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Bioreactor design for perfusion-based, highly-vascularized organ regeneration

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Abstract

Bioartificial or laboratory-grown organs is a growing field centered on developing replacement organs and tissues to restore body function and providing a potential solution to the shortage of donor organs for transplantation. With the entry of engineered planar tissues, such as bladder and trachea, into clinical studies, an increasing focus is being given to designing complex, three-dimensional solid organs. As tissues become larger, thicker and more complex, the vascular network becomes crucial for supplying nutrients and maintaining viability and growth of the neo-organ. Perfusion decellularization, the process of removing cells from an entire organ, leaves the matrix of the vascular network intact. Organ engineering requires a delicate process of decellularization, sterilization, reseeded with appropriate cells, and organ maturation and stimulation to ensure optimal development. The design of bioreactors to facilitate this sequence of events has been refined to the extent that some bioartificial organs grown in these systems have been transplanted into recipient animals with sustained, though limited, function. This review focuses on the state-of-art in bioreactor development for perfusion-based bioartificial organs and highlights specific design components in need of further refinement.

Keywords

Bioreactors; Tissue engineering; regenerative medicine; bioartificial organs; organ transplantation; perfusion decellularization

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Introduction

Advances in immunosuppression, surgical techniques, and donor/recipient patient selection have led to an increase in the number of patients considering organ transplantation as the optimal therapy for many types of organ failure. Organ transplantation leads to increased life expectancy and improved quality of life, and in many cases is the only durable long-term treatment [1,2]. As of December 2012, more than 116,000 people were waiting for an organ for transplantation, and the number grows larger every day [3]. This problematic trend is due in large part to the growing demand and limited supply of deceased donor organs and those from altruistic living donors. An estimated 18 people die every day due to organ failure [3]. Although bioartificial organs are still in their infancy, research in this field has expanded in the last few years and this technology has the potential to provide a new source of organs and tissues for patients in need of transplantation.

The premise of bioartificial organs is to strip an organ that is non-transplantable, due to parenchyma scarring or high fat content, of its cellular components using a process termed decellularization to yield a scaffold on which to develop a new organ (Figure 1). Important properties of these scaffolds are retention of native tissue architecture and maintenance of extracellular matrix (ECM) components and growth factors for proper cellular homing and differentiation. Scaffolds are then seeded with autologous or allogeneic cells to repopulate the matrix and return function to the organ.

These engineered organs are best grown in bioreactors that simulate the niche environment and optimize organ function; the presence of a native vascularized system allows for nutrients to be delivered to growing cells within the organ. Bioreactors are tailored to specific organs and an understanding of developmental biology, including specific chemical, mechanical, and electrical stimuli, is needed to optimize bioreactor performance to enhance the function of each engineered organ or tissue. Bioreactor design for organ engineering is a young, but rapidly growing field. The following sections cover the current state of bioreactor development and design parameters for the continuous perfusion of bioartificial organs during the different stages of organ regeneration.

Decellularization

The process of perfusion decellularization to produce a biological scaffold containing the structural proteins of an organ or tissue is well characterized for small animal models such as rodents. Physical and chemical methods have been used to remove cells and leave an intact ECM. The amount of DNA remaining within the ECM is typically used as a surrogate to measure efficiency of cell removal, and depends upon the cellular and extracellular composition of the organ or tissue, its geometry (planar or three dimensional) and method used. Baptista et al. and Soto-Gutierrez et al. reported removal of 97–99% of DNA from rodent livers using either a 1% Triton X-100/0.1% ammonium hydroxide combination [4] or 0.02% trypsin/0.05% ethylene glycol tetraacetic acid (EGTA)/3% Triton X-100 protocol with retrograde perfusion through the vena cava [5•], respectively. Bonvillain et al. used 0.1% Triton X-100/2% sodium deoxycholate (SDC)/1M hypertonic saline/30 µg/ml DNase to achieve ~85% DNA removal from macaque lungs [6]. The amount of growth factors retained also varies with the decellularization method. Soto-Gutierrez et al. demonstrated the presence of fibroblast growth factor (FGF, 13 ng/g-dry weight, reduced by ~60% after decellularization) and hepatocyte growth factor (34 ng/g-dry weight, reduced by ~50%) in liver scaffolds, but vascular endothelial growth factor (VEGF) could not be detected [5•]. Brown et al. detected FGF (1.8–2.5 ng/g-dry weight) in porcine adipose tissue decellularized using 0.02% trypsin/0.05% ethylene diamine tetraacetic acid (EDTA)/3% Triton X-100/4% SDC or 3 mg/g-dry weight collagenase/0.02% trypsin/0.05% EDTA, whereas the level was

greatly reduced for a protocol using 1% sodium dodecyl sulfate (SDS)/ 3 mg/g-dry weight collagenase/4% SDC/0.9% saline (0.05 ng/g-dry weight) [7]. In contrast to the liver, VEGF was detected in porcine adipose tissue decellularized using 0.02% trypsin/0.05% EDTA/3% Triton X-100/4% SDC or 3 mg/g-dry weight collagenase/0.02% trypsin/0.05% EDTA, but not with 1% SDS/ 3 mg/g-dry weight collagenase/4% SDC/0.9% saline [7].

Immunohistochemistry and immunofluorescence are commonly used methods to qualitatively evaluate the presence of growth factors within the ECM, while enzyme-linked immunosorbent assay can provide a quantitative analysis of retained growth factors [7]. More research is needed to understand the involvement of growth factors in cell homing and as cues for differentiation within tissue scaffolds. A recent multi-institutional review presents an in-depth analysis of the decellularization process and different strategies to remove cells [8•].

Organs are placed within containers specially designed for decellularization, allowing the organ to be perfused with solutions through its vasculature or submerged within fluid that is agitated by a stirrer or rocker. Decellularization is typically carried out at room temperature, but occasionally scaffolds are cooled to 4°C to enhance ECM preservation or warmed to 37°C when using enzymatic methods [4–8]. Bioreactors for organ decellularization have an inlet connected to the arterial or portal system (liver) for antegrade perfusion of the organ, and the fluid typically exits the organ through the venous system, draining into the bulk fluid and exiting via the bioreactor outlet line [9]. However, some investigators have used retrograde perfusion for the liver [5•] or the heart [10]. There are several design considerations to point out. As the organ decellularizes, cell fragments will be washed from the scaffold and debris may build up within the decellularization bioreactor. These fragments may be evacuated from the bulk fluid through the bioreactor outlet if the outlet line is large enough to prevent clogging, but care must be taken not to introduce cell fragments back into the organ scaffold if the perfusate is recirculated.

Scale-up of bioreactors to accommodate decellularization of large animal organs, while achieving the same level of DNA removal and retention of the ECM architecture and growth factors, presents additional challenges that have only begun to be addressed in porcine organs. Pig organs, which are nearly the same size as human organs, depending upon the weight and age of the animal, may represent a possible long term source of scaffolds for bioartificial organs. Porcine tissues such as small intestinal submucosa and urinary bladder matrix are currently used to augment surgical repair of tissue defects in patients. These acellular tissue substitutes have been shown to have low DNA content and are biocompatible with minimal inflammation [11].

The use of organs from large animals, such as porcine kidneys, leads to a significant increase in the volume of solutions needed to achieve effective cell removal and may be as great as 86 liters, including the decellularization detergents and saline needed to clear the organ of debris and residual decellularization agents [12•]. It is likely that the volume needed for effective decellularization and cleansing of the scaffold is close to 100 times the volume of the organ. Decellularization of porcine kidneys takes about four to seven days, including decellularizing and washing the scaffold [9,12], while lungs from rhesus macaques can be decellularized in three days [6]. Decellularization of rodent kidneys required five days [13], hearts five days [10], and lungs three days [14]. Cell removal and ECM damage both increase with the strength of the chemical solution, the flow rate, and the duration of treatment. After treatment with 0.5% SDS, 98% of the DNA had been removed from a porcine kidney, while only 90% was removed using 0.25% SDS [9]. However, the amount of collagen tended to be higher using 0.25% SDS. Taken together, the use of stronger detergents such as SDS may lead to effective decellularization over shorter time periods and

may be more useful for larger organs that need higher perfusion volumes. However, the use of stronger decellularization agents requires both a substantial organ rinse to remove these agents after decellularization and a close analysis of the ECM, including structural proteins, growth factors and glycosaminoglycans [8••].

Microenvironmental cues in the form of matrix-bound growth factors and signals initiated by adhesion receptors engaging matrix ligands in specific three-dimensional configurations likely play a large role in regulating cell growth, proliferation, function and phenotype in conjunction with a supportive cell population. One possibility is that preservation of a minimal level of these matrix-related proteins is needed to sustain early, proper organ recellularization. Growth factors in the matrix may be exogenously replenished by factors contained in cell culture media, but restoring the native, variable distribution within an organ matrix is challenging. However, new repopulating cells that take up residence within the organ niche environment will in turn secrete a new matrix milieu yielding a local environment that continually remodels.

Sterilization

Organ recellularization, cell growth, and maturation require several days to weeks in a bioreactor, depending upon the organ's size and cell source. The organ scaffold and bioreactor must each be sterilized prior to reseedling to prevent contaminants from growing within the scaffold. There are two common approaches for scaffold sterilization. Chemical sterilization typically relies upon acidified ethanol to sterilize the scaffold [5•]. Chemical agents are distributed throughout the scaffold and bioreactor perfusion circuit to reach all portions of the scaffold, tubing, and vessel walls. Ultraviolet light has also been used to sterilize scaffolds, but has limited tissue penetration and does not sterilize the perfusion circuit.

A common method of sterilization is gamma irradiation. One report indicates that a dose of 25 kGy is necessary to achieve complete scaffold sterilization [15]. Others have used 10–25 kGy, and the dose is typically reached using a powerful gamma radiation source such as a cobalt-60 irradiator [4,5,9,12]. Either the scaffold must be transferred from a temporary, clean container used for irradiation and then placed into the bioreactor, or the bioreactor housing the organ must fit into the irradiator. These design considerations must be reached early in bioreactor development. From a clinical standpoint, a single-use, disposable bioreactor would be desirable, and plastics would be attractive materials. However, plastics may become brittle when exposed to radiation [16•–19]. Low-density polyethylene, when irradiated, showed a decrease in the Young's modulus from 21.6 to 11–13.3 MPa, while high-density polyethylene and polypropylene showed an increase in Young's modulus due to crosslinking resulting from free radical formation on the repeat units of the polymer chain [16•]. Free radicals may damage the scaffold. Table 1 shows the effects of radiation on selected plastics that are commonly used in biomedical practice. Polycarbonate, polystyrene, and polysulphone are highly recommended if radiation is to be used for sterilization [18].

Reseeding

A general process flow diagram of a bioreactor circuit is shown in Figure 2A. Several methods are used to reseed scaffolds and dynamic methods tend to be more effective than static cell seeding [20••21]. The most common dynamic method for reseedling is to directly add cells at high concentration into the vascular perfusion line just upstream of the organ, allowing cells to travel directly through the vascular tree into the scaffold and parenchyma. This method is universally used to recellularize the vasculature of hearts [10], lungs [14], and livers [4,5,22]. Many investigators have delivered cells to an organ parenchyma through the scaffold vasculature; the cells are thought to traverse the vascular lining through holes or

pores created by the decellularization process. Low flow rates are used for reseeded to reduce shear stress on the cells. High seeding efficiency (86–96%) has been reported for directly injecting hepatocytes into the portal vein through the bioreactor inlet port; dividing the cells into multiple injections is superior to a single infusion with the same total number of cells [5•,22]. A second method to reseed the liver parenchyma is to inoculate cells into the bulk media and allow them to recycle through the circuit to reseed the organ, but this achieved a lower seeding efficiency (69%) compared to the multi-step process described above [5•]. Endothelial and organ parenchyma cells may be seeded together in a mixture [4,14] or via separate inoculations of pure cell populations [5•,22]. An alternative recellularization method is direct inoculation of cells into several locations of an organ parenchyma using a small gauge needle [10,23]. However, this method was less efficient than delivering hepatocytes through the vasculature [5•].

Although the vascular system is most commonly used to introduce cells into a scaffold, other routes may be needed to populate the diverse cell types that make up an organ. The lung is the most developed model for alternative delivery of specialized cells, with the tracheobronchial tree primarily used to deliver pneumocytes [14] or mesenchymal stem cells [6]. Delivery of cholangiocytes to the liver and urothelium to growing kidneys will likely require direct seeding through the bile duct or ureter, respectively, to deliver these specialized cells.

Figure 2B,C shows bioreactors that may be used for cell seeding. Each bioreactor has advantages and disadvantages. The cylindrical configuration shown in Figure 2B limits dead zones and promotes mixing, as determined by mathematical modeling and experimentation [24••]. However, placing the outlet port inline with and near the inlet caused channeling that decreased mixing with the surrounding bulk fluid [24••]. Effective mixing can be achieved by placing the exit 120° from the inlet (Figure 2B). An often-used bioreactor design is a spinner flask with a stir bar on the bottom (Figure 2C), which is especially useful when seeding cells into the bulk media. The continuous-flow stirred tank reactor (CSTR) (Figure 2C) keeps cells, which may have failed to lodge in the scaffold during a pass through the bioreactor, in suspension, allowing for multiple passes through the scaffold. However, cells used for recellularizing an organ are adhesion-dependent, so anoikis may occur if cells recirculate for extended periods of time. The organ is typically suspended on the inlet line, and stirring the bulk fluid may lead to adverse rotational shear on the organ. Suspending the stir bar will minimize lysis of cells caught between the vessel and the stir bar [25].

Scaffold porosity varies inversely with the cell density. When an organ is decellularized, the resistance to flow decreases [26]. However, as reseeded cells flow into the vascular tree, pores will fill with cells and the pressure drop will increase as the porosity decreases [24••]. For this reason it may be helpful to monitor perfusion pressure through a transducer placed before the inlet, as depicted in Figure 2A, or by using a micropipette transducer inserted into the organ parenchyma [26]. Increasing the cell seeding density increases the possibility of cell aggregates occluding vessels and forming thick polylayers during seeding. This may cause oxygen and nutrients to become mass transfer limited, leading to hypoxia and the development of a necrotic core [27–30].

Organ culturing and stimulation

Several different organs have been grown in perfusion bioreactors (Table 2). Tissue engineered livers and lungs have been implanted into recipient rodents with varying extent and duration of organ function. Ott and colleagues demonstrated that type II pneumocytes with appropriate differentiation markers could grow on rodent lung scaffolds within a perfusion bioreactor [31•]. Recipient animals breathing 100% oxygen that were transplanted

with these engineered lungs had a higher blood oxygen content at seven days after surgery compared to controls with a surgically removed lung.

The bioreactor environment should be tailored to the target organ function and be designed to mimic *in vivo* conditions that support the organ for the 1–3+ weeks required for organ maturation and development. Experimentation to determine the optimal media content is critical. Addition of growth factors may be necessary if sufficient levels are not retained in the ECM. However, maintaining a proper balance is important. VEGF is required for endothelial cells to seed the scaffold vasculature and form new vessels. However, when VEGF and FGF were added in high levels, giant cells and aggregates formed [32]. At times, modification of the oxygen level is needed. When seeding stem and progenitor cells, it is often desirable to use hypoxic conditions (~5% O₂), which have been shown to generally promote stem and progenitor cell expansion and to minimize differentiation into most mature cell types [33,34]. After sufficient expansion has been obtained, pO₂ can be increased to enhance tissue-specific differentiation. For example, shifting from 5% to 21% O₂ on day 8 during a culture of rat fetal liver cells on a collagen-coated polydimethylsiloxane membrane improved the functional (albumin synthesis), structural, and metabolic behavior of the culture, as compared to cultures continuously maintained at either 5% or 20% O₂ [35]. Employing sensors for pO₂ and pH in the reactor and inlet stream is essential to ensure an environment controlled at the desired conditions (Figure 2A).

The bioreactor may need to provide the growing organ with physical, electrical, or chemical stimulation, or a combination of these. One organ that requires special stimulation is the heart. Myocardium must be mechanically stretched and electrically stimulated. Mechanically stretching tissue promotes cell alignment, elongation, and expression of connexin-43, a cardiac marker [36]. Mechanical stretching can be induced by mounting wires to the organ or directly achieved through traction by motors with stress transducers. The strain required for optimal myocardial development is species-specific; for the rat model 30 kPa of stress resulted in elongation and promotion of connexin-43b [36]. Electrical stimulation causes cells to produce contractile forces and is critical for myocardium development. Tandon et al. incorporated carbon rods into the bioreactor to supply voltage. 4-cm carbon rods were fixed to the bottom of 1-cm petri dishes with a 1-cm gap to allow for 2-mm gaps between the rods and the top and bottom of the scaffold. Platinum wires were attached to each rod to supply voltage [37•]. For neonatal rat cardiomyocytes, carbon rods carried 3V/cm monophasic square waves at 3 Hz. With media perfusion and electrical stimulation, the excitation threshold voltage (2.5±0.5 V/cm) required to cause coordinated beating of cells was lower and the heart rate was faster (4.3±0.6 Hz) than that found without stimulation or perfusion (4.1±0.7 V/cm and 2.8±0.5 Hz, respectively) [37•]. Electrical stimulation can also be used in tandem with mechanical stretching [36–39].

The lung also requires special consideration for bioreactor design. As the lung is inflated and deflated, the ECM must retain appropriate mechanical compliance. A common method to achieve stretch in a bioreactor is to suspend the lung scaffold in a container of media and connect the lungs to a ventilator that matches the volume and respiratory rate of the animal [31•,40–42]. Song et al. ventilated the recellularized lung with media instilled into the bronchial tree at 1 day after seeding until epithelial cells reached a mature state (5 days), at which point the lungs were dry-ventilated with a respirator [31•]. With this bioreactor design it is possible to recellularize cadaveric lungs to the extent that improvement in oxygen exchange can be demonstrated in a rat transplanted with the recellularized lung at 7 days [31•,41].

The kidney does not require mechanical stimulation, but benefits from chemical stimulation. Humes et al. found that renal proximal tubule cells cultured as a monolayer formed lumens

with polarized epithelial layers, microvilli, and tight junction complexes when exposed to transforming growth factor β -1 and trans-retinoic acid, but not in the absence of these factors [43]. After culturing, the cells were incorporated into hollow fibers and connected through an extracorporeal circuit to dogs with renal failure. The hollow fibers containing proximal tubule cells increased the level of activated vitamin D (1–25 dihydroxy-Vitamin D₃) by 5.8 pmol/ml from the uremic dog's pre-treatment baseline over the course of 3 days, whereas dogs with sham-control hollow fibers had activated vitamin D levels decreased by 4.0 pmol/ml from their pre-treatment level [44].

Noninvasive monitoring and imaging

The next major advancement in bioreactor design will be the ability to monitor organ growth and development using noninvasive imaging detection that can provide a measurement of parenchymal growth as cells reconstitute an organ scaffold. The most useful metrics of organ growth typically require interrupting the bioreactor culture to perform an invasive analysis that may introduce infection into a long-term organ culture. This typically involves sampling the media to assess for synthesis and secretion of organ-specific proteins or performing a tissue biopsy on the growing organ for hematoxylin and eosin staining or assessment of other markers for cell survival, proliferation, and differentiation. Although biopsies are not useful for small rodent organs due to the organ size, for porcine organs they can provide many of the benefits of organ sectioning in a minimally invasive manner. Measurement of oxygen consumption within an organ can noninvasively provide information on changes in cell content and/or metabolic activity. Oxygen uptake can be measured by placing pO₂ probes at the inlet and outlet of the organ (Figure 2A).

New, noninvasive methods to evaluate organ and cell growth are near-infrared (IR) imaging and micro computed topography (CT). Bioreactors can be constructed to accommodate these imaging devices and it may not be necessary to remove the organ from the bioreactor for non-invasive organ assessment. IR imaging in the second near-infrared window allows for deep tissue imaging with limited tissue scattering and auto-fluorescence [45•]. The IR energy causes excitation of fluorescent particles injected into the organ. This allows for imaging in real time. With IR imaging it is possible to differentiate flow patterns within organs and determine leaky portions.

Micro CT can achieve a resolution of 50 μ m for tissue, but it may take minutes to complete a single scan. CT is based on x-ray penetration, so it has limited resolution of soft tissue. It is common to use a contrast agent made from an iodine salt to enhance resolution [46]. The use of micro CT to evaluate tissue grown in a bioreactor has been limited to date, but an early report from Porter et al. describes the use of this modality to follow the mineralization of bone fragments over time [47]. Using this information, the development and the structural integrity of an organ can be readily analyzed. Data gathered from these noninvasive image modalities can then be further interpreted using powerful computers to form mechanical models of the tissue [48]. Micro CT has also shown the capability to resolve ischemia within the liver at a level of definition matching that of magnetic resonance imaging [49], and may one day be used to detect regions of poor organ perfusion in a bioreactor.

Conclusions and future prospects

Bioartificial organs have the potential to bridge the gap between the supply of transplantable organs and the growing demand for them. In order for bioartificial organs to succeed and research in this area to expand, further development of bioreactors is critical. Bioreactors have a longstanding history in cartilage and bone engineering, but the development of complex culture systems for organ development is not yet well established in the literature.

Tissue engineering and regenerative medicine are broad fields and require the close collaboration of physicians, biological scientists, and engineers. Important features of bioreactor systems that will be required to maximize organ development include: 1) non-invasive monitoring of physiologically relevant parameters, 2) automation of critical parameters, 3) disposable or easily sterilized culture vessels, and 4) stimulation and flow dynamics for optimal organ maturation.

Incorporation of environmental sensors into bioreactor design, refinement of micropatterning techniques, and development of noninvasive monitoring of bioscaffold properties and organ growth will help develop culture conditions to better mimic *in vivo* organ development. The use of animal scaffolds from pigs and other large mammals will help in the establishment of organ models to complement improvements to bioreactor design [50–52]. Together, these model systems coupled with improvements in the selection of cells and techniques used to repopulate tissues will facilitate the translation of this technology to clinical applications [53,54].

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Abbreviations

ECM	Extracellular matrix
EGTA	Ethylene glycol tetraacetic acid
SDC	Sodium deoxycholate
FGF	Fibroblast growth factor
EDTA	Ethylene diamine tetraacetic acid
SDS	Sodium dodecyl sulfate
CSTR	Continuous stirred tank reactor
IR	Infrared
CT	Computed topography

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Highlights

- Decellularizing a non-transplantable organ provides the extracellular matrix scaffold for a new organ
- The vascular matrix facilitates cell and nutrient delivery to the developing organ
- Bioreactors must be tailored to individual organs including specific stimuli
- Recellularized bioartificial rat lungs transplanted into animals have demonstrated limited function
- Key challenges for bioreactor design include non-invasive monitoring of physiologically relevant parameters

Figure 1 (and Graphical abstract).

This illustration depicts the process of bioartificial organ engineering. Cells are removed from non-transplantable organs in a decellularization bioreactor. The resulting extracellular matrix scaffold is then reseeded within a specialized perfusion culture system that mimics the *in vivo* environment and provides for optimal organ development.

Figure 2. General bioreactor layout and design. A) Typical process flow diagram for a bioreactor circuit with sensors before, within or after the bioreactor system. B) Circular bioreactor. C) Dynamic reactor adapted from a CSTR. A stir bar is located in the bottom to mix the liquid.

Table 1
Stability of selected plastics to radiation [18]

This table lists commonly used polymers and their ability to withstand radiation. Adapted from “A Review of the Effects of Gamma Radiation on Pharmaceutical Materials” [18].

Material	Radiation Stability
Polycarbonate	Good
Polyethylene	Good
Polypropylene	Poor
Polystyrene	Excellent
Polysulphone	Excellent
Polytetrafluoroethylene	Poor
Polyvinylchloride	Good

Table 2
Current state of perfusion bioreactor organ engineering

This table illustrates the current state of perfusion bioreactor organ engineering. Hearts, livers and lungs from rodents and lungs from non-human primates have been recellularized in perfusion bioreactors. Rodent lungs and livers have been implanted into recipient rodents for a limited duration (liver 8 hours, lungs 7 to 14 days). Porcine kidneys have been decellularized in a perfusion system, but recellularization is complicated due to the specialized function of renal epithelial cells and difficulty in isolating renal progenitor cells in sufficient quantity.

Organ	Bioreactor Design	Implantation	State of development
Heart	Perfusion bioreactors for recellularization have been used for cardiac patches [36–39] and whole organ recellularization [10]. Most bioreactors incorporate electrical or mechanical stimulation to induce stretching [10,36–39].	Surgically created defects in the ventricle of rodent hearts have been repaired with tissue engineered myocardial patches in a rodent heterotopic heart transplant model [55]. Cardiac patches have also been used to repair infarcted heart muscle in rats [56].	Mechanical and electrical stimulation in a bioreactor enhanced the contractile function of cardiomyocytes almost to the level of native cells [36–39]. At one month, cardiac patches showed seamless integration and vascularization with surrounding normal tissue [55]. Patches placed on infarcted hearts showed decreased scarring, reduced dilation and improved ventricular function [56].
Lung	Both media infusion through the vasculature and gas distension of lung parenchyma in a perfusion bioreactor enhanced biomechanical properties of engineered lungs during recellularization [14,31].	Tissue engineered rat lungs were implanted into immunocompromised rodent recipients [14,31].	Rodents receiving a single tissue engineered lung transplant had superior oxygenation to pneumonectomy controls at day 7 while breathing 100% O ₂ [31•].
Liver	Rodent livers have been decellularized and recellularized in bioreactors. These reactors provided inflow through either the portal vein or the inferior vena cava [4,5,22].	Recellularized liver grafts have been implanted into anticoagulated rats for 8 hours [22].	Hepatocyte function was modestly reduced in liver scaffolds compared to collagen sandwich cultures [22].
Kidney	Large perfusion bioreactors have been constructed for porcine kidney decellularization consisting of multiple perfusion circuits allowing for simultaneous decellularization of several kidneys. Organs are perfused through the renal artery and fluid exits through the renal vein [9,12].	Decellularized pig kidneys were implanted into the abdominal cavity of age matched pigs and sutured to the recipient aorta and vena cava [12•].	The decellularized grafts maintained integrity, but were fully clotted upon retrieval. Decellularized grafts were perfused with increasing pressure <i>in vitro</i> to show that the scaffold could withstand physiological pressure [12•].