Review

Organ engineering based on decellularized matrix scaffolds

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End-organ failure is one of the major healthcare challenges in the Western world. Yet, donor organ shortage and the need for immunosuppression limit the impact of transplantation. The regeneration of whole organs could theoretically overcome these hurdles. Early milestones have been met by combining stem and progenitor cells with increasingly complex scaffold materials and culture conditions. Because the native extracellular matrix (ECM) guides organ development, repair and physiologic regeneration, it provides a promising alternative to synthetic scaffolds and a foundation for regenerative efforts. Perfusion decellularization is a novel technology that generates native ECM scaffolds with intact 3D anatomical architecture and vasculature. This review summarizes achievements to date and discusses the role of native ECM scaffolds in organ regeneration.

Clinical background

Cardiovascular disease, diabetes and chronic obstructive pulmonary disease are the three most common chronic diseases in the Western world [1]. End-organ failure is the inevitable clinical destination: nearly six million Americans suffer from heart failure with approximately 550 000 new cases diagnosed annually [2], 530 000 Americans suffer from end-stage renal disease and nearly 25 million Americans suffer from chronic obstructive pulmonary disease with an estimated 12 million new annual diagnoses [3]. Organ transplantation remains the only definitive treatment for end-stage organ disease. Yet, donor organs are in short supply, and the relatively few recipients face the sometimes harsh consequences of lifelong immunosuppression and chronic rejection. As a result, approximately 91 000 patients in the US are waiting for a heart, lung or kidney transplant, with a median waiting time of 0.6, 2.5 and 3.3 years, respectively [4]; in 2009, 5237 Americans died waiting for a donor organ [4].

Organ regeneration: a theoretical alternative to transplantation

Increasing numbers of patients facing end-organ failure, as well as the therapeutic challenges surrounding allo-transplantation, catalyzed the evolution of tissue engineering and regenerative medicine. Tissue engineering applies engineering principles to life sciences in an attempt to build biological substitutes for lost or failing tissues [5]. Regenerative medicine encompasses an even broader range of approaches to replace or regenerate human cells, tissues and organs to restore or establish normal function [6]. The regeneration of functional whole organs has not been accomplished to date, yet several intermediate milestones have been reached by tissue engineers in heart, lung, kidney and pancreas regeneration (Table 1). By integrating increasingly complex cell types, scaffold materials and culture environments, these efforts have successfully recapitulated different aspects of organ development and provided us with valid lessons for future studies. Although cells and culture conditions are equally important, we will focus this review mostly on scaffold-related aspects and the potential role of the native extracellular matrix (ECM) in regeneration.

Native ECM in physiologic organ development, repair and regeneration

Tissue and organ engineering attempt, at least in part, to recapitulate organ development by creating controlled environments in the laboratory setting. Our ability to do so is hindered by the complexity and fluidity of developmental regulation and our limited understanding of spatial and temporal changes during organogenesis. In the developing embryo, the native ECM plays a central role by mediating biophysical stimuli, biochemical and molecular signals and spatial organization. Its complexity gradually increases with the development from morula to blastocyst to germ layers and ultimately organogenesis [7,8]. The process of constant interchange between cells and the ECM, described as dynamic reciprocity, determines cell fate and triggers the shift from proliferation to structure formation [9]. Laminin, for instance, the first intercellular ECM protein produced in the eight-cell stage embryo, assists in cell adhesion and migration during gastrulation, whereas fibronectin, collagen IV and heparan sulfate glycosaminoglycans appear later in development [8,10]. In later stages of development, advanced matrix structures such as basement membranes are required for the formation of specialized tissue such as secondary epithelium from primary mesenchyme [8,11]. Specific binding sites within the matrix then guide further organ development and maturation. Fibronectin, for instance, guides branching morphogenesis as a ligand [12] and is essential for the formation of the heart, lung and kidney [12,13]. Periostin, as another example, supports the remodeling of endocardial cushions into properly functioning atrioventricular valves by guiding valve and chordae tendinae collagen expression and organization [14]. Beyond molecular cues, ECM-mediated stress and strain regulate cell proliferation and phenotype not only in development, but also later,

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Table 1. Milestones achieved in heart, lung, kidney and pancreatic tissue regeneration

Tissue Regeneration Milestones								
TIME	**HEART**	**Refs.**	**LUNG**	**Refs.**	**KIDNEY**	**Refs.**	**PANCREAS**	**Refs.**
1800							**1894:** Xenogenic tissue extracts treat diabetes	
1950	**1959:** *In vitro* cardiomyocyte aggregates	[33]						
1970			**1976:** *In vitro* culture of alveolar-like, surfactant producing pneumocyes	[53]				
1980							**1980:** Microencapsulated islerts corrected diabetic state in rats	[74]
			1983: *Ex vivo* culture of whole lung					
			1986: *In vitro* differentiation of type II pneumocytes into type I pneumocytes	[55]	**1987:** BAK Concept Pioneered	[66]		
1990	**1988:** Mechanical stretch mediated improved cardiomyocyte orientation	[35]						
					1996: *In vitro* tubulogenesis of adult renal proximal tubule cells	[63]		
	1997: *In vitro* force generating cardiomyocytes	[37]						
					1999: BAK replace physiologic renal tubule function in uremic dogs	[67]		
2000			**2001:** Disease specific bronchial mucosa generated *in vitro*	[56]				
	2002: Adult-like myocardium with electrophysiologic & pharmacologic properties						**2003:** Intraperitoneal growth, development, and differentiation of rat pancreatic anlagen	
			2004: Lung-blood barrier generated *in vitro*	[57]	**2004:** Multi-center, randomized, controlled clinical trial of BAK.	[68]		
	2004: Conductive myocardium with improved electrical physiology	[38]						
	2005: Re-engineered myocardium improve function *in vivo*	[39]			**2005:** Multi-center, randomized, controlled clinical trial of BAK.	[68]	**2005:** Biodegradable scaffolds improve transplant islet survivorship *in vivo*	
	2006: Channel matrices & oxygen carrietrs increase re-engineered myocardial tissue density	[40]	**2006:** *In vivo* pulmonary tissue angiogenesis	[58]				
					2007: *In vitro* differentiation of Wolffian Duct & metanephric mesencnyme		**2007:** Collagen IV & Laminin coated hydrogel encapsulation improve islet function	[75]
	2008: Rudimentary Pump-functioning bioartificial heart generated basedk upon perfusion-decellularized matrix	[44]	**2008:** *In vitro* pulmonary tissue formation				**2008:** Reprogramming of adult pancreatic exocrine cells into beta cells	
			2008: Successful transplantation of reseeded decellularized trachea in a human	[62]				
			2009: Bioartificial regenerated lung facilitate gas exchange *in vivo* when orthotopically transplanted	[60, 61]			**2009:** Islet chamber prevascularization improve survivorship & function	
							2010: Cytokine protective encapsulation of islet cells	
							2010: Bioartificial endocrine pancreas reverse diabetic state	[76]

during the processes of tissue repair and regeneration. In wound healing, the disruption of the ECM leads to the loss of mechanical support down to the cellular level. In acute tubular necrosis (acute kidney injury), for instance, necrotic tubular cells detach from the basement membrane, whereas surviving tubular epithelial cells dedifferentiate and proliferate, restoring tubular integrity [15]. This phenomenon is not seen in areas lacking intact basement membranes. By contrast, hyperactive reparative processes are characterized by the overproduction of ECM proteins, which in many instances distort tissue architecture, hinder repair and become a self-sustaining disease process [16]. The multifaceted role of the native ECM in repair and regeneration makes it an interesting graft material. Several products have been tested to date with promising results in multiple clinical applications.

Native ECM as a graft material

The successful recapitulation of development in the laboratory setting requires choosing an ideal scaffold material as a mediator of biochemical and biophysical signals. The advantage of synthetic scaffold materials lies in their controlled, well-characterized composition, degradation and physical properties. Their disadvantage lies in the relative paucity of organ-specific structure and cell type-specific niches. Native ECM or ECM-derived proteins alone or in combination with synthetic polymers provide a potential alternative. Biocompatible native ECM scaffolds can be generated by exposing cadaveric tissues to decellularization (the removal of cells without compositional, biological or mechanical disruption of the ECM) via physical (e.g. freeze/thaw cycles), enzymatic (e.g. trypsin) and chemical protocols (e.g. SDS) [17].

The rationale behind using native matrix materials is the isolation of ECM proteins that are site-specific and provide protein 'footprints' of previous resident cells. Because ECM proteins are among the most conserved proteins [18], the removal of xenogenic or allogenic cellular contents (thereby providing a limitless supply of scaffolds) via decellularization could theoretically produce an essentially minimally immunogenic scaffold with a native intact structure for new tissue regeneration. A wide diversity of decellularized native ECM products (both allogenic and xenogenic) are FDA-approved and clinically used. Alloderm® is one example of an acellular dermal matrix derived from human skin that has been proven useful in multiple applications (e.g. burn care [19], abdominal hernia repair [20] and cosmetic surgery [21]). Because it is fully biocompatible, it can be implanted safely, and it induces the formation of host-derived connective tissue while providing mechanical support and tissue augmentation. CryoValve® SG Pulmonary Valve, a decellularized human valve, is currently implanted in right ventricular outflow tract reconstruction in congenital cardiac surgery and the Ross Procedure [22]. Similar to acellular dermal matrix materials, acellular valves are repopulated by the cells of the host and are theoretically maintained similar to the native valves, a key feature to enable growth, resistance to infection and longevity. A good example of a native matrix as a graft material is the large-scale FDA-approved use of decellularized bone allografts in orthopedic surgery, which proves that decellularization protocols can be scaled to clinical use and become available to a large number of patients (Box 1).

Box 1. Decellularized bone as a prototype ECM graft

Aside from bone autografts, decellularized allografts currently represent the large majority of clinical bone grafts. Especially in cases requiring extensive grafting (such as bone reconstitution or limb salvage after metastatic tumor excision, arthroplasty or the repair of congenital bone defects), decellularized bone allografts are optimal given the limited availability of autograft material. Decellularized bone allografts are taken as building materials that can be customized to aid the surgical repair of bony defects (e.g. a decellularized rib allograft can be shaped to reconstruct bone ridge defects or facilitate cheekbone reconstructions). Several FDA-approved guidelines for both tissue recovery (donor medical and social evaluation, tissue packaging and shipment to processor) and processing (decellularization: cleaning, freeze-drying, disease testing, sterilization and further processing) are currently applied to generate decellularized bone allografts for clinical use. Several FDA-approved bone processing organizations use their own proprietary testing, sterilization and decellularization protocols to achieve the complete removal of cellular debris (decellularization) including blood and marrow components without damaging the bone ECM [86]. After meeting FDA recovery and processing requirements, decellularized bone allografts are further processed into nonproprietary allografts (including femoral/tibial/fibular/radial/ulnar sections, cancellous bone chips, cortical bone chips, tendons and tricortical wedges) or proprietary allografts (Graftech®, GraftCage®, BTB Select®, BioCAP Select™, MatriGRAFT® and ReadiGRAFT®). Decellularized bone allografts could also be converted into demineralized bone matrices via chemical digestion to remove calcium and other minerals while retaining bone ECM proteins. Such matrices are similarly distributed as nonproprietary allografts or further processed into proprietary mixes (Grafton®, Osteofil®, Allomatrix®, AlloGro®, Optium DBM® and OsteoBiologics I/C Graft Chamber®) by combining demineralized matrices with other chemicals, chemokines and biomaterials. The large number of decellularized bone allograft products and transplants that have been implanted safely since 1985 (the last documented transmission of HIV from a seronegative donor via unprocessed bone transplant [87]) suggest that decellularized tissue grafts can be safely derived from cadaveric tissues and brought to broad clinical application when controlled by the FDA.

Challenges in the isolation and sterilization of native ECM scaffolds

The optimal choice of recovery, processing and decellularization protocol to isolate the native ECM for clinical use depends on its intended purpose. Collagens, for instance, are resistant to ionic detergents, but succumb to enzymatic digestion [17]; thus, ionic detergent decellularization could be ideal in generating scaffolds that require collagen preservation. Any decellularization protocol requires a certain degree of ECM disruption to allow for adequate exposure to reagents and to provide a pathway for the removal of cellular debris [17]. Whereas chemical means of decellularization provide favorable antigenic profiles by removing cell surface antigens [23,24], they can also irreversibly damage ECM components, such as basement membrane proteins, that are essential for cellular growth, differentiation and repair.

Aside from structural concerns, the clinical applicability of the decellularized matrix depends on the ability to generate a pathogen-free graft material. The ideal decellularization process removes cellular components, but it might not provide sufficient sterilization. Most formal sterilization techniques used in medical device production and surgical instrument preparation carry the risk of ECM damage and toxic residues. Pressurized steam, dry heat and chemicals that have been tested clinically cannot be used because of the inevitable protein denaturation. Ethylene oxide gas exposure coupled with lyophilization does not denature the ECM or destroy common growth factors (e.g. basic fibroblast growth factors) [25], but it does decrease the helical stability of collagen fibers [26]. Gamma irradiation and electron beam irradiation are widely used in medical device manufacturing; however, they have been shown to potentially compromise the mechanical stability of collagen scaffolds [27]. Peracetic acid (PAA) submersion or perfusion seems to be an interesting alternative; it is bactericidal at 0.001%, fungicidal at 0.003% and sporicidal at 0.3% [28], and preserves ECM proteins such as glycosaminoglycans, laminin and fibronectin [29]. PAA does not

alter the mechanical characteristics of sterilized tissue [30] and it preserves ECM-bound growth factors [31,32]. The use of radiation, PAA and ethylene oxide does not address the risk of viral contamination, which remains an important concern in the translation of both human- and animal-derived tissue sources. This concern can only be addressed by thorough donor screening and via decellularization methods that break up and remove residual nucleic acids. To date, no direct comparison of the different sterilization protocols of decellularized whole organ scaffolds has been reported. However, from data points on tendon grafts and other native ECM products we can deduce that each protocol has its side effects on ECM properties and, therefore, needs to be tailored to the intended application. Clinically safe and well-standardized scaffolds are the foundation for organ engineering, but only the first step towards the creation of viable tissues. Finding the appropriate cell types, seeding strategies and culture conditions are the next challenges. Achievements to date provide us with many valuable data points, a few of which are summarized below.

Milestones in myocardial engineering

The first successful attempts of the *in vitro* culture of contractile cells were reported in 1959 when embryonic chick cardiomyocytes cultured in Erlenmeyer flasks under continuous gyration produced spontaneously contracting cell aggregates [33]. Although this showed that cells could be maintained viable in the laboratory, and function even after being taken out of their natural environment, it took nearly 30 years until the first attempts at reassembling functional cells to tissues were made. When seeded onto collagen matrixes, myotubule formation was observed, but cell orientation was not uniform enough to produce contractile function at a tissue level [34]. Repetitive cycles of mechanical stretch helped improve orientation and the formation of contractile elements [34–36]. The first spontaneously beating, force-generating myocardial tissue constructs were generated in 1997 by culturing embryonic chick cardiomyocytes between Velcro-coated glass tubes that moved rhythmically [37]. In addition to mechanical stretch, electrical field stimulation further improved cardiomyocyte biomimetic growth conditions by maturing construct ultrastructural organization (increased density of intercalated discs, gap junctions and t-tubules) [38]. As a first step showing potential clinical applicability in the treatment of heart failure, loops of regenerated myocardium (comprising rat neonatal cardiomyocytes, Matrigel® and liquid collagen I) were surgically grafted onto the damaged myocardium of infarcted rat hearts; this treatment improved left ventricular systolic and diastolic function [39]. The engineering of functional contractile tissue from single cells, and its successful application in a heart failure model, were groundbreaking. However, to treat heart failure in humans, myocardial patches of a much larger thickness needed to be generated. Using cell-seeded gels cultured in traditional cell culture dishes did not allow the formation of tissues beyond a few millimeters in thickness, simply due tobecause of the inability to support the high metabolic demands of cardiomyocytes [36,40]. New scaffolds and bioreactors were designed to improve metabolic exchange and oxygen delivery via pores or parallel channels [40], by stacking thin cardiac sheets [41,42] and through the use of artificial oxygen carriers [43]. Myocardial patches would not only need to be thick but also perfused immediately after implantation to avoid cell death in the newly formed tissue. Even porous scaffolds lacked the hierarchical vascular system (from large artery to smaller artery to capillary to veins) that could be connected surgically to the vasculature of the recipient similar to a transplanted organ. In a novel approach, the reseeding of perfusion-decellularized native heart ECM scaffolds that had preserved 3D anatomical structures enabled the formation of functional myocardia with greater thickness [44]. Because the acellular native ECM contains a coronary vascular basement membrane suitable for re-endothelialization, the antegrade perfusion of media paired with electrical and mechanical stimulation supported the engraftment and function of cardiomyocytes across larger thicknesses. In addition to a perfusable vascular bed, the acellular native ECM provides a blueprint for the entire organ, including ventricular geometry and heart valves, which transforms tissue contraction to actual pump function.

The ideal scaffold material for myocardial regeneration remains debatable: myocardial constructs have been generated on a variety of materials; natural components such as collagen [45], biodegradable gelatin [46], alginate [47] or fibronectin [48] might provide physiologic cell attachment sites, but lack organ-specific architecture. Decellularized tissues might retain a variety of physiologic niches and growth factors and provide organ structure and vasculature, but they are poorly characterized at the molecular level [49,50]. Likewise, synthetic polymers such as polyglycolic acid [51] and polymer poly(*N*-isopropylacrylamide) can be produced with defined mechanical properties and solubility, but they provide an environment that is entirely different from native ECM [52].

Milestones in pulmonary engineering

Similar to cardiac tissue engineering, the first attempts at lung tissue engineering reported the successful cultivation of lung cells that had been isolated from a developing organ and maintained in cell culture; when seeded onto gelatin discs, these cells produced surfactant secreting alveolar-like structures [53]. In addition to establishing basic cell culture conditions, these experiments highlighted the importance of cell–matrix interactions for tissue formation because the proteolytic digestion of cultured cells prevented the formation of 3D alveolar-like structures [53]. When culture conditions were subsequently enhanced by physiologic stimuli such as air–fluid interfaces, alveolar type II cells formed a surfactant-secreting monolayer with a histological resemblance to the native lung [54]. Similar to early experiments with cardiac cells, specific culture conditions combined with lung ECM proteins supported the differentiation of pneumocytes, thereby recapitulating the physiologic regenerative capacity of the lung *in vitro* [55].

Whereas *in vitro* culturing systems achieved milestones to generate disease models (e.g. asthmatic bronchial mucosa [56]), tissue engineering efforts aimed at generating constructs with a morphology and functional characteris-

tics of native lung tissue. The coculture of human epithelial cell lines (A549 and NCI H441) and primary human pulmonary microvascular endothelial cells generates a lung–blood epithelial barrier with intact intracellular junctions [57]. By mimicking the *in vivo* alveolar–capillary barrier, such constructs produced gas exchange *in vitro*. However, the augmentation of functional lung tissue *in vivo* using such artificial matrix constructs has not been reported to date. A polyglycolic acid mesh seeded with somatic lung progenitor cells promoted angiogenesis but not the regeneration of gas exchange tissue when implanted after lung reduction surgery [58]. When seeded with fetal rat cells and injected into adult rat parenchyma, Gelfoam® (an ECM substrate) enabled angiogenesis and the regeneration of porous alveolar-like structures but not fully integrated newly formed lung tissue [59]. Although some of these results show morphologic patterns resembling gas exchange tissue, functional maturation and connection to the pulmonary arterial, venous and bronchial system of the host have not been observed. Similar to engineered myocardium, the lack of the structure of engineered lung constructs limits clinical applicability given that regenerated airways and vessels must be ventilated and perfused by the host to mediate function.

Recently, two groups independently regenerated whole functioning lungs *in vitro* using a novel approach based on perfusion-decellularized whole rat lungs seeded with rat fetal lung cells cultivated under biomimetic conditions [60,61]. The native lung ECM provided a hierarchical system of airways and vasculature that could be connected to the pulmonary artery, vein and bronchus of the host, and supported physiologic functions *in vivo* when orthotopically transplanted. As a groundbreaking first step towards translation, a tracheal graft was generated using a similar approach and was successfully transplanted in a patient to replace part of the main airway. This indicates that *in vitro*-regenerated tissue based upon decellularized constructs could be safely translated to clinical practice [62]. Intermediate term results from this early clinical experience suggest that transplanted constructs that were regenerated *in vitro* can support host-derived revascularization and that they are maintained in a manner similar to native tissue.

Milestones in renal engineering

Isolated adult renal proximal tubule cells can be cultured with a high capacity for self-renewal and can differentiate into tubule structures when grown in 3D collagen gels [63,64]. When seeded onto matrix fibers, cells not only form a confluent layer, but also display functional transport capabilities [65]. The concept of the bioartificial kidney (BAK) takes advantage of these properties by combining the metabolic and endocrine functions of renal tubular cells cultured in a synthetic matrix construct with a traditional hemofiltration system [66]. The upscaling and refinement of a hemofilter in series with hollow polysulfone fibers coated with proximal tubule cells resulted in a BAK that replaced renal function in acutely uremic dogs [67]. Incorporated human proximal tubule cells in BAKs reached clinical application and temporarily improved patients with acute renal failure [68]. These results show that isolated cells can be expanded and function when seeded onto a suitable scaffold material. The next step is to provide a scaffold material that enables transplantation and full integration into a host system as a sustainable graft. The use of asymmetric membranes with hemocompatible surface and cell-compatible surfaces [69], collagen IV-coated thin film [70] and silk-based porous scaffolds with collagen Matrigel® suggests that improved function might be achieved with scaffold designs that are closer to the native ECM of the kidney. Indeed, early experiments showed that native kidney ECM isolated by the SDS submersion of cadaveric kidney sections was able to support fetal kidney cell engraftment [71].

Milestones in pancreatic engineering

In the past decade, human pancreatic islets were isolated and provided reproducible success in short-term transplantation for the treatment of insulin-dependent diabetes [72]. However, long-term independence from insulin cannot be achieved [73]. This lack of graft survival is probably related to decreased long-term islet survival outside of the natural environment, the islet 'niche', and because of chronic rejection, because the donor islets are not immunologically compatible with the recipient. Given the immediate clinical applicability, efforts have focused on providing a more physiologic environment as well as protecting them from the immune system of the host to improve the delivery, survival and long-term function of transplanted human islets. Microencapsulation and the addition of natural matrix substitutes such as collagen IV and laminin have provided an immunoprivileged islet niche while permitting waste and nutrient diffusion and improved islet function [74,75]. More recently, the perfusion decellularization of cadaveric rat and human pancreases was used to generate ECM scaffolds in an attempt to preserve the native islet cell niche to improve islet survival and function after transplantation. Seeding with human islets generated functional endocrine tissue *in vitro* and reversed the diabetic state after transplantation in a rat model *in vivo* [76]. An acellular native pancreatic ECM might provide an alternative to engineered matrix scaffolds and the advantage of an intact vascular bed. However, islets occupy only approximately 1–2% of the total pancreas. To benefit from the islet-specific niche, islets or insulin-producing cells would theoretically have to be seeded to the appropriate location within the matrix; this has not been achieved to date.

Whole organ regeneration based on perfusion-decellularized matrix scaffolds

In 2008, our group reported perfusion decellularization as a technique to generate acellular whole organ scaffolds from cadaveric organs. In this approach, decellularizing agents are delivered via the innate vasculature of the organ and are thereby equally distributed across the entire thickness of whole organs. Applying physiologic perfusion pressures, decellularization solutions can effectively permeate the tissue via arteries, arterioles and capillaries, and remove cellular debris via the venous system, thereby minimizing their exposure to scaffolds. A reduction in native ECM scaffold exposure to decellularization solutions lowers the

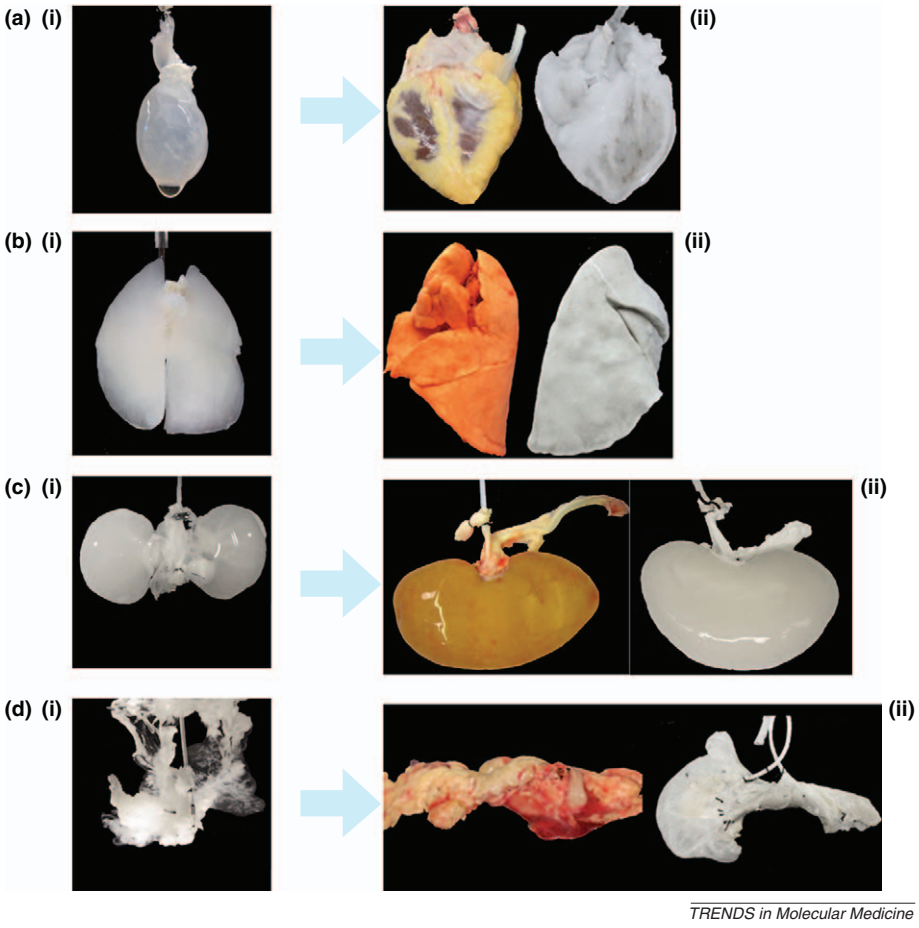

Figure 1. Perfusion-decellularized whole organ scaffolds. The native ECMs of cadaveric organs can be isolated by the perfusion of the native vascular system with detergent solutions. The resulting scaffolds are acellular, but maintain the structure of the native organ. **(a)** Rat heart scaffold generated from cadaveric heart by perfusion decellularization **(i)**. The ascending aorta was cannulated for perfusion. Cadaveric human heart before and after perfusion decellularization **(ii)**. **(b)** Rat lung scaffold generated from cadaveric lung by perfusion decellularization **(i)**. Perfusion was performed via the pulmonary artery. Cadaveric sheep lung before and after decellularization **(ii)**. **(c)** Rat kidney scaffold generated from cadaveric kidney by perfusion decellularization **(i)**. The abdominal aorta was cannulated for perfusion. Porcine kidney before and after perfusion decellularization **(ii)**. **(d)** Rat pancreas scaffold generated from cadaveric pancreas by perfusion decellularization **(i)**. The abdominal aorta was cannulated for perfusion. Human pancreas before and after perfusion decellularization **(ii)**.

risk of the chemical or physical alterations of ECM proteins and growth factor loss (thus facilitating the generation of a more biocompatible scaffold for regenerative endeavors). Although previous studies utilizing perfusion decellularization have reported a combination of SDS, Triton X-100 and PBS perfusion, the ideal detergent recipe must be tailored to the specific organ and application.

Perfusion decellularization generates acellular ECM scaffolds with intact 3D anatomical structures and patent vasculature conduits that can be re-endothelialized and perfused to enable the regeneration of tissues of clinically significant thickness [44,60]. Whole organ scaffolds have been generated from cadaveric hearts (rat, pig and human) [44], lungs (rat, pig, sheep and nonhuman primates) [60,61], liver (rat) [77], pancreas (rat, human) and kidney (rat, sheep, pig) (Figure 1). Notably, decellularized scaffolds are free of significant DNA content and nuclei, while retaining major ECM proteins (collagen I, III, laminin, fibronectin and glycosaminoglycans). The tensile strength testing of decellularized scaffolds revealed insignificant differences in membrane stiffness and preserved the 3D mechanical characteristics of cadaveric tissue (fiber direction). Several studies have shown that decellularized scaffolds can be repopulated with cells and cultured as a whole organ construct, thereby maintaining the structure of the organ. Decellularized rat heart scaffolds were recellularized with rat aortic endothelial cells and neonatal cardiomyocytes to produce functional tissue [44]. Decellularized rat liver scaffolds were reseeded with primary rat hepatocytes and endothelial cells and produced metabolically functional tissue [77]. Decellularized rat lungs were repopulated with endothelial and fetal rat lung cells to form lung constructs that enabled gas exchange after orthotopic transplantation [60,61]. Perfusion-decellularized rat and human pancreases were seeded with human islets and generated functional endocrine tissue that reversed the diabetic state of rats [76].

Concluding remarks

Perfusion decellularization allows tissue regeneration at a clinically relevant scale with an intact organ structure by meeting metabolic demands via intact vasculature and maintaining native ECM-contained cues. Although current and previous work in liver, heart, lung and pancreas are testament to this notion, a series of hurdles must be addressed to allow translation to the bedside (Box 2). All

Box 2. Outstanding questions

- Organ Scaffolds:
  - o type (engineered vs. native)
  - o tissue source (human vs. animal)
  - o standardized protocols for decellularization and sterilization
- Regenerative Cells:
  - o type (adult derived vs. embryonic)
  - o cell source (patient derived vs. banked)
  - o standardized protocols for expansion, differentiation and purification
- Bioreactor Design and Organ Culture:
  - o clinical grade bioreactors enabling safe and sterile whole organ culture
  - o standardized culture protocols using clinically applicable growth factors
- Transplantation:
  - o consensus on patient selection
  - o identification of intermediate products
  - o logistics of tissue/organ preservation and transport
  - o understanding of immunologic response to a regenerated organ
  - o optimization of graft longevity

regenerative efforts based on perfusion-decellularized whole organ scaffolds to date have been at the small animal scale, using cells of limited clinical applicability, and, aside from pancreatic tissue, displaying only short-term *in vivo* function. The first step in generating functional tissue that can be directly transplanted in humans will be the generation of perfusion-decellularized native ECM scaffolds that match human organs in size and structure. Although our group has shown that perfusion decellularization can be applied to porcine, primate and human heart, lung, pancreas and kidney (Figure 1), protocols need to be further refined and tailored to organ size, type, species and donor characteristics (e.g. age, ischemia time and body surface area). The resulting scaffolds need to be of reproducible clinical grade, sterile and preserved for further processing. Moreover, the immunogenicity of decellularized native scaffolds must be carefully assessed in model systems closer to clinical reality. ECM proteins are among the most conserved proteins [18]. As a result, decellularized ECM scaffolds (such as bovine type I collagen) have been widely successful in therapeutic applications [78]. Yet, decellularization can create or leave residual antigenic epitopes [79]. Animal organs could become a valid alternative to human tissue sources; however, species-specific antigens might have to be removed [79,80]. Human donor tissues used for dermal matrix products and bone allografts are considered relatively safe given the stringent donor screening and graft processing that dramatically reduce the risk of disease transmission and/or pathogen contamination [81]. The large-scale FDA-approved use of decellularized bone and dermal allografts demonstrates that decellularization protocols can be scaled to clinical use and become available to a large number of patients.

Bringing scaffolds to clinical quality and scale is only one of many steps towards the regeneration of viable and functional organs. At the current stage of technology, an attempt to recapitulate the entire process of embryogenesis from the single cell stage to organogenesis in the laboratory setting seems hardly realistic. Such a feat would require extensive culture periods to generate tissues of clinical size and a variety of tissues to enable crosstalk, and would lead to obvious ethical dilemmas. Native ECM scaffolds might offer a feasible shortcut from a stage of cell expansion to organ maturation, bypassing initial embryonic structure formation. Results thus far demonstrate that late fetal cells committed to cardiac or pulmonary phenotypes engraft onto native ECM scaffolds and form functional tissue, similar to fetal wound healing rather than true organogenesis [82]. Although ECM, spatial relationships and molecular stimuli shift rapidly in the early stages of embryonic development, the later fetal stages of organ maturation are better understood and easier to replicate. Mimicking this process by providing clinically relevant cell types of corresponding developmental stage and an environment of late organ development, including mechanical load and growth stimuli, might be a realistic approach. Indeed, because cell fate is more committed at this stage, the risk of differentiation down an incorrect path (e.g. thyroid vs. respiratory) could be smaller. The ideal clinically feasible cell source to derive such committed progenitor cell populations has yet to be identified. Embryonic stem cells carry ethical and supply issues and are immunogenic, thereby compromising their clinical value. Induced pluripotent stem cells are an adult-derived alternative, although disease-related mutations might have to be corrected (e.g. *BMPR2* mutations in pulmonary hypertension), and concerns surrounding genetic alterations have to be addressed. Creating the differentiated cell numbers required for the regeneration of human-sized organs exceeds current progenitor cell technology and poses significant challenges in phenotypic control at a large scale [83]. Many of the current efforts aimed at developing strategies for expansion and stemness (e.g. fibroblast reprogramming that skips pluripotent progenitor stages [84] and embryonic stem cell-derived protein-induced pluripotency [85]) might deliver novel solutions applicable to organ engineering in the foreseeable future. Solid organ regeneration based on perfusion-decellularized native ECM scaffolds holds great promise for patients suffering end-organ failure, but clearly remains an ambitious goal. Work towards that goal will span many disciplines and produce intermediate therapeutic products and milestones that improve our understanding of stem and progenitor cell fate in organ development and disease.

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