Bioengineering for Organ Transplantation: Progress and Challenges

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Abbreviations: ACL, anterior cruciate ligament; CYP 450, cytochrome P 450; DRT, decellularization-recellularization technology; ECM, extracellular matrix; ESC, embryonic stem cell; ESRD, end stage renal disease; HUVEC, human umbilical vein endothelial cell; iHIOs, induced human intestinal organoids; iMPC, induced multipotent progenitor cell; iPSC, induced pluripotent stem cell; MEMS, microelectromechanical systems; MSC, mesenchymal stem cell; RAD, renal assist device; SIS, small intestine submucosa; SNM, silicon nanopore membranes; VCA, vascularized composite allografts.

Organ transplantation can offer a curative option for patients with end stage organ failure. Unfortunately the treatment is severely limited by the availability of donor organs. Organ bioengineering could provide a solution to the worldwide critical organ shortage. The majority of protocols to date have employed the use of decellularization-recellularization technology of naturally occurring tissues and organs with promising results in heart, lung, liver, pancreas, intestine and kidney engineering. Successful decellularization has provided researchers with suitable scaffolds to attempt cell reseeding. Future work will need to focus on the optimization of organ specific recellularization techniques before organ bioengineering can become clinically translatable. This review will examine the current progress in organ bioengineering and highlight future challenges in the field.

Introduction

As of May 2015, over 123,000 people were on the waiting list for an organ in the USA alone.¹ As a result of this critical organ shortage, 6,885 patients died in the USA while awaiting an organ transplant in 2014.¹ Bioengineered organs could provide an inexhaustible organ source and carry the potential benefit of requiring an immunosuppression-free state.² ³ Successful outcomes have been reported with simple, hollow organs including their production and implantation.⁴ ⁶ The more complex modular organs have proved a greater challenge. There have been considerable advances in this new field but a clinically relevant model still remains elusive. This article explores the achievements of bioengineering organs to date and identifies future challenges.

Decellularization-Recellularization Technology (DRT)

Decellularization

Whole organ bioengineering first requires a scaffold to allow cells to develop and function.² ⁷ The extracellular matrix (ECM) performs this task in vivo. Current approaches to achieving a bioengineered organ construct have employed the use of a supporting structure generated by decellularization of naturally occurring human or animal tissues and organs.⁸ In doing so, allogeneic and xenogeneic cellular antigens are removed allowing the probable elimination of immunogenicity.⁸ Ott et al. (2008) have demonstrated a successful protocol for decellularization of a rat heart.⁹ In doing so, the ability to generate a biocompatible organ construct entered the realms of possibility. The technique produced a “complex, biocompatible cardiac ECM scaffold with a perfusable vascular tree, patent valves and a 4-chamber-geometry template for biomimetic tissue engineering.”⁹ Further protocols were subsequently developed but none were considered ideal for generating intact scaffolds with products either containing residual cell debris or resulting in destruction of ECM proteins.¹⁰ Effective decellularization protocols have since become established and in general, include the use of “physical, ionic, chemical and enzymatic methods,” with the organ’s own vascular network being used for detergent delivery.¹¹ ¹² The result is an intact acellular organ scaffold of ECM that retains the structure of the original organ that supports cell attachment, proliferation and integration.¹³

Recellularization

Once a satisfactory organ scaffold has been produced, recellularization is required to achieve a functional organ product for implantation. The principles of recellularization include obtaining a renewable source of cells, cell seeding onto the scaffold and finally organ specific culture.¹⁴ Significant work has already been carried out to establish effective and reproducible protocols that create functional organ products.
The first important goal is to identify an ethically approved and renewable source of cells for scaffold seeding. Fetal and adult cells, embryonic (ESC), mesenchymal (MSC) and induced pluripotent stem cells (iPSC) have all been used. MSCs have shown promise, being easily and ethically obtained from bone marrow stroma or adipose tissue. In addition, they have shown a good degree of differentiation, attachment and persistence on organ scaffolds. iPSCs, in particular, have also shown potential. They are created via the reprogramming of human somatic cells and exhibit characteristics that resemble embryonic stem cells. iPSCs have been efficiently differentiated into alveolar epithelial cells and myocytes but their adhesive potential to organ scaffolds has been inferior to that of MSCs. Interestingly, although immature iPSCs prompt a detrimental immune response, self tolerance has been achieved with terminally differentiated iPSCs, further paving the way for their use in organ bioengineering.

Cell seeding techniques are largely organ specific. Perfusion seeding via the vasculature is common to all organs but non-vascular routes (e.g. airway and ureter, in lung or kidney respectively) have also shown good results. The use of multiple perfusion routes, optimization of scaffold coating, mechanical environment and rate of cell perfusion have shown promise in improving cell concentration and diffusion over the scaffold.

Successful seeding of the scaffold vasculature has been essential to the survival of a bioengineered organ. The majority of recellularization protocols to date have used endothelial cells but more recent work has shown potential benefit with iPSCs. Interestingly, the success of vasculature seeding techniques has previously been shown to be largely dependent on perfusion flow rates.

Cell culture is required after sufficient cell seeding onto an organ scaffold. Delivery of nutrients to the entire 3-dimensional organ construct requires a bioreactor for perfusion and to supply an environment that encourages cell growth in an organ specific manner.

**Organ Specific Progress and Challenges**

**Heart**

The accomplishment of attaining a viable decellularized heart scaffold was a major turning point in the pursuit of achieving a bioengineered organ. In 2008, Ott and colleagues constructed a suitable heart scaffold from a rat heart, before reseeding and maturing the matrix to develop a structure capable of contracting and responding to drugs. This was the first protocol that produced a recellularized, functional organ scaffold. Further studies have demonstrated the potential of decellularized heart ECM to direct differentiation of progenitor cells, notably iPSCs, into those of cardiac lineage. Seeding techniques have employed the use of perfusion via the aorta and coronary arteries. However, there has been universal difficulty in establishing adequate cellularization of the ventricular wall. Direct injection attempts have resulted in dense cellularity at the injection site with poor distribution throughout the remainder. The first evidence of the potential of a scaled-up model was recently reported using porcine heart scaffolds. Successfully decellularized porcine hearts were reseeded with murine cardiomyocytes and human umbilical vein endothelial cells (HUVECs) resulting in a construct that exhibited intrinsic electrical activity. Further work is still required to achieve a fully functional bioartificial heart of appropriate clinical scale with focus on suitability of cardiac cell sources (including the use of conspecific cells), enhancing reseeding techniques to promote sufficient dispersion, and optimization of in vitro organ culture.

**Liver**

Optimization of protocols for the perfusion decellularization of other organs led to the generation of a 3 dimensional (3D), vasculature-intact liver scaffold from small animal and porcine models. The ECM composition and vasculature network was successfully retained while removing the cellular components, enabling adequate reseeding. HUVECs, human fetal liver cells or primary rat hepatocytes were perfused via the portal vein and/or vena cava resulting in the development of engrafted and functional cells typical of the native liver (e.g., CYP 450 and α-fetoprotein expression, production of urea and albumin). Liver bioengineering has been hindered by the struggle to achieve adequate hepatocyte proliferation once transplanted. Investigation of the suitability of iPSC derived adult hepatocytes have been disappointing in their ability to repopulate the liver. Human fibroblasts have since been reprogrammed to form an
induced multipotent progenitor cell (iMPC) and have shown promise in their ability to proliferate and successfully repopulate after transplantation. Further investigation is required to identify whether these iMPCs can successfully repopulate a decellularized liver matrix on a clinical scale and perform sufficient native hepatocyte function.

Pancreas

Attempts at bioengineering pancreatic tissue can be classified into 3 main areas: islet encapsulation, biomaterial carriers and whole-organ bioengineering. Islet encapsulation aims to isolate implanted islet cells from the recipient immune response by hiding the non-self antigens. Unfortunately, difficulties with biocompatibility of capsule materials, inadequate immunosolation and poor vascularization have limited successful clinical translation. Biomaterial carriers involve the use of bioartificial scaffolds that mimic native ECM to allow seeding of pluripotent cells and have yielded promising initial results. The decellularization-recellularization approach has since been applied to the pancreas. Successful decellularization and recellularization of mouse pancreata with both endocrine and exocrine cells has been achieved, with cell types displaying appropriate functionality (C-peptide and amylase secretion respectively) and differentiating in their appropriate locations. It was noted that insulin gene expression was greater in cells on the 3D scaffolds compared with 2D giving further evidence for possible benefits of whole organ bioengineering. Future directions will need to establish DRT methods to protect the bioengineered organ in vivo from the recipient autoimmune response in type 1 diabetic patients.

Intestine

Intestinal bioengineering remains in the very early stages of development. ECM scaffolds constructed from small intestine submucosa (SIS) have previously been implanted into dogs and have demonstrated development of tissue architecture comparable to native bowel. These tissues failed to demonstrate architectural organization or develop any enteric neurons thereby significantly limiting their function in vivo. The development of induced human intestinal organoids (iHIOs) has become a promising avenue for intestinal bioengineering as they contain enterocytes, enteroendocrine cells, goblet cells, Paneth and mesenchymal cells. These organoid units have been successfully incorporated into synthetic scaffolds of both small intestine and colon, and implanted into small animal models after resection with basic function intact. In addition, intestinal smooth muscle has been effectively engineered via fibroblast seeding of scaffolds and the use of growth factor. More recently, researchers have successfully decellularized small intestine segments and performed recellularization with bone marrow derived stem cells. Their work resulted in an intact mucosa, villi, crypts, blood vessels and abundant smooth muscle cells in the muscula-

Kidney

Several strategies have been employed in the development of a bioartificial kidney. Similarly to the other organs previously discussed, recellularization of decellularized scaffolds has shown promise. Decellularization of kidney tissue slices and whole organs has been documented with maintenance of ECM properties and characteristics. Song et al. (2013) decellularized rat, porcine and human kidneys creating intact vascular, glomerular and tubular compartments. They went onto achieve scaffold repopulation with cells that were capable of producing urine in vitro and in vivo. Interestingly, they observed site-specific adhesion and development of polarity during cell seeding. Glomerular filtration, glucose and electrolyte absorption, and macromolecular sieving was noted but found to be functionally immature when compared to cadaveric kidneys. To facilitate translation toward clinical use, further work is necessary to optimize seeding regimens and organ culture in human-sized scaffolds.

Other approaches have investigated the possibility of creating an ex vivo bioartificial device. Current haemodialysis techniques in ESRD focus on solute clearance but fail to address the metabolic, endocrine and transport functions physiologically provided by a normal kidney. The Renal Assist Device (RAD) was therefore developed by Humes et al. to address these shortcomings via cell seeding onto the inner surface of the hollow fibers used for microfiltration in a hemofilter. It was noted that the technique provided metabolic, endocrine and active transport functions in addition to solute transport and was associated with a significant mortality benefit at 28 d compared with standard haemofiltration in humans. Further efforts have investigated the feasibility of producing a device on a scale suitable for implantation by engaging the use of microelectromechanical systems (MEMS) technology and silicon nanopore membranes (SNM). Similarly to a natural kidney, this technology can be thought of as having 2 main functional units. Firstly, the filtration unit, containing SNM that is able to function like a physiological glomerulus by filtering substances depending on their molecular weight. Secondly, the reabsorption unit, where renal epithelial cells are seeded on a SNM scaffold and facilitate the reabsorption of water and solutes in addition to providing the metabolic and endocrine renal functions. Despite the progress, significant optimization of ex vivo bioartificial systems are required before the development of a clinically viable device.

Composite tissue bioengineering

The bioengineering of composite tissue grafts has become an avenue of interest, specifically to orthopaedic and plastic surgeons. Trauma to the hard and soft tissues often results in damage to multiple different tissue types. The construction of interfaces between musculoskeletal tissues and the integration of sufficient vascularity are complex tasks that hold the key to achieving functional integration. Similarly to whole organ bioengineering, work has focused on the production of scaffolds in order to provide structural support and proliferation cues to seeded cells by mimicking the role of the natural ECM.
The development of a tri-phasic scaffold by Spalazzi et al. has shown promise in the development of a ligament-bone interface (anterior cruciate ligament (ACL) to bone).

The scaffold consists of 3 different, but continuous phases that promote the formation of the distinct tissue regions required: fibroblast and soft tissue, fibrochondrocyte, and bone formation. These stratified scaffolds have demonstrated the ability to promote formation of the necessary fibrocartilage interfaces in ACL reconstruction grafts. They have also been successfully used to create both cartilage and bone on a single scaffold with subsequent steps made toward achieving an osteochondral interface.50

Tendon-bone interfaces have employed the use of biomimetic ECM nanofiber scaffolds and have shown potential, partly secondary to the ability to modulate cell development by adjusting nanofiber alignment.50

The recent success of clinical vascularized composite allografts (VCAs), including face, upper extremity, lower extremity and interdigital tissues.51 The difficulty in the engineering of such constructs lies in achieving the integration and cooperation of different tissue types in vivo, while maintaining a viable blood supply.51

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