

## Chapter 3

# Decellularized Scaffolds: Concepts, Methodologies, and Applications in Cardiac Tissue Engineering and Whole-Organ Regeneration

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Tissue engineering research, which aims to develop tissue/organ substitutes for treating pathological disorders and organ failures, has made many breakthroughs during the past three decades. The field still faces challenges such as identifying and optimizing scaffolds that must be biodegradable, non-immunogenic, and able to provide structural, mechanical, biological supports/cues for cell adhesion, proliferation, and differentiation. Recent accomplishments in tissue decellularization provide acellular tissue-derived scaffolds that retain the nature-designed structure from the whole organ level, to the microstructural scale, down to the nanoscale. The preservation of structurally organized entities such as collagen, elastin, glycosaminoglycans, and fibronectin enables a natural template that accommodates many tissue

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engineering and regeneration applications. This chapter discusses soft tissue-derived scaffolds from a structure-function perspective, with an emphasis on cardiac tissue engineering and whole organ regeneration applications.

## 1. Introduction

Combining fields of biology, chemistry, engineering, biotechnology and medicine, tissue engineering and regeneration offer a unique solution to repair damaged tissues or organs. The ultimate goal of these processes is to provide tissue constructs or regenerative implants that are viable, i.e., able to function and remodel, and that have potential to integrate into host organ systems.<sup>1-4</sup> Three methods of tissue engineering and regeneration have primarily been used to date: (i) implantation of cultured cells directly, (ii) regeneration of tissues *in situ*, and (iii) congregation of cells and scaffolds (*in vitro*).<sup>5-8</sup> Hereafter, we will focus on the third method that aims to engineer tissue constructs congregated by cells and scaffolds, specifically an approach that utilizes tissue-derived acellular scaffolds.

Tissue engineering research has made many breakthroughs during the past three decades; however, the field still faces the following challenges: (i) the growth of cells (often originating from stem cells) with the right phenotypes and organizations in suitable scaffolds; (ii) delivering and sustaining adequate oxygen and nutrients in tissue constructs; (iii) determining optimal engineering environments, such as bioreactors, growth media, physical simulations, for particular constructs; and (iv) functional integration of adaptable, durable tissue constructs with hosts.<sup>2-4</sup>

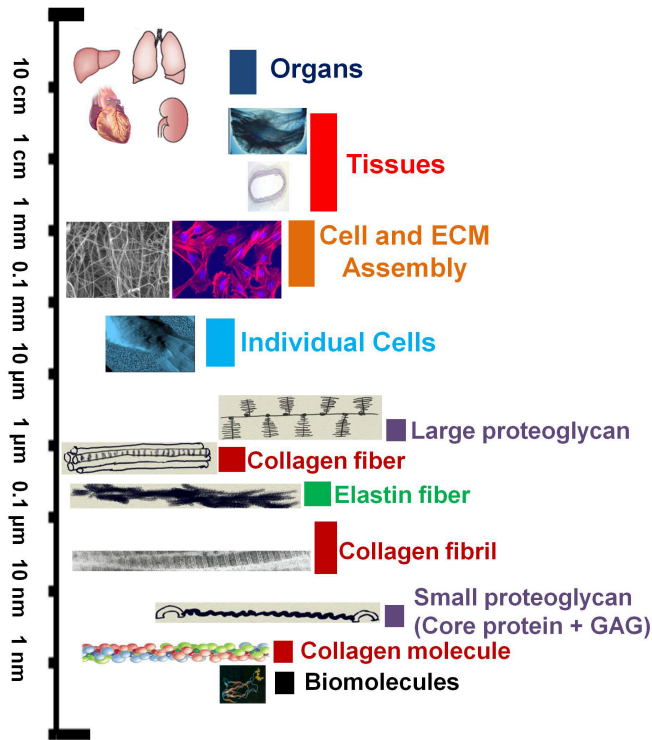
From the structure-function perspective, the above challenges all involve the recapitulation of the organization of target tissue or organs, implicating the importance of searching for biodegradable, non-immunogenic scaffolds that provide structural, mechanical, biological supports/cues for cell adhesion, proliferation, and differentiation.<sup>5</sup> The proper selection of scaffolds and cells warrants the later cell-scaffold/extracellular matrix (ECM) interactions that mediate favorable tissue organization and remodeling, as well as the cell-cell interactions that realize certain biological functionality.

Scaffolds used for tissue engineering and regeneration can be synthetic polymers, naturally derived proteins/polymers, and tissue-derived scaffolds produced by decellularization.<sup>9–15</sup> Commonly used synthetic scaffolds include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(caprolactone) (PCL), poly(hydroxyalkanoate), and poly ester (urethane urea) (PEUU).<sup>16,17</sup> Synthetic scaffolds can be tailored and reproduced readily with controlled mechanical properties, porosity, and surface topography. However, this approach faces limitations such as uncertainties regarding the degradation rate and products as well as cell compatibility.<sup>18–24</sup> Naturally derived proteins/polymers, such as collagen, fibrin, alginate, etc., possess intrinsic cell compatibility, but they are often weak in mechanical properties and difficult to manipulate.<sup>25–31</sup>

Decellularization, on the other hand, removes cellular components from tissues/organs to generate ECM templates, a complex mixture of structural and functional proteins that can be used for tissue engineering applications.<sup>13–15,32–42</sup> The removal of cellular content and antigens from the tissue-derived scaffolds reduces foreign body reaction, inflammation, and potential immune rejection.<sup>15,32</sup> Effective decellularization methods include chemical, enzymatic, physical or combinational approaches.<sup>14,15,43,44</sup> The working principle of these methods is that the cell membrane is disrupted, and the cellular contents are released and rinsed away.<sup>14,45</sup>

In terms of cell compatibility, tissue-derived acellular scaffolds are in some ways similar to naturally-derived proteins/polymers, and provide the best cellular recognition amongst all scaffolds.<sup>46–55</sup> In particular, decellularized tissue scaffolds preserve the overall structural compositions, shape compatibility, certain levels of mechanical integrity of the target tissues/organs, and bioactive molecules that benefit cell–cell interaction, cell-ECM adhesion, and new ECM formation.<sup>56–59</sup>

In other words, the acellular tissue scaffolds retain structures at multiple hierarchical levels, from the tissue level to the microstructural, and way down to the ultrastructural and nano-level. Notably, the preservation of structurally organized entities, such as collagen, elastin, glycosaminoglycans, and fibronectin, enables a natural



**Figure 1.** Length scale of various components in tissue engineering/regeneration *via* decellularization approach.

template/framework that accommodates many tissue engineering/regeneration applications, including those involving skin, urinary bladder, blood vessel, heart valve, and cardiac tissue.<sup>15,32,60–63</sup> Notably, various decellularized ECM scaffolds, including small intestine submucosa (SIS), pericardium, skin, and heart valves, have been granted U.S. Food and Drug Administration (FDA) approval and have been successfully used in both pre-clinical animal studies and in human clinical applications.<sup>27,39,64–71</sup>

In this chapter, we focus our discussion on acellular scaffolds derived from soft tissues that we have investigated. By examining the successes and failures of many efforts in the decellularization field, we hope this discussion will provide useful information regarding the

decellularization approach, which aims to harness the potential of nature-designed tissue templates/framework.

## 2. History and Current State of Decellularized Approach

In 1995, Badylak *et al.* successfully produced acellular small intestine mucosa (SIS) using chemical detergent treatment.<sup>32,72</sup> The acellular SIS was then used for shoulder injury repair, and astonishing clinical results were generated.<sup>32,73</sup> Successes using SIS stimulated a wave of investigation focusing on SIS scaffolds and other types of tissue-derived acellular scaffolds. The follow-up studies using SIS included tissue engineering in tendon/ligament, abdominal wall, blood vessel, heart valves, cartilage, and facial tissue regeneration.<sup>56,74–87</sup> Extending the SIS scaffolds research, bladder, blood vessel, heart valve, skin, esophagus, and other tissues were subjected to decellularization, and the corresponding scaffolds were used in tissue engineering and regenerative applications.<sup>13,56,88–90</sup> Recently, the decellularization approach has formed a line of research parallel to the approaches that use synthetic polymeric scaffolds and naturally-derived proteins/polymers.

In 2008, the decellularization field entered another phase of development that concentrates on whole-organ tissue engineering. Ott *et al.*'s study successfully produced an acellular whole rat heart that retained perfusable vascular architecture, competent acellular valves, and intact chamber geometry.<sup>91</sup> After being reseeded with cardiac and endothelial cells by a perfusion bioreactor, the revitalized rat heart was able to perform almost 2% of its normal physiological function, which ignited further enthusiasm in the decellularization field. More recent developments in whole-organ decellularization included a porcine liver study by Barakat *et al.*,<sup>92</sup> liver research by Uygun,<sup>93</sup> a rat lung study by Ott *et al.*,<sup>94</sup> a kidney study by Song *et al.*,<sup>63</sup> and a decellularized porcine heart effort by Wainwright *et al.*<sup>95</sup> These early-stage studies on whole-organ decellularization have revealed great potential as well as challenges that warrant further studies prior to engineering whole organs for clinical utilization.

As we mentioned above, decellularization approaches represent the use of a nature-designed ECM structure down to nano-level. The

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preserved structural components of the ECM, such as collagen, elastin, and proteoglycans, make the acellular tissues suitable scaffolds for load-bearing functionalities by providing the mechanical strength of the collagen fibers/fibrils, the elasticity of the elastin fibers, and the hydration/cushion/binding function of the proteoglycans.<sup>96</sup> Furthermore, the acellular scaffolds often retain bioactive molecules (fibronectin, laminins, glycoproteins, etc.) that acts as signals for various growth factors and other proteins/biofactors (e.g., integrins, cadherins, etc.) that are important for cell–cell interaction, cell–ECM interaction, and new ECM formation.<sup>15,57,97</sup>

Challenges exist for decellularization approaches. It is important to determine which decellularization protocols are optimal to completely remove cells, cell debris, chromosome fragments, and xenogeneic antigens to diminish immunogenicity.<sup>28,52,98</sup> The decellularization protocols, which are often disruptive to a certain degree, should preserve the needed structural and mechanical integrity that is important for target tissue functionalities; e.g., in the application of aortic valve tissue engineering, the scaffold template should maintain the aortic heart valve's anatomic shape, morphology, and fiber architecture, as well as be able to perform in aortic orifice.<sup>60</sup> Other desirable features needed to be retained in the decellularized scaffolds include bioactive molecules and proteins important for mediating cell behaviors.<sup>13,89</sup> Hence, researchers often have to find a balance between the removal of cellular content and preservation of favorable structural, mechanical, and biochemical characteristics.

Another major challenge lies in the recellularization of the acellular scaffolds, i.e., the revitalization of the tissue or organs in specific applications. As pointed out by Codina *et al.*,<sup>99</sup> the integration of ECM with cells, and then scaling it up to organ level, is still an uphill task. Researchers need to address issues such as how to effectively and efficiently deliver the correct type of cells into the acellular scaffold, how to promote proper cell–ECM and cell–cell interaction, and eventually how to generate functional tissues/organs that can integrate into the human body with remodeling potential and durability.

While the above discussed challenges are largely shared by tissue engineering approach that uses polymeric scaffolds, there are other

issues unique to the decellularization approaches. For instance, polymeric approaches have an option to assemble individual cells and individual nano-structural features together, such as seen by cell co-spray in the electrospinning technique.<sup>100–103</sup> However, in order to take advantage of the nature-designed organ morphology, architecture, and ultrastructure, researchers often work with scaffold templates/frameworks that take the form of real organs or tissues. The acellular templates/frameworks that they use might be relatively straightforward, such as acellular heart valves, or might be extremely complicated, such as acellular whole-heart scaffolds. The quantity and quality of cells that need to be delivered to various parts/subsystems of the organ constructs thus challenge the recellularization. In other words, the desired macro, micro, and nanostructures are well constructed in the templates/frameworks, but there are challenges such as (i) growing cells into the dense, collagenous network (e.g., acellular heart valves), (ii) delivering the correct types of cells to the appropriate subsystem (e.g., endothelial cells for vasculature network *vs.* cardiomyocytes for heart wall scaffolds in the whole-heart application), and (iii) precisely controlling cell distribution and tissue regeneration (e.g., inhomogeneous cell distribution, inhomogeneous ECM regeneration).<sup>28,52,98</sup>

### 3. Methodologies in Decellularization Approaches

#### 3.1. *The balance between cell removal and ECM preservation*

The primary advantage of the decellularization approaches is the potential to preserve the natural architecture and properties of tissue scaffolds after the removal of native cells that are immunologically troublesome. Although it is not trivial to remove all of the cellular components of a tissue while retaining the integrity of the ECM, a great deal of progress has been reported recently. In those applications, a wide range of decellularization techniques have been developed for various tissues/organs, often with purposes to remove cellular materials, nuclear content, and immunogenic antigens (as in xenogeneic

scaffolds), and meanwhile to preserve specific aspects of the ECM structure, such as the collagen fibrillar network, the elastin network, the 3D architecture, and desirable bioactive molecules.<sup>15,28,52,98</sup>

Decellularized tissues are prepared by chemical, enzymatic, or physical treatment methods, and procedures can range from few hours to days.<sup>9,13–15,39–42</sup> Traditionally, the tissue of interest is only subjected to a chemical or detergent solution, while accompanied with certain mechanical agitation or manual scrapping to facilitate the cellular content removal.<sup>14</sup> However, these traditional techniques are only effective in tissues up to a few millimeters thick.<sup>104</sup> Recently, perfusion decellularization has been successfully developed to produce anatomically intact structures, and has been widely adopted.<sup>41,54,91,94,105–108</sup> However, it is worthwhile to note that there is no “gold standard” approach for decellularization, and researchers will have to choose different combinations of techniques based on the needs for a specific tissue/organ, as well as continually explore new methods to refine these approaches.<sup>15,39,41,42,109,110</sup>

In the following section, we will briefly review the widely used methods in the decellularization research field.

### 3.2. Current state of decellularization methodologies

#### 3.2.1. Chemicals methods

Chemical methods include use of detergents, ionic solutions, or acid/base solutions, etc. for removal of cellular components.

##### 3.2.1.1. Ionic detergent (SDS)

Sodium dodecyl sulfate (SDS) is the most widely used/studied decellularization agent. It consists of an anionic hydrophobic ligand<sup>111</sup> that functions by concentrating charges on the scaffolds. At 0.1% concentration, the detergent is excellent at removing cells while preserving overall tissue dimension (free of swelling or contraction).<sup>60</sup> There is some degree of ECM damage associated with this technique due to the corrosive nature of SDS at high concentration.<sup>112</sup> The SDS treatment is often followed by some type of washing or detoxification by



chemicals such Triton X and peracetic acid.<sup>42,91,109,110</sup> Shaner *et al.* used a series of SDS concentrations (0.01%, 0.025%, 0.05%, 0.075% and 0.1%) and found 0.075% SDS to be appropriate for decellularization of small diameter veins (with more than 94% of the cells were removed).<sup>113</sup> SDS was also found to be effective in cardiovascular tissues, such as myocardium,<sup>114–116</sup> blood vessels,<sup>26,117,118</sup> and heart valves.<sup>60</sup> Wang *et al.* also reported the very thorough removal of Gal  $\alpha$  1-3Gal ( $\alpha$ -Gal) porcine antigen from the pig myocardium.<sup>114</sup> As to be expected, there were reports showing limitations of the SDS treatment, such as the removal of ECM growth factors,<sup>31</sup> calcification,<sup>119</sup> retention of excess SDS in ECM,<sup>44,120</sup> incomplete removal of cellular material,<sup>109,110</sup> and incomplete removal of porcine or bovine antigens from the scaffolds.<sup>121</sup> Other similar ionic detergents used in tissue decellularization include sodium hypochlorite,<sup>122,123</sup> sodium decholate,<sup>124</sup> and Triton X-200.<sup>15,41,125</sup>

### 3.2.1.2. Non-ionic detergents (Triton X-100)

Triton X is a mild detergent, soluble in water, biodegradable, highly effective in cell removal<sup>126,127</sup> and promoting cellular growth.<sup>31</sup> In most cases, Triton X-100 removes cellular components, leaving behind intact ECM and vascular architecture. However, some studies have reported variable results with some degree of ECM damage such as glycosaminoglycan (GAG) removal<sup>15,128,129</sup> and hence scaffold contraction.<sup>60</sup> Triton X-100 has been used as one of the chemicals to decellularize tissues such as blood vessels,<sup>25,26,130</sup> liver,<sup>58,131,132</sup> pericardium,<sup>133</sup> dermis,<sup>31</sup> and lungs.<sup>63,134</sup>

### 3.2.1.3. Chelating agent (EDTA)

Ethylenediaminetetraacetic acid (EDTA) is one of the widely used agents for decellularization, and it is usually used in combination with enzymes. As an example, Schenke-Layland *et al.* used a combination of 0.05% trypsin (enzyme) and 0.02% EDTA for different time periods (5 h, 8 h, and 24 h), followed by a phosphate-buffered saline (PBS) wash to remove the cellular components from the pulmonary

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valves.<sup>135</sup> However, prolonged exposure to EDTA led to reduction in the mechanical properties of the scaffolds.<sup>15,41,136</sup>

#### 3.2.1.4. Zwitterionic-based detergent

Zwitterionic-based detergents possess both anion and cationic properties, and they are best suited for thinner tissues that require mild treatment. Examples of Zwitterion detergents include 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), sulfobetaine-10 and 16 (SB-10 and SB-16).<sup>15,41</sup>

#### 3.2.1.5. Acids and bases

Acids and bases commonly used in decellularization include acetic acid, sodium hydroxide, calcium hydroxide, and ammonium hydroxide.<sup>25,31,58,131,132,137,138</sup> The acids and bases are able to remove cells' nuclear material; however, they also tend to denature the proteins and hence damage ECM structure.<sup>15,41</sup>

#### 3.2.1.6. Hypotonic and hypertonic solutions

Alteration of solute concentration effectively lyses the cells by breaking the cell membrane *via* osmotic shock.<sup>41</sup> This method has been widely used, along with chemicals for removal of cellular materials, to effectively lyse the cells.<sup>139,140</sup>

#### 3.2.1.7. Alcohols and acetone

Solvents such as alcohols and acetone are best suited for decellularization of dense tissues as they lyse cells by dehydration and remove lipids from the tissues. However, attention should be paid since these solvents also tend to form crosslinks and affect the mechanical properties of structural proteins.<sup>41</sup>

#### 3.2.2. Enzymatic methods

Trypsin is an enzymatic agent that has been extensively used to decellularize tissues such as arteries<sup>141,142</sup> and human amniotic

membrane.<sup>143–145</sup> However, at high concentration, damage to ECM is eminent due to trypsin proteolysis to structural proteins.<sup>126–128</sup> For example, a 0.5%-concentration trypsin treatment was found to severely disrupt the elastin network in aortic valve leaflets, resulting in a tissue with a “swelling” morphology due to lack of constriction of a healthy elastin network.<sup>60</sup> Nevertheless, a trypsin treatment can be used at low concentration (e.g., 0.01%), along with other detergents such as SDS (e.g., 0.1%); this combination has been highly effective to remove cellular contents in myocardium decellularization.<sup>114,116</sup>

Nuclease enzymes, such as DNase, RNase, endonuclease, and exonuclease, have often been used for cleaning nuclear/genetic material. Other protease such as dispase, which is able to degrade collagen IV and fibronectin, have also been applied in decellularization.<sup>15,41</sup>

### 3.2.3. *Physical methods and preservation methods*

Physical methods include scraping, solution agitation (stirring, rocking, etc.), sonication (ultrasound treatment), pressure gradient, snap freezing and supercritical fluid.<sup>91,106,116,144,146–149</sup> Scrapping helps remove cells that are located on the surface but results are variable. Methods like sonication and supercritical fluids were found to be effective,<sup>116,149</sup> but are not widely used yet.<sup>41</sup> Pressure gradient system was used by Weymann *et al.* to successfully decellularize whole heart.<sup>106</sup>

Snap freezing is adopted from the preservation method, in which biological tissues are frozen quickly at low temperatures (e.g.,  $-80^{\circ}\text{C}$ ). The quick freezing disrupts the cell membranes due to inevitable ice-crystal formation and volume expansion, hence promoting cell lyses. The side-effect of snap freezing is that the formation of ice crystal also disrupts ECM microstructures.<sup>39,150</sup>

### 3.2.4. *Combinations of various decellularization methods*

Many successful applications have shown that the most effective and efficient decellularization method includes a combination of treatment agents as well as physical means. For instance, Ketchedjian *et al.* devised a combination of cryopreservation with a N-lauroyl

sarcosinate (detergent)-benzoase (enzyme) treatment and obtained acellular arterial scaffolds that contained a minimum amount of cell debris, were appropriate for cellular growth and proliferation, and were free of calcification.<sup>151</sup> Moreover, many studies have reported that the combinations of various decellularization agents (e.g., SDS, Trypsin, EDTA), along with agitation or scrapping, achieved thorough decellularization of various tissues.<sup>124,144,145,152,153</sup>

In the successful decellularization of an intact rat heart, Ott *et al.* utilized fluid perfusion, a pressure gradient, and a combined SDS-Triton X treatment.<sup>91</sup> Wainwright *et al.*<sup>95</sup> and Weyman *et al.*<sup>106</sup> used a similar perfusion and pressure gradient method for the decellularization of a whole porcine heart. The combination of 1% Triton X-100 and 0.1% ammonium hydroxide has been perfused to obtain whole-organ decellularization in liver, kidneys, pancreas, and intestines.<sup>25,131,138,154,155</sup> These successful experiences have built a strong foundation for future innovative design in decellularization protocols.

### 3.2.5. Scaffold treatment — Sterilization, stabilization, coating, and storage/handling

#### 3.2.5.1. Sterilization

Sterilization helps to disinfect the scaffolds, preventing contamination in the recellularization phase and potential transmission of pathogens from the scaffolds to the host. Although most of the sterilization methods used by the medical-device industry lead to some sort of toxicity or damage to the ECM, some of them are still feasible and widely applied in the decellularization field. Some of the agents used for sterilization of the acellular scaffolds include ethanol,<sup>95,116</sup> peracetic acid,<sup>95,115,156</sup> ethylene oxide,<sup>82,141,156</sup> ultraviolet and gamma radiations.<sup>109,122,123</sup>

#### 3.2.5.2. Stabilization

Scaffold stabilization is another treatment often being used. The purpose of stabilization is to delay the degradation of scaffolds, deactivate or neutralize antigens, and stabilize the overall structure of the ECM. For example, penta-galloyl glucose (PGG) has recently been applied

to stabilize acellular bovine pericardium and acellular heart valves; tightly binding to elastin, PGG showed the benefits of stabilizing the acellular scaffolds, delaying the scaffold degradation rate, reducing calcification, as well as not affecting the recellularization potential.<sup>109,157–159</sup> Note that crosslinking stabilization often alters the mechanical properties to a certain degree, and those alterations must be considered in the design of functional tissue engineering.<sup>157,159,160</sup>

### 3.2.5.3. Coating

Similar to other tissue engineering endeavors, coating has been applied in acellular scaffolds with the hope to improve cell attachment, infiltration, and proliferation. Coating agents, such as chitosan, RGD polypeptides, silk fibroin, and heparin sodium, provide ligands that favor the attachment/interaction between cells and the surface of the scaffolds.<sup>109</sup>

### 3.2.5.4. Storage/handling

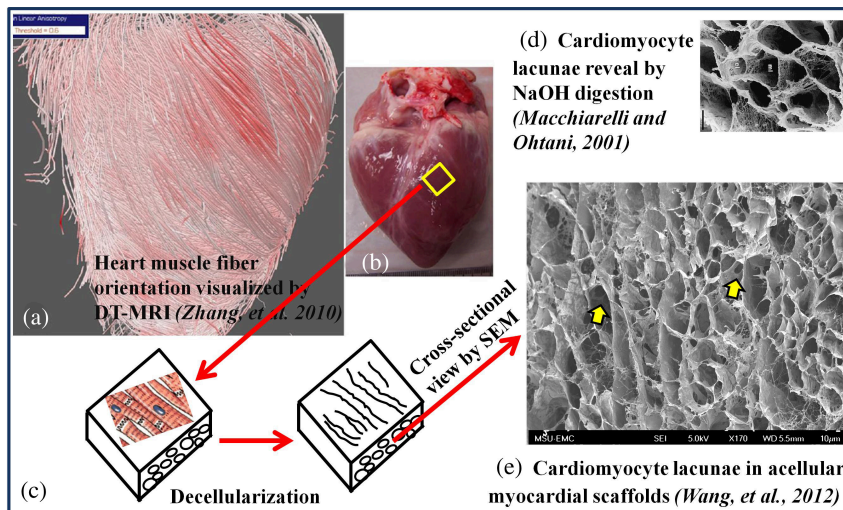
Cryopreservation and lyophilization have both been used in decellularization approaches for storage of scaffolds and/or facilitating easier handling. Cryopreservation is able to effectively preserve the acellular tissue scaffolds at sub-zero temperatures ( $-196^{\circ}\text{C}$ ) maintained by liquid nitrogen. Lyophilization (freeze-drying) is a preservation method in which biological tissues are frozen (e.g.,  $-80^{\circ}\text{C}$ ) and then vacuum-dried using a lyophilizer. The cryopreservation and lyophilization methods serve as methods to store the acellular scaffolds for a prolonged time period.

## 4. Application of Decellularized Scaffolds in Cardiac Tissue Engineering

### 4.1. *Cardiac tissue engineering*

The myocardium consists of special muscles that construct the heart walls; they are striated, and each cell contains sarcomeres with sliding filaments of actin and myosin. The myocardium, along with electrical

gap junctions and mechanical gap junctions, enables the heart to contract and expand in a coordinated fashion. The myocardium has a well-organized, multilayered helical architecture, as shown by the Diffusion Tensor-Magnetic Resonance Image (DT-MRI) in Figure 2a.<sup>161</sup> The multilayered helical structure has been found essential for global heart contraction, and this muscular architecture is mediated by a complicated 3D myocardial ECM.<sup>162</sup> However, the heart muscles die easily when the oxygen supply is low, as is usually the case during a heart attack.<sup>163</sup> Each year, approximately one million Americans suffer from myocardial infarction, (MI) with a 10% in-hospital mortality rate.<sup>164,165</sup> The progressive pathological changes resulting from MI include an initial inflammatory response,<sup>163</sup> loss of cardiomyocytes,<sup>166,167</sup> and degradation of the left ventricular (LV) ECM by matrix metalloproteases,<sup>168,169</sup> which leads to wall thinning, infarct expansion,<sup>170,171</sup> scar tissue formation, LV dilatation, and a decrease in cardiac function.<sup>172</sup>



**Figure 2.** (a) Multilayered helical structure of heart muscle fibers revealed by Diffusion Tensor-Magnetic Resonance Imaging (DT-MRI); (b) porcine heart subjected to DT-MRI imaging;<sup>161</sup> (c) schematic illustration of myocardium decellularization to obtain acellular myocardial scaffolds; (d) cardiomyocyte lacunae revealed by NaOH digestion;<sup>208</sup> (e) cardiomyocyte lacunae observed by SEM in acellular myocardial scaffolds.<sup>114</sup> Figures reproduced with permission.

If the amount of myocardium loss as a result of the MI is large, significant mechanical complications, such as left ventricular dilatation, arrhythmias, mitral regurgitation, and heart failure, can occur, each of which can be fatal.<sup>173–175</sup>

The main goal of all the treatments for MI is to prevent the adverse remodeling of the LV that leads to heart failure.<sup>176</sup> Standard treatments of acute MI include prompting revascularization as completely as possible with a fibrinolytic agent and surgery (angioplasty or coronary artery bypass grafting), which aim to limit the infarction size and prevent extension of the infarction.<sup>175</sup> For end-stage heart failure, the only successful treatment currently is total heart transplantation. Unfortunately, there is limited availability of suitable heart donors for patients who are progressing to severe cardiomyopathy, and some people die while waiting for a heart transplant. Recent research on MI treatment has focused on either avoiding scar formation<sup>177</sup> or replacing formed scar tissue with functioning cardiac muscle.<sup>178,179</sup> Still strategies have never come to the forefront as viable alternatives, such as cellular transplantation (myoblast/stem cell injection), intramyocardial gene transfer, and cardiac tissue engineering.<sup>180–182</sup> The ultimate goal of these interventions, as with the more traditional treatments, is to prevent further LV dilation and pathological remodeling.

Among these newer treatments, cellular transplantation and cardiac tissue engineering are two commonly studied approaches for which cardiac tissue regeneration therapy is utilized.<sup>45,180–184</sup> However, cell therapy *via* injection has met with limited success due to the lack of an appropriate extracellular environment for the retention of transplanted cells. The lack of extracellular environment also impedes the integration of the repair region with the host tissue, both structurally and biomechanically.<sup>185,186</sup> To overcome the hurdles that cellular transplantation has been experiencing, cardiac tissue engineering aims at fabricating tissue constructs that can restore basic cardiac function by integrating cellular components within scaffolds that serve as a structural guide.<sup>4,187–189</sup>

The ideal engineered cardiac tissues would provide optimal structural, mechanical, and electrophysiological properties maintained by viable transplanted cells, and might also stimulate the formation of vasculature that can supply oxygen and nutrients.<sup>190</sup> Various cell types,



such as bone marrow stem cells, umbilical stem cells, embryonic stem cells, cardiac stem cells, induced pluripotent cells, fetal cardiomyocytes, and skeletal myoblasts, have been investigated as cell sources for cardiac recellularization.<sup>191–195</sup>

For scaffolds used for cardiac tissue engineering, similar to other tissue engineering efforts, two major approaches have been investigating intensively: one is to use synthetic biodegradable material and the other is to use tissue-derived acellular scaffolds. Synthetic biodegradable polymers, such as polyglycolic acid (PGA), polylactic acid (PLLA), polyester (urethane urea), poly(caprolactone) (PCL), poly(ethylene oxide) (PEO), and poly(vinyl alcohol) (PVA), have been used as scaffold materials.<sup>37,196–200</sup> The advantages of polymeric materials include that they are biodegradable and that they can be reproduced with certain material and structural properties.<sup>196,201,202</sup> However, the synthetic polymeric approach is still facing issues such as inflammatory response, mismatched material properties, nonpliability, and challenges in controlling the degradation rates.<sup>200,203,204</sup> The tissue-derived acellular myocardial scaffolds, with its advantages and challenges, will be discussed in more detail in the following sections.

#### 4.2. Acellular myocardial scaffolds and applications

Myocardial ECM is an intriguing network that mediates the complex muscle fiber architecture in the heart and maintains unique cell-to-cell interconnections.<sup>205–208</sup> Composed of a collagen fiber network (type I and III), elastin, and proteoglycans,<sup>205–208</sup> the intriguing myocardial ECM network provides important functions in maintaining structural integrity, tethering myocytes and mediating their contraction/relaxation, and preventing excessive stretching.<sup>209–211</sup> Macchiarelli and Ohtani digested the native myocardium with NaOH and revealed, by using scanning electron microscopy (SEM), the well-organized lacuna morphology that houses cardiomyocytes (Figure 2d).<sup>208</sup> In other words, the unique, elegant ECM structure and design of the myocardium were evident, but so too were the challenges for scaffold fabrication using a polymeric approach. Thus, the decellularization might take better advantage of natural design.



Two lines of research are ongoing in myocardium decellularization: one is whole-heart decellularization, and the other is the use of the acellular myocardial scaffolds as a template for cardiac tissue engineering. In the field of whole-heart decellularization, Ott *et al.* reported that they successfully produced acellular, perfusable vascular architecture, competent acellular valves, and intact chamber geometry using a rat heart. The acellular intact rat heart was then reseeded with cardiac and endothelial cells by a perfusion bioreactor. By day eight, under physiological load and electrical stimulation, macroscopic contractions were observed with a pump function equivalent to about 2% of adult or 25% of 16-week fetal heart function.<sup>91</sup> The next and more challenging effort, currently being undertaken by Badylak *et al.* and Taylor *et al.*, is to apply this approach to larger xenogenous hearts, such as pig hearts that have similar size, anatomic geometry, and vasculature system as human hearts.<sup>147,212</sup> However, many questions and technical hurdles exist in the field of the whole-heart regeneration, such as reseeded homogeneity and thoroughness across the thick scaffolds, feasibility of using existing vasculature network, angiogenesis in the thick tissue construct, and ultimately functional integration. These issues need to be addressed before a scaled-up attempt will be successful.

This approach aims to harness the potential of acellular myocardial scaffolds as a template for cardiac tissue engineering; both Wang *et al.*<sup>116</sup> and Godier-Furnemont *et al.*<sup>213</sup> have developed the methods that completely remove cardiomyocytes and other types of cells from porcine myocardium and human myocardium, respectively. The preservation of myocardial ECM, its subtle 3D structure, and its mechanical properties was found to favor cell proliferation, ingrowth, differentiation, tissue remodeling, and angiogenesis.<sup>116,213</sup>

#### **4.3. Biomechanics of acellular myocardial scaffold and its implications**

The cardiomyocyte lacunae structure helps maintain the alignment and interaction of the heart muscle fibers. The tubular pores mainly align along the muscle fiber direction, exhibiting honeycomb morphology when viewed from cross-section (Figures 2d and 2e). This 3D

morphology of cardiomyocyte lacunae was revealed both by our SEM topographic views on decellularized myocardial scaffolds (Figure 2e)<sup>114</sup> and in an ultrastructural study by Macchiarelli and Ohtani in NaOH-digested ventricular tissue (Figure 2d).<sup>208</sup>

Consisting of dominantly type I and III collagen fibrils, the 3D cardiomyocyte lacunae network possesses a nonlinear, anisotropic mechanical behavior similar to other types of collagenous networks. We found that the mechanical properties of the acellular scaffolds is much stiffer along muscle fiber-preferred direction (note that muscle fibers removed already) than the cross-fiber preferred direction. The anisotropic behavior of the remaining collagenous network is most likely related to tubular lacunae orientation. The anisotropic, nonlinear mechanical behavior of the acellular myocardial scaffolds was validated independently in studies on porcine heart<sup>116</sup> and rat heart.<sup>214</sup>

According to Hanley *et al.*, collagen fibers in the native myocardium are wavy cords that straightened considerably as the sarcomere length was increased from  $1.85 \pm 0.06 \mu\text{m}$  (near-resting length) to  $2.30 \pm 0.04 \mu\text{m}$ .<sup>215</sup> Their study also implies that most of the collagen fibers run along the muscle fiber direction.<sup>215</sup> Using polarizing light microscopy, Wang *et al.* verified the existence of wavy patterns in the collagen network of both the native myocardium and the acellular myocardial scaffolds. It is well-known that wavy collagen fibers (collagen crimp) give rise to the flexible deformation in the toe region of the nonlinear stress-strain curve and then later on the much stiffer response in the linear part of the stress-strain curve.<sup>96</sup> This mechanism likely provides an easy lengthening of muscle fibers (along with the uncrimping of the collagen network) in the heart's diastolic phase but also an important lock-up by straightened and stretched collagen fibers to prevent overstretch of individual cardiomyocytes. This critical feature in the collagen network exists inherently in the acellular myocardial scaffolds but presents a challenge for the polymeric approach that tries to mimic native myocardial ECM structures, i.e., microstructurally replicating the honeycomb tubular pores and ultrastructurally mimicking a wavy collagen network with polymer fibers (or other elastomeric materials).

Wang *et al.* speculated that the capability of a cellular myocardial scaffolds being able to maintain open pores is due to the inherent properties of myocardial ECM.<sup>114</sup> The pore size of the porcine acellular myocardial scaffolds was estimated with a radius of  $21.4\ \mu\text{m} \pm 16.8\ \mu\text{m}$  for scaffolds generated by a frame-pin supporting system and  $19.5 \pm 17.9\ \mu\text{m}$  for scaffolds decellularized without any external support.<sup>114,116</sup> In another study using human acellular myocardial sheet, the majority of pores were found in the range of  $10\ \mu\text{m}$  to  $25\ \mu\text{m}$  diameter.<sup>213</sup> Apparently, the tissue shape and size before decellularization (tissue block *vs.* tissue thin sheet), and various decellularization protocols might result in various degrees of preservation of porous structures. Moreover, different imaging preparation routines might further distort the structure and make the estimation different.

The dimensional quantification of myocardium before and after decellularization can possibly be an indication of whether the internal porous structure is maintained or collapsed. Wang *et al.* found the overall maintenance of tissue volume after decellularization, confirming the internal structural preservation.<sup>114</sup> Another interesting feature shown in Wang *et al.*'s study is the interconnecting openings inside the cardiomyocyte lacunae (Figure 2e, arrows). It was reported that each ventricular myocyte is connected to other myocytes *via* gap junctions, with an average of 11.3 neighbors, 5.3 on the sides and 6.0 at the ends.<sup>216</sup> Those connections allow continuity of cardiac muscle fibers both physically and functionally. The preservation of the interconnecting passages will likely be helpful for functional interaction of reseeded cells. In a polymeric approach, Guan *et al.* showed a morphology of interconnected micropores in polyester (urethane urea) (PEUU) scaffolds fabricated by thermally-induced phase-separation techniques, and the pore size varied from several  $\mu\text{m}$  to more than  $150\ \mu\text{m}$ .<sup>217</sup> The follow-up study of this porous PEUU as a cardiac patch material in a rat model showed that the scaffolds were able to promote contractile phenotype smooth muscle tissue formation, consequently improving cardiac remodeling and contractile function of the patched heart.<sup>196</sup> The above observations all suggest the importance of accurately mimicking the physical and ultrastructural features in cardiac-scaffold design.

One more interesting feature that Wang *et al.* pointed out in the acellular myocardial scaffolds was cardiac elastin and the preservation of cardiac elastin structure after decellularization.<sup>114</sup> As discussed in a review paper by Fomovsky *et al.*, collagen, cardiac elastin, and proteoglycans are major structural components in cardiac ECM; however, the mechanical contributions of cardiac elastin and proteoglycans are poorly understood.<sup>218</sup> As we know, elastin fibers/sheets are often found in tissues experiencing dynamic cyclical loading (e.g., tendon/ligament, heart valves, blood vessels, bladder), providing resilience by storing and releasing energy in favor of passive recoil (elasticity).<sup>96</sup> From the structure-function perspective, then, a potential mechanical role of cardiac elastin is to assist the cardiac contraction cycles by providing microscale resilience.<sup>114</sup> Another type of elastin structure in the acellular myocardial scaffolds is in the cardiac vasculature. Well-preserved elastin in blood vessel networks in the acellular myocardial scaffolds might provide a favorable platform for later revascularization and angiogenesis, with structural cues essential to vascular smooth muscle cell differentiation and endothelialization.<sup>91,114,212,213</sup> The above elastin features, either in the form of cardiac elastin or vascular elastin, come with the nature-designed details and efficiency in the acellular myocardial scaffolds used for both whole-organ and partial (e.g., patch) repairing purposes. For the polymeric approach, those elastin features, however, present a challenge with questions, such as to what degree it is worthy to mimic those subtle structures.

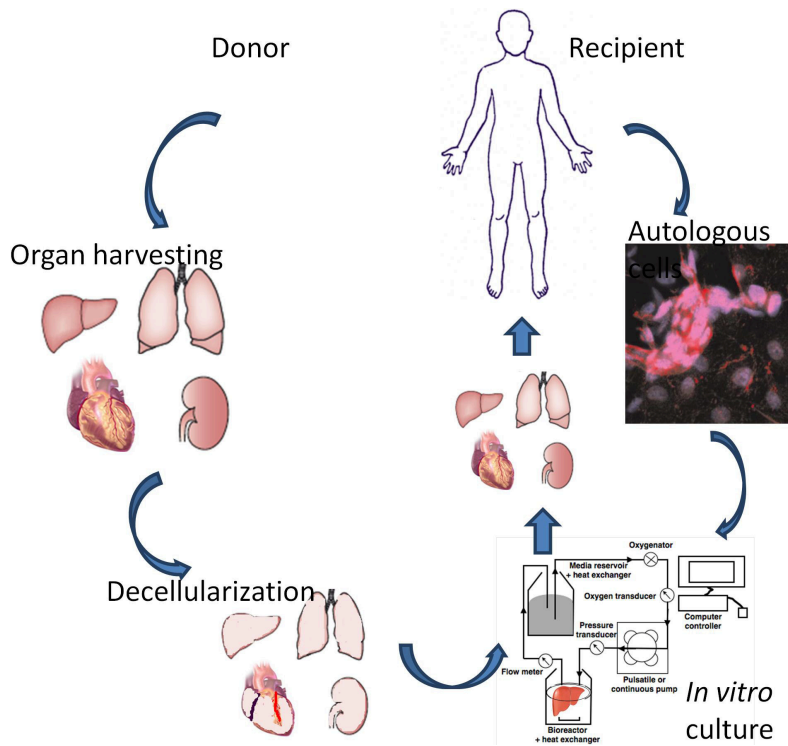
Note that the removal of cellular content turns the myocardium from a muscular tissue into collagenous scaffold with a highly porous structure. It is thus understandable that the acellular myocardial scaffolds showed stiffer tensile mechanical properties and a decreased shear resistance.<sup>114,116</sup> The hope is that, by recellularizing the empty lacunae, the cardiac tissue construct can regain its tensile property close to the native myocardium (due to refilling of cellular content) and recover shear resistance. The re-establishment of the physiologically relevant mechanical properties is essential to heart functionality, and it also provides an ECM environment that favors cell–cell interaction and cell–ECM coupling.<sup>91,116</sup>

## 5. Application of Decellularized Scaffolds in Whole-Organ Tissue Engineering

### 5.1. *Demands for whole-organ tissue engineering*

Organ transplantation is generally considered to be the gold standard of therapy for end-state organ failure, particularly for liver, kidney, and heart. The continually increasing number of patients alive with a functioning transplanted organ shows the success of transplantation. However, there is an ever-growing demand for cadaveric donor organs that is not being met by current availability. Nationally, more than 112,400 patients are waiting for solid organ transplants, yet the number of transplants performed annually falls short of this need by 75% according to the United Network for Organ Sharing. Meanwhile, since the late 1980s when the agencies of the US Department of Health and Human Services began recording statistics for organ transplantation<sup>219</sup> the average age of cadaveric donors has increased, which has led to a decline in the number of transplantable organs recovered per donor,<sup>220</sup> an increased prevalence of steatosis in liver grafts, and delayed graft function after transplantation.<sup>221</sup> The use of extended-criteria donors, organs from living donors, and split-liver transplantation are several methods that have been used to expand the number of usable organs for transplantation.<sup>222</sup> To be sure, these methods offer life-saving options, but they are unlikely to close the gap between the limited supply of useable cadaveric organs for donation and the growing demand for donor organs.

New sources of organs are urgently needed to solve the transplantable organ shortage, and the development of regeneration medicine is poised to potentially change the paradigm of organ transplantation. Recent progress in tissue engineering has established a foundation for the functional replacement of whole organs. Bioartificial organ ECM (acellular organ scaffold) could be developed by decellularization of the target organs. The acellular organ scaffold could then be recellularized with autologous cells and cultured in a physiologically appropriate bioreactor to create a functional organ *in vitro*.<sup>40,212,223</sup> Development of a tissue-engineered organ would allow for a potential reduction in waitlist mortality due



**Figure 3.** Whole-organ bioengineering.

to a decrease (or elimination) in waiting time if a bioartificial organ substitute can be produced “on demand.” Over the last several years, several organs, including livers, lungs, kidneys, and hearts, have been engineered in laboratory settings, implanted into rodent recipients, and shown some limited functions.<sup>212</sup> Here, we briefly summarize the most recent advances in whole-organ engineering and provide a review of the challenges that are now being addressed to translate this promising new field into a proven regenerative therapy that may one day be common for replacement of failing organs.

### 5.2. Whole-organ decellularization and its applications

Whole-organ decellularization can be realized by antegrade or retrograde perfusion of the decellularization solution through the vascular

bed to remove the cellular material from the tissues and produce an acellular, three-dimensional architecture of the organ that is capable of supporting the growth of donor cells for future recellularization.<sup>15,91</sup> Up until now, several tissue-engineered organs have been fabricated through the perfusion decellularization method and recellularized with cells through their inherent vascular channels with perfusion of media.

#### 5.2.1. Heart

As we mentioned earlier, Ott *et al.* decellularized a rat heart by perfusion with heparinized PBS with adenosine for 15 min, 1% SDS for 12 h, and 1% Triton X-100 for 30 min, with each step followed by a deionized-water rinse.<sup>91</sup> Their results also proved that the vascular network and the chamber geometry of the decellularized heart were intact despite the use of all the above detergents. They then recellularized the heart ECM with cardiac and endothelial cells by a perfusion bioreactor under physiological load and electrical stimulation, and they found that the ECM was amenable to cell seeding with cardiomyocytes that formed small foci of contractile muscle.<sup>91</sup>

#### 5.2.2. Lung

Another area of organ engineering that has been popularly investigated is the lung.<sup>94,224,225</sup> The specific function of the lung requires two accessible compartments with a short diffusion distance, i.e., the vascular network closely adjacent to the airway compartment with alveolar structures. Research has been mainly focused on perfusion of the decellularization agents into and through these two compartments. Ott *et al.* used vascular perfusion with 0.1% SDS for 2 h at physiological pressure to decellularize rat lungs.<sup>94</sup> Petersen *et al.* decellularized rat lungs by perfusion CHAPS in PBS through the airway compartment, along with perfusion through the vascular compartment, in order to maintain the whole decellularization pressure below 20 mmHg.<sup>224</sup> Both groups recellularized the lung ECM with cells isolated from the whole lung and implanted the engineered lung constructs in rats for a few hours.

Price and his colleagues perfused the lungs through both the airway and vascular compartments for delivery of decellularization agents, which included Triton X-100, deoxycholate, DNase solutions, and bleach.<sup>226</sup> Cortiella *et al.* perfused the lungs through the airway compartment using 1% SDS in a circulation bioreactor system for approximately five weeks.<sup>225</sup> They also recellularized the lung scaffold with embryonic stem cells from mice and found that the matrix was able to promote site-appropriate differentiation without any other specific differentiation cues. All the above studies showed the preservation of the major components of the ECM and general microstructure of the lung.<sup>94,225–227</sup> However, it was found that the decellularization process might cause some damage to the microvasculature channels.<sup>94,105</sup>

### 5.2.3. Liver

Liver engineering is another widely studied field of organ regeneration medicine. Basically, the liver is decellularized by perfusion of the decellularization agents into the portal vein and throughout the vasculature of the liver. SDS and Triton X-100 are the most commonly used combination for liver perfusion.<sup>93,228</sup> Studies of the decellularized liver have shown a translucent, white appearance and an absence of DNA by hematoxylin and eosin, DAPI, and quantitative DNA measurement. Moreover, the microvasculature constructs, ECM ultrastructure, and constituents, such as collagens I and IV, fibronectin, and laminin, were preserved.<sup>93,228</sup>

In several studies, liver scaffolds were also able to be recellularized with hepatocytes by portal vein perfusion.<sup>93,228,229</sup> However, only a short-term organ survival (i.e., several hours) has been demonstrated in small animal recipients due to hemorrhagic and thrombotic complications and technical difficulty of transplanting tiny engineered organs.<sup>93,94</sup> Therefore, the ability to sustain a long-term *in vivo* perfusion until the formation of a functional endothelialized vasculature is the key step at present to the whole organ engineering approach.



#### 5.2.4. Kidney

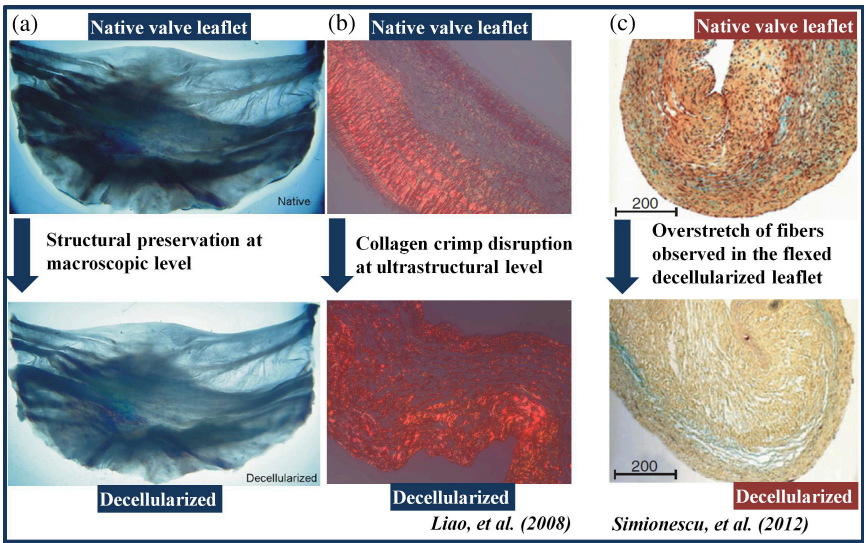
Several groups have also successfully decellularized kidneys using the perfusion method, with reports of intact vasculature preserved.<sup>131,230</sup> Some efforts have also been made in recellularization of the kidney ECM. Ross *et al.* recellularized the rat kidney ECM with mouse embryonic stem cells by infusing the cells through the renal artery and the ureter.<sup>230</sup> Their results showed stem cell proliferation and specific differentiation within the glomerular, vascular, and tubular compartments. Nakayama *et al.* decellularized kidneys of the rhesus monkey and examined the physical and biological properties of the acellular kidney scaffold. Their results showed that a 1% SDS solution could most effectively remove all cellular components at 48°C while preserving the native architecture; nevertheless, the mechanical properties after decellularization decreased in the compressive modulus compared to fresh kidneys. They also recellularized the kidney scaffold with non-human primates and fetal kidney cells and found good cell attachment and migration after a 5-day *in vitro* culture.<sup>231</sup>

Use of naturally derived ECM scaffolds populated with specific types of cells is a promising approach for future clinical treatment. To date, various engineered tissues have been successfully implanted into patients (e.g., trachea, bladder, cardiac patches). However, the development of vital organs is significantly less advanced due to the need to construct a complex, fine vascular network within the growing, engineered solid organ. The main barriers of organ regeneration include the choice of an optimal cell source for different organs, an effective recellularization method for reseeded of the thick tissue scaffold, the feasibility of using the existing vasculature network, and angiogenesis in the whole organ. Furthermore, only short-term organ survival (i.e., several hours) has been demonstrated in small animal recipients due to the complexity of these structures and the technical challenge of transplanting tiny, engineered organs.<sup>93,94</sup> Recent efforts have primarily focused on overcoming the above mentioned challenges in order to translate engineered organs into an accepted solution for replacing failed organs.

6. Discussions

6.1. 3D structural integrity

Decellularized tissues provide biological, chemical, and morphological cues, and most importantly, physical support to the tissue-engineered product and mechanical strength for the functional tissues.<sup>232</sup> In most cases, decellularization procedures were able to preserve the overall structural arrangement; consequently, for tissues dominated by ECM (e.g., heart valves), the mechanical properties of the acellular scaffolds showed an overall similarity to the native tissue but still sustained a certain degree of alteration.<sup>60</sup> Structural preservation at the macroscopic level seems very promising (Figure 4a), but it is often accompanied by various degrees of disruption at the ultrastructural or nanostructural levels. For example, the collagen crimp structure of heart valves was often found to lose long-range wavy organization (Figure 4b).<sup>60</sup> The loss of ultrastructural features, such as long-range



**Figure 4.** (a) Structural preservation of heart valve leaflet at macroscopic level; (b) local ultrastructural disruption — loss of long range collagen crimp organization;<sup>60</sup> (c) overstretch of fibers observed in the ventricularis layer of the decellularized leaflet.<sup>158</sup> Figures reproduced with permission.

organized crimp structure in heart valves (collagen fibers still locally crimped), incurs a larger tissue extensibility.<sup>60</sup> Moreover, Simionescu *et al.*'s study on tissue flexural behavior further showed that the acellular valve leaflet handles extreme flex relatively well, with no noticeable fiber buckling in the fibrosa layer (compressive region); however, fiber overstretch and disruption were observed in the ventricularis layer (tension region). (Figure 4c).<sup>158</sup>

Thus, it is important to understand how the altered ultrastructural features in the acellular scaffolds affect the behavior of the engineered tissues in terms of mechanical and functional performance, fatigue resistance, and long-term durability. It would also be interesting to know whether tissue remodeling and adaptation after implantation could fully restore the original structural integrity with all the key characteristics favoring the functional performance.

For tissues dominated by cells (e.g., heart muscles), the challenge shifts to maintaining the pores (cell lacunae) in the decellularized scaffolds. As we discussed in Section 5 and as various studies have shown, porous structure preservation may vary according to the type of tissue involved and the protocol used (Table 1).<sup>95,114,116,213</sup> Preventing the collapse of the internal porous structure is a foremost and significant challenge for both the whole-organ and scaffold-template approaches in decellularization. The open, interconnected pores are crucial for later successful reseeding and recellularization, as well as for harnessing the mechanical, microstructural, and potentially nanostructural cues preserved in the nature-designed cell environment.

## 6.2. *Recellularization and provision of oxygen and nutrients*

The major challenge in recellularization of acellular scaffolds is similar to other tissue engineering endeavors, i.e., how to achieve thorough, homogeneous reseeding with appropriate cell densities and cell phenotypes. For instance, in cardiac tissue engineering using myocardial scaffolds, it is a demanding task to achieve cell alignment, interconnection, cell density, and at the same time prevention of pore collapse in the reseeded constructs.<sup>116</sup> In tissue dominated by ECM, it was

**Table 1.** Tissue dimension and porous structure preservation were found to vary according to the type of tissue involved and the protocol used.

Scaffold	Decellularization agent	% Change of surface area	% Change of thickness	Pore size (μm)	Porosity (%)	Cell permeability (m <sup>4</sup> /Ns)	Reference No.
<b>Myocardium</b>							
Decellularized Porcine Myocardium	0.1% SDS + 0.01% Trypsin	-9.11 to -12.62	9.56 to 23.48	2-40	—	(no reseeding done)	114
<b>Heart Valves</b>							
Decellularized Porcine Aortic Valve	0.1% SDS + 0.01% EDTA	-0.86 to -1.93	7.27-9.76	—	—	(no reseeding done)	60
Decellularized Porcine Aortic Valve	0.5% Trypsin + 0.2% EDTA	-21.67 to -26.46	-10.64 to -12.82	—	—	(no reseeding done)	60
Decellularized Porcine Aortic Valve	1% Triton X-100 + 0.2% EDTA	16.92 to 19.38	44.44 to 53.85	—	—	(no reseeding done)	60

(Continued)

Table 1. (Continued)

Scaffold	Decellularization agent	% Change of surface area	% Change of thickness	Pore size (μm)	Porosity (%)	Cell permeability (m <sup>4</sup> /Ns)	Reference No.
<b>Blood Vessels</b>							
Decellularized Porcine Ascending Aorta (collagen scaffold)	1% SDS-Trypsin + Elastase	—	—	3–20	—	(40 μm) <sup>5</sup>	117
Decellularized Porcine Ascending Aorta (elastin scaffold)	Cyanogen bromide	—	—	10–120	—	(120 μm) <sup>5</sup>	117
Decellularized Ovine Carotid Artery	0.25% Trypsin + 1mM EDTA	(60 mm) <sup>1</sup>	3–4 <sup>2</sup>	23.4	~80	—	105
Decellularized Porcine Carotid Artery	1% SDS + 0.25% Trypsin + Collagenase	—	—	25–30, <sup>3</sup> 40–100 <sup>4</sup>	91.5– 99.25	(no reseeding done)	118

(Continued)

Table 1. (Continued)

Scaffold	Decellularization agent	% Change of surface area	% Change of thickness	Pore size (μm)	Porosity (%)	Cell permeability (m <sup>4</sup> /Ns)	Reference No.
Decellularized Canine Carotid Artery	0.5% Triton X-100 + 0.05% Ammonium Hydroxide	—	—	17.3	66.8	—	137
Decellularized Porcine Thoracic Aorta	0.1% SDS + 0.2% EDTA	5 <sup>6</sup> ,3 <sup>7</sup>	~20 <sup>8</sup>	—	—	(no reseeding done)	26
Decellularized Porcine Thoracic Aorta	0.1% Triton-X-100 + 0.2% EDTA	3 <sup>6</sup> ,3 <sup>7</sup>	~20 <sup>8</sup>	—	—	(no reseeding done)	26

<sup>1</sup>Length, <sup>2</sup>Lumen diameter or Internal diameter — of the tubular structure, <sup>3</sup>Short axis, <sup>4</sup>Long axis, <sup>5</sup>Distance infiltrated by fibroblast cells, <sup>6</sup>Longitudinal, <sup>7</sup>Circumferential, <sup>8</sup>Thickness change — percent change compared to the original.

found that improper recellularization resulted in valve-leaflet shortening, contraction, or thickening, and consequently a reduction in ECM formation.<sup>233–236</sup> Thorough, 3D quantitative studies on the degree of cell infiltration and repopulation are needed in the future as an objective assessment, and improvement of recellularization can thus be informed *via* those quantitative data.

In the acellular scaffolds, the features of pores that influence both reseeding potential and permeability include pore size, shape, alignment, interconnection, and mechanical resistance to collapse. For example, in dense ECM structures (lack of pores), cell infiltration is often a challenge, with most reseeding taking place in the peripheral range (surface and subsurface) such that the edges get clogged and encapsulated over time.<sup>117</sup> In large volume scaffolds (whole-organ), even with relatively big size pores (e.g., acellular myocardial scaffolds), cell infiltration into the center of the scaffolds is difficult to achieve, as is delivery of oxygen and nutrients.

In the case of tissue remodeling, porosity of the scaffolds decreases due to the new ECM deposition or contraction/collapse of pores, ultimately lowering migration ability of the cells and nutrient permeability; as a result, cell proliferation rate decreases and most cells die in the center of the tissue construct.<sup>237–239</sup> For instance, in tissue-engineered aortic valve leaflets, due to their relatively thin thickness (~500  $\mu\text{m}$ ), cells inside the tissue constructs might get enough oxygen and nutrients *via* diffusion mechanism; however, for large volume tissues, such as whole-heart and whole-liver, revascularization is crucial to provide oxygen and nutrients to the reseeded cells deep inside the tissue scaffolds. The need for revascularization comes not only from oxygen and nutrient provision into thick tissues, but also with a purpose of functional integration of the implant with the host. Considerable effort is needed to address the challenge of vascularization and integration of large-volume organs/tissues.<sup>131,132</sup>

### 6.3. Concluding remarks

The efforts of tissue engineering and regeneration *via* decellularization depend on to what degree we can revitalize the elegant,

nature-designed scaffolds/templates. Considering the above-discussed accomplishments, hurdles, and hopes, the challenge is still quite large, especially for whole-organ scaffold revitalization. Even envisioning an organ scaffold mostly recellularized with the correct types of cells, an important question still remains to be answered: Are we going to see these engineered organs as defective ones with unpredictable fates, or as less-than-perfect ones but with remodeling and “self-repairing” potential? With the research enthusiasm and efforts inspired by recent innovative conceptual developments and accomplishments,<sup>15,32,40,91,93–95,105,131,212,225,228–230</sup> we assert that eventually these questions can be answered, and from there, we can move forward to translational applications *via* the decellularization approach.

## Acknowledgments

This work was supported by NIH National Heart, Lung, and Blood Institute grant HL097321, and in part by Health Resources and Services Administration (DHHS R1CRH10429-01-00) and MAFES SRI (MIS-729110).

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