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REVIEW

Regenerative medicine as applied to solid organ transplantation: current status and future challenges

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Summary

In the last two decades, regenerative medicine has shown the potential for ''bench-to-bedside'' translational research in specific clinical settings. Progress made in cell and stem cell biology, material sciences and tissue engineering enabled researchers to develop cutting-edge technology which has lead to the creation of nonmodular tissue constructs such as skin, bladders, vessels and upper airways. In all cases, autologous cells were seeded on either artificial or natural supporting scaffolds. However, such constructs were implanted without the reconstruction of the vascular supply, and the nutrients and oxygen were supplied by diffusion from adjacent tissues. Engineering of modular organs (namely, organs organized in functioning units referred to as modules and requiring the reconstruction of the vascular supply) is more complex and challenging. Models of functioning hearts and livers have been engineered using ''natural tissue'' scaffolds and efforts are underway to produce kidneys, pancreata and small intestine. Creation of custom-made bioengineered organs, where the cellular component is exquisitely autologous and have an internal vascular network, will theoretically overcome the two major hurdles in transplantation, namely the shortage of organs and the toxicity deriving from lifelong immunosuppression. This review describes recent advances in the engineering of several key tissues and organs.

Introduction

In 2006, Atala *et al.* implanted bladders engineered *ex vivo* from the seeding of autologous cells onto artificial supporting scaffolds [1]. The recent report on the implantation of the trachea manufactured from human components, received the well-deserved coverage by the media across the world [2]. For the first time, an organ was produced from autologous differentiated cells and stem cells (SC). Enormous enthusiasm was generated also in the transplant community. Transplant specialists perceived for the first time that regenerative medicine (RM) has the potential to solve the problem of the shortage of organs available for donation. We believe that it is timely and critical to illustrate the state-of-the-art of the investigations in the field of RM as applied to solid organ transplantation.

Heart

One of the main objectives in cardiac restoration therapy is to augment the damaged cardiac muscle following an

infarct, by engineering functional myocardium. The earliest attempts at cardiac restoration therapy in humans were focused on the direct injection of either circulating progenitor cells or bone marrow-derived progenitor cells into the infarcted myocardium [3]. Although some studies showed an improvement in cardiac function following intravascular injection of such progenitor cells [4,5], the percent of surviving cells in the infarcted myocardium was generally very low [6,7]. The low cell survival following direct cell injection motivated the use of biomaterials.

The classic approach to use biomaterials for cardiac regeneration therapy has been to implant a cardiac patch made from a scaffold seeded with cardiac cells. Zimmermann used neonatal rat cardiomyocytes embedded in a collagen gel and subjected then to mechanical stimulation to improve the contractile properties of the patch [8]. Integration of the patch within the native muscle, as well as improvement in cardiac function was demonstrated. Later, Leor *et al.* used alginate sponges seeded with fetal cardiomyocytes and implanted into the infarcted rat myocardium [9]. After 9 weeks *in vivo*, only a small portion of the grafted patch was occupied by cardiomyocytes, whereas most of the alginate scaffold was filled with collagen fibers and scattered fibroblasts.

A cardiac tissue patch was also created using the cell self-assembly approach pioneered by Okano [10]. In this approach, cell sheets were cultivated and detached from their culture substrate by using a temperature-responsive polymer substrate. Using this multistep transplantation procedure, a 1-mm thick cardiac tissue sheets was implanted onto infarcted adult rat myocardia.

An emerging and promising field in cardiac bioengineering is injectable biomaterials for cellular cardiomyoplasty. Injection of a liquid biomaterial which can then be solidified *in situ* will not impose a fixed geometry on the heart muscle as with a cardiac patch. Moreover, injecting the biomaterial into the scar tissue allows for an intimate contact between the injected cells and the host tissue, and, more importantly, an injectable therapy can be administered using a less invasive procedure. Christman pioneered investigations on a fibrin glue biomaterial as an injectable scaffold to deliver myoblasts to the ischemic myocardium [11]. They reported that the fibrin significantly increased cell survival after 5 weeks.

Seliktar proposed a new type of injectable biosynthetic material based on fibrinogen to be used as a cell carrier in cardiac cell therapy. The biomaterial is made by conjugating poly-ethylene glycol (PEG) with fibrinogen to form a liquid precursor which is then assembled into a hydrogel matrix *in situ* using nontoxic photo-polymerization [12]. This formulation allows for the control of the hydrogel's degradation rate, while reducing the overall inflammatory response to the fibrinogen graft. Moreover, controlling the mechanical properties of the hydrogel by altering the composition of the matrix is also possible [13]. The PEGylated fibrinogen hydrogel was injected into infracted adult rat hearts together with neonatal rat cardiomyocytes or human embryonic stem cells (ESC) derived cardiomyocytes. When using the PEGylated fibrinogen biopolymers, cell survival was increased and the overall cardiac functionality was significantly improved after 30 days, as evaluated by echocardiography (26% improvement in percent factional shortening change) (M. Habib, K. Shapira-Schweitzer, O. Caspi, A. Gepstein, G. Arbel, D. Aronson, D. Seliktar, L. Gepstein, unpublished data).

More recently – and with a tremendous potential for the field of organ bioengineering – Ott *et al.* have published a novel method of perfusion decellularization that is able to generate whole organ scaffolds [14]. The insertion of a cannula into the ascending aorta allowed retrograde coronary perfusion with detergents. Such method achieved the complete removal of the cellular compartment of a whole heart that was later repopulated with neonatal rat cardiomyocytes. These latter were delivered within the heart scaffold through transmural injection, while endothelial cells were injected through the aorta. The construct was able to contract up to 2% of the normal contractile function.

Liver

Hepatocyte transplantation is the most valuable alternative to whole liver transplantation. Since its first attempt into a patient with familial hypercholesterolemia [15], several other cases have been performed to cure different livers diseases with nonconvincing results [16–25]. These failures may be attributed to the relatively small number of hepatocytes that engraft in the recipient because of the quality and quantity of infused cells, as well as immunosuppression-related toxicity. Nonetheless, transplantation of a number of hepatocytes corresponding to 1–5% of the total liver mass has been able to show a positive impact in transplanted patients [26].

As a result of the shortage of available human hepatocytes for transplantation, other cell sources have been identified and used. Specifically, bone marrow-derived mesenchymal SC [27], hematopoietic SC [28,29] and fetal liver progenitor cells [30,31] have shown to improve to a certain extent the condition of cirrhotic patients. Fetal liver progenitor cells also hold an enormous potential for cell/RM therapies because of their expansion and differentiation capabilities into hepatocytes and biliary epithelium [31]. While these alternative SC sources have been explored, significant advances were made using ESC and induced pluripotent SC (iPS) to create hepatic cells by using defined soluble growth factor signals that mimic

embryonic liver development [32,33]. Embryonic SC-derived hepatic cells, once transplanted into rodent livers, were able to engraft and express several normal hepatic functions [34]. However, more extensive characterization, as well as further safety evaluation, is needed to determine whether these cells will fully function as primary adult hepatocytes.

In addition to cell injection therapies, two experimental approaches may have a reasonable chance for clinical translation quicker than many others. The first experimental approach is the cell sheet technology developed by Okano in Japan [35]. Its simple configuration and fabrication allows for the stacking of up to four hepatocyte cell sheets that can readily engraft and provide a defined metabolic relief to the recipient [36]. More recently, Baptista *et al.* were able to use a perfusion decellularization technique to liver, pancreas, intestine and kidney generating decellularized organ scaffolds for organ bioengineering [37]. In an analogous fashion, Uygun *et al.* have just published a comprehensive approach of decellularizing rat livers and recellularize them with rat primary hepatocytes, showing promising hepatic function and the ability of heterotopically transplant these bioengineered livers into animals for up to 8 h [38]. This technology has the potential to translate into human liver bioengineering, which may offer readily available organs for drug discovery applications and for transplantation, overcoming organ availability.

Kidney

The complex anatomy and physiology of the kidney pose a tremendous challenge for scientists to develop functional self-sustaining renal substitutes. The current investigational approaches for renal regeneration are of three types: tissue engineering (TE), developmental biology and SC.

Four centers have decellularized rodent [37,39,40], porcine [37] (http://www.faqs.org/patents/app/20090202977) and rhesus monkey [41] kidneys to produce a scaffold with preserved extracellular matrix (ECM) and vasculature. As in the case of heart and liver bioengineering, kidney ECM maintains its natural characteristic in terms of protein and growth factors content. Ross *et al.* seeded rat renal ECM with murine ESC through the artery and ureter. Cells proliferated and repopulated within the glomerular, vascular, and tubular structures. Interestingly, cells lost their embryonic phenotype and turned on expression of Pax-2 and Ksp-cadherin, which are normally expressed in the ureteric bud (UB), the induced metanephric mesenchyme (MM) and the distal nephron tubular cells at late developmental stages [40]. Noteworthy, a perfectly intact vascular tree, present in the kidney ECM but lacking in artificial polymeric scaffolds, will grant the physiological delivery of oxygen and nutrients rather than only through diffusion [37].

Embryologic precursors of the urinary tract are being used to engineer kidneys, under specific culture conditions and the adoption of developmental biology technique. Kidneys develop from interactions between cellular components of two embryonic structures from which the entire adult nephron derives, namely the UB arising from the Wolffian duct and the MM. Combination of cells isolated and expanded from both UB/MM were seeded in a three-dimensional (3D) ECM gel, in the presence of conditioned media from an MM cell line or a medium containing hepatocyte growth factor and an epithelial growth factor receptor ligand [42,43]. Investigators were capable of inducing both branching morphogenesis in Wolffian duct tissue in cultures combining UB and MM cells, and MM epithelialization and tubulogenesis with apparent duct-like tubules with lumens. *In vitro*, culture of full UB and MM in presence of similar growth factors lead to a primordial kidney structure referred to as metanephroi, complete with its parenchyma and collecting system [43]. *In vivo* implantation of metanephroi in different rodents' models showed survival and generation of concentrated filtrate [44–46].

The human kidney has an intrinsic capability to repair after injury [47]. The repair process is accomplished by migration of new cells (stem/progenitor cells) into the damaged region, with eventual reconstitution of a functional epithelium. Such progenitors have been identified in resident epithelial cells, activated renal macrophages and glomerular parietal epithelial cells, but investigators believe that SC cells with broader regenerative properties are endowed in niches located in the proximal tubuli, glomeruli, papilla and peritubular capillaries, as well as urine itself [48]. Investigators are currently identifying niches within the kidney where SCs with regenerative capacities are most likely endowed. Such SC should express the phenotype for MM epithelial precursor cells.

Overall, RM for renal diseases is at its infancy and far from being established. The renal assist device is the only engineered kidney having completed phase II clinical trials in acute renal failure complicating sepsis, with encouraging *interim* data [49,50].

Pancreas

Regeneration of insulin-producing β-cells is a major goal for RM. The limited availability of human islets for transplantation and severe complication of immune-suppression therapy encourage scientist to search for a cell-based approach that produces large numbers of transplantable β-cells or islets to meet the needs of the many millions of diabetic patients.

Historically, attempts to produce large numbers of functional β-cells have utilized ESC and staged culture techniques that recapitulate the pancreatic ontogeny of β-cells [51]. Using ''cocktails'' of differentiation factors, ESC and other SC types can be directed to differentiate into endoderm, pancreatic progenitors, insulin-producing cells, and finally cells with some but not all of the phenotypic and functional characteristics of β-cells [52–54]. Chandra *et al.* found that adipose derived SC differentiated into insulin-producing cells in culture and normalized blood glucose levels after transplantation into diabetic mice [55]. Despite the inability to obtain actual β-cell or islets, human ESC and other SC types differentiated along the pancreatic lineage, display the ability to respond to glucose and secrete insulin when transplanted into mice, suggesting that the transplant ''environment'' provides the necessary signals to induce terminal differentiation [54–56].

Introduction of and ectopic expression of pancreatic transcription factors such as Pdx1 and MafA, known as direct reprogramming, increases the efficiency of derivation of pancreatic-like cells from SC. Reprogramming techniques could shorten the steps necessary to differentiate SC into terminally committed cell types. Chiou *et al.* found that constitutive expression of MafA facilitated differentiation of placental-derived SC into insulin-producing cells able to respond to high glucose levels *in vitro* and, after transplantation, restore euglycemia in diabetic mice [57]. Induced pluripotent SC and amniotic fluid SC might also differentiate into β-cells under the influence of appropriate genetic signaling. However, greater control over expression of introduced transcription factors is probably necessary to predictably guide SC to the desired cell phenotype [58].

Scaffolds or tissue constructs might serve as adjuncts to current cell-based approaches aiming to produce large numbers of functional pancreatic endocrine cells. An environment that allows 3D contacts may be necessary for appropriate terminal differentiation and functional phenotype *in vitro* and *in vivo* and appear to increase the efficiency of cell-based approaches for pancreatic islet TE [59,60]. However, islets, which have the ability to engraft and function within the liver, can simply be transplanted by percutaneous access to the portal vein, whereas a whole organ would require revascularization and possibly even exocrine drainage; which is clearly a more invasive procedure. Besides the challenge of obtaining large numbers of functional β-cells, cell-based therapies are faced with the risk of tumor formation and attack by the immune system (both allogeneic and autoimmune responses). Derivation of and the ability to differentiate SCs from patients may address some of these challenges, but the autoimmune nature of type 1 diabetes could still be problematic, and may require effective immunoisolation of the cells by microencapsulation prior to transplantation [61].

Airways

The larynx is a complex organ responsible for protecting the lungs from aspiration. Complete replacement will require the ability to engineer whole neuromuscular units, although there is immediate requirement for structural replacement following trauma, tumor or stenosis [62].

The respiratory organ on which RM has had most impact, however, is the trachea. After the first report of a clinically significant tracheobronchial defect treated with the implantation of an acellular bioartificial airway patch [63,64], Macchiarini *et al.* restored normal function to a 30-year-old mother whose tracheobronchial tree had been damaged by tuberculosis [2,65,66]. An SC-based bioengineered airway trachea was manufactured *ex vivo* and transplanted with immediate normalization of lung function. Briefly, a decellularized trachea retrieved form a deceased donor was seeded with autologous SC-derived chondrocytes and epithelial cells. The construct was allowed to mature into a bioreactor for 7 days, before implantation. Later, the same authors used an updated, intraoperative technique to graft a 7-cm trachea into an 11-year-old boy with congenital stenosis, with excellent early results. In this case, the new organ was implanted without undergoing any maturation period *ex vivo* (M. J Elliott, M. A Birchall, D. Roebuck, S. Speggiorin, A. Fierens, L. Cochrane, C. Doyle, D. Vondrys, P. DeCoppi, M. Lowdell, P. Macchiarini, unpublished data).

Lung bioengineering remains a major challenge, possibly in reason of the complexity of the tissue and the variety of cell types present in the lung. Lungs have been regenerated through the seeding of pulmonary epithelium and vascular endothelium on rat lung ECM [67,68]. Lung decellularization allows complete cell removal, while retains the hierarchical branching structures of airways and native vasculature. The mechanical characteristics of the above-mentioned constructs were comparable with those of native lungs, and when implanted into rats *in vivo*, the engineered lungs were effective in gas exchange.

Noteworthy, a biologically inspired bioengineering approach has been recently described by Ingber *et al.* [69]. By applying nano- and microscale engineering technologies, the authors developed a cutting-edge multifunctional microdevice able to reconstitute the functional alveolar-capillary interface and to reproduce complex responses to bacteria and inflammation. They first microfabricated a microfluidic system containing two closely apposed microchannels separated by a thin, porous, and

flexible membrane. Thereafter, both sides of membrane were coated with ECM, on which human microvascular endothelial and alveolar epithelial cells were eventually cultured. The engineered construct reproduced key structural, functional and mechanical properties of the fundamental functional unit of living lungs. In doing so, authors provided proof that development of cell-based biochips that reproduces complex organ-level responses could revolutionize fields that currently rely on animal testing and clinical trials [69].

Digestive tract

Despite the first pioneering investigations in the field of intestinal bioengineering date back to the 1980s [70], the initial excitement has been blunted by the considerable limitations and roadblocks encountered in the course of experimental investigations. The main culprit of such stagnation is the complexity of intestinal anatomy and the various functions of the intestine.

The early observation that enteric cells at the interface between a synthetic material used to patch a full thickness defect within the small intestine of a rodent and the native mucosa can migrate into the bare area to regenerate organized epithelium [71], paved the ground for future investigations. Vacanti's group in Boston processed neonatal rodent intestine to obtain partially digested pieces of intestine, referred to as organoid units. These units were seeded on a nonwoven polyglycolic acid (PGA) fiber and the constructs were implanted in rats having undergone the resection of 85% of their native intestine, to mimic short gut syndrome [72–75]. Bioengineered intestinal constructs were able to partially replace gut function in rats and, more recently, similar results have been described in pigs [76]. However, this technology is time consuming and expensive, as several centimeters of bowel are needed to obtain a sufficient number of organoid units able to repopulate just a few centimeters of engineered intestine. On the other hand, organoid units cannot be cultured and grown easily *in vitro*. In the future, the requirement for organoid units might be overcome by using SC to generate organoids [77].

Stem cells hold a great promise for intestinal bioengineering. As any other tissue in the body, the intestine hosts a population of slowly cycling SCs that maintain tissue homeostasis. The entire intestinal epithelium may be completely replenished every 3–4 days [78,79]. Intestinal SCs are believed to reside in the base of Lieberkuhn crypts and express Lgr5 [79,80]. Very recently, single Lgr5+ cells from intestinal crypts were capable of building crypt-villus structures *in vitro* without any mesenchymal niche [81]. As these cells can be reliably expanded in culture, they could represent the ideal source of progenitor cells for intestinal bioengineering. To further organize functional structures *in vitro* and *in vivo*, several synthetic or natural scaffolds have been adopted to support cell growth and differentiation, with unsatisfactory results [82–85]. To proceed toward clinical translation, it is possible that the engineering of less complex gut structures such as the esophagus may be desirable. A model of bioengineered esophagus has been recently developed in dogs. Oral keratinocytes and fibroblasts were first cultured on human amniotic membrane. Subsequently, the construct was laid over PGA scaffolds seeded with smooth muscle tissue and eventually rolled around a tube. Engineered scaffolds were then wrapped within the omentum and implanted intra-abdominally. Three weeks later, scaffolds developed into an esophagus-like tube showing a well-differentiated stratified squamous cell layer surrounded by a thick smooth muscle-like tissue. Thereafter, a segment of native esophagus was resected and replaced by the so-obtained graft in the same dogs [86].

Corneas

Cornea bioengineering presents three major challenges: functionality, strength, and transparency [87]. A number of reports have shown that progenitor cells, present at the basal surface of the limbus – namely, a specialized niche at the boundary between the cornea and conjunctival epithelium [88,89] – can be isolated and expanded in culture. These cells have been successfully used in animal models to restore corneal function [90–96]. Choi *et al.* investigated a clinically applicable and readily expandable source of human corneal endothelial cells (HCECs). Their data indicate that HCECs can be successfully isolated from residual human sclera rims obtained during routine endothelial keratoplasty [97].

The ideal cell carrier for corneal endothelium should be noncytotoxic, biodegradable, transparent, and have appropriate mechanical properties. In addition, it should be easily integrated into the surrounding tissue and permit sufficient fluid transport between the anterior chamber and the corneal stroma. Several graft materials have been proposed and used as scaffolds for corneal endothelium transplantation [98–106]. Natural scaffolds are normally difficult to handle during the implantation, whereas synthetic scaffolds often integrate poorly with host tissue [107]. In contrast, corneal stroma has a unique ECM organization which provides appropriate mechanical properties and transparency as well as inherent biological properties which are ideal to support cell functions [108]. Previous reports indicate that CECs grow best on corneal endothelial ECM [109,110]. For these reasons, Choi *et al.* developed corneal scaffolds derived from human corneal stromas for corneal endothelium transplantation. Human corneas can

be cut into 120–200 μm thick slices and decellularized. The corneal scaffolds retain ECM components that support cell growth and functions, and maintain basic biomechanical properties [97]. This potentially increases the number of transplants fourfold from each donated cornea and provides a human source of corneal tissue.

Immunology

Very little is understood regarding the host immune response to bioengineered constructs [111,112]. The available literature shows that the host response to the implantation of materials composed of ECM involves both innate and acquired immunity. Such response is affected by construct characteristics, including the source of the scaffolding material, the methods used for manufacturing and the degradation rates. The recipient characteristics such as species and site of implantation may also affect the response [111]. In general, it is assumed that reactions following the implantation of biomaterials include wounding, blood–material interactions, provisional matrix formation, and inflammation. The response of the immune system is vigorous as demonstrated by the onset of an early and acute inflammatory response consisting mostly of polymorphonucleate cells; moreover, cytokine and antibody analysis has revealed the presence of a Th-2 type response in the absence of a Th-1 response, consistent with tissue acceptance rather than rejection [113]. In some cases, such sequence of events leads to chronic inflammation with or without frank foreign body reaction, with formation of a fibrous capsule [114,115]. The consequences of the reaction to the material surface can be devastating, yet constructs may undergo a remodeling process which plays an important role in the successful clinical application of these devices. In fact, the rapid infiltration and proliferation of functional host cells at the implantation site, and the deposition and assembly of new replacement matrix, are essential for the integration of the engineered tissue into its new niche. It is important to note that such response is not comparable with that triggered by allogeneic cellular constructs where the mechanisms of immune rejection would destroy the whole engineered tissue, in the absence of any pharmacological suppression of the immune system.

Final remarks

The need for improved treatment modalities for patients with diseased or absent tissues or organs is evident. RM holds the promise of regenerating tissues and organs by either stimulating previously irreparable tissues to heal themselves, or manufacturing them *ex vivo*. In the first scenario, cells with regenerative potential are targeted to the diseased bodily district. Given the multitude of available sources of such cells, it remains unclear which is the most appropriate cell source. Although this may vary depending on the tissue or organ of interest, it is important to fully understand the biological mechanisms controlling differentiation along a specific lineage of all cell types. Ideally, it is desirable to have the ability to harvest autologous cells and employ them with minimal *ex vivo* manipulation. Ultimately, the goal is to identify cells that can be easily harvested and differentiated consistently along the lineage of interest.

In the second scenario, differentiated cells or SC are seeded on supporting scaffolds and allowed to mature in custom-made bioreactors. Human or animal-derived whole tissue ECM scaffolds are preferred, compared with artificial homogeneous materials, because they preserve an intact vascular network that will allow regeneration of the vascular system for optimal delivery of nutrients and oxygen. The utilization of autologous cells rules out immunological breakdowns and concerns, and limits the response of the immune system to a nonharmful inflammatory reaction.

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