Decellularization for the retention of tissue niches

Deana Moffat1, Kaiming Ye1,2 and Sha Jin1,2

Abstract
Decellularization of natural tissues to produce extracellular matrix is a promising method for three-dimensional scaffolding and for understanding microenvironment of the tissue of interest. Due to the lack of a universal standard protocol for tissue decellularization, recent investigations seek to develop novel methods for whole or partial organ decellularization capable of supporting cell differentiation and implantation towards appropriate tissue regeneration. This review provides a comprehensive and updated perspective on the most recent advances in decellularization strategies for a variety of organs and tissues, highlighting techniques of chemical, physical, biological, enzymatic, or combinative-based methods to remove cellular contents from tissues. In addition, the review presents modernized approaches for improving standard decellularization protocols for numerous organ types.

Keywords
Decellularization, extracellular matrix, tissue and organ, chemical method, physical method, biological method, enzymes

Date received: 30 March 2021; accepted: 1 May 2022

Introduction
To circumvent the disparity between the number of organ donors and transplant recipients, studies have been focused on using decellularized tissue scaffolds to help regenerate organs in vivo or in vitro. The decellularized tissues provide proper cues and anatomical structures for cells to proliferate, differentiate, and organize into adequately functioning tissues. Of all scaffolds used in regenerative medical research, those generated directly from or designed to mimic the qualities of the extracellular matrix (ECM) are considered the gold standard for making proper graft to repair damaged tissues or organs. The ECM contains physiochemical cues needed to regulate accurately cell phenotype and function, as well as to provide a foundation for the desired organ’s natural structure. Native ECM is ideal for regenerative medicine and tissue engineering approaches, as it retains an original tissue anatomical structure. Therefore, retention of native protein niches and physiochemical properties is the primary goal of decellularization. Often, a decellularization protocol must be customized in accordance with the origin of the tissue, as there is no singular ideal approach that can fit all. In addition, proteomic studies have revealed that the proteomic content of the matrisome varies according to the tissue from which the ECM is sourced.1 Hu et al.2 has implied that that age and sex may also play a lesser, but still notable role in the proteomic makeup of the ECM. This corroborates recent findings by Ozcebe et al.3 which indicate recellularization potential of ECM varies by donor age. To generate an ECM-derived scaffold, a plethora of decellularization protocols have been generated over the past decades, each with their own unique advantages and disadvantages. Some of these protocols often contradict to each other, due

1 Department of Biomedical Engineering, Binghamton University, State University of New York (SUNY), Binghamton, NY, USA
2 Center of Biomanufacturing for Regenerative Medicine, Binghamton University, State University of New York (SUNY), Binghamton, NY, USA

Corresponding author:
Sha Jin, Department of Biomedical Engineering, Thomas J. Watson College of Engineering and Applied Sciences, State University of New York, Biotechnology Building BI 2612, Binghamton, NY 13902, USA.
Email: sjin@binghamton.edu
to diverse experimental conditions including different tissue types, temperatures, flow applications, pH, etc. As a result, it is difficult to directly compare the efficiency of each individual component utilized. This review aims to highlight a variety of decellularization approaches, compare the methods used as a basis for decellularization of tissue to procure a well-decellularized ECM with minimal damage to the biomechanical structure and proteomic makeup. We provide a comprehensive analytical interpretation on the most recent advances in decellularization strategies for a variety of organs and tissues, highlighting techniques of chemical, physical, biological, enzymatic, or combinative-based methods to remove cellular content from tissues. In addition, we collectively present modernized approaches for improving standard decellularization protocols for various types of tissues and organs.

**Decellularization for ECM retrieval**

The ECM is a naturally occurring, dynamically active macromolecular network arranged in a highly organized tissue-specific manner. It is predominantly composed of collagens, proteoglycans, elastin, glycoproteins, and secreted factors based on tissue type (Figure 1). These components establish the ECM as a mechanically stable basement membrane that serves as a structural support for anchored cells. The bioactive molecules and growth factors secreted into the ECM by the cells allow for conformational changes in its structure, which induce chemical signals that regulate cell proliferation, differentiation, adhesion, migration, polarity, and apoptosis. ECM has been shown to preferentially promote the differentiation of cells from the same tissue origin. Stem cells or stem cell-derived cells seeded into the ECM have their cell morphology influenced toward the cell lineage of the ECM origin tissue, regardless of the origin of the cells being seeded. 

Interestingly, it has been shown that ECM from various species can support human stem cell proliferation and differentiation if the ECM constructs are sourced from the same tissue type as the organ of interest. Although the properties of ECM make it an ideal model for implantable scaffolds for tissue engineering purposes, the ECM’s...
intricate biomechanical and biochemical composition make it difficult to replicate its properties from inorganic materials. As such, it is of the utmost importance to develop methods to obtain naturally occurring ECM for tissue engineering. Decellularization, therefore, has been extensively studied in the past two decades.

Decellularized ECM has shown success as scaffolds for generation of a variety of tissue and organ types. Typically, decellularization results in the retention of larger macromolecules such as polysaccharides and constructive collagens due to their size and degree of crosslinking, while smaller ECM-associated components like growth factors, chemokines, and signaling molecules are more easily washed away. The loss of these molecules cripples the performance ability of the resulting tissue microenvironment to act as a tissue specific platform for recellularization. Different decellularization techniques vary in the rates of lost protein niches. Crap et al. has stated that suc-Zation. Different decellularization techniques vary in the performance ability of the resulting tissue microenvironment to act as a tissue specific platform for recellularization. Different decellularization techniques vary in the rates of lost protein niches. Crap et al. has stated that successful decellularization should be determined on the basis of producing (I) ECM which does not contain more than 50 ng of DNA per dry weight, (II) residual DNA fragments no longer than 200 bp, and (III) no visible nuclear components. Several strategies have since been able to successfully decellularize tissues to retrieve the ECM by this definition. Figure 1 illustrates two major decellularization processes, that is, minced tissue and whole organ decellularization, for ECM procurement and potential applications in tissue engineering and regenerative medicine. Minced or sectioned decellularized tissues can be reconstructed and used as cell culture substrates or as injectable hydrogels to improve cell proliferation, differentiation, and engraftment. Decellularized tissue ECM proteomics and subsequent bioinformatics analyses allow distinct applications, including but not limited to the understanding of tissue microenvironments associated diseases such as tumor metastasis and signaling molecules crucial to in vitro tissue generation (Figure 1). Hydrogels prepared from decellularized tissues can also be used as a bioink for 3D bioprinting of tissue or as a disease model. Decellularized animal whole organs retain their original 3D structure and vasculature network and thus are applied to repopulate human cells for organ regeneration.

**Decellularization techniques**

Common decellularization techniques utilize chemical, physical, biologic, or enzymatic methods to remove cellular content from the tissue in order to retrieve naturally occurring ECM. To overcome the deleterious effects associated with a single particular decellularizing method or agent, techniques are often used in combination. The specific tissue type being decellularized should also be taken into consideration, as the same decellularizing protocol can produce different results in different tissues. We discuss the decellularization techniques in details in the following.

**Chemical treatment-based decellularization**

The efficiency of chemical treatment-based decellularization varies according to the size, density, cellularity, thickness, and lipid content of the starting tissue. Chemical agents are a favorite among decellularization strategies, as there exists a plethora of evidence to validate their ability to quickly and efficiently rid tissue of native cells. Despite this, chemical agents usually are incapable of completely stripping cellular components and can leave trace amounts of dsDNA, mitochondrial DNA, mitochondria, and membranous phospholipids, the presence of which can jeopardize subsequent recellularization attempts and invoke immune responses. Moreover, chemical agents are typically cytotoxic and must be followed with additional washing steps to rid the decellularized construct of remnant chemical residues. Chemical agents can be compounded together, used in tandem, or combined with other decellularizing strategies to expedite the decellularization processes. In protocols where multiple decellularizing agents are used, the order by which a tissue sample is exposed to different chemical agents plays a critical role in the proteomic and biomechanical features of the resulting ECM. Chemical decellularizing agents are categorized as being either ionic detergents, non-ionic detergents, zwitterionic detergents, solvents, acids, bases, or hypertonic and hypotonic solutions.

Detergents, also known as surfactants, are the most commonly used chemical means for decellularization. Owing to their popularity, they are generally cheap, quick, and efficient decellularizing agents. The most common detergents for decellularization are the nonionic detergent Triton X-100 and the ionic detergent Sodium Dodecyl Sulfate (SDS). Triton X-100 removes cellular content by disrupting lipid-lipid and protein-lipid interactions without affecting protein-protein interactions, whereas SDS disrupts protein-protein interactions and solubilizes cell membranes. Ionic detergents like SDS have the benefit of being able to effectively remove nuclear materials in shorter time frames compared to other chemical treatments. This, however, is at the expense of greater damage to the ECM matrisome, as treatment of tissue with SDS can result in an altered microstructure that diminishes the biomechanical integrity of the ECM. The concentration of SDS and tissue exposure time must therefore be optimized for any SDS-based protocol, as increased exposure is directly linked to decreased ECM biomechanical properties. Also easily lost in SDS decellularizations are fibronectins, glycosaminoglycans (GAGs), proteoglycans, and ECM regulators and secreted factors. Thus, SDS is associated with removal of significant amounts of biochemical cues contained in a native ECM. Most successful...
SDS-based decellularizations use a concentration between 0.1% and 1% SDS.\textsuperscript{46-49} However, decellularization at SDS concentrations as low as 0.01% in ovine small intestine and as high as 2% in articular cartilage have been performed to varying degrees of success.\textsuperscript{44,50}

Remnant SDS within a decellularized matrix is difficult to remove, which is detrimental to recellularization due to its high cytotoxicity, potentially due to the exposure of the collagen C and N termini which reveal antigenic sites.\textsuperscript{51} As such, SDS treatment is commonly followed by Triton X-100. Alternatively, decellularizations using nonionic detergents like Triton X-100 are considered to be less harsh on tissue than ionic detergents, and are therefore useful for decellularizations where preservation of the ECM proteome is important.\textsuperscript{39} Although milder than ionic detergents, Triton X-100 is still itself a strong detergent and its use can lead to proteomic complications, especially at high concentrations. Normally decellularization protocols do not use concentrations of Triton X-100 in excess of 1%, though some protocols have found success with concentrations as high as 3% so long as exposure length was reduced accordingly.\textsuperscript{43,47,52} Like all other detergents, the adverse effects of Triton X-100 include, but are not limited to, significantly increased stiffness of the resulting matrix, lowered recellularization potential due to cytotoxic chemical remnants, and loss of bioactive molecules such as GAGs and proteoglycans.\textsuperscript{39}

In recent years, several new detergents have been discovered for decellularization protocols. The ionic detergents sodium deoxycholate (SDC) and sodium lauryl ether sulfate (SLES) have gained popularity upon discovery that they contain decellularizing abilities on par with or superior to SDS-based protocols. Both SDC and SLES have been shown to better preserve collagen and GAG content than SDS while showcasing superior biocompatibility following recellularization.\textsuperscript{39,53-56} SDC can successfully decellularize tissues at concentrations up to 4%,\textsuperscript{57,58} although increased concentration of SDC does not contribute to higher nuclear removal rates and results in increased damage to structural integrity, despite its inability to degrade collagens.\textsuperscript{57,59} Additionally, SDC decellularizations must be followed with agents such as Deoxyribonuclease (DNase) to reduce induction of DNA agglutination at the tissue surface.\textsuperscript{57} To date, the requirement of DNase for successful SDC decellularization has only been overcome in cardiac tissue, as described by Methé et al.\textsuperscript{56} Regardless, SDC has proven to be successful for decellularization of several tissue types, including rat peripheral nerve,\textsuperscript{57} auricular cartilage,\textsuperscript{58} murine ovary,\textsuperscript{53} and porcine heart valve,\textsuperscript{59} while SLES has successfully decellularized of rat heart, kidney, ovary, and bone tissue,\textsuperscript{51,60,61} demonstrating the versatility of both ionic surfactants. SLES is particularly intriguing, as Emami et al.\textsuperscript{16} found that SLES may be superior to not only SDS, but also other decellularizing agents, including Triton X-100 and enzymatic Trypsin/EDTA for decellularization of histologically dense bone tissue. Li et al.\textsuperscript{62} advised that the addition of dextrose perfusion pretreatment step prior to SLES exposure assists in further protecting collagens from degradation by the detergent. Potassium Laurate (PL), a naturally occurring ionic detergent, is also of interest. In 2019, Obata et al.\textsuperscript{63} was the first to describe its potential for decellularization of rat lung. They reported that PL was capable of sufficient cellular and DNA removal while significantly reducing ECM damage associated with SDS. Most ostensibly, these results indicate the potential for the development of PL based protocols to permit effective detergent based decellularization of easily damaged thin tissues. As such, further investigations into the potential of PL to act as a decellularizing agent are necessary. The only nonionic detergents other than Triton X-100 to be investigated for use in decellularization are Tween 20 and Tween 80, both of which consistently produce inadequate decellularizations apart from a study by Chaschin et al.\textsuperscript{67} who found that inclusion of Tween 80 in decellularization of human aorta by supercritical carbon dioxide (scCO2) was beneficial.\textsuperscript{64-66}

Zwitterionic detergents share properties with both ionic and nonionic detergents. They have been shown to preserve the ECM ultrastructure but tend to be limited in their ability to completely remove cellular content.\textsuperscript{39,60} For this reason, they are typically followed by treatment with enzymatic techniques to complete the decellularization. They are known to target and break protein-protein bonds in a similar fashion to ionic detergents but are far less aggressive. 3-\{(3-cholamidopropyl) (dimethylammonio)-1-propane sulfonate (CHAPS) decellularizes tissues by disrupting lipid-protein and lipid-lipid interactions.\textsuperscript{68} Its reduced permeating qualities limit CHAPS’ ability to remove nuclear DNA, thereby making CHAPS more applicable for decellularization of thin tissues.\textsuperscript{68-70} CHAPS also highly retains the biomechanical properties of native tissue, making it ideal for tissues, which must be capable of contracting with ease such as heart or lung.\textsuperscript{70} Other zwitterionic detergents SulfoBetaine 10 and SulfoBetaine 16 (SB10 and SB16, respectively) induce cell apoptosis, resulting in improved cell removal, which eliminates the need for vigorous washing steps.\textsuperscript{71} Physical agitation is used to gently assist in coaxing apoptotic cells from the matrix.\textsuperscript{71,72} SB-10 and SB-16 have been shown to result in better retention of ECM basement membrane integrity and higher rates of cell removal when compared to ionic detergents.\textsuperscript{73} Both detergents are typically followed by enzymatic treatments, such as DNase, to promote DNA fragmentation and reduce immunogenicity of the ECM construct.\textsuperscript{39,71} In the case of CHAPS treatment, immunogenicity can also be reduced by induction of a physiologically accurate pH during decellularization to reduce inflammation upon later implantation.\textsuperscript{74,75} Song et al.\textsuperscript{71} describes that cellular apoptosis is more quickly induced...
when SB-10 and SB-16 are combined with another apoptosis-inducing agent, camptothecin, while McCrary et al.\textsuperscript{57} found that SB-10 and SB-16 treatment could improve SDC decellularizations.

Solvents, another category of chemically-based decellularization techniques, are generally inadequate for decellularization. They can be used as an initiating step for decellularization to remove lipids and reduce fat content of the tissue.\textsuperscript{76–78} Exercising caution when using solvent-based techniques is encouraged, as the use of solvents often results in damage to the 3D microstructure and reduces the likelihood of successful recellularization.\textsuperscript{17} Solvents for decellularization include alcohols, acetone, tri-n-butyl phosphate (TNBP), dimethyl ether (DME), and urea. Alcohols dehydrate native cells to lyse them and have been shown to delipidate tissues. The use of alcohols is effective for the removal of fat from thick, dense muscle tissue. However, they can crosslink and precipitate collagen, altering the structural integrity of the 3D proteomic network.\textsuperscript{17} Alcohols such as isopropanol,\textsuperscript{77,79} glycerol,\textsuperscript{80} ethanol,\textsuperscript{81–83} and methanol\textsuperscript{81} have all proven to be effective for removal of lipid content, though some reports indicate that isopropanol may be a superior agent for this purpose.\textsuperscript{84} Like alcohols, acetone removes cellular content from ECM by acting on lipids. It is typically used in conjunction with ethanol, albeit these ethanol-acetone solutions dehydrate the ECM and result in significant increases in stiffness.\textsuperscript{85,86} Because acetone has a dehydrating effect on ECM, some studies use it to chemically dry and sterilize collagen matrices rather than to decellularize them.\textsuperscript{87} Due to its sterilizing properties, it has reduced immunogenicity compared to detergent-based techniques.\textsuperscript{81} Typically, acetone results in extensive adverse impacts on the biomechanics of ECM and is not recommended for use in tissues which are contracting or load bearing.\textsuperscript{87,88}

TNBP, which does not share the same dehydrating effect as alcohols or acetone, has been shown to be capable of successful removal of DNA content from the ECM membrane when used in tandem with multiple enzymatic and chemical decellularizing agents. Recently, TNBP has been utilized to assist in physical scCO\textsubscript{2} decellularization strategies, though these processes can be lengthy.\textsuperscript{39,89,90} Unlike alcohols and acetone, TNBP is not useful for decellularization of dense muscle tissues, as increased concentrations or exposure times do not improve nuclear removal rates, but also do not further damage the matrix of the ECM.\textsuperscript{91,92} Unlike alcohols and acetone, TNBP may promote collagen crosslinking as opposed to degrading them.\textsuperscript{90,91} It can also act as a principal decellularizing agent, and has been effective in protocols for decellularization of porcine diaphragm,\textsuperscript{91} rabbit tendon,\textsuperscript{90} porcine trabeculae,\textsuperscript{90} and human vein.\textsuperscript{91}

Dimethyl ether (DME) and urea are decellularizing solvents which are not yet extensively studied, and it is therefore difficult to infer their effect on the ECM. Kanda et al. demonstrated that DME under subcritical temperatures, followed by DNA fragmentation with DNase, can effectively replace SDS in decellularization protocols for porcine aorta and ostrich carotid artery.\textsuperscript{94,95} In addition to reducing structural damage to the ECM, DME may also reduce the immunogenic response properties. Urea is a powerful solubilizing agent with a high affinity for antigen removal. Addition of urea to decellularization protocols showed severe alterations to the histoarchitecture, elastin, and GAG content of the ECM in bovine bone and pericardium.\textsuperscript{86,97} These findings indicate that urea may not be an ideal agent for use in future decellularization attempts.

Protocols which utilize acids and bases for decellularization have also been developed. Peracetic acid (PAA) can be used as both a decellularizing and disinfecting agent. In this way, it is much like alcohols and acetone, though it may result in fewer significant impacts to the biomechanical structure of the ECM.\textsuperscript{98} Similar to but stronger than PAA is acetic acid (AA), which is more likely to damage the structural integrity of the ECM by destroying or removing collagens, although it has a negligible effect on smaller bioactive molecules such as GAGs.\textsuperscript{99} For tendon, acetic acid or hydrochloric acid can be utilized to successfully strip calcium from the matrix prior to decellularization by detergent.\textsuperscript{100} Ammonium-hydroxide, a mildly basic compound, disrupts the cell membrane and breaks down the cell wall while disrupting hydrogen bonds to induce cell lysis. Typically, following ammonium-hydroxide treatment, an ionic or nonionic detergent solubilizes the cell membrane and separates the proteins from the matrix. Ammonium-hydroxide has been reported to have been utilized in protocols to successfully decellularize liver,\textsuperscript{101–104} urological tissue,\textsuperscript{94} and mesenchymal stem cell derived ECM.\textsuperscript{105} It is considered to work best in tandem with Triton X-100 under perfusion or static conditions.\textsuperscript{101,106,107} Treatment with enzymes like DNase following decellularization with ammonium hydroxide and Triton X-100 has been recorded to result in up to 100% DNA removal in thin cell sheets.\textsuperscript{106}

Unlike all other types of chemical decellularizations, hypertonic and hypotonic solution-based decellularizations are consistently described as having little to no negative impact on the proteome of the ECM along with powerful removal of cellular DNA.\textsuperscript{7,18} Hypertonic solution rinses followed by hypotonic solution rinses induce cell lysis by an osmotic shock to decellularize tissue. This technique seeks to capitalize on the initial incubation in the hypertonic water step used in many decellularization strategies and limit exposure to harsh chemicals known to strip ECM of native proteins. Previously, most attempts to utilize this technique resulted in subpar immunogenic conditions for implantation into a host biosystem.\textsuperscript{108,109} However, recent advancements in the technique have increased removal rates of cellular content, thereby making the resulting constructs more biocompatible. Recent advances
by our group have shown that NaCl and distilled water changes under agitation can produce porcine pancreatic ECM, which meets criteria for successful decellularization. Interestingly, this protocol resulted in DNA removal rates similar to or exceeding those of an SDS and Triton X-100 based techniques. Similar studies which use hypertonic NaCl solution to decellularize cornea have indicated a downside with this technique, in which the tissue grafts produced by these decellularized corneal grafts remained transparent in rabbit eyes for 6 months, which is approximately half the amount of time that grafts produced by SDS-containing isotonic buffer decellularized corneal grafts. Due to the lack of decellularizing strength associated with this technique, only minced or sectioned tissue samples have been successful. Thus, no hypertonic/hypotonic solution change protocol has been developed to date for whole organ decellularization. Table 1 summarizes numerous chemical methods that have been developed and utilized to decellularize tissues based on the classification of the chemicals with comparison of their advantages and disadvantages.

Physical/Mechanical decellularization

Physical methods of removing cellular content from tissue work by disrupting cell membranes and creating unfavorable cellular environments that can induce apoptosis. Physical decellularization has the advantage of producing a uniform effect throughout tissue. Moreover, their effect is more predictable than chemical or enzymatic decellularizing agents. Physical treatment alone is often insufficient for decellularization. While they can induce cell lysis, they are ineffective for complete removal of cell or nuclear remnants. Nonetheless, they can be used in conjunction with chemical, biological, or enzymatic decellularizing agents to reduce exposure times and aid in the retention of ECM proteomic content. Vacuums, high hydrostatic pressure (HHP), freeze-thaw cycles, scCO2, and sonication are all physical methods commonly used for decellularization.

Vacuum-assisted decellularizations refer to any decellularization technique that is aided or accelerated by the usage of a negative pressure. Though the use of negative pressure systems is ineffective for decellularization on its own, it has been found to be effective when used in combination with other physical methods as well as chemical or enzymatic methods. These systems can significantly reduce the decellularization time without sacrificing additional proteins, allowing for more efficient processes and reduced exposure to potentially damaging agents.

HHP bursts cells with minimal risk of the type of protein denaturation associated with nonphysical strategies. Pressures over 150 MPa are required to achieve adequate cell death, though pressures over 500 MPa can potentially result in ECM protein denaturation. Supercooling pretreatment of tissue before HHP decellularization can also assist in reducing the likelihood of protein denaturation. HHP has the disadvantage of not retaining the biomechanical properties of the original tissue as well as other physical methods like freeze-thaw cycles. However, it has been found to produce immunologically superior decellularized matrices that increase likelihood of achieving recellularization.

Freeze-thaw cycles result in thermal shock-induced cell death upon immersing tissue in liquid nitrogen. Freeze-thaw is used as a precursor step to decellularization and cannot effectively decellularize any kind of tissue alone. Therefore, freeze-thaw cycles have been followed by washes with detergents such as Triton X-100, solvents like isopropanol, and enzymes such as trypsin. This method assists in retaining a majority of the ECM’s 3D structural integrity and allowing for reduced exposure required for adequate cell removal by chemical or enzymatic agents. Freeze-thaw cycles are particularly applicable for use in tissues when treat with highly damaging agents known to harm ECM components, such as SDS and SDC. However, it should be carried out with caution, as they can also cause main components of the ECM to rupture and make recellularization difficult.

scCO2 is notable for its ability to decellularize tissues in a fraction of the time that it takes for most chemical agents. The carbon dioxide used in this technique is an ideal gas for decellularization, as it is nontoxic, inflammable, relatively inert, and cost effective. The exact mechanism by which scCO2 removes cells and cellular content from a tissue has been widely disputed, although previous beliefs that it is the result of high pressure induced cell bursting have been disproven. Recently, attempts to identify the main mechanism by which it decellularized tissue has brought claims that it may induce hypoxia, though this is still uncertain. Previous studies found scCO2 to be inadequate for developing viable scaffolds, as the final matrices were often too dehydrated for reseeding. Pre-saturating scCO2 with water overcomes tissue dehydration. In addition, combination of scCO2 with 2% PAA can successfully decellularize tissues without damaging the vasculature or proteome of their ECM. PAA was found to be superior to all other solvents when paired with scCO2. Combination of scCO2 with ethanol also results in the production of successfully decellularized and immunologically inert acellular matrix from pig esophagus, albeit, to a lesser extent than detergent-based approaches.

Sonication is a technique that allows decellularizing agents to better permeate a tissue. Sonication has been used to successfully assist in decellularization of aorta, larynx, and cartilage. The cavitation intensity during the course of sonication is influenced by pH, temperature, viscosity, diffusion rate of dissolved oxygen and vapor pressure, and solubility of gas in liquid. These conditions are heavily influenced by the concentration of
| Classification   | Decellularizing agent                  | Mechanism                          | Advantages                                                                 | Disadvantages                                                                 | References                                                                 |
|------------------|----------------------------------------|------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Ionic Detergents | Sodium Dodecyl Sulfate (SDS)           | Breaks non-covalent bonds          | Consistently removes over 90% of cellular content                          | Requires vigorous rinsing                                                      | Alizadeh et al.⁴⁰, Xu et al.⁴¹, Elder et al.⁴⁴, Wang et al.⁴⁸,               |
|                  |                                        |                                    |                                                                             | Damages biomechanical integrity                                              | Schmid et al.¹¹³, Dal Sasso et al.¹¹⁴, Barnard et al.¹¹⁵,                  |
|                  | Sodium Deoxycholate (SDC)              | Disrupts cell membrane             | Higher retention of GAGs and collagen than SDS                             | Induces immune response                                                       | Simsa et al.³⁹, Alshaikh et al.⁵³, McCrary et al.⁵⁷, Rahman et al.⁵⁸,     |
|                  |                                        |                                    |                                                                             | Induces DNA agglutination                                                      | Hwang et al.⁵⁹                                                          |
|                  | Sodium Lauryl Ester Sulfate (SLES) &   | Disrupts cell membrane             | Higher retention of GAGs and collagen than SDS                             | Removable collagen and GAGs                                                    | Emami et al.¹⁶, Ma et al.⁵⁴, Keshvari et al.⁵⁵, Kawasaki et al.⁶¹         |
|                  | Sodium Lauryl Sulfate (SLS)            |                                    |                                                                             | Higher recellularization potential than SDS                                 |                                                                             |
|                  |                                        |                                    |                                                                             | Preserves biomechanical integrity and microarchitecture                      |                                                                             |
|                  | Potassium Laurate (PL)                 | Solubilizes membrane proteins      | Better retention of GAGs, elastin, and collagen than SDS                   | Not extensively studied                                                        | Obata et al.⁶³                                                        |
| Nonionic Detergents | Triton X-100                          | Disrupts lipid-lipid and lipid-protein interactions | Higher recellularization potential than SDS and SDC                        | Increases stiffness                                                           | Simsa et al.³⁹, Xu et al.⁴¹, Luo et al.⁴⁹, Liao et al.¹¹⁶               |
|                  |                                        |                                    |                                                                             | Does not require extensive rinsing                                            |                                                                             |
|                  | Tween 20 & Tween 80                    | Induce cell lysis                  | Protects proteins from denaturation                                         | Insufficient for decellularization alone                                      | Aeberhard et al.⁶⁴, Heidarzadeh et al.⁶⁵, O'Neil et al.⁶⁶               |
| Zwitterionic Detergents | CHAPS                                  | Disrupts lipid-lipid and lipid-protein interactions | Retains biomechanical integrity                                            | Minimally impactful                                                           | Mendibil et al.⁶⁸, Marin-Tapia et al.⁶⁹, Qi et al.⁷⁰, Tsuchiya et al.⁷⁴, Zvarova et al.⁷⁵ |
|                  |                                        |                                    |                                                                             | Reduces biomechanical integrity                                               |                                                                             |
|                  | SB10 & SB16                            | Induces apoptosis                  | Retains small bioactive molecules                                          | Insufficient for decellularization alone                                      |                                                                             |
|                  |                                        |                                    |                                                                             | Unable to permeate tissue                                                     |                                                                             |
|                  |                                        |                                    |                                                                             | pH dependent                                                                  |                                                                             |
|                  |                                        |                                    |                                                                             | Not extensively studied                                                        |                                                                             |

*(Continued)*
| Classification               | Decellularizing agent | Mechanism                | Advantages                                                                 | Disadvantages                                                                 | References                                                                 | Notes                                                                 |
|-----------------------------|-----------------------|--------------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------|
| Solvents                    | Alcohols              | Dehydrate and lyse cells | Effective for removing fat from thick tissues                              | Crosslinks and precipitates collagens                                          | Crapo et al.\(^a\), Lumpkins et al.\(^b\), Kabirian and Mozafari\(^c\)   |                                                                       |
|                             |                       |                          |                                                                             | Increases stiffness                                                             |                                                                          |                                                                       |
|                             | Acetone               | Acts on lipids           | Sterilizes ECM                                                               |                                                                              |                                                                          |                                                                       |
|                             |                       |                          |                                                                             | Increases stiffness                                                             |                                                                          |                                                                       |
|                             | Tri(n)butyl Phosphate (TNBP) | Disrupts protein-protein interactions | Les structurally damaging than alcohols and acetone                          | Insufficient for decellularization alone                                        | Simsa et al.\(^d\), Xing et al.\(^e\), Duarte et al.\(^f\), Deeken et al.\(^g\), Yang et al.\(^h\) |                                                                       |
|                             |                       |                          | Promotes collagen crosslinking                                               |                                                                              |                                                                          |                                                                       |
|                             |                       |                          | Increases protein retention and recellularization potential                  |                                                                              |                                                                          |                                                                       |
|                             |                       |                          | Compatible with detergent-based methods and physical methods                 |                                                                              |                                                                          |                                                                       |
|                             | Urea                  | Disrupts non-covalent bonds | Reduces immunogenicity                                                        | Primarily used as an antigen removal agent as opposed to a decellularization agent | Wong et al.\(^a\), Wong et al.\(^b\)                                      |                                                                       |
|                             |                       |                          |                                                                              | Removes cytotoxic detergents                                                  |                                                                          |                                                                       |
|                             |                       |                          |                                                                              | Disrupts biomechanical integrity                                              |                                                                          |                                                                       |
|                             |                       |                          |                                                                              | Disrupts collagen organization                                                |                                                                          |                                                                       |
|                             | dimethyl ether (DME)  | Acts on lipids           | May reduce immunogenicity                                                    | Insufficient for decellularization alone                                        | Kanda et al.\(^a\), Kanda et al.\(^b\)                                    |                                                                       |
|                             |                       |                          |                                                                              |                                                                              |                                                                          |                                                                       |
|                             | Acids                 | Solubilizes cell membrane and disrupts nucleic acids                         | Retains biomechanical integrity                                              | Damages collagen                                                              | Syed et al.\(^a\), Abaci and Guvendir\(^b\), Zhao et al.\(^c\), Dong et al.\(^d\), Datta et al.\(^e\) |                                                                       |
|                             |                       |                          | Negligible reduction of small bioactive molecules such as GAGs              |                                                                              |                                                                          |                                                                       |
|                             |                       |                          | Can strip calcium from bone tissue                                           |                                                                              |                                                                          |                                                                       |
|                             | Bases                 | Denature chromosomal and plasmid DNA                                       | Can achieve 100% DNA removal in thin tissue samples                          | Removes growth factors                                                         | Kajbafzadeh et al.\(^a\), Farag et al.\(^b\), Poomejad et al.\(^c\)    |                                                                       |
|                             |                       |                          |                                                                              | Reduces biomechanical integrity                                               |                                                                          |                                                                       |
|                             | Hypotonic & Hypertonic Solutions | Induce cell lysis by osmotic shock                                      | Gentler removal of cells than detergent-based methods                        | Difficult to achieve acceptable cellular removal                              | Hu et al.\(^a\), Dahl et al.\(^b\), Woods and Gratzer\(^c\), Lee et al.\(^d\) |                                                                       |
|                             |                       |                          | High retention of ECM components                                            | Ineffective for whole organ decellularization                                 |                                                                          |                                                                       |
|                             |                       |                          |                                                                              | Can result in ECM swelling                                                   |                                                                          |                                                                       |
the decellularizing agent. Sonication protocols use low concentrations of SDS, as this agent can aggressively solubilize cellular content.145 However, sonication is itself a physically aggressive process, which may or may not cause structural damage to the ECM.146 Ultrasonic baths are better able to allow smooth penetration of chemical agents into tissue than sonicators while also causing less damage to the ECM ultrastructure. This, however, is at the cost of longer protocols.147 Table 2 highlighted decellularizing methods with advantages and disadvantages of each physical method.

**Biological treatment-based decellularization**

Biological treatment-based decellularization aims to induce apoptosis of cells. These apoptosis-inducing agents are looked upon favorably for decellularization protocols because they result in contained cell debris that can more easily be washed away. Few studies have investigated the role of apoptosis-inducing agents, but those which indicate that they likely involve complicated mechanisms, leaving the field open for further exploration. Cytotoxic drugs, hydrogen peroxide, and hypoxia are means of inducing apoptosis in tissue to facilitate the removal of cells from the tissue.

To date, cytotoxic drugs are generally utilized only to improve the efficiency of zwitterionic detergent decellularizations and are not effective decellularizing agents alone.114,151 Camptothecin, a cytotoxic drug, is employed in decellularization as it inhibits DNA topoisomerase I. It has been utilized in several studies and has shown great promise for decellularization of sciatic nerve, as it preserves anatomical architecture while retaining small bioactive molecules.71,151 Cornelison et al.151 reported that camptothecin was necessary for cellular removal through hypotonic buffers. Further treatment with DNase over hypotonic buffers led to effective DNA removal for nerve tissue.

Rotenone, another cytotoxic compound and a strong mitochondrial class I inhibitor, mediates apoptosis by inducing oxidative stress.150,152,153 Treatment of tissues with rotenone for up to 24 h prior to detergent based decellularization of cell sheets has shown no significant benefit in terms of DNA removal, although this could be due to the already efficient strategy to decellularize cell sheets.150 The effect of rotenone in decellularization of tissues thicker than cell sheets has not yet been reported and is worth investigation. The cytotoxic drug Latrunculin B induces changes in cell shape and actin organization. It has been identified as an efficient DNA removal agent, and has even been found to produce greater reduction of DNA in skeletal muscle than standard Triton X-100 and SDS/Triton X-100 methods. However, this was at the cost of notable reduction in structural integrity.154–156 Other apoptotic inducing compounds for decellularization, including analogs of those previously listed, have not yet been extensively tested.

Apart from cytotoxic drugs, hydrogen peroxide or hypoxia can also be used to induce apoptosis for decellularization purposes. In addition to being a strong induction of apoptosis, hydrogen peroxide (H$_2$O$_2$) also shows promise as a sterilizing agent, which may make it useful for recellularization or implantation purposes. H$_2$O$_2$ cannot act as the major component of a decellularization protocol, as it has been found to be largely unhelpful when tested in combination with PAA/ethanol and scCO$_2$ mediated decellularization strategies.155 Isolation of tissue under hypoxic conditions can be used to assist in the removal of cellular content from tissue. N$_2$ is particularly useful as a hypoxic agent for decellularization. It has been utilized with semi-successful results in porcine cornea but has not been tested in any other tissue.159,160 Table 3 showed advantage and disadvantages of the biological decellularization techniques.

**Enzymatic decellularization**

Enzymatic digestion can improve decellularization by digesting tissues with one or a combination of several enzymes to allow for decellularizing agents to diffuse through the tissues more easily. This makes enzymatic treatments particularly alluring for use in dense tissues that are difficult to decellularize. Despite the benefits associated with enzymatic digestion, these approaches are difficult to reproduce. They also magnify the risk of significantly altering the structural and proteomic composition of the ECM.

Trypsin is an aggressive enzyme that specifically cleaves at the C-terminus of lysine and arginine to disrupt the tissue microstructure, allowing for accelerated solubilization by detergents or enzymatic chelating agents.163,164 As these bonds help crosslink collagens and elastin, trypsin is rarely used as a principal agent for decellularization.114 Use of trypsin (0.05%–0.2%) typically is restricted to an initial pretreatment step before decellularization with chemical agents.114,164,165 Higher concentrations or lengthy exposure to trypsin can result in detrimental damage to biomechanics of ECM. Attempts to utilize trypsin as a principal decellularizing agent have resulted in inadequate DNA removal and severely damaged biomechanical properties of ECM.166 Ethylenediamine tetra acetic acid (EDTA) is a chelating agent that decellularizes ECM by targeting the Ca$^{2+}$ and Mg$^{2+}$ ions that maintain bonds between the ECM and native cells. Most protocols that use EDTA also include trypsin to help cleave the bonds between cells.37 Though most protocols pair EDTA with trypsin, its versatility allows it to also be paired with ionic detergents and non-ionic detergents.37,167 Miranda et al.37 coupled EDTA with Tris buffer and used it for murine skeletal muscle decellularization by induction of an osmotic
pressure above 600MPa. High hydrostatic pressure can also be used as a replacement for TNBP, which similarly decreases decellularization time without causing ECM damage. However, their use is limited by the biological properties and GAG content.

### Table 2. Advantages and disadvantages of physical decellularizing methods.

| Decellularizing agent | Mechanism | Advantages | Disadvantages | References |
|-----------------------|-----------|------------|---------------|------------|
| High Hydrostatic Pressure | Induces necrosis | Reduced likelihood of protein denaturation | Proteins can denature at pressures above 600MPa | Le et al.124, Frey et al.125, Zemmyo et al.128, Watanabe et al.129 |
| scCO2 | N/A | Decellularizes tissue quickly | Reduces biomechanical properties | White et al.130, Dillow et al.131, Lencschmidt et al.132, Sawada et al.133 |
| Freeze-Thaw Cycles | Induces necrosis by thermal shock | Leaves majority of ECM components intact | Insufficient for decellularization alone | Leverson et al.134, Thibault et al.135, Li et al.136, Liu et al.137 |
| Vacuums | Negative pressure system aids decellularization | Reduces decellularization time | Insufficient for decellularization alone | Butler et al.138, Wang et al.139 |
| Sonication | Rupture cell membrane | Helps decellularizing agents permeate tissue | Can impact microarchitecture and biomechanics of ECM | Azhim et al.140, Yusof et al.141, Rabbani et al.142, Manalastas et al.143 |

Enzymatic digestion can also be carried out by proteases and esterases, such as dispase, collagenase, phospholipase A2, and chondroitinase ABC. Dispase is a neutral protease that dissociates cells quickly but gently from a tissue by selectively cleaving at fibronectin and collagen IV. This makes it ideal for decellularizing the basement membrane of tissues, which are predominantly composed of collagen IV and laminin. Dispase is also good for preventing cell aggregation. It is generally used to dissociate cells from thinner tissue membranes such as lung or cornea, but it can be used in succession with other chemical or enzymatic agents to decellularize denser tissues. Collagenase treatment can be used to selectively metabolize collagens from the ECM, permitting better proteolytic analysis of other components of the ECM matrix using mass spectrometry. Kuljanin et al.144 found that the use of collagenase depleted the relative abundance of collagen in the ECM of bone and adipose tissue from 90% to less than 10%. Phospholipase A2 is an enzyme which hydrolyses the phospholipids in cells but does not disrupt collagen and proteoglycan content, allowing for insignificant structural damage to the ECM but noticeable reduction of GAG content.145 Phospholipase A2 can assist in the removal of lipid content but is inadequate for removal of cellular content from a tissue. Thus, it is commonly used in combination with chemical detergent or non-detergent methods to produce adequate decellularized ECM. Chondroitinase ABC can aggressively digest proteoglycans, making it useful for the decellularization of dense cartilage tissue. As such, it can be utilized to enhance the removal of cellular remains from the cartilage ECM. Chondroitinase ABC is directly associated with the ECM and is useful for removing proteoglycans from the tissue matrix.
with extreme reduction of GAG content, which changes the mechanical properties of the ECM and increases stiffness.\textsuperscript{186,187} However, for cartilage decellularization, this may be ideal, as the reduction of GAG content can improve later recellularization efforts and subsequently be restored.\textsuperscript{186–188} Apart from cartilage, Chondroitinase ABC has also been utilized for decellularization of tissues associated with the peripheral nervous system, such as sciatic nerve and peripheral nerve. It is able to digest the chondroitin sulfate proteoglycans which inhibit neuronal repair after injury, thereby permitting axonal growth.\textsuperscript{188–191} Chondroitinase ABC treated ECM scaffolds appear to permit adequate recellularization, which indicates great potential for use in tissue engineering.\textsuperscript{186–188,192,193} Table 4 outlined types of enzymes widely used in decellularization, their mechanism, advantages, and disadvantages.

**Recent novel approaches to decellularization by tissue type**

Figure 2 summarizes a variety of novel approaches that have been recently developed and tailored for decellularization of various tissue types.

**Bone**

Organic bone ECM is a complex heterogeneous composite material composed predominantly of collagen types I, III, and V as well as low levels of proteoglycans, glycoproteins, and small signaling molecules.\textsuperscript{201} Decellularization of bone has a variety of uses, as it contains the ideal properties to be developed into 3D bioinks and hydrogel scaffolds for tissue engineering, and surgical meshes. In clinical settings, decellularized bone allografts have demonstrated excellent bone regeneration capability comparable to that of autologous bone grafts, which signifies the importance of the ECM in naturally occurring bone reconstruction.\textsuperscript{202} For these reasons, optimizing decellularization protocols for bone tissue is highly appealing. During decellularization, pretreatment of bone tissue with chelating agents or acids can be performed to first demineralize the bone. As these agents can break down several key ECM components that contribute to the biomechanics of bone, the concentration and exposure time must be taken into consideration.\textsuperscript{203} Many decellularization protocols use combinative chemical and biological decellularizing approaches. Multiple chemical reagents are effective for decellularizing bone tissue, but the most commonly utilized are SDS and Triton X-100. Addition of acids to these decellularization processes is also possible, although they tend to result in lower success rates.\textsuperscript{204} While these agents have been able to successfully decellularize bone tissue, they all can destruct essential ECM components such as collagens, GAGs, and growth factors. If not washed away properly, they can also cause increased immunogenicity due to the retention of cellular waste.\textsuperscript{16}

Emami et al.\textsuperscript{16} recently investigated the effectiveness of multiple detergents for bone decellularization. They found 0.5% SLES to outperform typical SDS and trypsin/EDTA protocols in regard to DNA removal rates, retention of critical ECM proteins, and recellularization potential. Rasch et al.\textsuperscript{205} investigated sonication as a potential new means of bone decellularization. They determined sonication to be a superior means of removing DNA from bone tissue when compared to SDS treatment. They also found the final ECM product to have good recellularization potential and biocompatibility. These characteristics led to the conclusion that sonication produces a matrix comparable to commercially available products. However, the effectiveness of retaining the native architecture and biomechanical properties was not evaluated and is worth further evaluation.\textsuperscript{205} Hashimoto et al.\textsuperscript{206} was the first to report that a high hydrostatic pressure (HHP) of 980 MPa followed by treatment with nucleases could produce decellularized ECM from bone (Figure 2). However, Nakamura et al.\textsuperscript{207} later found HHP to be inferior for overall cell removal compared to SDS, but superior for retaining the ECM microenvironment.

**Cartilage**

Naturally occurring cartilage has highly limited healing capabilities, making the generation of scaffolds for tissue

---

**Table 3. Advantages and disadvantages of biological strategies for tissue decellularization.**

| Agent            | Mechanism         | Advantages                                                                 | Disadvantages                                                                 | References                                                                 |
|------------------|-------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Cytotoxic Drugs  | Induce apoptosis  | Retains small bioactive molecules                                           | Insufficient decellularization, can cause damage to structural integrity,     | Song et al.\textsuperscript{71}, Novoseletskaia et al.\textsuperscript{159}, Cornelison et al.\textsuperscript{151}, Giordano et al.\textsuperscript{153}, Reyna et al.\textsuperscript{154}, Fishman et al.\textsuperscript{155}, Desouza et al.\textsuperscript{156}, Gosztyla et al.\textsuperscript{157}, Hennessy et al.\textsuperscript{158} |
| Hydrogen Peroxide| Induce apoptosis  | Sterilizes ECM                                                               | Efficient as a principal decellularizing agent, decreases immunogenicity     | Amano et al.\textsuperscript{159}, Isidan et al.\textsuperscript{160}, Han and Flynn\textsuperscript{161}, Colombo et al.\textsuperscript{162} |
| Hypoxia          | Induce apoptosis  | Can be used to assist in recellularization following decellularization.      | Efficient as a principal decellularizing agent, only somewhat successful in assisting decellularization | |
engineering by decellularization alluring. Different types of cartilage have different structural composition and permeabilities, and thus require individualized decellularization protocols. Cartilage is a highly dense tissue, making decellularization and subsequent recellularization highly difficult. Despite this, several successful decellularization strategies have been developed for various cartilaginous structures. Generally, pretreatment with physical methods such as freeze thawing, snap-freezing, or tissue smash can be performed to assist in the decellularization, as the ice crystals they produce can create more pores in the tissue, thereby reducing the exposure time to damaging reagents. Other pretreatments that have been explored to optimize decellularization of hyaline cartilage include pulverization of the tissue, which increases the surface area and promotes slightly better permeation of chemical agents into the tissue. These steps are commonly followed by combinative chemical-enzymatic treatments. The chemical detergents used most in cartilage decellularization are SDS and Triton X-100, although some studies have utilized SLES. These detergents are then quickly followed by treatment with enzymes such as trypsin-EDTA, DNase, or RNase to completely remove cellular DNA and prevent nucleic waste from sticking to the matrix. Due to the detrimental nature of these compounds on the ECM matrisome, the exposure time to these agents must be highly controlled. Since some types of cartilage are denser than others and therefore require longer subjection to decellularizing agents, the specific tissue type must be considered when determining the necessary exposure time. Hyaline cartilage, which is present between most joint surfaces, is the most extensively studied cartilage for decellularization. The ECM of hyaline cartilage consists of predominantly collagens, particularly collagen II. It is also extensively composed of GAGs and laminin. Auricular cartilage, present within the outermost part of the ear, is highly elastic and flexible as a result of more elastic fibers in its ECM composition compared to hyaline cartilage. Fibrous cartilage makes up the intervertebral disks and menisci in ligaments and tendon. Its ECM composition is unlike any other cartilage in that fibrous cartilage contains collagen I only, as collagen II was not detected.

Decellularization of cartilage tissue should aim to retain as much of the original GAG content as possible, as it is predominantly the GAG content which grants cartilage its unique mechanical properties. Unfortunately, exposure to common decellularizing agents tend to result in the destruction of GAGs, causing an increase in matrix stiffness. Despite this setback, reduction of GAG content has resulted decellularized matrix. This echoed several years of similar findings by studies, which analyzed the effects of detergents and enzymes on other tissue types as well. As such, investigation into alternative strategies for decellularization of cartilage tissue is encouraged.

### Adipose

Adipose tissue is important for cushioning and supporting the internal organs and is made up of adipocytes, which are dependent upon the basement membrane to provide mechanical support as well as facilitate adipogenesis. This basement membrane is predominantly made up of collagen types I and IV, laminin, and proteoglycans. Mechanical disruption or dilapidation steps should be performed prior to cell removal in order to permit proper invasion by decellularizing agents. While decellularization of adipose is not as extensively studied as many other tissue types, it has been shown that adipose tissue can be decellularized using detergent, enzymatic, or solvent-based techniques. Thomas-Porch et al. sought to compare several previously described combinative chemical and enzymatic protocols for adipose decellularization and found that while all were capable of decellularizing adipose tissue, the agents used influenced the proteome of the resulting decellularized matrix. This echoed several years of similar findings by studies, which analyzed the effects of detergents and enzymes on other tissue types as well. As such, investigation into alternative strategies for decellularization of adipose is encouraged.

A detergent-free decellularization protocol for adipose tissue, described by Flynn et al. involved freeze-thaw cycles followed by isopropanol treatment, which was then followed by enzymatic digestion with trypsin-EDTA, DNase, RNase, and lipase to remove cellular content and...
remnant lipid content. This protocol retained adequate levels of collagen IV and laminin to support adipocytes in the future. Since then, few studies have been published which claim to have developed adequate decellularization by alternative means. For this reason, most recent advancements in adipose decellularization have focused on optimizing or comparing existing approaches. Despite these attempts, detergents and acids of varying concentrations and exposure times have consistently proven to be too harsh on adipose tissue, resulting in full decellularization being accompanied by a reduction or complete removal of structurally supportive laminin. One successful attempt to develop a detergent-free method of decellularizing adipose tissue has been reported recently. This protocol, developed by Wang et al., utilized scCO2 to adequately decellularize adipose tissue with ethanol as a modifying agent (Figure 2).

**Cornea**

The ECM of the cornea is composed of water, inorganic salts, proteoglycans, glycoproteins, and several types of...
collagens. Similar to other tissues, a successful decellularization should aim to preserve as much of the native ECM components as possible. Unlike other tissues, decellularization of cornea must aim to produce a fully transparent matrix. Several conventional chemical and physical decellularization methods have been applied to corneal tissue, although most cause some undesirable results such as reduced transparency, damage to the ECM microstructure, dehydration, and/or edema. As such, several studies focus on uncovering newer unconventional decellularizing techniques. For instance, some studies indicate that phospholipase A2 or human serum with electrophoresis are capable of maintaining ECM structural proteins and transparency, albeit no protocol has been optimized to attain adequate cell removal. Perhaps the most promising recent advancement, described by Lin et al., indicates that decellularization of cornea can be performed using glycerol with chemical crosslinking (Figure 2). The application of glycerol to the tissue through a pressure based osmotic system followed by gamma-ray irradiation preserved the fibrous collagen morphology and GAG content. Moreover, it provided a fully transparent and non-immunogenic decellularized graft with long-term stability.

**Respiratory organs**

Due to the importance of the lungs to be able to expand and collapse, it is critical that a lung decellularization protocol be optimized to retain as many of the native organ’s mechanical properties as possible. In particular, the ultrathin “air-blood barrier” between the alveoli and capillaries are of the utmost importance to preserve during decellularizations, as this permits the exchange of gases between the blood and the lungs. The thinness of the lungs allows them to be decellularized quickly by a variety of agents. As such, several protocols have been published which indicate that the lungs can be decellularized by chemical, enzymatic, physical, and combinative methods. Most commonly, lung tissue is decellularized by perfusion through the airways, vasculature, or both, with combinations of detergents such as SDC and Triton X-100, or individually by low concentrations of SDS or CHAPS. Protocols have utilized different concentrations for each reagent, exposure times to reagents, routes for perfusion, and order of reagent administration, each with differing levels of success. Tebyanian et al. found that a detergent-based approach using 2 mM CHAPS and 0.1% SDS for 24h maintained the microarchitecture of rat lung better than 2 mM CHAPS and 0.5% Triton X-100 for 96h. It
is important to note that a donor tissue source must be taken into consideration when choosing a means of introducing decellularizing agents into the tissue, as lung from species of differing sizes cannot be decellularized under the same conditions. For instance, pressure-based perfusion of decellularizing agents into the tissue should be avoided for lungs sourced from larger donors, such as human or pig, but are acceptable for lungs sourced from smaller donors such as mouse or rat. Palma et al.\(^\text{235}\) overcame this barrier by developing a constant pressure-based perfusion system to introduce SDS into horse lung that was able to maintain most of the native collagen, elastin, fibronectin, and GAG content, although the procedure resulted in the collagen area being significantly reduced and increased stiffness of the ECM compared to native tissues. Obata et al.\(^\text{63}\) found that decellularization of rat lung by the naturally occurring detergent, potassium laurate, could significantly reduce the damage to the ECM microstructure often caused by commonly used ionic and non-ionic detergent-based protocols. Interestingly, the use of potassium laurate for decellularization resulted in an increased recellularization potential and a significantly reduced immune response upon implantation compared to lung decellularized by SDS.\(^\text{63}\) Song et al.\(^\text{71}\) found that the zwitterionic detergent, SB10 following treatment with the cytotoxic drug, camptothecin, was able to successfully decellularize rat lung while significantly reducing the damage to the ECM collagen structure when compared to a commonly utilized Triton X-100 and SDS treatment.\(^\text{71}\) The potential of using potassium laurate and SB10 for detergent-based decellularization of thin tissues is worth further investigation (Figure 2).

**Cardiovascular tissue**

ECM sourced from the heart is composed mostly of collagens, fibronectin, and elastin. These components permit the heart to be durable, strong, and flexible.\(^\text{237}\) Cardiovascular tissue remains to date one of the most extensively studied organs for decellularization, which has allowed for the development of several protocols capable of adequate cell removal as well as retention of biomechanical properties. While cardiovascular tissue can be decellularized using several combative detergent-based approaches, protocols have been developed with the express purpose of enhancing the effectiveness of individual detergents alone. The most common detergents used are SDS, SDC, and Triton X-100. Several studies claim to have discovered an optimized protocol for whole organ, valve, and sectioned cardiovascular tissue decellularizations across several species.\(^\text{238–240}\) These protocols typically utilize either physical methods, such as osmotic shock, or enzymatic methods, such as treatment with trypsin-EDTA, prior to decellularization with detergents in order to improve results. Post-decellularization, treatment with low concentrations of enzymes is used to wash away remnant nuclear waste from the matrix. Sokol et al.\(^\text{241}\) tested multiple standardized detergent-based pericardium decellularization protocols and found that while multiple detergent-based protocols adequately removed cellular content, protocols which use single step detergent or enzyme decellularization were most likely to negatively impact the collagen structure of the resulting matrix. Single step decellularization also led to higher levels of trace nucleic acid in the matrix than combative approaches.\(^\text{241}\) SDS and Trypsin enzyme combative approaches in particular are of interest, as they were shown to result in the most optimal proteomic and biomechanical results.\(^\text{241}\)

Despite the heart being a relatively durable organ and the adequate decellularizations performed by combative detergent-based approaches, the potential for detergents to damage the mechanical properties of the ECM, particularly GAG content that provides much of the heart’s mechanical strength, remains indisputable. Thus, recent attempts to do away with detergents for cardiovascular decellularization are becoming increasingly popular. Decellularization by scCO2 treatment has been studied as a potential means for avoiding detergent induced damage to ECM components, however, this method can dehydrate the ECM.\(^\text{242}\) Cesar et al.\(^\text{243}\) found that a combative Triton X-100 and scCO2 method could overcome the weaknesses associated with both decellularizing techniques.

**Artery**

Arteries contain three layers: the intima, media, and adventitia, each with different ECM composition. The tunica intima consists mainly of laminin and collagen IV, the media consists mostly of collagen II, elastin, glycoproteins, and GAGs, and the tunica adventitia is predominantly collagen I, elastin, and proteoglycans.\(^\text{244}\) Because this tissue is so thin, elastic, and structurally complex, it is considered difficult to decellularize while maintaining its ECM components. To attain the best results, it has been suggested that prior to decellularization, an additional step may be taken to begin cell lysis, such as washing in water under physical agitation or introducing freeze-thaw cycles.\(^\text{136,245}\) Similar to other tissues, artery is most commonly decellularized using detergents, including SDS, EDTA, SDC, CHAPS or Triton X-100. These detergents can be used alone or in combination and produce satisfactory results. Additionally, some protocols have shown that use of either trypsin or hypo/hypertonic solutions in tandem with these detergents can produce greater success rates.\(^\text{246,247}\) While many studies have reported successful decellularization of blood vessels by detergent-based methods, they have been applied under varying experimental conditions, making it difficult to assess the usefulness of each individual detergent. Simsa et al.\(^\text{39}\) sought to overcome these discrepancies in experimental conditions to determine the efficacy of individual agents in commonly utilized detergent-based protocols. Although protocols which use Triton
X-100, SDS, SDC, and CHAPS as the main decellularizing reagent all produced sufficiently decellularized matrices with acceptable biodegradability and mechanical properties, Triton X-100 based protocols were shown to result in the greatest recellularization potential. However, it was noted that it is essential for Triton X-100 decellularization be followed up with enzymatic treatment with DNase to ensure removal of nuclear remnants.39 Most recently, attempts to overcome the need for detergents using scCO2 as a decellularizing agent have been described (Figure 2). Gil-Ramirez et al.248 indicated that scCO2-ethanol mediated decellularizing agents have been described (Figure 2). Gil-Ramirez et al.248 indicated that scCO2-ethanol mediated decellularizations followed by enzymatic treatment with benzonase may be a potential means for detergent-free arterial decellularization. scCO2 was shown to successfully decellularize arterial tissue, although it resulted in some external damage to the tissue. This is to be expected, as scCO2 is known to dehydrate the ECM, and thus it may not be ideal for procedures that require an intact final matrix. Another issue with arterial decellularizations is the fact that many arterial walls are protected by a watertight lining that spans the length of the arterial wall. Tuan-Mu et al.249 recently discovered that pretreatment of umbilical cord with collagenase permitted better decellularization of the arterial walls within. Collagenase treatments were able to remove the watertight lining of the abluminal surface, in turn allowing for improved perfusion of 1% SDS detergent and reducing the overall decellularization process to less than 24 h.249

Decellularization of thinner tissues, such as artery, may be improved by the development of a camptothecin and SB10 approach. Blood vessels are known to be best decellularized using combinative detergent-based and enzymatic techniques, but the thinness of these tissues can result in overexposure to harmful agents, resulting in unwanted removal of protein niches. Attempts to utilize non-detergent based methods are difficult as well, as several of these non-detergent agents can dehydrate the ECM, reducing elasticity and damaging the matrix. To date, no studies have been published showing the utilization of SB10 or SB16 as the main decellularizing agents for thin elastic tissues such as artery. Camptothecin, which generally mediates apoptosis, followed by rinses with SB10, a zwitterionic detergent shown to effectively remove cellular content with a low risk of resulting in unwanted protein removal, may prove successful. However, this likely would still require further enzymatic or chemical treatment to adequately remove cellular content, which may decrease the mechanical strength of arterial ECM.71,151,250 It is also necessary for satisfactory cellular removal with commonly used detergents such as Triton X-100.39 For this reason, thin tissue decellularized with SB10 or SB16 could potentially require shorter posttreatment exposure times to highly damaging enzymes for adequate cell removal.

**Dermis**

Dermal tissue, like arterial tissue, contains multiple layers in order to perform a multitude of functions. These layers make decellularization by gentle decellularizing agents difficult, as the density of dermal tissue makes infiltration of non-surfactant agents nearly impossible. As such, decellularization of dermal tissue generally requires treatment with aggressive decellularizing agents such as surfactants and enzymes. Dermis must first be delipidated before decellularization that is most commonly carried out using combinative detergent and enzymatic treatments. While surfactant-enzymatic methods are adequate for decellularization, recently, multiple protocols have demonstrated that dermal ECM can be procured by using detergents in combination with hypo/hypertonic solution changes to induce osmotic shock, as opposed to enzymatic treatment.251–253 Apart from osmotic shock-based methods, other attempts to remove or reduce the need for enzymatic exposure step from dermal tissue decellularization protocols have not necessarily proven to be more effective than standard chemical and enzymatic treatment protocols.254,255 Because surfactant-enzymatic methods have been thus far the most practical and common decellularization protocols for dermal decellularization, recent advancements by Koo et al sought to reduce exposure time to these harsh chemicals by altering standard chemical-enzymatic treatment to include hypo/hypertonic pretreatments along with physical sonication and electroporation methods.256 The results of the study show promise in the premise of combining standard detergent-enzymatic decelluarizations with physical decellularization methods, and further investigation into combinative physical/chemical/ enzymatic treatment is worth exploring.

**Pancreas**

Pancreatic decellularization is typically carried out by Triton X-100, SDS, or enzymatic agents. Attempts to define the best detergent reagents for decellularization consistently indicate that Triton X-100 is superior to other detergents in that it typically results in far less structural damage to the ECM components of the matrix.257 This may be due to the ability of Triton X-100 to interrupt lipid-lipid and lipid-protein bonds. Reagents used for pancreatic decellularization must be capable of breaking the protein-protein and protein-lipid bonds within the tissue, though this aspect of pancreatic decellularization is not widely reported on. Still, Triton X-100, as well as several other detergent-based protocols are well documented for the proteomic makeup of pancreatic ECM.257,258 Recent advancements in pancreatic decellularization tend to focus on either developing protocols to optimize maintenance of the pancreatic ECM proteome or overcoming lipid barriers to successfully decellularize agents homogeneously throughout the tissue. For whole organ decellularization, Sackett et al.259 developed a protocol that used a novel homogenization step followed by SDC treatment under agitation to successfully delipidate and decellularize human pancreas samples that contained up to 70% lipid content by weight. The subsequent matrix was determined to be non-immunogenic and had excellently
retained collagen and laminin content, but only partially retained GAG content. A study performed by Panjota et al. echoed this conclusion that 4% SDC could better preserve structural pancreatic ECM components than Triton X-100 or SDS in canines. SDC is known to be incapable of degrading collagens, which may explain its ability to better retain the fibrous structural components of pancreas. Elebring et al. indicated that detergent-based techniques could be optimized by adjusting the temperature at which detergent-based decellularization occurs. They reported that the use of “cold-perfusion,” in which a 4% SDC and 6% Triton X-100 reagent mixture was perfused through pancreas at a constant temperature of 4°C, assisted in the maintenance of pancreatic ECM ultrastructure. This may have been due to the fact that the low temperature inhibits the activation of the enzymatic proteases native to the pancreas which can be triggered by exposure to detergents (Figure 2). Bi et al. and Hu et al. demonstrated that effective rat and porcine pancreatic decellularization with maintenance of key ECM proteins could be achieved through nondetergent-based methods. Their studies indicated that hyper/hypotonic solution changes under agitation were able to sufficiently remove up to 99% and 98% DNA from rat and porcine, respectively, while maintaining collagen, laminin, and GAG content, though investigation into potential impact on the biomechanics and ultrastructure were not reported.

Kidney

Recent studies have demonstrated progress in optimizing kidney decellularization to better maintain an intact vascular tree by means of physical, chemical, and biological methods. Both Yang et al. and Feng et al. investigated the usefulness of several different cryoprotectants to reduce the damage caused by the freezing process to the kidney’s vascular network. While kidneys have been successfully decellularized by a variety of detergents, they are most commonly decellularized by Triton X-100, SDS, or combinative SDS and Triton X-100 protocols. Multiple studies have indicated that the addition of a freeze-thaw step to kidney decellularization by Triton X-100 allows for relatively short treatment time with the chemical agent. However, freeze-thaw is also known to cause damage to the overall structure of the ECM as well as the vascular network by expanding blood vessels by nearly 200% of their original size. Several cryoprotectant agents could be used to optimize the freeze-thaw decellularization process. The study results indicated that conditions such as crystalization temperature, freeze-thaw temperature, rate of cooling, and concentration of each cryoprotecting agent must be considered before choosing which cryoprotectant is best suited for use in a freeze-thaw decellularization protocol. Regarding recent advancements in renal decellularization by chemical methods, Poornejad et al. optimized a whole organ SDS detergent protocol by inducing osmotic shock on porcine kidney. By cycling between perfusion of SDS-containing NaCl solution at gradually increasing flow rates, they were able to significantly reduce chemical treatment time and increase preservation of collagens, and GAGs. Despite being relatively successful, there remains the potential for kidney decellularization for better retention of ECM ultrastructure. More specifically, establishing a protocol that better preserves the vasculature of native kidney is of the utmost importance.

Liver

The liver is mainly composed of laminin, elastin, fibronectin, and collagen types I, III, and IV, as well as sulfated GAGs. Decellularization of liver has been extensively studied. Livers that have been decellularized by the techniques discussed above maintain their structural and proteomic components, promote cell proliferation, attachment, and migration, and show acceptable biocompatibility. Typically, for liver, whole organ decellularization is performed using perfusion methods, but can also be achieved using immersion-based methods at the cost of losing the 3D architecture and vascular network. Whole liver decellularization usually use various combinations of chemical, physical, and biological methods such as detergents like Triton X-100 or SDS, hypo/hypertonic solution changes involving NaCl, and enzymatic treatment with DNase. Currently, the most vital obstacle to overcome for liver decellularization is inefficient diffusion of decellularizing reagents through the native whole organ. Several recent advances have been made in liver decellularization—many of which have determined that the addition of external pressure or forces acting on the liver during decellularization are beneficial. The first to report that pressure-based decellularizations could produce optimized liver scaffolds. They reported that the application of controlled oscillating pressures from 0 to 35 mbar during perfusion of detergents into porcine liver assisted in decreasing the amount of time required for decellularization to be achieved. Similarly, found that decellularization carried out at constant pressure of 120 mmHg can assist in reducing the required exposure time to Triton X-100, thereby minimizing the damage incurred on essential ECM components. Mazza et al. applied high shear stress during decellularization to produce ECM scaffolds from human liver (Figure 2). They found that agitation the liver at 45X g significantly decreased the time required to achieve successful decellularization of sectioned tissue. These results indicate that further exploration into the application of external forces on tissue during decellularization are necessary.

Reproductive organs

SDS appears to be the commonly utilized reagent for decellularization of ovary and uterus, but is well known to be
cytotoxic if inadequately cleared from the final ECM product.11,274 As a result, some attempts have been made to find alternative detergents for ovarian decellularization. Alshaikh et al. determined that decellularization of murine ovary by 2% SDC better preserved GAGs, collagen, and elastin fibers than 0.5% SDS treatment at the cost of retaining slightly higher DNA content. The inclusion of a DNase enzymatic treatment step further improved DNA removal for all decellularization protocols.282

Nervous tissue

ECM sourced from nervous tissue is mainly composed of collagen types II and IV, laminin, and fibronectin.283,284 As is the case with most tissues, chemical methods, namely detergents, are the favored decellularizing agents for nerve. Traditionally, the most widely utilized protocol for decellularization was pioneered by Sondell et al.285 which has demonstrated sufficient myelin and Schwann cell removal as well as adequate neural regeneration with optimal biocompatibility. This protocol used a combinatorial detergent effort using Triton X-100 SDC to effectively remove cellular content. Later, the decellularization protocol was optimized by Hudson et al.73 who removed SDC entirely in favor of using the less aggressive zwitterionic detergents SB10 and SB16, replaced Triton X-100 with the now discontinued Triton X-200, and added multiple shorter wash steps. These adaptations have since been made to this protocol and multiple studies have sought to find entirely new means of nervous tissue decellularization. Nieto-Nicolau et al.286 developed a successful combinatorial multi-chemical and enzymatic approach that preserved collagen IV and laminin content, known to make up the basal lamina that is essential for axon growth. This technique, which used SB 10 and 16, Triton X-100, hypertonic NaCl, and DNase treatment steps left no cytotoxic remnants and removed the MHC II receptor, known to be the antigen most responsible for inducing immune response and thereby demonstrating the biocompatibility of the matrix.286 Suss et al.287 found that sonication during chemical decellularization does not help to remove DNA content, but can assist in removing cellular debris and myelin-sheaths. Cornelison et al.151 developed a novel apoptosis-inducing decellularization protocol using camptothecin and hyper/hypotonic solution treatment, indicating the potential to overcome structural damage and immunogenicity concerns associated with detergents (Figure 2).

Bladder

Urinary bladder regeneration requires a scaffold that can produce tissue with the biomechanical properties to easily expand and contract. An engineered bladder must also contain a muscular wall and urothelium which responds to dynamic changes in sensory and autonomic provocation.288 As it is an extensively studied organ for decellularization, several protocols have been developed and found to be effective. Distension of the native bladder prior to beginning decellularization can assist the process due to the reduced thickness of the tissue wall.289–291 Decellularizations are most commonly carried out by PAA and ethanol or SDS and Triton X-100, although trypsin-EDTA and SDC protocols have been developed.292–297 Recent updates in the field indicate that PAA and ethanol may not be ideal for decellularization of bladders.49 Only Kao et al.298 has recently sought to overcome this obstacle by optimizing SDS protocols (Figure 2). To enhance the efficacy of the detergent, SDS was prepared in a buffer to ameliorate its degradative characteristics. Subsequently, 1% SDS treated bladder-derived hydrogel scaffolds surpassed the decellularization standards set forth by PAA protocols within the realm of cytotoxicity, immunogenicity, and cell removal.288 In the future, it may be beneficial to decellularize urinary bladder with other ionic surfactants under organ distension. For instance, SLES has consistently shown to remove cellular and nuclear material on par with SDS while also better maintaining the biomechanics of the ECM. Alternatively, since the issue associated with PAA is its inability to fully remove cellular content, perhaps the stronger analog, acetic acid may prove useful for correcting this issue, though exposure time would likely need to be optimized to discourage dehydration of the ECM. Figure 2 highlights recently developed approaches for improving decellularization efficacy specific to a tissue type. Table 5 exhibited some examples of novel step-by-step protocols for tissue
Table 5. Step-by-step protocols for decellularizing different types of tissues.

| Organ | Donor species | Protocol | Comments | Reference |
|-------|---------------|----------|----------|-----------|
| Bone  | Homo sapien  | 2 h 750 uL PBS and agitate [30 m wash in deionized water 15 m centrifuge at 1850xg] (x3) 10 m 20 kHz sonication in 1 mL 3% hydrogen peroxide 10 m 20 kHz sonication in 1 mL 70% ethanol 10 m deionized water 15 m centrifuge at 1850xg 30 m wash in deionized water | Acceptable biocompatibility Comparable to commercially available bone ECM products | Rasch et al.205 |
| Bone  | Bovine       | Wash in PBS 48 h 0.5N HCl wash in PBS 24 h 0.5% SLES wash in PBS 24 h 1% Triton X-100 24 h wash in PBS | Greater preservation of ECM proteins compared to other surfactants | Emami et al.16 |
| Heart | Murine       | soak in 2 mL ethanol 6 h 35 MPa scCO2 wash 5 days in PBS & DNase | Retains significantly more collagens and GAGs compared to SDS and Triton X-100 | Seo et al.141 |
| Heart | Caprine      | 2 h 0.1% Triton X-100 Wash with PBS 1 h scCO2 0.25% trypsin-EDTA 0.1% SDS in 70% isopropanol 12 h 1% Triton X-100 in 70% isopropanol 12 h 100% isopropanol Rinse with deionized water x3 | Does not dehydrate the ECM Avoids structural damage caused by long exposure to Triton X-100 Avoids enzymatic digestion Preserves more GAGs, collagen, and elastin than several other strategies Maintains similar biomechanical properties to native tissue | Cesur and Laçin243 Greco et al.253 |
| Dermis| Porcine      | 6 h deionized water 12 h hypertonic solution (1 M NaCl, 10 mM EDTA, 50 mM Tris-HCl) 8 h wash buffer 12 h hypotonic solution (5 mM EDTA, 10 mM Tris-HCl) 8 h wash buffer Rinse with deionized water x3 6 h 0.25% trypsin-EDTA Rinse with deionized water x3 6 h 0.1% SDS in 70% isopropanol Rinse with deionized water x3 12 h 1% Triton X-100 in 70% isopropanol Rinse with deionized water x3 12 h 100% isopropanol Rinse with deionized water x3 | Effectively decellularizes skin while maintaining ECM proteomic content Sufficiently biocompatible Combinative surfactant-alcohol approach increases ECM protein retention compared to surfactant alone Addition of hypertonic solution changes, sonication, and electroporation reduce exposure time to decellularizing agents Maintained structural integrity better than standard surfactant-enzymatic dermal decellularizations | Ventura et al.77 Greco et al.253 |
| Dermis| Homo sapien  | Rinse with deionized water 1 M NaCl solution 0.05% trypsin-EDTA 2% SDS 1% Triton X-100 Rinse again 2 h 40 kHz sonication Electric stimulation Sonication Rinse again 2 h 40 kHz sonication Electric stimulation Sonication | | Koo et al.256 |
| Lung  | Murine       | 0.0035% Triton X-100 rinse in PBS and 1 M NaCl 0.15% PL & 0.5% Triton X-100 Rinse in PBS | Preserved microstructure Decreased immunogenicity compared to SDS based approaches | Obata et al.63 |
decellularization. The outcomes of these protocols were highlighted as well.

**Future directions**

Even though the ECM matrisome of various tissues are similar, the varied abundance of the ECM proteins maintains the uniqueness of the tissue type and plays a substantial role in directing cell fate. The proteome of the ECM can be difficult to analyze due to the complexity of large, insoluble, crosslinked, and glycosylated proteins. Thus, it is difficult to determine whether decellularization results in the retention of the entire proteome. Decellularized ECM must be characterized following decellularization to confirm that the properties of the resultant scaffold will be able to promote re-seeded cell proliferation and not invoke an immune response upon implantation into a host biosystem. Residual DNA, cytotoxic chemicals, or disruption of the native mechanical structure could all result in unfavorable outcomes upon recellularization or implantation of the final decellularized construct.

Currently, no singular decellularizing agent can be used to retrieve a decellularized ECM scaffold with perfectly identical proteome to that of native ECM, even though tremendous strategies for tissue- and organ-specific decellularization have been developed. For this reason, decellularization protocols must cautiously determine which agents are best for use with a particular tissue of interest. Many decellularization protocols can therefore unintentionally increase the risk of inefficient nuclear removal rates, produce unintentionally cytotoxic constructs, or induce strong immune responses from the host system upon implantation. Therefore, further studies must be performed to determine the extent to which these obstacles can be overcome. For instance, recent investigations have sought to introduce drugs, such as Rosiglitazone and Honokiol to decellularized ECM to reduce immunogenicity. Raptinal has been identified as a possible apoptosis-inducing decellularizing agent. Additionally, while many techniques described throughout this review have been shown to reduce GAG content, some studies have suggested that partial loss of GAGs may be beneficial for recellularization. Investigation into the level of acceptable GAG removal should be performed to better characterize the usefulness of individual decellularizing agents.

While the importance of using tissues from an organ of interest has been stressed, recent findings have indicated that decellularized plant could potentially be used as an alternative to some types of decellularized animal tissues for scaffolding. Current research progresses on plant decellularization and their potential applications of plant-derived scaffolding have been introduced and summarized elsewhere. Taken together, we provide a comprehensive and up to date review focusing on a variety of decellularization techniques that have been extensively investigated lately. Further advancements in decellularization strategies would facilitate the clinical applications of the decellularized biomaterials for tissue repair and disease treatment.

**Author contributions**

DM wrote the manuscript. SJ and KY revised the manuscript. All authors read and approved the final manuscript.

---

**Table 5. (Continued)**

| Organ | Donor species | Protocol | Comments | Reference |
|-------|---------------|----------|----------|-----------|
| Ovary | Murine | 16 h 2% SDC wash in deionized water | Preserved collagen fiber networks, GAGs, and elastin | Alshaikh et al. |
| | | 30 m 40 U/mL DNase | | |
| | | 24 h wash in deionized water | | |
| | | 30 m 0.1% PAA | | |
| | | 24 h wash in PBS | | |
| Nerve | Murine | 1d 5uM camptothecin | Cells in early stages of apoptosis more easily washed away than cells in secondary stages | Cornelison et al. |
| | | 24 h hypertonic 4X PBS | | |
| | | 30 m wash in 2X PBS | | |
| | | 30 m wash in 1X PBS (x2) | | |
| | | 36 h 75 U/mL DNase | | |
| | | 30 m wash in 1X PBS (x2) | | |
| Cornea | Porcine | Wash in PBS | Produced high transparency graft | Lin et al. |
| | | 4h immerse in % glycerol | | |
| | | Wash in preservation solution | | |
| | | 2h glycerol buffer solution | | |
| | | Wash in preservation solution | | |
| | | Irradiate at 25KGy | | |

Irradiate at 25KGy

Table 5. (Continued)
Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work is partially supported by the National Institutes of Health for the research, authorship, and/or publication of this article: This article is funded by the National Science Foundation CBET1928855 and CBET1919830.

ORCID iD

Sha Jin https://orcid.org/0000-0002-8033-8110

References

1. Goddard ET, Hill RC, Barrett A, et al. Quantitative extracellular matrix proteomics to study mammary and liver tissue microenvironments. *Int J Biochem Cell Biol* 2016; 81:223–232.
2. Hu M, Bi H, Moffat D, et al. Proteomic and bioinformatic analysis of decellularized pancreatic extracellular matrices. *Molecules* 2021; 26:6740.
3. Ozcebe SG, Bahcecioglu G, Yue XS, et al. Effect of cellular and ECM aging on human iPSC-derived cardiomyocyte performance, maturity and senescence. *Biomaterials* 2021; 268:120554.
4. Nakayama KH, Hou L and Huang NF. Role of extracellular matrix signaling cues in modulating cell fate commitment for cardiovascular tissue engineering. *Adv Healthc Mater* 2014; 3:628–641.
5. Nicolas J, Magli S, Rabbachin L, et al. 3D extracellular matrix mimics: fundamental concepts and role of materials chemistry to influence stem cell fate. *Biomacromolecules* 2020; 21:1968–1994.
6. Brown BN and Badyal SF. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. *Transl Res* 2013; 163:268–285.
7. Bi H, Karanth SS, Ye K, et al. Decellularized tissue matrix enhances self-assembly of islet organoids from pluripotent stem cell differentiation. *ACS Biomater Sci Eng* 2020; 6:4155–4165.
8. Hoshiba T and Yamaoka T. *Decellularized*. London: Royal Society of Chemistry, 2020.
9. Robertson MJ, Dries-Devlin JL, Kren SM, et al. Optimizing recellularization of whole decellularized heart extracellular matrix. *PLoS One* 2014; 9:e90406.
10. Song JJ, Guyette JP, Gilpin SE, et al. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. *Nat Med* 2013; 19:646–651.
11. Laronda MM, Jakus AE, Whelan KA, et al. Initiation of puberty in mice following decellularized ovary transplant. *Biomaterials* 2015; 50:20–29.
12. Guo Y, Wu C, Xu L, et al. Vascularization of pancreatic decellularized scaffold with endothelial progenitor cells. *J Artif Organs* 2018; 21:230–237.
13. De Waele J, Reekmans K, Daans J, et al. 3D culture of murine neural stem cells on decellularized mouse brain sections. *Biomaterials* 2015; 41:122–131.
14. Ahmadipour M, Duchesneau P, Taniguchi D, et al. Negative pressure cell delivery augments recellularization of decellularized lungs. *Tissue Eng Part C Methods* 2021; 27:1–11.
15. Baptista PM, Siddiqui MM, Lozier G, et al. The use of whole organ decellularization for the generation of a vascularized liver organoid. *Hepatology* 2011; 53:604–617.
16. Emami A, Talaei-Khozani T, Vojdani Z, et al. Comparative assessment of the efficiency of various decellularization agents for bone tissue engineering. *J Biomed Mater Res Part B Appl Biomater* 2021; 109:19–32.
17. Crapo PM, Gilbert TW and Badyal SF. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2011; 32:2323–2343.
18. Bi H, Ye K and Jin S. Proteomic analysis of decellularized pancreatic matrix identifies collagen V as a critical regulator for islet organogenesis from human pluripotent stem cells. *Biomaterials* 2020; 233:119673.
19. Belviso I, Romano V, Sacco AM, et al. Decellularized human dermal matrix as a biological scaffold for cardiac repair and regeneration. *Front Bioeng Biotechnol* 2020; 8:229.
20. Pu W, Han Y and Yang M. Human decellularized adipose tissue hydrogels as a culture platform for human adipose-derived stem cell delivery. *J Appl Biomater Funct Mater* 2021; 19:228080020988141.
21. Sawkins MJ, Bowen W, Dhadda P, et al. Hydrogels derived from demineralized and decellularized bone extracellular matrix. *Acta Biomater* 2013; 9:7865–7873.
22. Ravichandran A, Murekatete B, Moedder D, et al. Photocrosslinkable liver extracellular matrix hydrogels for the generation of 3D liver microenvironment models. *Sci Rep* 2021; 11:15566.
23. Karanth SS, Sun S, Bi H, et al. Angiopoietins stimulate pancreatic islet development from stem cells. *Sci Rep* 2021; 11:13558.
24. Hoshiba T. Decellularized extracellular matrix for cancer research. *Materials* 2019; 12:1311.
25. Wishart AL, Conner SJ, Guarin JR, et al. Decellularized extracellular matrix scaffolds identify full-length collagen VI as a driver of breast cancer cell invasion in obesity and metastasis. *Sci Adv* 2020; 6:eabc3175.
26. Winkler A, Abisoye-Ogunniyan A, Metcalf KJ, et al. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun* 2020; 11:5120.
27. Kort-Mascot J, Bao G, Elkashoty O, et al. Decellularized extracellular matrix composite hydrogel bioinks for the development of 3D bioprinted head and neck in vitro tumor models. *ACS Biomater Sci Eng* 2021; 7:5288–5300.
28. Pati F, Jung J, Ha DH, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Sci Rep* 2021; 11:15566.
29. Toprakhisar B, Nadernezhad A, Bakirci E, et al. Development of bioink from decellularized tendon extracellular matrix for 3D bioprinting. *Macromol Biosci* 2018; 18:e1800024.
30. Li Z, Zhang S, Chen Y, et al. Gelatin methacryloyl-based tactile sensors for medical wearables. *Adv Funct Mater* 2020; 30:2003601.
32. Yu C, Ma X, Zhu W, et al. Scanningless and continuous 3D bioprinting of human tissues with decellularized extracellular matrix. *Biomaterials* 2019; 194:1–13.
33. Khan AA, Vishwakarma SK, Bardia A, et al. Repopulation of decellularized whole organ scaffold using stem cells: an emerging technology for the development of neo-organ. *J Artif Organs* 2014; 17:291–300.
34. Baptista PM, Orlando G, Mirmalek-Sani SH, et al. Whole organ decellularization - a tool for bioscaffold fabrication and organ bioengineering. *Ann Int Conf IEEE Eng Med Biol Soc* 2009; 2009:6526–6529.
35. Lu TY, Lin B, Kim J, et al. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. *Nat Commun* 2013; 4:2307.
36. Roosens A, Padma AM, Dehlin M, et al. Decellularization and recellularization of the ovary for bioengineering applications; studies in the mouse. *Reprod Biol Endocrinol* 2020; 18:75.
37. Miranda CMF, Leonel LCP, Cañada RR, et al. Effects of chemical and physical methods on decellularization of murine skeletal muscles. *An Acad Bras Cienc* 2021; 93:e20190942.
38. Gilpin A and Yang Y. Decellularization strategies for regenerative medicine: from processing techniques to applications. *Biomed Res Int* 2017; 2017:1–13.
39. Simsa R, Padma AM, Heher P, et al. Systematic in vitro molecular assessment of decellularization processes for porcine tissues for heart valve engineering. *Ann Biomed Eng* 2016; 44:2827–2839.
40. Alizadeh M, Rezakhani L, Soleimannejad M, et al. Evaluation of vacuum washing in the removal of SDS from decellularized bovine pericardium: method and device description. *Helyon* 2019; 5:e02253.
41. Lin CH, Hsia K, Su CK, et al. Sonication-assisted method for decellularization of human umbilical artery for small-caliber vascular tissue engineering. *Polymers* 2021; 13:1699.
42. Casali DM, Yost MJ and Matthews MA. Eliminating glutaraldehyde from crosslinked collagen films using supercritical CO₂. *J Biomed Mater Res* 2018; 106:86–94.
43. Xu H, Xu B, Yang Q, et al. Comparison of decellularization protocols for preparing a decellularized porcine annulus fibrosus scaffold. *PloS One* 2014; 9:e86723.
44. Elder BD, Eleswarapu SV and Athanasiou KA. Extraction techniques for the decellularization of tissue engineered articular cartilage constructs. *Biomaterials* 2009; 30:3749–3756.
45. Xing Q, Yates K, Tahtinen M, et al. Decellularization of fibroblast cell sheets for natural extracellular matrix scaffold preparation. *Tissue Eng Part C Methods* 2015; 21:77–87.
46. Schmitt A, Csiki R, Tron A, et al. Optimized protocol for whole organ decellularization. *Eur J Med Res* 2017; 22:31.
47. Luo Z, Bian Y, Su W, et al. Comparison of various reagents for preparing a decellularized porcine cartilage scaffold. *Am J Transl Res* 2019; 11:1417–1427.
48. White LJ, Taylor AJ, Faulk DM, et al. The impact of detergents on the tissue decellularization process: A ToF-SIMS study. *Acta Biomater* 2017; 50:207–219.
49. Wang Z, Li Z, Li Z, et al. Cartilaginous extracellular matrix derived from decellularized chondrocyte sheets for the reconstruction of osteochondral defects in rabbits. *Acta Biomater* 2018; 81:129–145.
50. Rashbar M, Hadjati J, Al J, et al. Characterization of decellularized ovine small intestine submucosal layer as extracellular matrix-based scaffold for tissue engineering. *J Biomed Mater Res B Appl Biomater* 2018; 106:933–944.
51. Chakraborty J, Roy S and Ghosh S. Regulation of decellularized matrix mediated immune response. *Biomater Sci* 2020; 8:1194–1215.
52. Tehyanian H, Karami A, Motavallian E, et al. Histologic analyses of different concentrations of tritonx-100 and sodium dodecyl sulfate detergent in lung decellularization. *Cell Mol Biol* 2017; 63:46–51.
53. Alshaikh AB, Padma AM, Dehlin M, et al. Decellularization and recellularization of the ovary for bioengineering applications; studies in the mouse. *Biorep Biol Endocrinol* 2020; 18:75.
54. Ma J, Ju Z, Yu J, et al. Decellularized rat lung scaffolds using sodium laurel ether sulfate for tissue engineering. *ASAIO J* 2018; 64:406–414.
55. Keshvari MA, Afshar A, Daneshi S, et al. Decellularization of kidney tissue: comparison of sodium laurel ether sulfate and sodium dodecyl sulfate for allotransplantation in rat. *Cell Tissue Res* 2021; 386:365–378.
56. Methe K, Bäckdahl H, Johansson BR, et al. An alternative approach to decellularize whole porcine heart. *Biores Open Access* 2014; 3:327–338.
57. McCrery MW, Vaughan NE, Hlavac N, et al. Novel sodium deoxycholate-based chemical decellularization method for peripheral nerve. *Tissue Eng Part C Methods* 2020; 26:23–36.
58. Rahman S, Griffin M, Naik A, et al. Optimising the decellularization of human elastic cartilage with trypsin for future use in ear reconstruction. *J Biomed Mater Res* 2018; 8:3097.
59. Hwang J, San BH, Turner NJ, et al. Molecular assessment of collagen denaturation in decellularized tissues using a collagen hybridizing peptide. *Acta Biomater* 2017; 53:268–278.
60. Hassanpour A, Talaei-Khozani T, Kargar-Abarghoei E, et al. Decellularized human ovarian scaffold based on a sodium laureyl ester sulfate (SLES)-treated protocol, as a natural three-dimensional scaffold for construction of bioengineered ovaries. *Stem Cell Res Ther* 2018; 9:252.
61. Kawasaki T, Kirita Y, Kami D, et al. Novel detergent for whole organ tissue engineering. *J Biomed Mater Res* 2015; 103:3364–3373.
62. Li Y, Wu Q, Li L, et al. Decellularization of porcine whole lung to obtain a clinical-scale bioengineered scaffold. *J Biomed Mater Res* 2021; 109:1623–1632.
63. Obata T, Tsuchiya T, Akita S, et al. Utilization of natural detergent potassium laurate for decellularization in lung bioengineering. *Tissue Eng Part C Methods* 2019; 25:459–471.
64. Aebischer P, Grognuz A, Peneveyre C, et al. Efficient decellularization of equine tendon with preserved biomechanical properties and cytocompatibility for human tendon surgery indications. *Artif Organs* 2020; 44:E161–E171.
65. Heidarzadeh M, Rahbarghazi R, Saberianpour S, et al. Distinct chemical composition and enzymatic treatment induced human endothelial cells survival in acellular ovine aortae. *BMC Res Notes* 2021; 14:126.
66. O’Neill JD, Anfang R, Anandappa A, et al. Decellularization of human and porcine lung tissues for pulmonary tissue engineering. Ann Thorac Surg 2013; 96: 1046-1055.

67. Chaschin IS, Britíkov DV, Khugaev GA, et al. Decellularization of the human donor aortic conduit by a new hybrid treatment in a multicomponent system with supercritical CO2 and Tween 80. J Supercrit Fluids 2022; 180:105452.

68. Mendibil U, Ruiz-Hernandez R, Retegi-Carrion S, et al. Tissue-specific decellularization methods: Rationale and strategies to achieve regenerative compounds. Int J Mol Sci 2020; 21:5447.

69. Marin-Tapia HA, Romero-Salazar L, Arteaga-Arcos JC, et al. Micro-mechanical properties of corneal scaffolds from two different bio-models obtained by an efficient chemical decellularization. J Mech Behav Biomed Mater 2021; 119:104510.

70. Qiu X, Lee BLP, Wong SY, et al. Cellular remodeling of fibrotic conduit as vascular graft. Biomaterials 2021; 268:120565.

71. Song YH, Maynes MA, Hlavac N, et al. Development of novel apoptosis-assisted lung tissue decellularization methods. Biomater Sci 2021; 9:3485–3498.

72. Philips C, Campos F, Roosens A, et al. Qualitative and quantitative evaluation of a novel detergent-based method for decellularization of peripheral nerves. Ann Biomed Eng 2018; 46:1921–1937.

73. Hudson TW, Zawko S, Deister C, et al. Optimized acellular nerve graft is immunologically tolerated and supports regeneration. Tissue Eng 2004; 10:1641–1651.

74. Tsuchiya T, Balestrini JL, Mendez J, et al. Influence of pH on extracellular matrix preservation during lung decellularization. Tissue Eng Part C Methods 2014; 20:1028–1036.

75. Zvarova B, Uhl FE, Uriarte JJ, et al. Residual detergent detection method for nondestructive cytocompatibility evaluation of decellularized whole lung scaffolds. Tissue Eng Part C Methods 2016; 22:418–428.

76. Prasertsung I, Kanokpanont S, Bunaprasert T, et al. Development of acellular dermis from porcine skin using periodic pressurization technique. J Biomed Mater Res B Appl Biomater 2008; 85B:210–219.

77. Ventura RD, Padalhin AR, Park CM, et al. Enhanced decellularization technique of porcine dural ECM for tissue engineering applications. Mater Sci Eng C 2019; 104:109841.

78. Choi YJ, Kim TG, Jeong J, et al. 3D cell printing of functional skeletal muscle constructs using skeletal muscle-derived bioink. Adv Healthc Mater 2016; 5:2636–2645.

79. Porziano A, Sfriso MM, Macchi V, et al. Decellularized omentum as novel biologic scaffold for reconstructive surgery and regenerative medicine. Eur J Histochem 2013; 57:4.

80. Lin Y, Zheng Q, Hua S, et al. Cross-linked decellularized porcine corneal graft for treating fungal keratitis. Sci Rep 2017; 7:9955.

81. Van de Walle AB, Uzarski JS and McFetridge PS. The consequence of biologic graft processing on blood interface biocompatibility and mechanics. Cardiovasc Eng Technol 2015; 6:303–313.

82. Mangold S, Schrammel S, Huber G, et al. Evaluation of decellularized human umbilical vein (HUV) for vascular tissue engineering - comparison with endothelium-denuded HUV. J Tissue Eng Regen Med 2015; 9:13–23.

83. Uzarski JS, Van De Walle AB and McFetridge PS. Preimplantation processing of ex vivo-derived vascular biomaterials: effects on peripheral cell adhesion. J Biomed Mater Res A 2013; 101A:123–131.

84. Flynn LE. The use of decellularized adipose tissue to provide an inductive microenvironment for the adipogenic differentiation of human adipose-derived stem cells. Biomaterials 2010; 31:4715–4724.

85. Lumpkins SB, Pierre N and McFetridge PS. A mechanical evaluation of three decellularization methods in the design of a xenogeneic scaffold for tissue engineering the temporomandibular joint disc. Acta Biomater 2008; 4:808–816.

86. Kabirian F and Mozafari M. Decellularized ECM-derived bioinks: prospects for the future. Methods 2020; 171:108–118.

87. Gorschewsky O, Klakow A, Riechert K, et al. Clinical comparison of the Tutoplast allograft and autologous patellar tendon (bone-patellar tendon-bone) for the reconstruction of the anterior cruciate ligament: 2- and 6-year results. Am J Sports Med 2005; 33:1202–1209.

88. Gorschewsky O, Puetz A, Riechert K, et al. Quantitative analysis of biochemical characteristics of bone-patellar tendon-bone allografts. Biomed Mater Eng 2005; 15:403–411.

89. Xing S, Liu C, Xu B, et al. Effects of various decellularization methods on histological and biomechanical properties of rabbit tendons. Exp Ther Med 2014; 8:628–634.

90. Duarte MM, Ribeiro N, Silva IV, et al. Fast decellularization process using supercritical carbon dioxide for trabecular bone. J Supercrit Fluids 2021; 172:105194.

91. Deeken CR, White AK, Bachman SL, et al. Method of preparing a decellularized porcine tendon using tributyl phosphate. J Biomed Mater Res Part B Appl Biomater 2011; 96B:199–206.

92. Cartmell JS and Dunn MG. Development of cell-seeded patellar tendon allografts for anterior cruciate ligament reconstruction. Tissue Eng 2004; 10:1065–1075.

93. Kumar Kuna V, Xu B and Sumitran-Holgersson S. Decellularization and recellularization methodology for human saphenous veins. J Vis Exp 2018; 137:57803.

94. Kanda H, Ando D, Hoshino R, et al. Surfactant-free preparation of an ostrich carotid artery scaffold using liquefied dimethyl ether. Acta Biomater 2010; 6:10543–10547.

95. Kanda H, Ando D, Oya K, et al. Surfactant-free decellularization protocols for production of tubular small intestine submucosa scaffolds for use in oesophageal tissue engineering. Acta Biomater 2014; 10:5043–5054.
99. Abaci A and Guvendiren M. Designing decellularized extracellular Matrix-Based bioinks for 3D bioprinting. *Adv Healthc Mater* 2020; 9:e2000734.

100. Zhao F, Cheng J, Zhang J, et al. Comparison of three different acidic solutions in tendon decellularized extracellular matrix bio-ink fabrication for 3D cell printing. *Acta Biomater* 2021; 131: 262–275.

101. Kajbafzadeh AM, Javan-Farazmand N, Monajemzadeh M, et al. Determining the optimal decellularization and sterilization protocol for preparing a tissue scaffold of a human-sized liver tissue. *Tissue Eng Part C Methods* 2013; 19:642–651.

102. Ahmed E, Saleh T, Yu L, et al. Micro and ultrastructural changes monitoring during decellularization for the generation of a biocompatible liver. *J Biosci Bioeng* 2019; 128:218–225.

103. Zhou P, Huang Y, Guo Y, et al. Decellularization and recellularization of rat livers with hepatocytes and endothelial progenitor cells. *Arfii Organs* 2016; 40:E25–E38.

104. Coronado RE, Somarakis-Cormier M, Natesan S, et al. Decellularization and solubilization of porcine liver for use as a substrate for porcine hepatocyte culture: method optimization and comparison. *Cell Transplant* 2017; 26:1840–1854.

105. Lu H, Hoshita T, Kawazoe N, et al. Comparison of decellularization techniques for preparation of extracellular matrix scaffolds derived from three-dimensional cell culture. *J Biomed Mater Res A* 2012; 100: 2507–2516.

106. Farag A, Hashimi SM, Vaquette C, et al. Assessment of static and perfusion methods for decellularization of PCL membrane-supported peridontal ligament cell sheet constructs. *Arch Oral Biol* 2018; 88: 67–76.

107. Ng CP, Mohamed Sharif AR, Heath DE, et al. Enhanced ex vivo expansion of adult mesenchymal stem cells by fetal mesenchymal stem cell ECM. *Biomaterials* 2014; 35: 4046–4057.

108. Dahl SLM, Koh J, Prabhakar V, et al. Decellularized native and engineered arterial scaffolds for transplantation. *Cell Transplant* 2003; 12: 659–666.

109. Woods T and Gratzer PF. Effectiveness of three extraction techniques in the development of a decellularized bone–anterior cruciate ligament–bone graft. *Biomaterials* 2005; 26: 7339–7349.

110. Oh JY, Kim MK, Lee HJ, et al. Processing porcine cornea for biomedical applications. *Tissue Eng Part C Methods* 2009; 15: 635–645.

111. Choi HJ, Kim MK, Lee HJ, et al. Efficacy of pig-to-rhesus lamellar corneal xenotransplantation. *Investig Ophthalmol Vis Sci* 2011; 52: 6643–6650.

112. Zhou Y, Wu Z, Ge J, et al. Development and characterization of acellular porcine corneal matrix using sodium dodecylsulfate. *Cornea* 2011; 30: 73–82.

113. Schmid M, Prinz TK, Stäbler A, et al. Effect of sodium sulfite, sodium dodecyl sulfate, and urea on the molecular interactions and properties of whey protein isolate-based films. *Front Chem* 2017; 4:49.

114. Dal Sasso E, Menabò R, Agillo D, et al. RegenHeart: a time-effective, low-concentration, detergent-based method aiming for conservative decellularization of the whole heart organ. *ACS Biomater Sci Eng* 2020; 6:5493–5506.

115. Fernandez-Perez J and Ahearne M. The impact of decellularization methods on extracellular matrix derived hydrogels. *Sci Rep* 2019; 9:14933.

116. Liao J, Joyce EM and Sacks MS. Effects of decellularization on the mechanical and structural properties of the porcine aortic valve leaflet. *Biomaterials* 2008; 29:1065–1074.

117. Yang G, Rothrauff BB and Tuan RS. Tendon and ligament regeneration and repair: clinical relevance and developmental paradigm. *Birth Defects Res C Embryo Today* 2013; 99:203–222.

118. Dong X, Wei X, Yi W, et al. RGD-modified acellular bovine pericardium as a bioprosthesis scaffold for tissue engineering. *J Mater Sci Mater Med* 2009; 20:2327–2336.

119. Datta N, Holtorf HL, Sikavitas VI, et al. Effect of bone extracellular matrix synthesized in vitro on the osteoblastic differentiation of narrow stromal cells. *Biomaterials* 2005; 26:971–977.

120. Poornnejad N, Schaumann LB, Buckmiller EM, et al. The impact of decellularization agents on renal tissue extracellular matrix. *J Biomater Appl* 2016; 31:521–533.

121. Lee W, Miyagawa Y, Long C, et al. A comparison of three methods of decellularization of pig corneas to reduce immunogenicity. *Int J Ophthalmol* 2014; 7:587–593.

122. Butler CR, Hynds RE, Crowley C, et al. Vacuum-assisted decellularization: an accelerated protocol to generate tissue-engineered human tracheal scaffolds. *Biomaterials* 2017; 124:95–105.

123. Wang Z, Sun F, Lu Y, et al. Rapid preparation method for preparing tracheal decellularized scaffolds: vacuum assistance and optimization of dnase I. *ACS Omega* 2021; 6:10637–10644.

124. Le TM, Morimoto N, Ly NTM, et al. Hydrostatic pressure can induce apoptosis of the skin. *Sci Rep* 2020; 10:17594.

125. Frey B, Janko C, Ebel N, et al. Cells under pressure—treatment of eukaryotic cells with high hydrostatic pressure, from physiologic aspects to pressure induced cell death. *Curr Med Chem* 2008; 15:2329–2336.

126. Zemmyo D, Yamamoto M and Miyata S. Efficient decellularization by application of moderate high hydrostatic pressure with Supercooling Pretreatment. *Microachines* 2021; 12:1486.

127. Watanabe N, Mizuno M, Matsuda J, et al. Comparison of high-hydrostatic-pressure decellularized versus freeze-thawed porcine menisci. *J Orthop Res* 2019; 37:2466–2475.

128. Levorson EJ, Hu O, Mountzias PM, et al. Cell-derived polymer/extracellular matrix composite scaffolds for cartilage regeneration, Part 2: construct devitalization and determination of chondroinductive capacity. *Tissue Eng Part C Methods* 2014; 20:358–372.

129. Thibault RA, Mikos AG and Kasper FK. Winner of the 2013 annual meeting and exposition, April 10-13, 2013, Boston, Massachusetts. Osteogenic differentiation of mesenchymal stem cells on demineralized and devitalized biodegradable polymer and extracellular matrix hybrid constructs. *J Biomed Mater Res A* 2013; 101A:225–1236.

130. Cheng J, Wang C and Gu Y. Combination of freeze-thaw with detergents: a promising approach to the decellularization of porcine carotid arteries. *Biomedi Mater Eng* 2019; 30:191–205.
131. Roth SP, Glauche SM, Plenge A, et al. Automated freeze-thaw cycles for decellularization of tendon tissue - a pilot study. *BMC Biotechnol* 2017; 17:13.
132. Roth SP, Erbe I and Burk J. Decellularization of large tendon specimens: combination of manually performed freeze-thaw cycles and detergent treatment. *Methods Mol Biol* 2018; 1577:227–237.
133. Jiang X, Lai XR, Lu JQ, et al. Decellularized adipose tissue: A key factor in promoting fat regeneration by recruiting and inducing mesenchymal stem cells. *Biochem Biophys Res Commun* 2021; 541:63–69.
134. Li N, Li Y, Gong D, et al. Efficient decellularization for bovine pericardium with extracellular matrix preservation and good biocompatibility. *Interact Cardiovasc Thorac Surg* 2018; 26:768–776.
135. Liu H, Yang L, Zhang E, et al. Biomimetic tendon extracellular matrix composite gradient scaffold enhances ligament-to-bone junction reconstruction. *Acta Biomater* 2017; 56:129–140.
136. White A, Burns D and Christensen TW. Effective terminal sterilization using supercritical carbon dioxide. *J Biotechnol* 2006; 123:504–515.
137. Dillow AK, Dehghani F, Hrkach JS, et al. Bacterial inactivation by using near- and supercritical carbon dioxide. *Proc Natl Acad Sci USA* 1999; 96:10344–10348.
138. Isenschmid A, Marison IW, von Stockar, et al. The influence of pressure and temperature of compressed CO2 on the survival of yeast cells. *J Biotechnol* 1995; 39:229–237.
139. Casali DM, Handleton RM, Shazly T, et al. A novel supercritical CO2-based decellularization method for maintaining scaffold hydration and mechanical properties. *J Supercrit Fluids* 2018; 131:72–81.
140. Harris AF, Lacombe J, Liyanage S, et al. Supercritical carbon dioxide decellularization of plant material to generate 3D biocompatible scaffolds. *Sci Rep* 2021; 11:3643.
141. Seo Y, Jung Y and Kim SH. Decellularized heart ECM hydrogel using supercritical carbon dioxide for improved angiogenesis. *Acta Biomater* 2018; 67:270–281.
142. Azhim A, Syazwani N, Morimoto Y, et al. The use of sonication technique to decellularize aortic tissues for preparation of bioscaffolds. *J Biomater Appl* 2014; 29:130–141.
143. Syazwani N, Azhim A, Morimoto Y, et al. Decellularization of aorta tissue using sonication treatment as potential scaffold for vascular tissue engineering. *J Med Biol Eng* 2015; 35:258–269.
144. Hung SH, Su CH, Lee FP, et al. Larynx decellularization: combining freeze-drying and sonication as an effective method. *J Voice* 2013; 27:289–294.
145. Mardhiyah A, Sha‘ban M and Azhim A. Evaluation of histological and biomechanical properties on engineered meniscus tissues using sonication decellularization. *Annu Int Conf IEEE Eng Med Biol Soc* 2017; 2016:2064–2067.
146. Yusof F, Sha‘ban M and Azhim A. Development of decellularized meniscus using closed sonication treatment system: potential scaffolds for orthopedics tissue engineering applications. *Int J Nanomedicine* 2019; 14:5491–5502.
147. Rabbani M, Forouzesh F and Bonakdar S. A comparison between ultrasonic bath and direct sonicator on osteochondral tissue decellularization. *J Med Signals Sens* 2019; 9:227–233.
148. Sawada K, Terada D, Yamaoka T, et al. Cell removal with supercritical carbon dioxide for acellular artificial tissue. *J Chem Technol Biotechnol* 2008; 83:943–949.
149. Manalastas TM, Dogus N, Ramos G, et al. Effect of decellularization parameters on the efficient production of Kidney Bioscaffolds. *Appl Biochem Biotechnol* 2021; 193:1239–1251.
150. Novoseletskaya E, Grigorieva O, Nimiritsky P, et al. Mesenchymal stromal cell-produced components of extracellular matrix potentiate multipotent stem cell response to differentiation stimuli. *Front Cell Dev Biol* 2020; 8:555378.
151. Cornelison RC, Wellman SM, Park JH, et al. Development of an apoptosis-assisted decellularization method for maximal preservation of nerve tissue structure. *Acta Biomater* 2018; 77:116–126.
152. Jiang D, Gao F, Zhang Y, et al. Mitochondrial transfer of dopamine on cellular bioenergetics and cell death. *PLoS One* 2012; 7:e44610.
153. Giordano S, Lee J, Darley-Usmar VM, et al. Distinct effects of rotenone, 1-methyl-4-phenylpyridinium and 6-hydroxydopamine on cellular bioenergetics and cell death. *Cell Death Discov* 2019; 2016; 7:e2467.
154. Reyna WE, Pichika R, Ludvig D, et al. Efficiency of skeletal muscle decellularization methods and their effects on the extracellular matrix. *J Biomech* 2020; 110:109961.
155. Fishman JM, Ansari T, Sibbons P, et al. Decellularized rabbit cricoarytenoid dorsalis muscle for laryngeal regeneration. *Ann Otol Rhinol Laryngol* 2012; 121:129–138.
156. Desouza M, Gunning PW and Stehn JR. The actin cytoskeleton as a sensor and mediator of apoptosis. *BioArchitecture* 2012; 2:75–87.
157. Gosztyla C, Ladd MR, Werts A, et al. A comparison of sterilization techniques for production of decellularized intestine in mice. *Tissue Eng Part C Methods* 2020; 26:67–79.
158. Hennessy RS, Jana S, Tefft BJ, et al. Supercritical carbon dioxide-based sterilization of decellularized heart valves. *JACC Basic Transl Sci* 2017; 2:71–84.
159. Amano S, Shimomura N, Yokoo S, et al. Decellularizing corneal stroma using N2 gas. *Mol Vis* 2008; 14:878–882.
160. Isidan A, Liu S, Li P, et al. Decellularization methods for developing porcine corneal xenografts and future perspectives. * Xenotransplantation* 2019; 26:e12564.
161. Han TTY and Flynn LE. Perfusion bioreactor culture of human adipose-derived stromal cells on decellularized adipose tissue scaffolds enhances in vivo adipose tissue regeneration. *J Tissue Eng Regen Med* 2020; 14:1827–1840.
162. Colombo JS, Jia S and D’Souza RN. Modeling hypoxia induced factors to treat pulpal inflammation and drive regeneration. *J Endod* 2020; 46:S19–S25.
163. Olsen JV, Ong SE and Mann M. Trypsin cleaves exclusively C-terminal to arginine and lysine residues. *Mol Cell Proteomics* 2004; 3:608–614.
164. Sajith S. Comparative study of two decellularization protocols on a biomaterial for tissue engineering. *J Clin Exp Cardiolog* 2017; 08:1000523.
165. Grauss R, Hazeckamp M, Oppenhuizen F, et al. Histological evaluation of decellularised porcine aortic valves: matrix changes due to different decellularisation methods. *Eur J Cardiothorac Surg* 2005; 27:566–571.
166. Zou Y and Zhang Y. Mechanical evaluation of decellularized porcine thoracic aorta. *J Surg Res* 2012; 175:359–368.

167. Haupt J, Lutter G, Gorb SN, et al. Detergent-based decellularization strategy preserves macro- and microstructure of heart valves. *Interact Cardiovasc Thorac Surg* 2018; 26:230–236.

168. Bousalis D, McCrary MW, Vaughn N, et al. Decellularized peripheral nerve as an injectable delivery vehicle for neural applications. *J Biomed Mater Res A* 2022; 110:595–611.

169. Feng H, Xu Y, Luo S, et al. Evaluation and preservation of vascular architectures in decellularized whole rat kidneys. *Cryobiology* 2020; 95:72–79.

170. Negishi J, Funamoto S, Kimura T, et al. Porcine radial artery decellularization by high hydrostatic pressure. *J Tissue Eng Regen Med* 2015; 9:E144–E151.

171. Batioglu-Karaaltin A, Ovali E, Karaaltin MV, et al. Decellularization of trachea with combined techniques for tissue-engineered trachea transplantation. *Clin Exp Otorhinolaryngol* 2019; 12:86–94.

172. Ramm R, Goecke T, Theodoridis K, et al. Decellularization combined with enzymatic removal of N-linked glycans and residual DNA reduces inflammatory response and improves performance of porcine xenogeneic pulmonary heart valves in an ovine in vivo model. *Xenotransplantation* 2020; 27:e12571.

173. Liu J, Li Z, Li J, et al. Application of benzonase in preparation of decellularized lamellar porcine corneal stroma for lamellar keratoplasty. *J Biomed Mater Res A* 2019; 107:2547–2555.

174. Godehardt AW, Ramm R, Gulich B, et al. Decellularized pig pulmonary heart valves—depletion of nucleic acids measured by proviral PERV pol. *Xenotransplantation* 2020; 27:e12565.

175. Dong M, Zhao L, Wang F, et al. Rapid porcine corneal decellularization through the use of sodium N-lauroyl glutamate and supernucllease. *J Tissue Eng* 2019; 10:2041731419875876.

176. Oliveri M, Daga A, Cantoni C, et al. Dnase I mediates intermuneososomal DNA degradation in human cells undergoing drug-induced apoptosis. *Eur J Immunol* 2001; 31:743–751.

177. Asadi M, Khalili M, Lotfi H, et al. Liver bioengineering: Recent trends/advances in decellularization and cell sheet technologies towards translation into the clinic. *Life Sci* 2021; 276:119373.

178. Keane TJ, Swinehart IT and Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. *Methods* 2015; 84:25–34.

179. Chen RN, Ho HO, Tsai YT, et al. Process development of an acellular dermal matrix (ADM) for biomedical applications. *Biomaterials* 2004; 25:2679–2686.

180. Kuljanin M, Brown CFC, Raleigh MJ, et al. Collagenase treatment enhances proteomic coverage of low-abundance proteins in decellularized matrix bioscaffolds. *Biomaterials* 2017; 144:130–143.

181. Wu Z, Zhou Y, Li N, et al. The use of phospholipase A(2) to prepare acellular porcine corneal stroma as a tissue engineering scaffold. *Biomaterials* 2009; 30:3513–3522.

182. Chen L, Wei L, Shao A, et al. Immune risk assessment of residual αGal in xenogeneic decellularized cornea using GTKO mice. *Regen Biomater* 2020; 7:427–434.

183. Gessner RC, Hanson AD, Feingold S, et al. Functional ultrasound imaging for assessment of extracellular matrix scaffolds used for liver organoid formation. *Biomaterials* 2013; 34:9341–9351.

184. Huang M, Li N, Wu Z, et al. Using acellular porcine limbal stroma for rabbit limbal stem cell microenvironment reconstruction. *Biomaterials* 2011; 32:7812–7821.

185. Li N, Wang X, Wan P, et al. Tectonic lamellar keratoplasty with acellular corneal stroma in high-risk corneal transplantation. *Mol Vis* 2011; 17:1909–1917.

186. Bautista CA, Park HJ, Mazur CM, et al. Effects of chondroitinase ABC-Mediated proteoglycan digestion on decellularization and recellularization of articular cartilage. *PLoS One* 2016; 11:e0158976.

187. Natoli RM, Revell CM and Athanasiou KA. Chondroitinase ABC treatment results in greater tensile properties of self-assembled tissue-engineered articular cartilage. *Tissue Eng Part A* 2009; 15:3119–3128.

188. Bian L, Crivello KM, Ng KW, et al. Influence of temporary chondroitinase ABC-induced glycosaminoglycan suppression on maturation of tissue-engineered cartilage. *Tissue Eng Part A* 2009; 15:2065–2072.

189. Neubauer D, Graham JB and Muir D. Chondroitinase treatment increases the effective length of acellular nerve grafts. *Exp Neurol* 2007; 207:163–170.

190. Boyer RB, Sexton KW, Rodriguez-Feo CL, et al. Adjuvant neurotrophic factors in peripheral nerve repair with chondroitin sulfate proteoglycan-reduced acellular nerve allografts. *J Surg Res* 2015; 193:969–977.

191. Bradbury EJ, Moon LDF, Popat RJ, et al. Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* 2002; 416:636–640.

192. Shojaie S, Ermini L, Ackerley C, et al. Acellular lung scaffolds direct differentiation of endoderm to functional airway epithelial cells: requirement of matrix-bound HS proteoglycans. *Stem Cell Reports* 2015; 4:419–430.

193. Tamez-Mata Y, Pedroza-Montoya FE, Martinez-Rodriguez HG, et al. Nerve gaps repaired with acellular nerve allografts recellularized with Schwann-like cells: Preclinical trial. *J Plast Reconstr Aesthet Surg* 2022; 75:296–306.

194. Sart S, Jeske R, Chen X, et al. Engineering stem cell-derived extracellular matrices: decellularization, characterization, and biological function. *Tissue Eng Part B Rev* 2020; 26:402–422.

195. Wallis JM, Borg ZD, Daly AB, et al. Comparative assessment of detergent-based protocols for mouse lung de-cellularization and re-cellularization. *Tissue Eng Part C Methods* 2012; 18:420–432.

196. Rademacher F, Dreyer S, Kopfnagel V, et al. The antimicrobial and immunomodulatory function of RNase 7 in skin. *Front Immunol* 2019; 10:2553.

197. Spurr SJ and Gipson IK. Isolation of corneal epithelium from mice. *Invest Ophthalmol Vis Sci* 1985; 26:818–827.

198. Gonzalez-Andrades M, de la Cruz Cardona J, Ionescu AM, et al. Generation of detergent-based protocols for mouse lung de-cellularization and re-cellularization. *Biomaterials* 2004; 25:2679–2686.

199. Palka JA and Phang JM. Prolidase activity in fibroblasts is regulated by interaction of extracellular matrix with cell surface integrin receptors. *J Cell Biochem* 1997; 67:166–175.
200. Shaya D, Hahn BS, Bjerkman TM, et al. Composite active site of chondroitin lyase ABC accepting both epimers of uronic acid. Glycobiology 2008; 18:270–277.

201. Lin X, Patil S, Gao YG, et al. The bone extracellular matrix in bone formation and regeneration. Front Pharmacol 2020; 11:757.

202. Mansour A, Mezour MA, Badran Z, et al. Extracellular matrices for bone regeneration: A literature review. Tissue Eng Part A 2017; 23:1436–1451.

203. Pang S, Su FY, Green A, et al. Comparison of different protocols for demineralization of cortical bone. Sci Rep 2021; 11:7012.

204. Bronstein JA, Woon CYL, Farnebo S, et al. Physicochemical decellularization of composite flexor tendon-bone interface grafts. Plast Reconstr Surg 2013; 132:94–102.

205. Rasch A, Naujokat H, Wang F, et al. Evaluation of bone allograft processing methods: impact on decellularization efficacy, biocompatibility and mesenchymal stem cell functionality. PLoS One 2019; 14:e0218404.

206. Hashimoto Y, Funamoto S, Kimura T, et al. The effect of decellularized bone/bone marrow produced by high-hydrostatic pressure on the osteogenic differentiation of mesenchymal stem cells. Biomaterials 2011; 32:7060–7067.

207. Nakamura N, Kimura T, Nam K, et al. Induction of in vivo ectopic hematopoiesis by a three-dimensional structured extracellular matrix derived from decellularized cancellous bone. ACS Biomater Sci Eng 2019; 5:5669–5680.

208. Nouri Barkestani M, Naserian S, Uzan G, et al. Post-decellularization techniques ameliorate cartilage decellularization process for tissue engineering applications. J Tissue Eng 2021; 12:2041731420935362.

209. Kim YS, Majid M, Melchiorri AJ, et al. Applications of decellularized extracellular matrix in bone and cartilage tissue engineering. Bioeng Transl Med 2019; 4:83–95.

210. Kheir E, Stapleton T, Shaw D, et al. Development and characterization of an acellular porcine cartilage bone matrix for use in tissue engineering. J Biomed Mater Res A 2011; 99A:283–294.

211. Utomo L, Pleumeekers MM, Nimeskern L, et al. Preparation and characterization of a decellularized cartilage scaffold for ear cartilage reconstruction. Biomed Mater 2015; 10:015010.

212. Tavassoli A, Matin MM, Niaki MA, et al. Mesenchymal stem cells can survive on the extracellular matrix-derived decellularized bovine articular cartilage scaffold. Iran J Basic Med Sci 2015; 18:1221–1227.

213. Kang H, Peng J, Lu S, et al. In vivo cartilage repair using adipose-derived stem cell-loaded decellularized cartilage ECM scaffolds. J Tissue Eng Regen Med 2014; 8:442–453.

214. Yang Q, Peng J, Guo Q, et al. 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 2008; 29:2378–2387.

215. Ghassemi T, Saghatoleslami N, Mahdavi-Shahri N, et al. A comparison study of different decellularization treatments on bovine articular cartilage. J Tissue Eng Regen Med 2019; 13:1861–1871.

216. Xia C, Mei S, Gu C, et al. Decellularized cartilage as a prospective scaffold for cartilage repair. Mater Sci Eng C 2019; 101:588–595.

217. Eyre DR and Muir H. The distribution of different molecular species of collagen in fibrous, elastic and hyaline cartilages of the pig. Biochem J 1975; 151:595–602.

218. Naumann A, Dennis JE, Awadallah A, et al. Immunohistochemical and mechanical characterization of cartilage subtypes in rabbit. J Histocomp Cytochem 2002; 50:1049–1058.

219. Arokoski JPA, Hytinen MM, Helminen HJ, et al. Biomechanical and structural characteristics of canine femoral and tibial cartilage. J Biomed Mater Res 1999; 48:99–107.

220. Vas WJ, Shah M, Blacker TS, et al. Decellularized cartilage directs chondrogenic differentiation: creation of a fracture callus mimetic. Tissue Eng Part A 2018; 24:1364–1376.

221. Shen W, Berning K, Tang SW, et al. Rapid and detergent-free decellularization of cartilage. Tissue Eng Part C Methods 2020; 26:201–206.

222. Ojima K, Oe M, Nakajima I, et al. Dynamics of protein secretion during adipocyte differentiation. FEBS Open Bio 2016; 6:816–826.

223. Thomas-Porch C, Li J, Zanata F, et al. Comparative proteomic analyses of human adipose extracellular matrices decellularized using alternative procedures. J Biomed Mater Res A 2018; 106:2481–2493.

224. Giatsidis G, Succar J, Haddad A, et al. Preclinical optimization of a Shelf-Ready, injectable, Human-Derived, decellularized allograft adipose matrix. Tissue Eng Part A 2019; 25:271–287.

225. He Y, Lin M, Wang X, et al. Optimized adipose tissue engineering strategy based on a neo-mechanical processing method. Wound Repair Regen 2018; 26:163–171.

226. Brown BN, Freund JM, Han L, et al. Comparison of three methods for the derivation of a biologic scaffold composed of adipose tissue extracellular matrix. Tissue Eng Part C Methods 2011; 17:411–421.

227. Choi JS, Kim BS, Kim JY, et al. Decellularized extracellular matrix derived from human adipose tissue as a potential scaffold for allograft tissue engineering. J Biomed Mater Res A 2011; 97A:292–299.

228. Wang L, Johnson JA, Zhang Q, et al. Combining decellularized human adipose tissue extracellular matrix and adipose-derived stem cells for adipose tissue engineering. Acta Biomater 2013; 9:8921–8931.

229. Young DA, Ibrahim DO, Hu D, et al. Injectable hydrogel scaffold from decellularized human lipopasipirite. Acta Biomater 2011; 7:1040–1049.

230. Wang JK, Luo B, Guneta V, et al. Supercritical carbon dioxide extracted extracellular matrix material from adipose tissue. Mater Sci Eng C 2017; 75:349–358.

231. Espana EM and Birk DE. Composition, structure and functionality of human stromal refractive lenticules for corneal tissue engineering. J Tissue Eng Regen Med 2020; 50:633–643.

232. Lynch AP, Wilson SL and Ahearne M. Dextran preserves native corneal structure during decellularization. Exp Eye Res 2020; 198:108137.

233. Yam GHF, Yusoff NZBM, Goh TW, et al. Decellularization protocol for use in tissue engineering. FEBS Open Bio 2020; 10:015010.

234. Ohata K and Ott HC. Human-scale lung regeneration based on decellularized matrix scaffolds as a biologic platform. J Tissue Eng Regen Med 2018; 12:15:2041731418810164.
236. Tebyanian H, Karami A, Motavallian E, et al. Rat lung decellularization using chemical detergents for lung tissue engineering. *Biotech Histocomp* 2019; 94:214–222.
237. Ke P, Hong Y and Zhang G. Cardiac tissue-derived extracellular matrix scaffolds for myocardial repair: advantages and challenges. *Regen Biomater* 2019; 6:185–199.
238. Di Meglio F, Nurzynska D, Romano V, et al. Optimization of human myocardium decellularization method for the construction of implantable patches. *Tissue Eng Part C Methods* 2017; 23:525–539.
239. Perea-Gil I, Uriarte JJ, Pratt-Vidal C, et al. In vitro comparative study of two decellularization protocols in search of an optimal myocardial scaffold for recellularization. *Am J Transl Res* 2015; 7:558–573.
240. Tenreiro MF, Almeida HV, Calmeiro T, et al. Interindividual heterogeneity affects the outcome of human cardiac tissue decellularization. *Sci Rep* 2021; 11:20834.
241. Sokol AA, Grekov DA, Yemets GI, et al. Comparison of bovine pericardium decellularization protocols for production of biomaterial for cardiac surgery. *Biopolym Cell* 2020; 36:392–403.
242. Guler S, Aslan B, Hosseinian P, et al. Supercritical carbon dioxide-assisted decellularization of aorta and cornea. *Tissue Eng Part C Methods* 2017; 23:540–547.
243. Cesur NP and Laçin NT. Decellularization of ram cardiac tissue via supercritical CO$_2$. *J Supercrit Fluids* 2022; 180:105453.
244. Xu J and Shi GP. Vascular wall extracellular matrix proteins and vascular diseases. *Biochim Biophys Acta* 2014; 1842:2106–2119.
245. Cai Z, Gu Y, Cheng J, et al. Decellularization, cross-linking and heparin immobilization of porcine carotid arteries for tissue engineering vascular grafts. *Cell Tissue Bank* 2019; 20:569–578.
246. Remlinger NT, Wearden PD and Gilbert TW. Procedure for decellularization of porcine heart by retrograde coronary perfusion. *J Exp Vis* 2012; e50059.
247. Lin CH, Hsia K, Tsai CH, et al. Decellularized porcine coronary artery with adipose stem cells for vascular tissue engineering. *Biomed Mater* 2014; 13:1845014.
248. Gil-Ramirez A, Rosmark O, Spégel P, et al. Pressurized carbon dioxide as a potential tool for decellularization of pulmonary arteries for transplant purposes. *Sci Rep* 2020; 10:4031.
249. Tuan-Mu HY, Chang YH and Hu JJ. Removal of an abluminal lining improves decellularization of human umbilical arteries. *Sci Rep* 2020; 10:10556.
250. Yamanaka H, Morimoto N and Yamaoka T. Decellularization of submillimeter-diameter vascular scaffolds using peracetic acid. *J Artif Organs* 2020; 23:156–162.
251. Brouki Milan P, Pazouki A, Joghataei MT, et al. Decellularization and preservation of human skin: A platform for tissue engineering and reconstructive surgery. *Methods* 2020; 171:62–67.
252. Kamalvand M, Biazar E, Daliri-Joupari M, et al. Design of a decellularized fish skin as a biological scaffold for skin tissue regeneration. *Tissue Cell* 2021; 71:101509.
253. Greco K, Francis L, Somasundaram M, et al. Characterisation of porcine dermis scaffolds decellularised using a novel non-enzymatic method for biomedical applications. *J Biomater Appl* 2015; 30:239–253.
254. Salinas F, Robla D, Meana À, et al. Novel technique of development of human derived acellular dermal matrix. *Cell Tissue Bank* 2021. DOI: 10.1007/s10561-021-00954-4.
255. Gzik-Zroska B, Joszko K, Wolański W, et al. Assessment of the impact of decellularization methods on mechanical properties of biocomposites used as skin substitute. *Materials* 2021; 14:4785.
256. Koo MA, Jeong H, Hong SH, et al. Preconditioning process for dermal tissue decellularization using electroporation with sonication. *Regen Biomater* 2022; 9:rbab071. DOI: 10.1093/rr/rrbab071
257. Gaetani R, Aude S, DeMaddalena LL, et al. Evaluation of different decellularization protocols on the generation of pancreas-derived hydrogels. *Tissue Eng Part C Methods* 2018; 24:697–708.
258. Berman A, Klak M, Adamiok A, et al. The influence of the flow of detergent and donor characteristics on the extracellular matrix composition after human pancreas decellularization. *Transplant Proc* 2020; 52:2043–2049.
259. Sackett SD, Tremmel DM, Ma F, et al. Extracellular matrix scaffold and hydrogel derived from decellularized and delipidized human pancreas. *Sci Rep* 2018; 8:10452.
260. Pantoja B, Silva D, Silva M, et al. Ultrastructural analysis of decellularized diabetic and non-diabetic canine pancreas for the production of biological scaffolds. *Cytotherapy* 2021; 23:37.
261. Elebring E, Kuna VK, Kvarnström N, et al. Cold-perfusion decellularization of whole-organ porcine pancreas supports human fetal pancreatic cell attachment and expression of endocrine and exocrine markers. *J Tissue Eng* 2017; 8:2041731417738145.
262. Yang J, Xu Y, Luo S, et al. Effect of cryoprotectants on rat kidney decellularization by freeze-thaw process. *Cryobiology* 2022; 105:71–82.
263. Zambon J, Ko IK, Abolbashari M, et al. Optimization of kidney decellularization methods utilizing a quantitative vascular casting technique for long-term implantation. *Urol J* 2019; 201:e1129–e1130.
264. Zambon JP, Ko IK, Abolbashari M, et al. Comparative analysis of two porcine kidney decellularization methods for maintenance of functional vascular architectures. *Acta Biomater* 2018; 75:226–234.
265. Kajbafzadeh AM, Khorramirouz R, Nabadvazade B, et al. Whole organ sheep kidney tissue engineering and in vivo transplantation: Effects of perfusion-based decellularization on vascular integrity. *Mater Sci Eng C* 2019; 98:392–400.
266. Tajima K, Kuroda K, Otaka Y, et al. Decellularization of canine kidney for three-dimensional organ regeneration. *Vet World* 2020; 13:452–457.
267. Poornejad N, Frost TS, Scott DR, et al. Freezing/thawing without cryoprotectant damages native but not decellularized porcine renal tissue. *Organogenesis* 2015; 11:30–45.
268. Poornejad N, Momtahan N, Salehi AS, et al. Efficient decellularization of whole porcine kidneys improves reseeded cell behavior. *Biomed Mater* 2016; 11:025003.
269. Pozzi A, Yurchenco PD and Iozzo RV. The nature and biology of basement membranes. *Matrix Biol* 2016; 57-58:1–11.
270. Naba A, Clauser KR, Whitaker CA, et al. Extracellular matrix signatures of human primary metastatic colon cancers and their metastases to liver. *BMC Cancer* 2014; 14:518.
271. Strucl B, Butter A, Hillebrandt K, et al. Improved rat liver decellularization by arterial perfusion under oscillating...
pressure conditions. *J Tissue Eng Regen Med* 2017; 11:531–541.

272. Willems J, Verstegen MMA, Vermeulen A, et al. Fast, robust and effective decellularization of whole human livers using mild detergents and pressure controlled perfusion. *Mater Sci Eng C* 2020; 108:110200.

273. Mazza G, Al-Akkad W, Telese A, et al. Rapid production of human liver scaffolds for functional tissue engineering by high shear stress oscillation-decellularization. *Sci Rep* 2017; 7:5534.

274. Jakus AE, Laronda MM, Rashedi AS, et al. “Tissue Papers” from organ-specific decellularized extracellular matrices. *Adv Funct Mater* 2017; 27:1700992.

275. Alshaikh AB, Padma AM, Dehlin M, et al. Decellularization of the mouse ovary: comparison of different scaffold generation protocols for future ovarian bioengineering. *J Ovarian Res* 2019; 12:58.

276. Daryabari SS, Kajbafzadeh AM, Fendereski K, et al. Development of an efficient perfusion-based protocol for whole-organ decellularization of the ovine uterus as a human-sized model and in vivo application of the bioscaffolds. *J Assist Reprod Genet* 2019; 36:1211–1223.

277. Padma AM, Alshaikh AB, Song MJ, et al. Decellularization protocol-dependent DAMPs in rat uterus scaffolds differentially affect the immune response after transplantation. *J Tissue Eng Regen Med* 2021; 15:674–685.

278. Hellström M, El-Akouri RR, Sihlbom C, et al. Towards the development of a bioengineered uterus: comparison of different protocols for rat uterus decellularization. *Acta Biomater* 2014; 10:5034–5042.

279. Hellström M, Moreno-Moya JM, Bandstein S, et al. Bioengineered uterine tissue supports pregnancy in a rat model. *Fertil Steril* 2016; 106:487–496.e1.

280. Padma AM, Carrière L, Kroksström Karlsson F, et al. Towards a bioengineered uterus: bioactive sheep uterus scaffolds are effectively recellularized by enzymatic perconditioning. *NPJ Regen Med* 2021; 6:26.

281. Pennarossa G, Ghiringhelli M, Gandolfi F, et al. Whole-ovary decellularization generates an effective 3D bioscaffold for ovarian bioengineering. *J Assist Reprod Genet* 2020; 37:1329–1339.

282. Eivazkhan F, Ahtai NS, Tavana S, et al. Evaluating two ovarian decellularization methods in three species. *Mater Sci Eng C* 2019; 102:670–682.

283. Burnside ER and Bradbury EJ. Review: manipulating the extracellular matrix and its role in brain and spinal cord plasticity and repair. *Neuropathol Appl Neurobiol* 2014; 40:26–59.

284. Dityatev A, Schachner M and Sonderegger P. The dual role of the extracellular matrix in synaptic plasticity and homeostasis. *Nat Rev Neurosci* 2010; 11:735–746.

285. Sondell M, Lundborg G and Kanje M. Regeneration of the rat sciatic nerve into allografts made acellular through chemical extraction. *Brain Res* 1998; 795:44–54.

286. Nieto-Nicolau N, López-Chicón P, Farías O, et al. Effective decellularization of human nerve matrix for regenerative medicine with a novel protocol. *Cell Tissue Res* 2021; 384:167–177.

287. Suss PH, Ribeiro VST, Motooka CE, et al. Comparative study of decellularization techniques to obtain natural extracellular matrix scaffolds of human peripheral-nerve allografts. *Cell Tissue Bank* 2021. DOI: 10.1007/s10561-021-09977-x.

288. Serrano-Arocá Á, Vera-Donoso CD and Moreno-Manzano V. Bioengineering approaches for bladder regeneration. *Int J Mol Sci* 2018; 19:1796.

289. Bolland F, Korossis S, Wilshaw SP, et al. Development and characterisation of a full-thickness acellular porcine bladder matrix for tissue engineering. *Biomaterials* 2007; 28:1061–1070.

290. Farhat W, Chen J, Erdeljan P, et al. Porosity of porcine bladder acellular matrix: impact of ACM thickness. *J Biomed Mater Res* 2003; 67A:970–974.

291. Freytes DO, Badyal SF, Webster TJ, et al. Biaxial strength of multilaminated extracellular matrix scaffolds. *Biomaterials* 2004; 25:2353–2361.

292. Freytes DO, Martin J, Velankar SS, et al. Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. *Biomaterials* 2008; 29:1630–1637.

293. Saldiv LT, Cramer MC, Velankar SS, et al. Extracellular matrix hydrogels from decellularized tissues: Structure and function. *Acta Biomater* 2017; 49:1–15.

294. Yang B, Zhang Y, Zhou L, et al. Development of a porcine bladder acellular matrix with well-preserved extracellular bioactive factors for tissue engineering. *Tissue Eng Part C Methods* 2010; 16:1201–1211.

295. Moreno-Manzano V, Zaytseva-Zotova D, López-Mocholi E, et al. Injectable gel form of a decellularized bladder induces adipose-derived stem cell differentiation into smooth muscle cells in vitro. *Int J Mol Sci* 2020; 21:8608.

296. Sabetkish S, Sabetkish N and Kajbafzadeh AM. In vivo regeneration of bladder muscular wall with whole decellularized bladder matrix: A novel hourglass technique for duplication of bladder volume in rabbit model. *J Pediatr Surg* 2020; 55:2226–2232.

297. Dahms SE, Piechota HJ, Dahiya R, et al. Composition and biomechanical properties of the bladder acellular matrix graft: comparative analysis in rat, pig and human. *Br J Urol* 1998; 82:411–419.

298. Kao CY, Nguyen HQ and Weng YC. Characterization of porcine urinary bladder matrix hydrogels from sodium dodecyl sulfate decellularization method. *Polymers* 2020; 12:3007.

299. Hynes RO and Naba A. Overview of the matriosome—an inventory of extracellular matrix constituents and functions. *Cold Spring Harb Perspect Biol* 2012; 4:a004903.

300. Han X, Liao L, Zhu T, et al. Xenogeneic native decellularized matrix carrying PPARγ activator RSG regulating macrophage polarization to promote ligament-to-bone regeneration. *Mater Sci Eng C* 2020; 116:111224.

301. Zhu S, Chen P, Chen Y, et al. 3D-Printed extracellular matrix/polyethylene glycol diacrylate hydrogel incorporating the anti-inflammatory phytomolecule honokiol for regeneration of osteochondral defects. *Am J Sports Med* 2020; 48:2808–2818.

302. Schwarz S, Koerber L, Elsässer AF, et al. Decellularized cartilage matrix as a novel biomatrix for cartilage tissue-engineering applications. *Tissue Eng Part A* 2012; 18:2195–2209.

303. Zhu Y, Zhang Q, Wang S, et al. Current advances in the development of decellularized plant extracellular matrix. *Front Bioeng Biotechnol* 2021; 9:712262.