment, e.g. sodium dodecyl sulfate (SDS), the cell components and antigens may be removed, but GAG is also stripped from the ECM, leading to reduced compressive stiffness and potential mechanical failure. Insufficient antigen removal with a gentle decellularization protocol, such as hypotonic solutions, may lead to immune reaction and graft degradation in vivo. The ideal antigen removal protocol will remove the major antigenic components of the cartilage explant, while preserving its ECM composition, architecture, and mechanical properties.

Fithian et al., 1990). Heart valves consist of type I and type III collagens of differing densities and alignments depending on the layer of the valve (Hinton and Yutzey, 2011). The fibrosa (outflow) layer of heart valves contains the greatest proportion of collagen, where highly parallel, circumferentially oriented fibers provide tensile stiffness (Hinton and Yutzey, 2011; Liao et al., 2008; Schenke-Layland et al., 2003). The atrialis or ventricularis (inflow) layer of heart valves is less densely populated with collagen (Schenke-Layland et al., 2003). Articular hyaline cartilage is composed of finer type II collagen fibers that also exhibit different orientations depending on the cartilage layer (Almarza and Athanasiou, 2004). In the superficial zone of articular cartilage, collagen fibers are aligned tangential to the joint surface, helping provide resistance to shear stress. In the middle zone, collagen fibers are arranged in a looser, random network, providing a scaffold for the attachment of proteoglycans. Proteoglycans make up ~20% of the ECM dry matter in articular hyaline cartilage (Lohmander, 1988) and less than 1% of the ECM dry matter in the menisci (Fithian et al., 1990) and tendons (Aparecida de Aro et al., 2012; Gillard et al., 1977). Heart valves and ligaments also contain proteoglycans and associated glycosaminoglycans (GAG) in relatively small amounts compared to hyaline cartilage (Cigliano et al., 2012; Gratzer et al., 2006). The type and distribution of proteoglycans also differ between tissues and influence a tissue's mechanical properties. In articular hyaline cartilage, the primary proteoglycan is aggrecan, consisting of a large protein core with many GAG side chains. This results in a large, negative fixed charge density in hyaline cartilage, which serves to bind and hold water within the ECM. Together with collagen fibers, the proteoglycans restrict water movement out of the cartilage ECM, providing the tissue's compressive stiffness (Almarza and Athanasiou, 2004). Large proteoglycans serve a similar role in fibrocartilage, helping to resist compressive stress (Fithian et al., 1990). Proteoglycans are found in the greatest portion in the middle, spongiosa layer of heart valves, where they provide flexibility and resistance to compression (Hinton and Yutzey, 2011; Liao et al., 2008). Depletion or excessive accumulation of proteoglycans in heart valves results in valve incompetence (Hinton and Yutzey, 2011). In tendons, smaller proteoglycans, such as fibromodulin and decorin, predominate. In addition to having a possible regulatory role in the formation of the collagen fiber architecture of the tendon, proteoglycans accumulate at sites in tendons that are subject to bending and compression (Aparecida de Aro et al., 2012).

Elastin is found in both heart valves and tendons, where it allows elongation of the collagen fiber network without excessive stretching and damage of the tissue (Aparecida de Aro et al., 2012; Hinton and Yutzey, 2011). Elastin is primarily found in the atrialis or ventricularis layers of heart valves, where the radially oriented elastin filaments permit normal valve motion (Hinton and Yutzey, 2011; Schenke-Layland et al., 2003). In heart valves, elastin contributes tensile stiffness at low strains (Vesely, 1998). As with collagen, the content and orientation of elastin fibers determine its contribution to the mechanical properties of heart valves, and these properties vary with location and layer (Vesely, 1998).

The macromolecule content, distribution, and organization within the ECM of a tissue is essential to the tissue's mechanical function. To capitalize on the inherent mechanical properties of xenogeneic tissues, a proposed antigen removal protocol must preserve the concentration of major ECM molecules, including collagen, GAG, and elastin. It is also important to preserve the microarchitecture of the collagen fiber network and the anistotropy of the tissue ECM to maintain the tissue's functional properties.

4. Effects of antigen removal protocols on tissue biomechanical properties

In order to reduce the antigen content of xenogeneic tissues, a physical or chemical treatment must disrupt the cells within the tissue and then remove the cell components as well as any immunogenic molecules that may be present in the ECM. Chemical treatments aim to rupture cells using osmotic pressure (hypotonic solutions) or by solubilizing the cells' lipid membranes (detergents and organic solvents). Hypotonic solutions alone are not efficient at removing antigens from tissues (Meyer et al., 2006; Wong et al., 2011) and are often used in combination with detergents, enzymes, or other solution components that facilitate removal of proteins and other cell components (Whitlock et al., 2012; Woods and Gratzer, 2005; Yang et al., 2009). Cell components are then removed from the tissue,

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Table 1
Effects of chemical antigen removal treatments on the functional properties of xenogeneic tissues.

Treatment	Species	Tissue	Effect on mechanical properties[a]	Effect on ECM composition	Reference(s)
Non-ionic detergent					
1% Triton X-100	Porcine	ACL	No change in tensile stiffness or failure load	No change in collagen or GAG	Woods and Gratzer (2005)
1% Triton X-100	Porcine	Diaphragm tendon	Unreported	Disrupted collagen architecture	Deeken et al. (2011)
0.5% Triton X-100	Murine	Heart valve	Unreported	No change in ECM architecture; Decreased elastin	Meyer et al. (2006)
1% Triton X-100	Porcine	Heart valve	Decreased flexural stiffness	Disrupted ECM architecture	Liao et al. (2008)
1% Triton X-100	Bovine	Pericardium	No change in E_Y, σ_{max}, or ε_{max}	Preserved ECM architecture	Yang et al. (2009)
Ionic detergents					
0.1% SDS	Porcine	Meniscus	No change in indentation deformation, UTS, or ε_{UTS}	Decreased GAG	Stapleton et al. (2008)
2% SDS	Bovine	Articular hyaline cartilage	Decreased aggregate modulus	No change in collagen; Decreased GAG	Elder et al. (2010)
0.1–1% SDS	Porcine	ACL	Increased tensile stiffness	No change in collagen; Decreased GAG	Vavken et al. (2009), Woods and Gratzer (2005)
0.1% SDS	Porcine	Heart valve	Decreased flexural stiffness; Increased extensibility	Disrupted collagen crimp	Liao et al. (2008)
0.1–1% SDS	Bovine	Pericardium	No change in E_Y, UTS, or ε_{UTS}	No change in collagen; Decreased elastin; Disrupted ECM architecture	Wong et al. (2011), Wong et al. (2013), Zhou et al. (2010)
2% SDS	Equine	SDF tendon	No change in E_Y, UTS, or ε_{UTS}	No change in collagen; Decreased GAG	Youngstrom et al. (2013)
1% SDC	Bovine	Pericardium	Unreported	Preserved ECM components and architecture	Zhou et al. (2010)
4% SDC	Murine	Trachea	Decreased E_Y; No change in UTS or strain at failure	Decrease GAG	Zang et al. (2012)
0.5% SDS+0.5% SDC	Bovine	Pericardium	Decreased E_Y; Increased elongation; No change in UTS	Unreported	Hulsmann et al. (2012)
Non-ionic plus ionic detergents					
0.05% Triton X-100+0.05% SDC+0.05% IGEPAL	Porcine	Heart valve	No change in maximum tension or ε_{max}	Unreported	Seebacher et al. (2008)
0.25% Triton X-100+0.25% SDC	Porcine	ACL	Unreported	No change in collagen; Decreased GAG	Vavken et al. (2009)
0.5% Triton X-100+0.5% sodium deoxycholate	Bovine	Pericardium	Decreased E_Y, σ_{max}, and ε_{max}	Disrupted ECM architecture	Yang et al. (2009)
Organic solvents					
Tri (*n*-butyl) phosphate	Equine	SDF tendon	Unreported	No change in collagen; Decreased GAG	Youngstrom et al. (2013)
Tri (*n*-butyl) phosphate	Porcine	Diaphragm tendon	No change in E_Y or strength at yield; Increased strain at yield	No change in collagen architecture	Deeken et al. (2011)
Enzymatic digestion					
0.1% Trypsin	Porcine	ACL	Unreported	No change in collagen; Decreased GAG	Vavken et al. (2009)
0.05% Trypsin	Bovine	Pericardium	Decreased E_Y; Increased elongation; No change in UTS	Decreased GAG; Decreased elastin; Disrupted ECM architecture	Hulsmann et al. (2012), Zhou et al. (2010)
1% Trypsin	Bovine	Pericardium	Decreased E_Y, σ_{max}, and ε_{max}	Disrupted ECM architecture	Yang et al. (2009)
0.05% Trypsin	Porcine	Heart valve	Decreased tensile strength	Disrupted collagen fiber pattern; Decreased GAG; Decreased elastin	Schenke-Layland et al. (2003), Yu et al. (2013)
0.5% Trypsin	Porcine / Murine	Heart valve	Decreased flexural stiffness; increased extensibility; Increased maximum tangential modulus in circumferential direction	Decreased elastin; Disrupted ECM architecture; Depletion of spongiosa layer; Loss of regional variations in alignment	Liao et al. (2008), Meyer et al. (2006)
Sequential protein solubilization					
Hypotonic buffer+KCl+Dithiothreitol	Bovine	Pericardium	No change in E_Y, UTS, or ε_{max}	No change in collagen or elastin content; Decreased GAG	Wong et al. (2011)
Amidosulfabetaine-14	Bovine	Pericardium	No change in E_Y, UTS, or ε_{max}	No change in collagen or elastin content; Decreased GAG	Wong et al. (2013)

ECM, extracellular matrix; ACL, anterior cruciate ligament; GAG, glycosaminoglycans; E_Y, Young's modulus; σ_{max}, maximum stress; UTS, ultimate tensile stress; ε_{max}, maximum strain; ε_{UTS}, strain at UTS; SDS, sodium dodecyl sulfate; SDC, sodium deoxycholate; IGEPAL, octylphenyl-polyethylene glycol; SDF, superficial digital flexor.

[a] Mechanical properties included here are as reported by the original authors.

primarily by diffusion. Physical processes alone have not been shown to eliminate antigens from a tissue and are typically used in combination with some chemical treatment. To achieve a xenogeneic tissue with reduced antigenicity and maintained functional properties, the effects of the antigen removal process on the tissue's hierarchy of ECM composition and architecture, and the resulting mechanical properties must be examined. Table 1 provides an overview of the effects of various chemical antigen removal strategies on tissue functional properties.

4.1. Detergents

The effects of detergents on the ECM depend on the specific detergent, its concentration, and the tissue to which it is applied. The most commonly used non-ionic detergent for antigen removal research is octyl phenol ethoxylate (Triton X-100). Commonly used ionic detergents include sodium dodecyl sulfate (SDS) and sodium deoxycholate (SDC).

Triton X-100 has been examined for its ability to decellularize and reduce antigen content in xenogeneic heart valve (Liao et al., 2008; Meyer et al., 2006; Yu et al., 2013), pericardium (Yang et al., 2009), tendon (Deeken et al., 2011), and ligament (Woods and Gratzer, 2005) with varying effects on tissues' mechanical properties and ECM. The ECM architecture was preserved and no change in the tensile properties was observed in bovine pericardium treated with 1% Triton X-100 (Yang et al., 2009). Similarly, no change in collagen content or tensile properties was found when 1% Triton X-100 was used to decellularize porcine anterior cruciate ligament (ACL) (Woods and Gratzer, 2005). Also, the collagen fiber pattern was preserved in rat heart valves treated with Triton X-100 (Meyer et al., 2005). No change in GAG content was found in porcine ACL tissue treated with Triton X-100 (Woods and Gratzer, 2005). However, Triton X-100 is not without deleterious effects on the ECM of some tissues. Porcine heart valves decellularized with Triton X-100 demonstrated decreased flexural stiffness, increased extensibility, and altered maximum tangential moduli associated with disrupted collagen crimp, depletion of the spongiosa layer, and loss of regional variation in collagen fiber alignment (Liao et al., 2008). Triton X-100 was also found to reduce the elastin content of rat heart valves (Meyer et al., 2006) and to disrupt the collagen architecture of porcine diaphragmatic tendon (Deeken et al., 2011), but mechanical testing was not performed in these studies. Varying results associated with the use of Triton X-100 may be due to different tissue types, different testing modalities, and/or slight differences in the antigen removal protocol between studies.

SDS has been extensively studied for its potential to remove antigens from xenogeneic tissues, although it has been found to have negative effects on nearly every tissue investigated. For example, the GAG content of tissue ECM is particularly susceptible to treatment with SDS. SDS has been shown to reduce the GAG content in porcine ACL (Vavken et al., 2009; Woods and Gratzer, 2005), bovine pericardium (Wong et al., 2013), bovine articular cartilage (Elder et al., 2010), porcine meniscus (Stapleton et al., 2008), and equine superficial digital flexor (SDF) tendon (Youngstrom et al., 2013). A concentration of SDS as low as 0.1% was found to deplete GAG in some tissues (Stapleton et al., 2008; Vavken et al., 2009). Other deleterious effects of SDS include depletion of elastin (Meyer et al., 2006; Wong et al., 2011, 2013) and disruption of the ECM architecture (Kasimir et al., 2003; Zhou et al., 2010). In contrast, no change in collagen content was observed in any of these tissues treated with SDS (Elder et al., 2010; Stapleton et al., 2008; Wong et al., 2011, 2013; Youngstrom et al., 2013). SDS caused no change in compressive stiffness in porcine meniscus (Stapleton et al., 2008), but a marked reduction of compressive stiffness in bovine articular cartilage (Elder et al., 2010) despite similar reductions of GAG content in both tissues. When applied to bovine cancellous bone, SDS caused a treatment duration and concentration dependent decrease in the Young's modulus under uniaxial compressive testing (Shahabipour et al., 2013). Porcine heart valve treated with 0.1% SDS exhibited decreased flexural stiffness and increased extensibility associated with disrupted collagen crimp (Liao et al., 2008), underscoring the importance of examining tissue mechanical properties and ultrastructure in order to fully understand the effects of an antigen removal agent. No change in tensile properties was observed in bovine pericardium (Wong et al., 2011, 2013), bovine articular cartilage (Elder et al., 2010), or equine SDF tendon (Youngstrom et al., 2013) following treatment with SDS. Interestingly, treatment with SDS caused an increase in tensile stiffness in porcine ACL (Woods and Gratzer, 2005). When combined with 0.5% Triton X-100, 0.25% SDS did not affect the ultimate tensile strength (UTS) or strain at failure for bovine pericardium (Nam et al., 2012). To summarize, SDS causes depletion of GAG across all tissues, decreases elastin, and disrupts the ECM architecture, resulting in undesirable effects on mechanical properties of some tissues, including cartilage, heart valve, and bone. Due to its potential for harmful effects on the functional properties of multiple biomechanical tissues, its use should be avoided for antigen removal, particularly if GAG content needs to be preserved. If SDS is used as part of an antigen removal protocol in any tissue with a mechanical role, the resulting antigen-removed graft material should be examined with appropriate mechanical testing and ultrastructural analyses prior to in vivo application.

SDC is infrequently used by itself for antigen removal, but is often used in combination with another detergent, such as Triton X-100 or SDS. When used by itself to remove antigens from bovine pericardium, 1% SDC was found to preserve the major ECM components and architecture, but no mechanical testing was performed (Zhou et al., 2010). SDC (4%) applied to murine trachea decreased the Young's modulus and GAG content of the tissue, but did not influence the UTS or strain at failure (Zang et al., 2012). When combined with Triton X-100, SDC had no effect on the matrix architecture of porcine heart valve (Kasimir et al., 2003) or collagen content of porcine ACL (Vavken et al., 2009). The combination of Triton X-100 and SDC significantly reduced the GAG content of porcine ACL (Vavken et al., 2009) and reduced the tensile properties associated with disrupted ECM in bovine pericardium (Yang et al., 2009). At a low concentration, 0.05% SDC combined with 0.05% Triton X-100, and 0.05% octylphenyl–polyethylene glycol, a non-ionic detergent, did not alter the tensile properties of porcine heart valve (Seebacher et al., 2008). SDC (0.5%) combined with 0.5% SDS resulted in decreased tensile stiffness, increased strain at failure, but no change in UTS when used to remove antigens from bovine pericardium (Hulsmann et al., 2012). Given the relative lack of mechanical testing and variable results obtained from existing studies using SDC for antigen removal, further research is needed to characterize its effects on ECM composition and mechanical properties in biomechanical tissues.

4.2. Organic solvents

Organic solvents are capable of solubilizing lipid membranes and hydrophobic proteins, making them potentially useful for antigen removal. The organic solvent tri(*n*-butyl)phosphate (TnBP) has been used alone and in combination with other factors for antigen removal from tendon (Cartmell and Dunn, 2000; Deeken et al., 2011; Youngstrom et al., 2013), ligament (Woods and Gratzer, 2005), and engineered cartilage (Elder et al., 2009). TnBP (1%) applied to equine SDF tendon (Youngstrom et al., 2013) and porcine diaphragmatic tendon (Deeken et al., 2011) had no effect on collagen content in either tissue and no effect on tensile properties in porcine diaphragm tendon. However, TnBP caused a significant reduction in GAG content in equine SDF tendon (Youngstrom et al., 2013).

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Using 2% TnBP for antigen removal from engineered articular cartilage did not influence the collagen content, but caused near complete loss of GAG as well as significant reductions of aggregate modulus and Young's modulus (Elder et al., 2009). Furthermore, TnBP did not achieve reductions in cell counts or DNA content in engineered articular cartilage (Elder et al., 2009). A two-step antigen removal protocol, consisting of 1% Triton X-100 followed by 1% TnBP, applied to porcine ACL resulted in no change in collagen content, tensile stiffness, or ultimate failure load compared to native tissue controls or samples treated with Triton X-100 alone (Woods and Gratzer, 2005). The preservation of collagen content and tensile properties following antigen removal with TnBP may be useful for application to some tissues. The possible effect on tissue GAG content should be considered when choosing an antigen removal protocol for a given tissue that includes TnBP. Additional research is needed to examine the utility of TnBP for antigen removal from fibrocartilage, heart valve, or pericardium.

4.3. Sequential protein solubilization

Due to the potentially harmful effects of detergents and enzymes on tissue functional properties, an alternative strategy based on targeted protein solubilization has been proposed and applied to bovine pericardium (Goncalves et al., 2005; Wong et al., 2011, 2013). This strategy involves sequential removal of water-soluble antigens followed by lipid-soluble antigens. In the first step, a hypotonic buffer with added electrolytes and a reducing agent induces cell lysis and disrupts molecular interactions, allowing removal of water-soluble antigens (Wong et al., 2011). In the second step, a novel, zwitterionic detergent, amidosulfabetaine-14 (ASB-14), is used to solubilize and remove lipid-soluble antigens (Wong et al., 2013). Although both steps were found to decrease the GAG content of bovine pericardium, no changes in tensile properties, collagen content, or elastin were found and the ECM architecture was preserved better following treatment with ASB-14 than with SDS (Wong et al., 2011, 2013). Future studies examining a sequential protein solubilization strategy for antigen removal in other tissues types are indicated.

4.4. Enzymatic digestion

Enzymes are employed in many antigen removal protocols to break down proteins and other macromolecules that cause an immune reaction. Most antigen removal protocols include nucleases (i.e., ribonuclease and deoxyribonuclease) in the main antigen removal solution or as an additional step in order to degrade and facilitate removal of RNA and DNA from the xenogeneic tissue. Although the effect of nucleases on the ECM composition and tissue mechanical properties has not been examined, nucleases have been used in multiple studies that did not find negative effects of the antigen removal protocol (Woods and Gratzer, 2005; Yang et al., 2009; Zhou et al., 2010), suggesting these enzymes do not have a significant impact on ECM composition or mechanical properties. Enzymes are also used to remove specific antigens. For example, α-galactosidase, which is used to remove α-gal (Goncalves et al., 2005; Nam et al., 2012; Yoshida et al., 2012), has been shown to have no effect on the UTS or strain at failure of bovine pericardium (Nam et al., 2012).

Trypsin is the protease most commonly used for enhancing antigen removal from xenogeneic tissues. Trypsin, in concentrations from 0.05% to 1%, causes negative effects on the functional properties of porcine ACL (Vavken et al., 2009), bovine pericardium (Hulsmann et al., 2012; Yang et al., 2009; Zhou et al., 2010), murine heart valve (Meyer et al., 2006), and porcine heart valve (Liao et al., 2008; Schenke-Layland et al., 2003; Yu et al., 2013). Specifically, 0.05% trypsin decreased the Young's modulus and increased the strain at failure associated with disrupted ECM architecture and reductions in GAG and elastin content in bovine pericardium (Hulsmann et al., 2012; Zhou et al., 2010). A concentration of 1% trypsin caused severe disruption of bovine pericardial ECM organization and reduced its Young's modulus, maximal stress, and strain at failure (Yang et al., 2009). Trypsin (0.05–0.5%) caused similar loss of GAG and elastin, disruptions of the ECM collagen fibers, and decreased tensile strength and flexural stiffness in heart valves (Liao et al., 2008; Meyer et al., 2006; Schenke-Layland et al., 2003; Yu et al., 2013). Depletion of the spongiosa ECM and loss of the normal regional variations in collagen fiber alignment were also observed in porcine heart valves following application of 0.5% trypsin (Liao et al., 2008). When applied to porcine ACL, 0.1% trypsin caused a 83.6% decrease in the tissue GAG content; mechanical testing was not performed (Vavken et al., 2009). Overall, the results obtained from using trypsin as an antigen removal agent suggest a consistent, deleterious effect on tissue functional properties.

Limited research has examined the use of other proteases or trypsin in combination with other antigen removal agents. Pepsin has been used with 75% ethanol for antigen removal from ovine carotid arteries and shown to preserve collagen and elastin within the ECM resulting in no change in artery burst pressure or suture retention (Zhao et al., 2010). When combined with hypotonic Tris buffer and Triton X-100, trypsin caused no change in type II collagen and an apparent, but not statistically significant, reduction of GAG in powdered bovine articular cartilage (Yang et al., 2010). A similar protocol applied to human Achilles tendon included an additional step subjecting samples to peracetic acid and found an increase in tendon strain at failure, but did not change significantly the Young's modulus or UTS (Whitlock et al., 2012). Trypsin combined with 1% SDC applied to porcine heart valves showed increased spacing between collagen fibers, but mechanical testing was not pursued (Yu et al., 2013). Overall, trypsin has a negative effect on xenogeneic tissue properties. Further testing is indicated to determine if other proteases or the combination of trypsin with other factors may preserve tissue ECM and mechanical properties better than trypsin used alone. Given the known impact of trypsin on xenogeneic tissues, it is especially important that tissue composition, architecture and mechanical properties be thoroughly evaluated for any proposed antigen removal protocol that includes proteolytic enzymes.

4.5. Physical processes for antigen removal

Physical processes that have been described for antigen removal from biomechanically functional tissues include irradiation, photo-oxidation, sonication, and freeze-thaw cycles. Although physical processes alone are not capable of removing antigens from a tissue, they can mask residual antigens, induce cell lysis, and enhance the ability of subsequent chemical treatments to remove antigens from the tissue. The effect of physical processes on biomechanical tissue properties has not been widely investigated, but multiple studies have shown improved antigen removal when a physical process is combined with a chemical treatment. The advantage of physical processes is that a chemical residue, such as solvents, detergents, or enzymes, is generally not left behind, making them an appealing alternative to potentially harmful chemical treatments, such as glutaraldehyde.

Irradiation and photo-oxidation both act to cross-link collagen and other proteins within a tissue and provide a possible alternative to chemical fixation using glutaraldehyde. Gamma-irradiation has been performed as an adjunct to decellularization of porcine heart valves using a hypotonic solution (Sarathchandra et al., 2012). In that study, irradiation was found to cause "a marked reduction in the expression of collagen types I and III, hyaluronan, chondroitan

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6-sulphate, and versican" compared to native heart valves or heart valves undergoing only decellularization (Sarathchandra et al., 2012). Irradiation also increased the spacing between collagen fibrils and decreased the density of pyridinoline collagen cross-links compared to controls, suggesting additional disruption of the ECM (Sarathchandra et al., 2012). In a separate study of the effect of gamma-irradiation on porcine heart valves, a protocol consisting of immersing the valves in 80% polythethylene glycol followed by irradiation resulted in no change in maximum load, collagen content, or histologic appearance of the ECM compared to controls (Ota et al., 2007). Photo-oxidation involves applying methylene blue and exposing a tissue to visible light, which alters the amino acids of collagen resulting in increased cross-links (Akens et al., 2002). This process has been applied to bovine osteochondral explants and used for xenogeneic transplantation into sheep and rabbits (Akens et al., 2001, 2002; Hetherington et al., 2007). Although positive results have been reported from in vivo studies (Akens et al., 2001, 2002; Hetherington et al., 2007) and the photo-oxidation process is believed to increase the stiffness of the tissue (Akens et al., 2002), examination of the ECM composition and mechanical properties has not been reported. Further research is necessary, but these techniques have the potential for masking the antigenicity of ECM components that remain in the tissue following initial antigen removal while maintaining or improving tissue mechanical properties.

Sonication has been investigated for its ability to improve antigen removal from fibrocartilaginous tendon–bone interfaces (entheses) and from the nucleus pulposus of intervertebral discs. When applied to human flexor tendon entheses, sonication followed by antigen removal with peracetic acid and SDS decreased the number of visible cells and antigen content in the interface region without affecting the UTS compared to native tissue (Bronstein et al., 2013). A trend was observed for greater cell removal from entheses with increasing power of sonication, with no difference in tensile properties when samples were treated with high power sonication (Bronstein et al., 2013). Sonication applied to porcine nucleus pulposus in conjunction with Triton X-100 and SDC caused a 41–49% decrease in GAG content, but did not influence the compressive stiffness or viscosity of the tissue (Mercuri et al., 2011). The reduced GAG content associated with sonication was significantly different from native nucleus pulposus, whereas no significant difference in GAG content occurred following treatment with only Triton X-100 and SDC (Mercuri et al., 2011). Sonication appears to be promising for enhancing antigen removal from tissues that may be difficult to decellularize by chemical processes alone. It may be possible to titrate the power and duration of sonication to maximize antigen removal while preserving desirable tissue properties. Additional research is warranted to determine the effects of sonication on antigen removal and functional properties of biomechanical tissues.

Cyclic freezing and thawing is incorporated into many antigen removal protocols with the intention of disrupting cells within the tissue, aiding the removal of cell components and other antigens (Burk et al., 2013; Dutra and French, 2010; Kheir et al., 2011; Ning et al., 2012; Shahabipour et al., 2013; Stapleton et al., 2011, 2008; Youngstrom et al., 2013). Freeze-thaw cycles were found to be beneficial for removal of cells and DNA content from equine SDF tendon compared to the use of detergents alone without causing any histologic ECM disruption (Burk et al., 2013). Tendon slices treated only with freezing-thawing and nuclease digestion exhibited a 30% decrease in Young's modulus, but no significant change in UTS or strain at failure compared to native tissue controls (Ning et al., 2012). In many of these studies, the freeze-thaw process was applied to all treatment groups and the specific effects of freezing and thawing cannot be isolated. The efficacy of freeze-thaw cycles to enhance antigen removal and to preserve the tissue ECM should be compared to other methods of cell lysis, such as hypotonic/hypertonic solutions and sonication.

5. Future directions

Expanding the clinical use of xenogeneic tissues as scaffolds and biologic grafts requires development and assessment of antigen removal strategies that preserve the functional properties of a tissue while removing sufficient antigen content to avoid immune rejection. A proposed general strategy to develop antigen-removed xenogeneic tissues for clinical applications is depicted in Fig. 2. Essentially, after a xenogeneic tissue undergoes antigen removal treatment, it must be assessed for residual antigen content, quantification of ECM structural macromolecules, and ECM microarchitecture. The antigen-removed graft material should also undergo mechanical testing as appropriate for the intended function of the graft. Finally, if the functional properties of the xenogeneic graft have been sufficiently preserved and the antigen content reduced, evaluation of the material in an animal model must be performed to ensure maintenance of graft function in vivo.

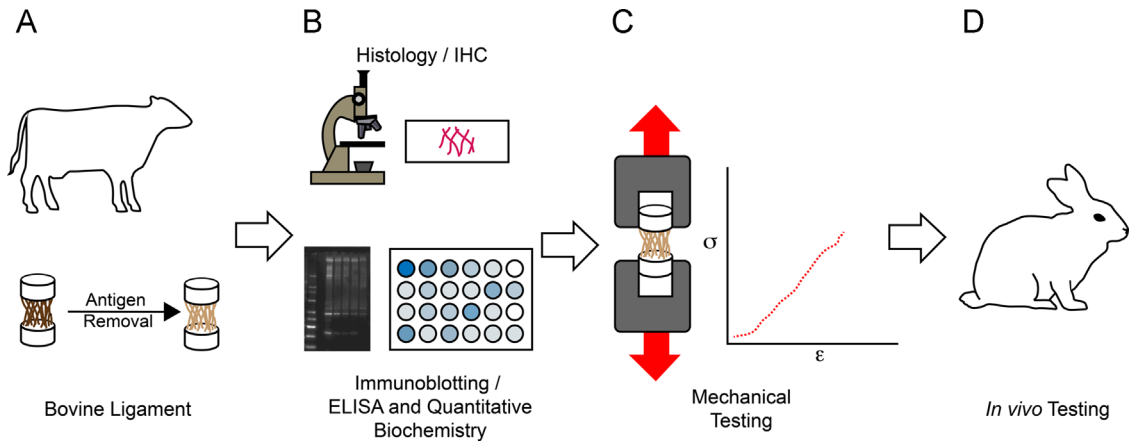

Fig. 2. A xenogeneic ligament is subject to an antigen removal protocol (A). The antigen-removed ligament must be evaluated for successful reduction of antigens by immunoblotting, ELISA, and/or immunohistochemistry (IHC) as well as preservation of the matrix composition and organization by quantitative biochemistry and histology (B). Mechanical testing is then performed to verify maintenance of appropriate mechanical properties (C) prior to evaluation of immunogenicity, function, and durability in vivo (D).

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Development of antigen removal procedures should include novel applications of known antigen removal protocols as well as discovery of new agents and processes. New antigen removal strategies may involve applying existing, successful protocols to different tissues. Other opportunities for improving antigen removal include combining agents or processes with different mechanisms of action that can be applied sequentially or simultaneously to enhance removal of antigens with varied properties, such as solubility. For example, a physical process, such as sonication, may be combined with hypotonic solutions and detergents to efficiently lyse cells and remove both cytosolic and membrane-associated antigens. For maintaining biomechanical functionality, it is thus important to develop new antigen removal agents and protocols that only reduce antigens without significantly affecting macromolecules that have an important structural role in the tissue ECM.

In some cases, it may be impossible to remove antigens without compromising tissue biomechanics. Thus, another exciting avenue for future research is the examination of treatments that may restore functional properties that are unavoidably diminished by the antigen removal process. Such treatments may include cross-linking agents that mask any residual antigens as well as increase tissue stiffness. Recellularization of antigen-removed xenogeneic tissues using allogeneic or autologous cells with the intention of restoring tissue functional properties has also been the subject of considerable research (Ingram et al., 2007; Iwai et al., 2007; Martinello et al., 2012; Ota et al., 2007; Syedain et al., 2013; Wang et al., 2010), and the ability of recellularization to restore tissue ECM composition and mechanical properties should be examined. Successful identification of processes that improve tissue functional properties may permit more aggressive antigen removal protocols to be used that would otherwise deteriorate a tissue's ECM and prevent its use in vivo.

Considering the variation in current attempts to quantify antigen content, it is essential to adopt a measure of success for assessing the residual antigen content of a tissue following antigen removal. Because cell or nuclei counts do not necessarily correlate with residual antigen content in an antigen-removed tissue, success criteria should assess more directly the tissue antigen content. Antigen content can be quantitatively or qualitatively examined using methods such as ELISA, immunoblotting, immunohistochemistry (IHC), monocyte activation, or lymphocyte response tests. ELISA and immunoblotting have been performed for specific antigens, such as α-gal and major histocompatibility complexes, as well as entire tissue proteomes (Goncalves et al., 2005; Nam et al., 2012; Pei et al., 2010; Raghavan et al., 2012; Syedain et al., 2013; Wong et al., 2011, 2013). IHC is typically performed to evaluate the distribution of known antigens within a tissue following antigen removal. Building on antigen quantification, it is important to identify thresholds of antigen content that are tolerated in vivo. It is likely that the maximum allowable antigen content for a xenogeneic graft will differ depending on the tissue type, species of origin, and ultimate site of implantation. Meaningful measures of antigen content and establishing targets for acceptable residual antigenicity will help advance the translation of antigen-removed xenogeneic tissues.

Successful antigen removal is not useful unless the desirable properties of the original xenogeneic tissue are preserved. A core set of functional properties for each tissue should be defined by collaborations between engineers and surgeons to ensure that in vitro tests are practical and relevant toward the tissue's ultimate function in vivo. Such a core of functional properties should include appropriate mechanical testing and examine the hierarchy of ECM properties that contribute to the tissue's function. For example, appropriate testing of an antigen-removed tissue might include compressive and tensile testing; concentrations of major ECM macromolecules, such as GAG and collagen; distribution of those macromolecules; and examination of the ECM microstructure and ultrastructure. The choice to report material or structural mechanical properties should depend on how the graft will be applied in vivo. For example, if an entire xenogeneic ligament will be used to repair an injured ligament in a patient, describing the structural properties of the antigen-removed ligament would be most appropriate. Therefore, collecting and reporting both structural and material properties is warranted.

In reporting the tissue properties, when possible, mechanical testing should adhere to appropriate standards to allow comparison among different studies. For tissues such as cartilage, ACL, and heart valve, U.S. Food and Drug Administration (FDA) guidance documents exist that guide researchers in the appropriate choice of assays and tests to conduct (FDA, 1997, 2010, 2011). For a cartilage repair product, the FDA recommends assessment of static mechanical properties, including aggregate modulus, shear modulus, and permeability, as well dynamic mechanical behavior, such as complex shear modulus (FDA, 2011). The FDA recommends tensile testing to failure as well as tensile fatigue testing for devices intended to replace the ACL; other tests may be required for a finished device (FDA, 1997). At a minimum, the treated tissue must be compared to untreated, native tissue controls tested in the same manner. Comparison of xenogeneic tissue properties to the human tissue properties they are intended to replace is also essential. Some xenogeneic tissues have greater mechanical properties than their human counterparts (Athanasiou et al., 1991), making some reduction in mechanical properties during the antigen removal process acceptable. Every study that is conducted using xenogeneic grafts must refer to mechanical testing standards, such as those set forth by the FDA or by the American Society for Testing and Materials.

Once a promising antigen removal strategy has been examined in vitro, testing in an appropriate xenogeneic animal model must be performed for final evaluation of the immune reaction as well as tissue function in vivo. Considerations for choosing an appropriate animal model include cost, size of the donor tissue and recipient site, ease of surgical implantation, and site of implantation. Due to potential differences in immune reaction associated with different implantation sites, orthotopic implantation of the antigen-removed tissue should be performed when possible. For technically difficult or costly implantation procedures, it may be preferable to first evaluate the antigen-removed xenogeneic graft in a heterotopic site to characterize any possible immune reaction prior to orthotopic implantation to evaluate the graft's function. Evaluation of an antigen-removed graft's function should include its ability to integrate with the surrounding adjacent tissues and to maintain its mechanical properties over time. Inasmuch as no appropriate in vitro models exist to assess the complex in vivo immunological and mechanical events that take place, it is imperative that appropriate animal models be employed.

The combination of residual antigen quantification, ECM characterization, and mechanical testing will allow researchers to screen antigen removal agents for their likelihood of success prior to implantation. Furthermore, standardized testing will allow comparison of results between different antigen removal protocols, researchers, and tissues. Ultimately, in vivo studies must be conducted that finally determine the immunogenicity, function, and durability of antigen-removed xenogeneic tissues, especially in load-bearing tissues.

Conflict of interest statement

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

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References

Ahmed, T.A., Hincke, M.T., 2010. Strategies for articular cartilage lesion repair and functional restoration. Tissue Eng. Part B 16, 305–329.

Akens, M.K., von, R.B., Bittmann, P., Nadler, D., Zlinszky, K., Auer, J.A., 2002. In vitro studies of a photo-oxidized bovine articular cartilage. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 49, 39–45.

Akens, M.K., von Rechenberg, B., Bittmann, P., Nadler, D., Zlinszky, K., Auer, J.A., 2001. Long term in-vivo studies of a photo-oxidized bovine osteochondral transplant in sheep. BMC Musculoskelet Disord. 2, 9.

Almarza, A.J., Athanasiou, K.A., 2004. Design characteristics for the tissue engineering of cartilaginous tissues. Ann. Biomed. Eng. 32, 2–17.

Aparecida de Aro, A., Vidal Bde, C., Pimentel, E.R., 2012. Biochemical and Anisotropical Properties of Tendons, 43. Micron, Oxford, England, pp. 205–214. (1993).

Arai, S., Orton, E.C., 2009. Immunoblot detection of soluble protein antigens from sodium dodecyl sulfate- and sodium deoxycholate-treated candidate bioscaffold tissues. J. Heart. Valve. Dis. 18, 439–443.

Athanasiou, K.A., Rosenwasser, M.P., Buckwalter, J.A., Malinin, T.I., Mow, V.C., 1991. Interspecies comparisons of in situ intrinsic mechanical properties of distal femoral cartilage. J. Orthop. Res. 9, 330–340.

Bronstein, J.A., Woon, C.Y., Farnebo, S., Behn, A.W., Schmitt, T., Pham, H., Castillo, A. B., Chang, J., 2013. Physicochemical decellularization of composite flexor tendon-bone interface grafts. Plast. Reconstr. Surg. 132, 94–102.

Burk, J., Erbe, I., Berner, D., Kacza, J., Kasper, C., Pfeiffer, B., Winter, K., Brehm, W., 2013. Freeze-Thaw cycles enhance decellularization of large tendons. Tissue Eng. Part C.

Cartmell, J.S., Dunn, M.G., 2000. Effect of chemical treatments on tendon cellularity and mechanical properties. J. Biomed. Mater. Res. 49, 134–140.

Cigliano, A., Gandaglia, A., Lepedda, A.J., Zinellu, E., Naso, F., Gastaldello, A., Aguiari, P., De Muro, P., Gerosa, G., Spina, M., Formato, M., 2012. Fine structure of glycosaminoglycans from fresh and decellularized porcine cardiac valves and pericardium. Biochem. Res. Int. 2012, 979351.

Courtman, D.W., Pereira, C.A., Kashef, V., McComb, D., Lee, J.M., Wilson, G.J., 1994. Development of a pericardial acellular matrix biomaterial: biochemical and mechanical effects of cell extraction. J. Biomed. Mater. Res. 28, 655–666.

Deeken, C.R., White, A.K., Bachman, S.L., Ramshaw, B.J., Cleveland, D.S., Loy, T.S., Grant, S.A., 2011. Method of preparing a decellularized porcine tendon using tributyl phosphate. J. Biomed. Mater. Res. Part B 96, 199–206.

Dienst, M., Burks, R.T., Greis, P.E., 2002. Anatomy and biomechanics of the anterior cruciate ligament. Orthop. Clin. N. AM. 33, 605–620. (v).

Dutra, T.F., French, S.W., 2010. Marrow stromal fibroblastic cell cultivation in vitro on decellularized bone marrow extracellular matrix. Exp. Mol. Pathol. 88, 58–66.

Elder, B.D., Eleswarapu, S.V., Athanasiou, K.A., 2009. Extraction techniques for the decellularization of tissue engineered articular cartilage constructs. Biomaterials 30, 3749–3756.

Elder, B.D., Kim, D.H., Athanasiou, K.A., 2010. Developing an articular cartilage decellularization process toward facet joint cartilage replacement. Neurosurgery 66, 722–727. (discussion 727).

Elkins, R.C., Dawson, P.E., Goldstein, S., Walsh, S.P., Black, K.S., 2001. Decellularized human valve allografts. Ann. Thorac. Surg. 71, S428–S432.

FDA, 1997. Guidance Document for the Preparation of IDE and PMA Applications for Intra-Articular Prosthetic Knee Ligament Devices. Retrieved from: www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm081345.pdf.

FDA, 2010. Draft Guidance for Industry and FDA Staff: Heart Valves—Investigational Device Exemption (IDE) and Premarket Approval (PMA) Applications. Retrieved from: www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM198043.pdf.

FDA, 2011. Guidance for Industry: Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage. Retrieved from: www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM288011.pdf.

Fithian, D.C., Kelly, M.A., Mow, V.C., 1990. Material properties and structure-function relationships in the menisci. Clin. Orthop. Relat. Res., 19–31.

Galili, U., LaTemple, D.C., Walgenbach, A.W., Stone, K.R., 1997. Porcine and bovine cartilage transplants in cynomolgus monkey: II changes in anti-Gal response during chronic rejection. Transplantation 63, 646–651.

Gilbert, T.W., Sellaro, T.L., Badylak, S.F., 2006. Decellularization of tissues and organs. Biomaterials 27, 3675–3683.

Gillard, G.C., Merrilees, M.J., Bell-Booth, P.G., Reilly, H.C., Flint, M.H., 1977. The proteoglycan content and the axial periodicity of collagen in tendon. Biochem. J 163, 145–151.

Goncalves, A.C., Griffiths, L.G., Anthony, R.V., Orton, E.C., 2005. Decellularization of bovine pericardium for tissue-engineering by targeted removal of xenoantigens. J. Heart. Valve. Dis. 14, 212–217.

Gong, Y.Y., Xue, J.X., Zhang, W.J., Zhou, G.D., Liu, W., Cao, Y., 2011. A sandwich model for engineering cartilage with acellular cartilage sheets and chondrocytes. Biomaterials 32, 2265–2273.

Gratzer, P.F., Harrison, R.D., Woods, T., 2006. Matrix alteration and not residual sodium dodecyl sulfate cytotoxicity affects the cellular repopulation of a decellularized matrix. Tissue Eng. 12, 2975–2983.

Gulotta, L.V., Rodeo, S.A., 2007. Biology of autograft and allograft healing in anterior cruciate ligament reconstruction. Clin. Sports. Med. 26, 509–524.

Henaine, R., Roubertie, F., Vergnat, M., Ninet, J., 2012. Valve replacement in children: a challenge for a whole life. Arch. Cardiovasc Dis. 105, 517–528.

Hetherington, V.J., Kawalec-Carroll, J.S., Nadler, D., 2007. Qualitative histological evaluation of photooxidized bovine osteochondral grafts in rabbits: a pilot study. J. Foot Ankle Surg. Official publication of the American College of Foot and Ankle Surgeons 46, 223–229.

Hinton, R.B., Yutzey, K.E., 2011. Heart valve structure and function in development and disease. Annu. Rev. Physiol. 73, 29–46.

Huey, D.J., Hu, J.C., Athanasiou, K.A., 2012. Unlike bone, cartilage regeneration remains elusive. Science 338, 917–921.

Hulsmann, J., Grun, K., El Amouri, S., Barth, M., Hornung, K., Holzfuss, C., Lichtenberg, A., Akhyari, P., 2012. Transplantation material bovine pericardium: biomechanical and immunogenic characteristics after decellularization vs. glutaraldehyde-fixing. Xenotransplantation 19, 286–297.

Ingram, J.H., Korossis, S., Howling, G., Fisher, J., Ingham, E., 2007. The use of ultrasonication to aid recellularization of acellular natural tissue scaffolds for use in anterior cruciate ligament reconstruction. Tissue Eng. 13, 1561–1572.

Iwai, S., Torikai, K., Coppin, C.M., Sawa, Y., 2007. Minimally immunogenic decellularized porcine valve provides in situ recellularization as a stentless bioprosthetic valve. J. Artif. Organs The official journal of the Japanese Society for Artificial Organs 10, 29–35.

Kang, H., Peng, J., Lu, S., Liu, S., Zhang, L., Huang, J., Sui, X., Zhao, B., Wang, A., Xu, W., Luo, Z., Guo, Q., 2012. In vivo cartilage repair using adipose-derived stem cell-loaded decellularized cartilage ECM scaffolds. J. Tissue. Eng. Regen. Med..

Kasimir, M.T., Rieder, E., Seebacher, G., Silberhumer, G., Wolner, E., Weigel, G., Simon, P., 2003. Comparison of different decellularization procedures of porcine heart valves. Int. J. Artif. Organs 26, 421–427.

Katta, J., Stapleton, T., Ingham, E., Jin, Z.M., Fisher, J., 2008. The effect of glycosaminoglycan depletion on the friction and deformation of articular cartilage. Proc. Inst. Mech. Eng. H J. Eng. Med. 222, 1–11.

Kheir, E., Stapleton, T., Shaw, D., Jin, Z., Fisher, J., Ingham, E., 2011. Development and characterization of an acellular porcine cartilage bone matrix for use in tissue engineering. J. Biomed. Mater. Res. Part A 99, 283–294.

Liao, J., Joyce, E.M., Sacks, M.S., 2008. Effects of decellularization on the mechanical and structural properties of the porcine aortic valve leaflet. Biomaterials 29, 1065–1074.

Lohan, A., Stoll, C., Albrecht, M., Denner, A., John, T., Kruger, K., Ertel, W., Schulze-Tanzil, G., 2013. Human hamstring tenocytes survive when seeded into a decellularized porcine Achilles tendon extracellular matrix. Connect. Tissue Res..

Lohmander, S., 1988. Proteoglycans of joint cartilage. Structure, function, turnover and role as markers of joint disease. Baillieres Clin. Rheumatol 2, 37–62.

Manji, R.A., Zhu, L.F., Nijjar, N.K., Rayner, D.C., Korbutt, G.S., Churchill, T.A., Rajotte, R.V., Koshal, A., Ross, D.B., 2006. Glutaraldehyde-fixed bioprosthetic heart valve conduits calcify and fail from xenograft rejection. Circulation 114, 318–327.

Martinello, T., Bronzini, I., Volpin, A., Vindigni, V., Maccatrozzo, L., Caporale, G., Bassetto, F., Patruno, M., 2012. Successful recellularization of human tendon scaffolds using adipose-derived mesenchymal stem cells and collagen gel. J. Tissue. Eng. Regen. Med..

Mercuri, J.J., Gill, S.S., Simionescu, D.T., 2011. Novel tissue-derived biomimetic scaffold for regenerating the human nucleus pulposus. J. Biomed. Mater. Res. Part A 96, 422–435.

Meyer, S.R., Chiu, B., Churchill, T.A., Zhu, L., Lakey, J.R., Ross, D.B., 2006. Comparison of aortic valve allograft decellularization techniques in the rat. J. Biomed. Mater. Res. Part A 79, 254–262.

Meyer, S.R., Nagendran, J., Desai, L.S., Rayat, G.R., Churchill, T.A., Anderson, C.C., Rajotte, R.V., Lakey, J.R., Ross, D.B., 2005. Decellularization reduces the immune response to aortic valve allografts in the rat. J. Thorac. Cardiov. Sur. 130, 469–476.

Nam, J., Choi, S.Y., Sung, S.C., Lim, H.G., Park, S.S., Kim, S.H., Kim, Y.J., 2012. Changes of the Structural and Biomechanical Properties of the Bovine Pericardium after the Removal of alpha-Gal Epitopes by Decellularization and alpha-Galactosidase Treatment. Korean J. Thorac. Cardiov. Sur. 45, 380–389.

Ning, L.J., Zhang, Y., Chen, X.H., Luo, J.C., Li, X.Q., Yang, Z.M., Qin, T.W., 2012. Preparation and characterization of decellularized tendon slices for tendon tissue engineering. J. Biomed. Mater. Res. Part A 100, 1448–1456.

Ota, T., Taketani, S., Iwai, S., Miyagawa, S., Furuta, M., Hara, M., Uchimura, E., Okita, Y., Sawa, Y., 2007. Novel method of decellularization of porcine valves using polyethylene glycol and gamma irradiation. Ann. Thorac. Surg. 83, 1501–1507.

Pei, M., Yan, Z., Shoukry, M., Boyce, B.M., 2010. Failure of xenoimplantation using porcine synovium-derived stem cell-based cartilage tissue constructs for the repair of rabbit osteochondral defects. J. Orthopaed. Res. 28, 1064–1070.

Raghavan, S.S., Woon, C.Y., Kraus, A., Megerle, K., Choi, M.S., Pridgen, B.C., Pham, H., Chang, J., 2012. Human flexor tendon tissue engineering: decellularization of human flexor tendons reduces immunogenicity in vivo. Tissue Eng. Part A 18, 796–805.

Reinhardt, K.R., Hetsroni, I., Marx, R.G., 2010. Graft selection for anterior cruciate ligament reconstruction: a level I systematic review comparing failure rates and functional outcomes. Orthop. Clin. N. AM. 41, 249–262.

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Remlinger, N.T., Czajka, C.A., Juhas, M.E., Vorp, D.A., Stolz, D.B., Badylak, S.F., Gilbert, S., Gilbert, T.W., 2010. Hydrated xenogeneic decellularized tracheal matrix as a scaffold for tracheal reconstruction. Biomaterials 31, 3520–3526.
Revell, C.M., Athanasiou, K.A., 2009. Success rates and immunologic responses of autogenic, allogenic, and xenogenic treatments to repair articular cartilage defects. Tissue Eng. Part B 15, 1–15.
Rieder, E., Seebacher, G., Kasimir, M.T., Eichmair, E., Winter, B., Dekan, B., Wolner, E., Simon, P., Weigel, G., 2005. Tissue engineering of heart valves: decellularized porcine and human valve scaffolds differ importantly in residual potential to attract monocytic cells. Circulation 111, 2792–2797.
Ruzmetov, M., Shah, J.J., Geiss, D.M., Fortuna, R.S., 2012. Decellularized versus standard cryopreserved valve allografts for right ventricular outflow tract reconstruction: a single-institution comparison. J. Thorac. Cardiov. Sur. 143, 543–549.
Sarathchandra, P., Smolenski, R.T., Yuen, A.H., Chester, A.H., Goldstein, S., Heacox, A. E., Yacoub, M.H., Taylor, P.M., 2012. Impact of gamma-irradiation on extracellular matrix of porcine pulmonary valves. J. Surg. Res. 176, 376–385.
Schenke-Layland, K., Vasilevski, O., Opitz, F., Konig, K., Riemann, I., Halbhuber, K.J., Wahlers, T., Stock, U.A., 2003. Impact of decellularization of xenogeneic tissue on extracellular matrix integrity for tissue engineering of heart valves. J. Struct. Biol. 143, 201–208.
Scott, M., Vesely, I., 1995. Aortic valve cusp microstructure: the role of elastin. Ann. Thorac. Surg. 60, S391–S394.
Seebacher, G., Grasl, C., Stoiber, M., Rieder, E., Kasimir, M.T., Dunkler, D., Simon, P., Weigel, G., Schima, H., 2008. Biomechanical properties of decellularized porcine pulmonary valve conduits. Artif. Organs 32, 28–35.
Shahabipour, F., Mahdavi-Shahri, N., Matin, M.M., Tavassoli, A., Zebarjad, S.M., 2013. Scaffolds derived from cancellous bovine bone support mesenchymal stem cells' maintenance and growth. In vitro cellular & developmental biology. Animal 49, 440–448.
Silver, F.H., Freeman, J.W., Seehra, G.P., 2003. Collagen self-assembly and the development of tendon mechanical properties. J. Biomech. 36, 1529–1553.
Stapleton, T.W., Ingram, J., Fisher, J., Ingham, E., 2011. Investigation of the regenerative capacity of an acellular porcine medial meniscus for tissue engineering applications. Tissue Eng. Part A 17, 231–242.
Stapleton, T.W., Ingram, J., Katta, J., Knight, R., Korossis, S., Fisher, J., Ingham, E., 2008. Development and characterization of an acellular porcine medial meniscus for use in tissue engineering. Tissue Eng. Part A 14, 505–518.
Syedain, Z.H., Bradee, A.R., Kren, S., Taylor, D.A., Tranquillo, R.T., 2013. Decellularized tissue-engineered heart valve leaflets with recellularization potential. Tissue Eng. Part A 19, 759–769.
Trivedi, H.L., 2007. Immunobiology of rejection and adaptation. Transplan. Proc. 39, 647–652.
Vavken, P., Joshi, S., Murray, M.M., 2009. TRITON-X is most effective among three decellularization agents for ACL tissue engineering. J. Orthop. Res.Official publication of the Orthopaedic Research Society 27, 1612–1618.
Vesely, I., 1998. The role of elastin in aortic valve mechanics. J. Biomech. 31, 115–123.
Wang, B., Borazjani, A., Tahai, M., Curry, A.L., Simionescu, D.T., Guan, J., Elder, F., Liao, J., S.H., 2010. Fabrication of cardiac patch with decellularized porcine myocardial scaffold and bone marrow mononuclear cells. J. Biomed. Mater. Res. Part A 94, 1100–1110.
Whitlock, P.W., Seyler, T.M., Parks, G.D., Ornelles, D.A., Smith, T.L., Van Dyke, M.E., Poehling, G.G., 2012. A novel process for optimizing musculoskeletal allograft tissue to improve safety, ultrastructural properties, and cell infiltration. J. Bone Joint Surg. Am. 94, 1458–1467.
Williamson, A.K., Chen, A.C., Sah, R.L., 2001. Compressive properties and function-composition relationships of developing bovine articular cartilage. J. Orthop. Res.Official publication of the Orthopaedic Research Society 19, 1113–1121.
Wong, M.L., Leach, J.K., Athanasiou, K.A., Griffiths, L.G., 2011. The role of protein solubilization in antigen removal from xenogeneic tissue for heart valve tissue engineering. Biomaterials 32, 8129–8138.
Wong, M.L., Wong, J.L., Athanasiou, K.A., Griffiths, L.G., 2013. Stepwise solubilization-based antigen removal for xenogeneic scaffold generation in tissue engineering. Acta Biomater. 9, 6492–6501.
Woods, T., Gratzer, P.F., 2005. Effectiveness of three extraction techniques in the development of a decellularized bone-anterior cruciate ligament-bone graft. Biomaterials 26, 7339–7349.
Yang, M., Chen, C.Z., Wang, X.N., Zhu, Y.B., Gu, Y.J., 2009. Favorable effects of the detergent and enzyme extraction method for preparing decellularized bovine pericardium scaffold for tissue engineered heart valves. J. Biomed. Mater. Res. Part B 91, 354–361.
Yang, Q., Peng, J., Guo, Q., Huang, J., Zhang, L., Yao, J., Yang, F., Wang, S., Xu, W., Wang, A., Lu, S., 2008. A cartilage ECM-derived 3-D porous acellular matrix scaffold for in vivo cartilage tissue engineering with PKH26-labeled chondrogenic bone marrow-derived mesenchymal stem cells. Biomaterials 29, 2378–2387.
Yang, Z., Shi, Y., Wei, X., He, J., Yang, S., Dickson, G., Tang, J., Xiang, J., Song, C., Li, G., 2010. Fabrication and repair of cartilage defects with a novel acellular cartilage matrix scaffold. Tissue Eng. Part C 16, 865–876.
Yoshida, R., Vavken, P., Murray, M.M., 2012. Decellularization of bovine anterior cruciate ligament tissues minimizes immunogenic reactions to alpha-gal epitopes by human peripheral blood mononuclear cells. The Knee 19, 672–675.
Youngstrom, D.W., Barrett, J.G., Jose, R.R., Kaplan, D.L., 2013. Functional characterization of detergent-decellularized equine tendon extracellular matrix for tissue engineering applications. PLoS One 8, e64151.
Yu, B.T., Li, W.T., Song, B.Q., Wu, Y.L., 2013. Comparative study of the Triton X-100-sodium deoxycholate method and detergent-enzymatic digestion method for decellularization of porcine aortic valves. Eur. Rev. Med. Pharmacol. Sci. 17, 2179–2184.
Zang, M., Zhang, Q., Chang, E.I., Mathur, A.B., Yu, P., 2012. Decellularized tracheal matrix scaffold for tissue engineering. Plast. Reconstr. Surg. 130, 532–540.
Zhai, W., Chang, J., Lin, K., Wang, J., Zhao, Q., Sun, X., 2006. Crosslinking of decellularized porcine heart valve matrix by procyanidins. Biomaterials 27, 3684–3690.
Zhao, Y., Zhang, S., Zhou, J., Wang, J., Zhen, M., Liu, Y., Chen, J., Qi, Z., 2010. The development of a tissue-engineered artery using decellularized scaffold and autologous ovine mesenchymal stem cells. Biomaterials 31, 296–307.
Zhou, J., Fritze, O., Schleicher, M., Wendel, H.P., Schenke-Layland, K., Harasztosi, C., Hu, S., Stock, U.A., 2010. Impact of heart valve decellularization on 3-D ultrastructure, immunogenicity and thrombogenicity. Biomaterials 31, 2549–2554.
Zhu, W., Mow, V.C., Koob, T.J., Eyre, D.R., 1993. Viscoelastic shear properties of articular cartilage and the effects of glycosidase treatments. J. Orthop. Res. Official publication of the Orthopaedic Research Society 11, 771–781.

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