

Tissue Engineering of Tubular and Solid Organs: An Industry Perspective

Joydeep Basu and John W. Ludlow
*Bioprocess Research and Assay Development, Tengion Inc., North Carolina,
USA*

1. Introduction

Tissue engineering and regenerative medicine (TE/RM) represents a broad spectrum of cell and biomaterial based approaches aiming to repair, augment and regenerate diseased tissues and organs. The successful recent clinical implantation of tissue engineered trachea, bladder and bladder derivative (Neo-Urinary Conduit™) has highlighted the emergence of common methodological frameworks for the development of TE/RM approaches that may be broadly applicable towards the regeneration of multiple, disparate tubular organs.

Similarly, recent progress towards regeneration of heart, kidney, liver, pancreas, spleen and central nervous system is identifying shared methodologies that may underlie the development of foundational platform technologies broadly applicable towards the regeneration of multiple solid organ systems. Central themes emerging for both tubular and solid neo-organs include the application of a biodegradable scaffold to provide structural support for developing neo-organs and the role of committed or progenitor cell populations in establishing the regenerative micro-environment of key secreted growth factors and extra-cellular matrix critical for catalyzing *de novo* organogenesis.

However, aspects of these strategies currently under active development for tissue engineering of tubular and solid neo-organs may not be relevant for successful *commercialization* of neo-organs as novel TE/RM *products* for clinical application. For example, difficulties in large scale sourcing and quality control of biomaterials derived from decellularization of cadaveric organs imply that such biomaterials may be less suitable for incorporation into TE/RM products when compared to biomaterials of synthetic origin. Similarly, TE/RM technologies that attempt to leverage populations of stem and progenitor cells are less likely than platforms focused on committed cell populations or acellular biomaterials to facilitate rapid development of viable products.

In this chapter, we will present our experience in the development of the Neo-Bladder Augment™, Neo-Urinary Conduit™ and Neo-Kidney Augment™ to identify elements of this foundational organ regeneration technology platform that may be broadly applicable towards the design and development of additional tubular neo-organ *products*, including the esophagus and small intestine, the lung, the vasculature and the male or female genitourinary tract as well as additional solid neo-organs. We will focus specifically on highlighting aspects of these neo-organ regenerative platforms conducive to the commercial viability of such technologies.

2. Tissue engineering of tubular organs

Two significant recent studies have brought the concept of *de novo* regeneration of tubular organs within humans towards proof-of-practice in the clinic. In the first example, seeding of tubular, biodegradable scaffolds with autologous urothelial and smooth muscle cells was shown to catalyze regeneration of a complete bladder with laminarly organized histology and associated urologic functionality upon implantation within seven pediatric patients (Atala et al., 2006). In the second example, a tissue engineered, functional human trachea was created using a decellularized, cadaveric tracheal segment as scaffold and seeded with autologous respiratory epithelial cells and chondrocytes generated by the directed differentiation of the patient's own bone marrow cells (Asnaghi et al., 2009; Macchiarini et al., 2008).

Both studies leveraged a scaffold seeded with autologous cells to trigger a regenerative response within the patient, ultimately leading to complementation of organ functionality with concomitant histogenesis of laminarly organized tissue structures. These approaches have focused on the regeneration of tubular organs and, taken together with additional recent developments in the regeneration of additional hollow organs, provide perspective into overlapping technology platforms and insight into key methodological differences that may impact the commercial feasibility of tubular organ engineering. In the following section (based on Basu & Ludlow, 2010), we discuss recent developments in tissue engineering of tubular organs.

2.1 A Platform technology for tubular organ regeneration

Organ regeneration technologies aim to restore the original structure and functionality of a diseased organ. Typically, healing responses within mammals are characterized by fibrosis and scar tissue formation, not regeneration. Tubular organ regeneration involves a specific, temporal sequence of cellular infiltration, vasculogenesis, neurogenesis and the defined differentiation of mucosal, stromal and parenchymal laminar tissue architectures (reviewed by Basu & Ludlow, 2010). Strategies for organ and tissue regeneration must therefore achieve the dual objectives of triggering a true regenerative response while ameliorating any tendency towards fibrotic repair. The methodology first pioneered for regeneration of the bladder (illustrated in Figure 1) may serve as a foundational platform for the regeneration of any tubular organ.



Fig. 1. Tension Neo-Bladder Augment™ (NBA). Dome shaped biodegradable bladder scaffold (left panel) is placed in a support prior to seeding with cells and incubated in a bioreactor. The bioreactor is transported to the clinical site and the supported seeded scaffold removed (middle panel) and positioned for patient implantation (right panel).

Examination of the temporal sequence of neo-bladder regeneration in dogs illustrates the dichotomy in outcomes between implantation of acellular and cellularized scaffolds. This distinction is a principal theme of most studies describing the regeneration of tubular organs. Cell seeded scaffolds mediated a regenerative response within one month post-implantation, characterized by induction of heavily vascularized, smooth muscle-like parenchyma. In contrast, acellular scaffolds triggered a principally fibrotic, reparative outcome characterized by randomly organized collagen fibers with minimal vascularization.

Baseline urodynamics were reconstituted within four months of implantation with cell seeded scaffold, whereas the urodynamic profile of animals implanted with acellular scaffold remained abnormal throughout the trial period (Jayo et al., 2008a).

In a related dog study, restoration of tri-laminar bladder wall architecture occurred within three months post-implantation and normal compliance characteristics of a urinary bladder wall developed by 12 months. Regenerated bladders were functionally and structurally stable for up to two years post-implantation. Importantly, although the construct volume was constant at implantation within variably sized dogs, the ratio of the regenerated bladder's volume to host body weight adapted to the recipient animal's size, demonstrating that the neo-organ responds to homeostatic mechanisms regulating organ volume (Jayo et al., 2008b).

Although these canine studies utilized both bladder-derived urothelial and smooth muscle cells, urothelial cells have been shown to *not* be essential for bladder regeneration, thereby greatly facilitating process development and commercialization (Jack et al., 2009). However, use of bladder-derived smooth muscle cells is problematic in patients presenting with bladder related malignancies. Therefore, a number of alternate sources of smooth muscle cells have been investigated. Such alternate cell sources may have broad application in the regeneration of additional, laminarily organized tubular organs.

One possible alternate source of smooth muscle cells may be the directed differentiation of mesenchymal stem cells (MSC) with recombinant, inductive cytokines such as transforming growth factor β (TGF- β). Such MSC may be procured from bone marrow or adipose (Tian et al., 2010a,b; Sakuma et al., 2009). Additionally, autologous smooth muscle cells may be isolated from the vasculature of omentum or other adipose tissue as well as directly from the mononuclear fraction of peripheral blood (Basu et al., 2011a; Toyoda et al., 2009; Lin et al., 2008; Metharom et al., 2008).

Although the use of MSC or other stem cell populations (adult-derived, embryonic or iPS (induced pluripotent stem cell)) for organ regeneration is informative as proof-of-concept, from a process development perspective focused on eventual manufacturing and product release, the requirements to monitor and control stem cell potential and directed differentiation towards a smooth muscle lineage substantially complicates efforts to streamline, standardize and assure quality, as well as leading to significant increases in cost of goods. To simplify bioprocessing procedures, enhance product consistency, reduce the risk of immune rejection and to ensure a robust supply of cellular raw material, we are focused on developing classes of *committed* smooth muscle cells for tubular organ regeneration (Basu et al., 2011a).

An alternative platform technology for regeneration of bladder and related tubular organs including vas deferens and uterus is based around application of the peritoneal cavity as a living bioreactor to trigger the encapsulation of a tubular scaffold with myofibroblasts, as

demonstrated in rabbit and rat models (Campbell et al., 2008). Two to three weeks after implantation, tubular constructs were observed to be heavily encapsulated with myofibroblasts. These tissue engineered composites were anastomosed to the native organ and allowed to mature *in vivo* for up to 14 months. Histological analysis of regenerated tissue was performed together with evaluation of organ functionality. Bladder function was confirmed by normal urine flow, vas deferens function by ejaculation and uterine function by the ability to sustain pregnancy.

With all three organ systems, an epithelial layer and laminarly organized musculature was observed with minimal evidence of inflammation. However, this approach may be impractical for widespread clinical application, as the cell encapsulation protocol represents an additional major surgical event. *In vitro* scaffold cell seeding is a simple and straightforward methodology sufficient to induce neo-organ regeneration *in vivo*. Use of alternate, non-bladder sources of smooth muscle-like cells will likely eliminate any requirement for peritoneal cavity based tissue engineering of bladder or bladder-like derivatives.

2.2 Trachea

Tissue engineered trachea illustrates another iteration of the foundational, bladder-based platform for tubular organ regeneration. Here, a decellularized, cadaveric tracheal segment was used as a scaffold. The recipient's own epithelial cells seeded the luminal surface, while MSC derived from the recipient's bone marrow was differentiated towards a chondrocytic lineage with recombinant cytokines prior to application on the abluminal surface. Although the patient showed reconstitution of pulmonary function within four months, detailed histological examination of the regenerated organ was not possible (Macchiarini et al., 2008).

However, in a swine model of this tissue engineered trachea, both the chondrocytes derived from differentiated MSC as well as the epithelial cells were needed for host survival (Go et al., 2010). While this methodology is valuable as proof of concept, it is clearly not amenable to large-scale process development. Decellularization of cadaveric scaffolds is an uncontrolled methodology that may not entirely alleviate immune response (Kasimir et al., 2006; Zhou et al., 2010), as well as increasing cost of goods by the requirement to stringently monitor the decellularization protocol. Broader application requires synthetic biodegradable scaffold materials, facilitating reproducible manufacturability at large scale with defined chemical and physical properties. Furthermore, the application of directed differentiation strategies on cells destined for patient implantation may trigger additional regulatory hurdles, further complicating commercialization efforts. To this end, chondrocytes derived from autologous tracheal explants may be leveraged (Komura et al., 2008 a,b).

2.3 Gastro-intestinal tract (GI)

Individual components of the GI represent locally specialized iterative variations of essentially the same laminarly organized tubular histologic architecture as the bladder. However, from a commercial perspective, the small intestine represents by far the most pressing current clinical need, with small bowel transplantation being an unsatisfactory current standard of care for pediatric small bowel syndrome (SBS) (<http://digestive.niddk.nih.gov/ddiseases/pubs/shortbowel/>). Esophageal replacement for esophageal cancer may also be commercially viable.

The intestinal epithelium is the most highly regenerative tissue within adult mammals and may therefore be expected to be highly amenable towards tissue engineering or regenerative medicine methodologies. In one such study, *in vivo* derived organoid units, formed from incompletely disassembled clusters of epithelial and mesenchymal cells generated through partial digestion of intestinal epithelium (and therefore likely incorporating resident intestinal stem cells) were used to seed PLLA scaffold tubes that were subsequently matured within the peritoneal cavity of pigs. Seven weeks post-implantation, this tissue-engineered small intestine recapitulated the gross overall laminar organization of native small intestine (SI) (Sala et al., 2009).

Significantly, acellular scaffolds did not result in the regeneration of tissue engineered gastrointestinal structures. These data notwithstanding, anastomosis of these tissue engineered small intestines to host SI within a large animal model remains to be demonstrated. Additionally, up to 10cm of host SI was harvested to derive donor organoids that are not readily expandable *in vitro*. Whether organoid units capable of seeding a scaffold structure may be isolated from diseased human intestine, and how much diseased donor material may be needed, remain factors yet to be elucidated. The requirement to leverage the peritoneal cavity as a bioreactor for tissue engineering may also impede widespread application of this approach.

The bladder-derived organ regeneration platform of biopolymeric scaffold seeded with smooth muscle cells may be applicable for regeneration of SI. To this end, stomach derived smooth muscle cells were used to seed a collagen-based scaffold prior to implantation within surgically isolated ileal loops of dogs for eight weeks, prior to re-anastomosis to the native intestine. Acellular collagen scaffold was used as a control. By 12 weeks post-surgery, macroscopic analysis of the cell seeded scaffold implantation site demonstrated regeneration of neo-mucosa resembling native mucosa. However, in animals containing an acellular scaffold, the implant site remained ulcerated up to 12 weeks post-implantation. Additional histological data showed significantly enhanced vascularization, epithelialization and organization of the circular muscle layer at the cell seeded scaffold defect site relative to acellular control (Nakase et al., 2006).

Increasing the number of smooth muscle cells seeded onto the scaffold increased the area of regenerated SI tissue, although no concomitant increase in the thickness of the smooth muscle layer was observed (Nakase et al., 2007). Nevertheless, these data suggest that a simple regenerative platform composed of biodegradable scaffold nucleated with smooth muscle cells may be adequate to facilitate SI regeneration. Although this approach must be reproduced using a directly anastomosed tubular scaffold and alternate sources of smooth muscle cells, if successful, this methodology represents the most straightforward, clinically relevant and commercially viable strategy for regeneration of the SI.

This organ regeneration platform technology may also be leveraged for regeneration of the esophagus. In one such example, patch defects were created in the abdominal esophagus of 27 female rats, subsequently implanted with gastric acellular matrix (GAM). Of the 24 survivors, none showed evidence of regeneration of the lamina muscularis mucosa even 18 months post-implantation (Urita et al., 2007).

In contrast, a study of a canine model of esophageal resection and replacement demonstrated that PGA tubes seeded with a mixture of keratinocytes and fibroblasts triggered regeneration of smooth muscle laminar organization similar to native esophagus within 3 weeks post-implantation, whereas acellular PGA tubes formed esophageal

strictures associated with near complete obstruction within two to three weeks (Nakase et al., 2008).

In another dog study, cervical esophageal defects were patched with either small intestinal submucosa (SIS) alone, or SIS seeded with autologous oral mucosal epithelial cells. After four weeks, dogs implanted with cell seeded SIS showed almost complete re-epithelialization and minimal evidence of inflammation and, by eight weeks post surgery, regeneration of the underlying smooth muscle layer. Acellular SIS grafted animals presented only partial re-epithelialization and a more extensive inflammatory response by four weeks, and no muscular regeneration by eight weeks post-implantation. Attempts to introduce an acellular SIS tubular construct into the cervical esophagus of piglets were also unsuccessful, demonstrating scarification and a minimal regenerative response (Doede et al., 2009).

Progress has also been made in efforts to tissue engineer the stomach. Stomach derived organoid units, (analogous to the SI organoids used to tissue engineer the SI) upon seeding of a biopolymeric scaffold, triggered reconstitution of the gastric and muscularis mucosa in stomach tissue engineered within the peritoneal cavities of swine (Sala et al., 2009). In another study, circular defects were created in the stomach of seven dogs and a composite biodegradable scaffold ("New-sheet"), soaked with either autologous peripheral blood or bone marrow aspirate, was sutured over the defect. By 16 weeks post implantation, the defect site had formed regenerated stomach with evidence of re-epithelialization, formation of villi, vascularization and fibrosis within the submucosal layer. However, minimal regeneration of the smooth muscle layer was observed, as shown by expression of smooth muscle α -actin, without expression of calponin, a marker of mature smooth muscle cells (Araki et al., 2009).

Though strictly not a tubular organ, the anal sphincter is a component of the gastrointestinal tract and is critical in regulating patency of the large intestine. Recent efforts to engineer the anal sphincter leverage the same general platform used to catalyze bladder regeneration. To this end, smooth muscle cells isolated from human internal anal sphincter were seeded onto fibrin gels poured around a central mold. Cell mediated contraction of the gel around the mold resulted in the formation of a three-dimensional cylindrical tube of sphincteric smooth muscle tissue. Although *in vivo* anastomosis remains to be demonstrated, this bio-engineered anal sphincter demonstrated contractile properties and response to defined neurotransmitters consistent with the functionality of native anal sphincter (Hashish et al., 2010; Somara et al., 2009). Use of alternatively sourced smooth muscle cells may facilitate the transition of engineered sphincter towards commercial production.

2.4 Neo-vessel

Neo-blood vessels represent a major commercial opportunity for application of a tubular organ platform technology towards patients receiving bypass surgery. In a recently published clinical trial of vascular grafts composed of autologous bone marrow aspirate seeded onto a PGA/PCL scaffold and implanted into a cohort of 25 patients presenting with single ventricle physiology, all patients were asymptomatic 30 days post implantation and 24/25 patients were alive at one year post-implantation (Hibino et al., 2010).

Efforts to seed PGA tubular neo-vessel scaffolds with smooth muscle cells derived from the directed differentiation of bone marrow or adipose derived MSC with TGF- β have also been described (Wang et al., 2010; Gong and Niklason, 2008). The requirement to induce directed differentiation with TGF- β or related agents as well as the prolonged maturation period

under pulsatile conditions needed to achieve a mature smooth muscle phenotype will likely make this approach impractical for commercial application. A reliable source of committed smooth muscle cells (e.g., the vascular fraction of adipose or omentum) may represent a more commercially feasible platform.

2.5 Lung

Lung may be regarded as a highly specialized tubular organ amenable to regeneration with the bladder-based platform outlined above. Evidence to this effect was provided by a study examining the regenerative potential of PGA felt sheets seeded with adipose derived stromal cells in triggering pulmonary regeneration within a rat lung lobectomy model (Shigemura et al., 2006). The cell seeded PGA sheet was sealed onto the remaining lung lobe. Alveolar and vascular regeneration was observed within 1 week of implantation, with concomitant recovery of pulmonary functionality. Paracrine action by secreted factors including VEGF, HGF and FGF was suggested as a possible mechanism of action for triggering the regenerative effect.

In another study, fetal rat lung cells were seeded onto gelfoam sponge-based scaffolds and implanted into adult rat lung. Alveolar-like structures with apparent vascular networks were observed to regenerate within degrading scaffold by 4 months post-implantation (Andrade et al., 2007). Importantly, the formation of these alveolar like networks was observed to be strongly dependant on prior seeding of the scaffold with lung cells. As with the previous study, the authors suggest paracrine action of secreted factors from the seeded lung cells acting to facilitate regenerative in-growth of lung cells from the surrounding native lung tissue as the likely mechanism of action.

Growth of lung cells in three-dimensions is essential to induce expression of epithelial genes related to lung morphogenesis, including FGFR2 (Mondrinos et al., 2006). Appropriate combinations of exogenous fibroblast growth factors chosen to target specific receptor isoforms may facilitate appropriate lung epithelial and mesenchymal cell behavior conducive to tissue regeneration (Mondrinos et al., 2007). Finally, tissue engineered lungs created from recellularized scaffolds derived from decellularized lung have also been recently characterized (Ott et al., 2010; Petersen et al., 2010; Cortiella et al., 2010).

2.6 Genito-urinary system

The recent reports of functional regenerated neo-phallus and neo-vagina within a rabbit model illustrate how foundational principles of tubular organ regeneration pioneered for bladder may be extrapolated to facilitate organogenesis of functionally distinct tubular organs (De Filippo et al., 2008). Decellularized corpora cavernosa was used as a collagen-based scaffold matrix for seeding autologous corporal smooth muscle and endothelial cells in a rabbit model of penile replacement. Implantation of decellularized matrix alone led to formation of a non-functional, fibrotic phallus. However, cell seeded scaffolds regenerated corporal tissue organization histologically similar to native controls within 3-6 months post-implantation. Tissue engineered phallus was functional as demonstrated by the ability of recipient animals to copulate normally (Chen et al., 2010).

For the neo-vagina, autologous vaginal epithelial and smooth muscle cells were seeded onto the luminal and abluminal surfaces of PGA tubular scaffolds, preconfigured to resemble native rabbit vagina. Seeded composites were implanted in place of the native vagina of nine rabbits, with unseeded controls introduced into six other animals. As has been

observed for multiple organs, unseeded scaffold failed to trigger a regenerative response, whereas cell seeded scaffolds generated stage specific histogenesis, vascularization, innervation and regeneration of a patent neo-vagina by 6 months post-implantation with a defined muscular layer and a luminal invaginated epithelium. Organ functionality was confirmed by a graded contractile response of the musculature to electrical stimulation in a manner paralleling native tissue (De Filippo et al., 2008).

2.7 Mechanism of action for tubular organ regeneration

The lineage of cell populations constituting a regenerated neo-organ remains unclear. What is the contribution of the original cell population used to seed the scaffold compared to that derived from cellular migration from the surrounding tissue and/or omentum typically used to wrap neo-organs at the time of implantation as a source of neo-vascularization? Data addressing this issue was provided for the regenerated neo-vagina, where both the epithelial and smooth muscle cell populations used to seed the neo-vagina were labeled independently with fluorochromes and tracked to three months post-surgery. Labeled cells of both types were found to compose over 85% of the regenerated neo-organ (De Filippo et al., 2008).

However, implantation of a human bone marrow derived cell seeded neo-vessel scaffold into immuno-deficient mice led to loss of all seeded human cells within days, followed by re-population of the scaffold with mouse monocytes and subsequent re-population with mouse smooth muscle cells and endothelial cells recruited via the monocyte chemoattractant protein MCP-1 (Roh et al, 2010). No direct contribution of the seeded bone marrow cells to the regenerated neo-vessel was observed.

Organ specific mechanistic differences notwithstanding, these and results from other tubular organs demonstrate that defined populations of stem and progenitor cells are not required to trigger neo-organ formation. Smooth muscle cells and other committed cell types appear capable of mechanistically substituting for stem cells, by initiating cellular scaffold repopulation and catalyzing a host specific regenerative response that may additionally mediate paracrine signaling pathways to modulate recruitment of resident stem and progenitor populations, and regulate inflammation, immune response, apoptosis and fibrosis (Caplan, 2009). The ability to engineer neo-organs *without* a requirement for the isolation, expansion and manipulation of defined stem cell populations has greatly facilitated the commercial viability of this platform technology and will continue to do so in the future (Basu and Ludlow, 2010).

2.8 Role of extracellular matrix (ECM) in regeneration

An analysis of the bladder submucosal matrix generated by bladder smooth muscle cells identified multiple key paracrine signaling factors including VEGF, BMP4, PDGF- $\beta\beta$, KGF, TGF- β 1, IGF, bFGF, EGF and TGF- α as resident components (Chun et al., 2007). This observation might suggest that the presence of living cells is entirely superfluous for triggering regeneration, which would greatly lower cost-of-goods for neo-organ production. A synthetic scaffold may be matured with an autologous, committed cell population to facilitate deposition of ECM, decellularized and implanted within the host to induce organ regeneration. Alternatively, defined ECM components or growth factors may be introduced into the scaffold to recapitulate key aspects of organogenesis (Sahoo et al, 2010). However, studies to date confirm that ECM alone in the context of, for example, an acellular matrix

such as small intestinal submucosa or bladder mucosa, is insufficient to induce organogenesis *in vivo* (Doede et al., 2009; Dorin et al., 2008). It is likely that living cells are required to modulate a more sustained, physiologically relevant regenerative response.

2.9 Conclusions & clinical outlook

We may summarize the key factors affecting the commercial viability of any tubular organ tissue engineering or regenerative technology platform as follows:

- A biodegradable, synthetic scaffold based on well-characterized materials (e.g., PGA, PLGA) that is reproducibly manufactured with defined characteristics.
- A population of committed cells (typically smooth muscle cells), easily isolatable and expandable, is required for scaffold seeding to trigger regeneration *in vivo*.
- A purified population of defined stem cells is neither needed nor desirable. Cost of goods increases significantly with the requirements to monitor stem cell potential and to control directed differentiation.
- Engineering an entire organ *in vitro* or within the peritoneal cavity is neither needed nor desirable. Instead, an *in vitro* cell seeded scaffold is adequate to trigger the body's innate regenerative response, resulting in *de novo* organogenesis *in vivo*.

The outlook for tubular organ regeneration in the clinic is promising. We have seen how the foundational technology platform consisting of a biodegradable scaffold nucleated with a population of committed smooth muscle cells, first pioneered for the bladder, may be applied towards regeneration of multiple, functionally disparate tubular organs. Proof of concept studies in human patients have already been successfully completed with neo-bladder, trachea and vascular grafts. However, the neo-bladder continues to represent the pioneering regenerative neo-organ, with Phase 2 clinical trials currently underway [www.clinicaltrials.gov/ct2/show/NCT00512148?term=tengion&rank=4; www.clinicaltrials.gov/ct2/show/NCT00419120?term=tengion&rank=5]. Additionally, clinical trials are underway for the development of a neo-urinary conduit, a bladder derivative neo-organ designed to facilitate urinary diversion and developed through foundational principles closely resembling those used to engineer the neo-bladder replacement [www.clinicaltrials.gov/ct2/show/NCT01087697?term=Tengion&rank=3].

3. Tissue engineering of solid organs

3.1 Introduction

Though mammalian fetuses are capable of spontaneous regeneration of damaged organs and tissues until the third trimester, adult mammals typically respond to trauma by initiating reparative healing mechanisms. These are characterized by wound contraction, extensive fibrosis and scar tissue formation mediated by the migration and re-organization of myo-fibroblasts, as demonstrated during the response of the heart to ischaemic injury (Boudoulas and Hatzopoulos, 2009). True organ regeneration as commonly observed in certain species of fish and amphibian is associated with an absence of the fibrotic response and concomitant reconstitution of the three dimensional laminar or parenchymal organization of the regenerating organ, as exemplified by the regeneration of adult mammalian liver in response to lobectomy.

The objective of *de novo* organ regeneration through tissue engineering or regenerative medicine is to use defined combinations of cells and biomaterials to catalyze a principally regenerative response towards organ trauma while simultaneously ameliorating any

tendency towards fibrotic repair. The *de novo* regeneration of a complete organ in mammals has been demonstrated for laminarily organized, tubular organs including bladder, trachea and vessels of the vasculature (Basu and Ludlow, 2010) as well as recently for the small intestine (Basu et al., 2011c). However, the regeneration of solid organs presents a unique challenge, requiring the organization of highly specialized cell types into complex three dimensional micro-architectures within a parenchymal matrix. Additionally, the regenerating solid neo-organ must synergize with developing elements of the vasculature, lymphatic and nervous systems throughout neo-organ morphogenesis.

In this section, we will highlight the latest progress towards regeneration of solid organs in mammals, with an emphasis on identifying commonalities of methodology that may drive the creation of an organ regeneration platform broadly applicable towards multiple solid organ systems (Figure 2). We will focus on identifying aspects of the regenerative methodology most conducive towards translation into commercial process development and manufacture.

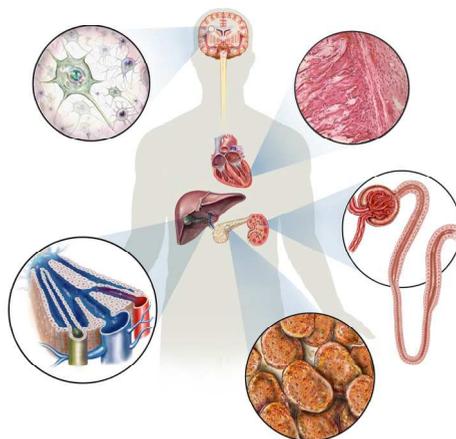


Fig. 2. Solid organ systems potentially amenable to commercial tissue engineering strategies. From top, clockwise: central nervous system, heart, kidney, pancreas, liver.

3.2 Kidney

Kidney provides an excellent illustration of the considerable technical difficulties associated with solid organ regeneration. Numerous specialized cell types including podocytes, mesangial cells, endothelial cells, fibroblasts, epithelial cells and numerous stem and progenitor cell populations are organized across the renal parenchyma into discrete, specialized functional units or nephrons, which serve to selectively filter electrolytes from the vasculature (Vize et al., 2003; Basu et al., 2010).

Efforts to trigger regeneration within animal models of ischaemic or chronic renal disease have typically centered around the isolation, expansion and re-introduction of defined populations of mesenchymal, embryonic or renal stem cells, potentially capable of site-specific engraftment and directed differentiation along multiple renal lineages as well as facilitating the creation of a regenerative micro-environment through paracrine signaling mechanisms (reviewed by Sagrinati et al., 2008; Hopkins et al., 2009).

Such cell therapy methodologies have had generally mixed results, with little if any evidence supporting site specific engraftment and directed differentiation as a mechanism of action by exogenously applied stem or progenitor populations. Introduction of stem cells within the kidney by direct injection into the renal vasculature or renal parenchyma leads to apoptosis or efflux of the majority of applied cells from the target organ within days of implantation (Togel *et al.*, 2008). Uncontrolled differentiation of stem cells that actually do engraft may also represent a significant technical challenge and potential regulatory obstacle against successful commercialization (Kunter *et al.*, 2007).

Reconstitution of kidney mass, a central prerequisite for true *de novo* organ regeneration, will likely require a cell/biomaterial scaffold composite facilitating the morphogenesis of glomeruli and tubules derived from seeded primary renal cell populations, as well as the migration and directed differentiation of host derived renal stem and progenitor cells within the scaffold framework, followed by progressive vascularization and innervation of the developing neo-organ, deposition of extracellular matrix and the reconstitution of a renal parenchyma.

To this end, it may be reasoned that biomaterials derived from the native kidney are best suited to retain renal specific elements of the extracellular matrix (ECM) potentially capable of modulating renal morphogenesis as well as the three dimensional parenchymal micro-architecture unique to the kidney. Application of sodium dodecyl-sulphate (SDS) detergent to whole rat kidneys in one recent report was shown to facilitate the removal of renal cells while maintaining the functional integrity of the ECM as well as the overall three dimensional parenchymal organization of the kidney. Murine pluripotent embryonic stem (ES) cells were applied to this renal scaffold via the renal artery or ureter, and the cell seeded biomaterial composite matured under static or pulsatile culture *without* the application of inductive differentiation agents.

This approach permitted an evaluation of the specific effect of native renal-derived ECM in modulating the directed differentiation of the ES cell population. After arterial seeding, it was observed that ES cells localized within the Bowman's capsule by 4 days post-seeding and within the associated vasculature and renal cortex by day 10. Niche specific localization of ES cells was accompanied by concomitant acquisition of the renal markers Pax-2 and Ksp-cadherin as evidenced by histochemical and RT-PCR approaches (Ross *et al.*, 2009). The effectiveness of SDS relative to other detergents in preserving components of the ECM as well as details of the renal micro-architecture was confirmed independently in comparative studies of the effect of decellularization within monkey kidneys with multiple detergent agents (Nakayama *et al.*, 2010).

Although it is not unreasonable to assume that the organ specific ECM and three dimensional histo-architecture associated with scaffolds procured by decellularization of native organs may be ideally suited to direct the potential regeneration of that specific organ type, this strategy may not be appropriate for a commercially viable solid organ regeneration platform. Apart from difficulties associated with the procurement of cadaveric organs for scaffold assembly, decellularization is a generally uncontrolled process not easily subject to scale-up, process development and quality assurance. Monitoring and verification of the extent of cell loss will substantially increase cost of goods associated with manufacture. Furthermore, native scaffolds retain the potential for immunogenicity despite the presumed absence of cells (Zhou *et al.* 2010). Reliable and reproducible manufacturability of solid neo-organs will likely require the application of synthetic

biomaterials with defined physical and chemical properties. With these considerations granted, progress towards creation of biosynthetic scaffolds appropriate for the regeneration of renal architecture was provided by the demonstration that tubular and glomerular structures spontaneously self-assembled from primary rat renal cell populations within one week of growth in collagen I gels (Joraku *et al.*, 2009).

The ability to introduce specific biomimetic peptides and defined proteolytic cleavage sites within the context of gel based biomaterials raises the intriguing possibility of controlling the morphogenesis of tubules, glomeruli or other renal structural units to modulate defined functional outcomes. For example, polyethylene glycol (PEG)-based hydrogels engineered with protease degradation sites and controlled densities of RGD peptide or laminin bioligands have been found to regulate epithelial morphogenesis of cysts derived from MDCK cells, such that cysts grown within ligand functionalized gels demonstrated an increased frequency of lumen formation and unambiguous baso-lateral polarization compared to those grown in unmodified hydrogels (Chung *et al.*, 2008). Therefore, a true renal augment designed to trigger regeneration of glomeruli, tubules, EPO secreting fibroblasts (Paliege *et al.*, 2010) or other key renal cell populations may potentially be envisioned as an injectable hydrogel containing functionalized matrix optimized to catalyze this defined regenerative outcome. Such methodologies alleviate potential concerns regarding the requirement for major surgical intervention within the diseased organ.

Alternatively, a neo-kidney augment may be contemplated as a semi-permanent, implantable, cell/biomaterial composite that upon introduction within or adjacent to the renal parenchyma of a diseased organ, provides a foundational framework for regeneration of tubular or other renal superstructures as well as potentially establishing a regenerative microenvironment through paracrine mediated recruitment of native stem and progenitor populations as well as amelioration of inflammatory, fibrotic and apoptotic cascades (Caplan 2009). To this end, certain species of polyester fleeces have demonstrated the capacity to facilitate the growth of renal tubules within the context of a fibrous artificial interstitium.

In this system, a heterogenous primary renal cell population incorporating stem and progenitor cells was extracted from the sub-capsular space of embryonic rabbit kidneys. The isolated cell population was sandwiched between two layers of polyester fleece and maintained within a perfusion culture system in the presence of aldosterone, a hormone involved in the renin/angiotensin axis (Vize *et al.*, 2003). Spontaneous generation of tubular structures was observed with concomitant expression of key functional markers including cingulin and Na⁺/K⁺ ATPase. The regenerated tubules appeared to interact with the polyester fibers within the context of the artificial interstitium.

The authors speculate that these cell seeded polyester scaffolds may be multiplexed by horizontal "tiling" or "paving" as well as vertical "piling" to create renal superstructures supporting the continued morphogenesis of renal tubules *in vitro*. The authors suggest that these compounded renal units could potentially form the basis of a true neo-kidney augment upon implantation within the sub-capsular space between the renal capsule and the renal parenchyma (Roessger *et al.*, 2009; Minuth *et al.*, 2008), although our observations of the mechanical properties of the renal capsule associated with diseased, fibrotic human kidneys suggest that this approach may not be feasible.

Nevertheless, this methodology illustrates one key criterion for commercial viability; synthetic polyester fleeces are leveraged for regeneration of renal tubular superstructures,

without the requirement for extracellular matrix components derived from decellularized kidney or from other naturally occurring sources. In addition, the application of a defined, serum/BPE (Bovine Pituitary Extract) free media for tubule growth and maintenance additionally serves to facilitate large scale process development. Conversely, it remains to be ascertained whether cells derived from human kidney tissue in general and from diseased human organs in particular, are capable of supporting the spontaneous assembly of tubular structures *de novo* within the context of polyester or other synthetic polymer based biomaterial.

At Tengion, development of the Neo-Kidney Augment (NKA™), a cell/biomaterial composite for renal tissue engineering, has focused on step-wise identification of bioactive cellular components and biomaterials amenable to implantation within renal parenchyma. To this end, we have leveraged principles discussed throughout this chapter, identifying lineage committed, primary renal cell populations as therapeutically bioactive agents capable of mediating functional rescue of aspects of disease phenotype within small animal clinical models of chronic kidney disease (Kelley et al., 2010; Presnell et al., 2010). Similarly, our evaluation of biomaterials compatible with renal parenchyma has led us towards application of gelatin hydrogels as the best tolerated biomaterial scaffold for renal tissue engineering (Basu et al., 2011b). Studies are currently underway to evaluate the ability of bioactive, primary renal cell/hydrogel biomaterial composite constructs (NKA™) to catalyze functional rescue of disease in small and large animal clinical models of chronic kidney disease.

3.3 Heart

Mammalian heart provides one of the clearest demonstrations of the inability of most mammalian solid organs to regenerate. Cardiac ischemia typically results in extensive fibrosis, scarification and loss of function (Boudoulas and Hatzopoulos, 2009). In contrast, zebrafish and other non-mammalian vertebrates are capable of complete regeneration and reconstitution of function upon removal of up to 20% of the ventricle, leading to speculation that an understanding of the mechanism of action underlying regeneration within model organisms such as zebrafish may identify analogous mechanisms that may be leveraged within mammals to trigger cardiac regeneration. To this end, dedifferentiation and proliferation of existing cardiomyocytes was shown to be the principal mechanism of regeneration following ventricular amputation within zebrafish (Jopling *et al.*, 2010).

This modality of action notwithstanding, tissue engineering approaches towards construction of functional mammalian heart have generally focused on decellularization of cadaveric organs to provide scaffold structures for reseeded and implantation. For example, neonatal rat cardiac or aortic endothelial cells were used to seed a decellularized cadaveric rat heart. Upon growth within customized bioreactors for 8 days, evidence of spontaneous contractility was observed. Pump functionality of up to 2% of adult was successfully reconstituted (Ott *et al.*, 2008).

However, although of interest as proof of concept, the requirement for cadaveric organs as a source of biomaterials may ultimately limit the usefulness of this methodology for commercial development and application within the clinic. As we have seen with the kidney, decellularization is a difficult procedure to monitor during quality assurance regimens, and there can be no guarantee that the resultant tissue engineered composite will lack immunogenicity upon implantation (Zhou *et al.* 2010). Furthermore, it remains to be

demonstrated whether cardiac cells derived from adult human tissue are capable of repopulating a scaffold to regenerate a functional organ. Finally, the requirement for tissue maturation within pulsatile bioreactors will likely substantially increase cost of goods for tissue engineered cardiac neo-organs.

These criticisms aside, the engineering of synthetic scaffolds that recapitulate defined cardiac structures such as valves and chambers remains technically challenging (Fong *et al.*, 2008). We speculate that triggering the dedifferentiation and subsequent proliferation of existing cardiomyocyte populations by synthetic biomaterial composites containing defined biomimetic peptides and/or autologously derived lineage committed cell populations with smooth muscle cell-like properties may ultimately prove to be the more commercially feasible approach for regeneration of cardiac substructure and ultimately neo-organ regeneration.

Evidence supporting the idea that biomaterial composites seeded with lineage committed cell populations may represent a potential solid organ regeneration platform comes from studies of biodegradable scaffolds nucleated with human ES cell derived cardiomyocytes alone or cardiomyocytes, endothelial cells and embryonic fibroblasts. Upon implantation within rat heart, more extensive vascularization was observed from tri-culture seeded constructs when compared to scaffolds seeded with ES cell derived cardiomyocytes alone (Lesman *et al.*, 2010). In addition, acellular or mesenchymal stem cell (MSC) seeded SIS (small intestinal submucosa) grafts have been implanted on the epicardial surface of a rabbit model of myocardial infarct. Resultant ventricular functionality and histopathology were significantly more improved in MSC seeded relative to acellular grafts.

Finally, the use of injectable gels derived from decellularized human or porcine pericardium to trigger migration and proliferation of cardiomyocytes and cardiac progenitors has also been explored (Seif-Naraghi *et al.*, 2010). Such synthetic hydrogels incorporating biomimetic peptides or containing committed smooth muscle, endothelial or other fully differentiated cell type may serve as a commercially feasible organ regeneration platform to trigger cardiac regeneration. These regenerative platforms most likely leverage paracrine and ECM-based signaling to recreate a regenerative micro-environment and thereby facilitate the regenerative response.

3.4 Liver

The regenerative potential of the liver is unparalleled among mammalian organs. Adult liver progenitors are thought to be defined by the population of "oval cells" capable of reconstituting both hepatocytes and biliary epithelium upon mobilization by an appropriate regenerative signal (Zaret & Grompe, 2008). However, as with the heart, liver regeneration does not appear to leverage discrete, organ specific pools of stem and progenitor cells, rather, operating through the increased proliferation of existing, mature hepatocytes. Unfortunately, this magnified proliferative capacity has not translated into the ready expandability of hepatocyte populations *in vitro*. Mammalian hepatocytes remain notoriously difficult to maintain and expand in culture (Underhill *et al.*, 2007). Regardless, regenerative medicine and tissue engineering approaches to reconstitute the liver have typically focused on the isolation and expansion of mature, adult hepatocytes as a cell source for biomaterials seeding or, alternatively, on the directed differentiation of ES, iPS, adipose or bone marrow derived MSC towards a hepatic lineage followed by 3D culturing. Morphogenesis of the mammalian liver is triggered by induction of the embryonic endodermal epithelium by adjacent mesodermal populations (reviewed by Zaret & Grompe,

2008). Mimicking these early developmental signaling events through co-culture of hepatocytes with mesenchymal cell populations such as bone marrow-derived stem cells (BMSC) or even 3T3 fibroblasts results in significant enhancement of hepatic functionality as evidenced by prolonged maintenance of hepatocyte specific morphology and enhanced secretion of albumin (Nahmias *et al.*, 2006). This observation raises the possibility that biomaterials seeded with mesenchymal cell populations may function as a potential solid organ regeneration platform, acting to facilitate the proliferation and functionality of hepatocytes *in vivo*.

Evidence to this effect was provided by studies of Nagase analbuminea rats implanted with coralline hyaluronic acid (HA) ceramic disks seeded either with freshly isolated rat hepatocytes alone, or rat hepatocytes together with bone marrow derived mononuclear cells (Takeda *et al.*, 2005). Hepatocytes cultured in the presence of BMSC secreted significantly more albumin into the media during *in vitro* culture relative to hepatocytes in monoculture. These effects were recapitulated *in vivo* within 4 weeks in analbuminic rats, within which HA-based biomaterials seeded with both BMSC and hepatocytes were associated with significantly greater blood serum albumin levels relative to monoculture controls. Finally, implantation of co-cultured biomaterials within mice presenting chemically induced liver damage resulted in complementation of blood serum albumin as well as increased levels of serum IL-6.

This reconstitution of function notwithstanding, no evidence was provided regarding tissue regeneration *in vivo* within the context of the biomaterial, or whether the presence of hepatocytes is in fact essential. A clear demonstration of hepatic tissue regeneration and functional complementation using synthetic biomaterials seeded with BMSC or some other mesenchymal cell population would be of significant commercial interest. As with heart and kidney, we speculate that paracrine action by factors released from the seeded mesenchymal cells may be adequate to trigger angiogenesis and vascularization of the biomaterials as well as to stimulate the proliferation of the resident hepatocyte population. In keeping with the suggestion of paracrine mechanisms, it may additionally be possible to leverage the resident pluripotent progenitor populations normally resident in the liver by implantation of a biomaterial within the canals of Hering to provide additional space for the development of regenerated tissue (Zhang *et al.*, 2008).

Alternate cell sources of hepatocyte like cells may offer a potential substitute for mediating catalysis of liver regeneration. Hepatocyte like cells may be derived from bone marrow or adipose primary cells through a multi-step directed differentiation regimen attempting to phenocopy key signaling events during hepatic morphogenesis through the controlled application of recombinant bioactive factors and small molecules including hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF) and oncostatin-M (OMS) (Talens-Visconti *et al.*, 2006). These pseudo-hepatocytes display a characteristic polygonal morphology, demonstrate expression of key hepatocyte associated transcriptional and protein markers and secrete albumin into the media.

Similarly, cells derived from the stromal vascular fraction of adipose can be driven to acquire hepatocyte-like characteristics by application of analogous multi-stage differentiation protocols. Importantly, both adipose derived hepatocytes as well as undifferentiated adipose stromal vascular fraction derived cells are capable of engraftment within chemically damaged liver *in vivo*, where they appear to form cords of tissue within the hepatic parenchyma and acquire hepatocyte specific functionality, as demonstrated by the *in situ* expression of albumin (Ruiz *et al.*, 2010). In this study, engraftment by human adipose derived stromal cells within

the liver of a SCID mouse recipient was examined, with functionality being monitored through the use of antibodies specifically recognizing human albumin. The differentiation of bone marrow derived primary cells into hepatocyte-like cell types appears to be facilitated by growth in three dimensions, as shown by the enhanced secretion of albumin, urea, transferrin, serum glutamic pyruvic transaminase and serum oxaloacetate aminotransferase from hepatocyte-like cells grown in three-dimensions on polycaprolactate (PCL) scaffolds relative to similar populations maintained in two-dimensional monoculture (Ruiz *et al.*, 2010).

The trans-differentiation of bone marrow cells towards a hepatic phenotype may also be accomplished by leveraging paracrine and ECM mediated signaling mechanisms between existing hepatic cells and bone marrow cells. For example, human bone marrow MSC may be incubated in plates containing HepG2 derived ECM and HepG2 derived conditioned media for up to 30 days to facilitate the acquisition of a hepatocyte-like phenotype (Tai *et al.*, 2009). These hepatocyte like cells were seeded onto an RGD (arginine-glycine-aspartic acid) modified chitosan alginate polyelectrolyte fibrous non-woven scaffold prior to implantation within a rat liver lobectomy model presenting with 70% removal of liver mass. Post-implantation analysis of the cell seeded biomaterials within 1-2 weeks confirmed the expression of key hepatic markers. Detection of human albumin within rat serum was also demonstrated. No histological evidence for regeneration was provided, although this outcome is unlikely over such a short time period. Taken together, these results provide evidence for functional complementation *without* clear demonstration of *de novo* hepatic tissue regeneration.

An alternative, methodology for regeneration of hepatic tissue is based on the engineering of contiguous monolayers of primary hepatocytes through culturing on temperature responsive surfaces (Ohashi *et al.*, 2007). The temperature sensitive polymer PIPAA_m, upon decrease in temperature to below 32°C, rapidly hydrates triggering the spontaneous detachment of cultured hepatocytes in the form of discrete, uniform sheets. Stacking of multiple hepatic tissue sheet monolayers within the subcutaneous space of mice resulted in the formation of a significant hepatic tissue mass, with histologically meaningful micro-architecture, vascularization and functionality as assayed by the presence of glycogen. Importantly, ectopic engineered hepatic tissue was capable of reacting to a regenerative stimulus (2/3 liver resection) by significantly increased levels of proliferative activity. Furthermore, hepatocytes organized as stacked monolayer sheets generated significantly greater functional neo-organ volume compared to the same number of hepatocytes introduced into the subcutaneous cavity within an injectable Matrigel matrix. Such injectable hepatocyte matrices have been proposed as alternate regenerative stimuli for liver (Fiegel *et al.*, 2009).

3.5 Pancreas

Strategies for regeneration of pancreas have focused almost exclusively on the *de novo* regeneration of pancreatic β -cell populations. Morphogenesis of the pancreas broadly resembles hepatogenesis, with induction of the endodermal epithelium of embryonic foregut triggered by adjacent mesenchymal cell populations (Zaret & Grompe, 2008). As a result, methodologies developed to mediate the directed differentiation of ES or adult derived stem and progenitor cells towards a pancreatic lineage leverage many of the same key developmental morphogens as those formulated to mediate acquisition of hepatic phenotype. The forced expression of certain pancreatic transcription factors and/or treatment with defined growth factors is sufficient to trigger acquisition of insulin transcription within non-

pancreatic cell populations as well as within pancreatic ductal and acinar cells, though typically not at levels comparable to true β -cells. Lineage transdifferentiation and dedifferentiation towards a progenitor phenotype have been proposed as mechanisms of action underlying the observed *de novo* presentation of β -cell specific phenotypes *in vitro*. Evidence is also accumulating that these mechanisms may operate *in vivo* to modulate true pancreatic regeneration (reviewed by Juhl *et al.*, 2010).

Strategies to specifically target pancreatic ductal and acinar cells for delivery of defined transcription factors selected to mediate dedifferentiation and reacquisition of a β -cell specific phenotype, while useful as proof of concept, may not represent a commercially viable solid organ regeneration platform. Similarly, methodologies oriented around the directed differentiation of ES or adult stem cells will considerably increase cost of goods, with process development and quality assurance issues focused on the evaluation of stem cell potential and the extent and completeness of the directed differentiation process (see Table 1). To this end, it may be worthwhile exploring whether tissue engineering approaches for regeneration of pancreas may be designed to leverage the innate regenerative potential inherent within the organ itself. Although not as dramatic as the liver, regeneration of mammalian pancreas has been observed under certain circumstances (Cano *et al.*, 2008).

Stem Cells	Committed Cells
<p>Considerable initial variability in proliferative capacity and multi-lineage differentiation potential</p> <p>Expansion in culture leads to loss of differentiation potential. Monitoring multi-lineage differentiation potential is lengthy and expensive</p> <p>Directed differentiation is an uncontrolled, inefficient process. Only a proportion of cell population acquires lineage specific characteristics</p> <p>Requirement for inductive cytokines to direct lineage specific differentiation leads to significant increase in cost of goods</p> <p>Multiple molecular, proteomic and functional tests required to evaluate stem and differentiation potential. Substantial increase in time, cost, labor. Tests may be misleading and unreliable (Montzka <i>et al.</i>, 2009)</p> <p>Long term effects of inductive cytokines on cells implanted <i>in vivo</i> not known. Potential for transformation, teratoma formation</p>	<p>Straightforward, readily isolatable cell sources, less subject to donor variability</p> <p>Readily expanded in culture without loss of lineage specific characteristics</p> <p>No requirement to monitor stem cell potential or directed differentiation</p> <p>No requirement for recombinant cytokines, substantially decreasing cost of goods</p> <p>No regulatory concerns regarding effects of recombinant cytokines on cell transformation</p> <p>Significant reduction in time required to expand committed cell population</p> <p>Application of committed smooth muscle cells (example) for organ regeneration demonstrated across multiple organ systems within <i>in vivo</i> models</p> <p>No possibility of abnormal <i>in vivo</i> differentiation or teratoma formation</p>

Table 1. Potential of Stem and Committed Cell Populations for Application in Commercially Viable Solid Organ Regeneration Platforms

Autologously derived pancreatic islet cells or alternate cell sources presenting a β -cell like phenotype may be engineered within an appropriate gelatinous matrix or other biosynthetic scaffold. Considerable effort is currently being invested in developing pancreatic islet cell encapsulation technology, which may be manifested as a vascular device, microcapsule, tubular or planar membrane chamber or sheet architecture (reviewed by Sambanis, 2007). Encapsulation techniques facilitate the delivery of allogeneic or cadaveric islets cells by modulation of the host immune response. Recent developments in encapsulation methodologies include the application of bioactive hydrogels with functionalized moieties designed to improve β -cell survival and secretion of insulin (Lin and Anseth 2009) as well as to modulate inflammation (Su *et al.*, 2010). Implantation of PEG tubes containing rat islet cultures maintained on acellular pancreatic matrix was observed to lead to partial rescue of insulin secretory activity (De Carlo *et al.*, 2010).

Alternatively, pancreatic islet cells may be expanded through growth over polyglycolic acid (PGA) scaffolds. Scaffold seeded cells may then be further matured into tissue engineered islets within a thermo-responsive gel prior to harvesting and implantation beneath the kidney capsule of streptozotocin (STZ) induced diabetic mice, triggering a subsequent return to the normo-glycaemic condition (Kodama *et al.*, 2009). Similarly, transplantation of pancreatic islets grown over a biodegradable scaffold composed of a vicryl fleece with polydioxanone backing and implanted within a canine total pancreatectomy model resulted in normo-glycaemia without the requirement for exogenous insulin injection ($n=4$), in one case up to 5 months post-implantation. In contrast, dogs receiving an equivalent mass of islets without scaffold did not become normo-glycaemic at any time (Kin *et al.*, 2008).

The sheet architecture solid organ regeneration platform pioneered for application in liver regeneration (Ohashi *et al.*, 2007) has also been applied towards ectopic regeneration of pancreatic tissue (Shimizu *et al.*, 2009). In this study, isolated rat pancreatic islets were expanded over laminin 5 coated PIPAA_m plates. Implantation of the tissue engineered pancreatic sheets within the subcutaneous space of rats led to reconstitution of pancreatic-like tissue structures within 7 days post-implantation. As with the liver, the authors speculate that stacking of pancreatic islet cell sheets may lead to regeneration of an increased mass of pancreatic tissue. It is noteworthy that none of these studies have examined the regeneration of native pancreas *in situ*. This may be a function of the additional challenges inherent in modulating regeneration *in situ*, including the requirement for vascularization and oxygenation specific to the internal volume of regenerating solid organs located deep within the peritoneal cavity. It will be of significant interest to evaluate whether islet cells implanted within biomaterials and ligated to lobectomized pancreas are capable of catalyzing partial *de novo* organ regeneration.

3.6 Spleen

Although the regeneration of spleen may not be clinically or commercially relevant, the principles developed for engineering these organs may have broader implications for solid organ regeneration. To this end, progress towards regeneration of spleen has been reported through the leveraging of platform technologies successfully applied towards engineering of small intestine and stomach, these representing examples of tubular, laminarily organized organs with fundamentally distinct micro- and macro-architecture compared to the spleen or other solid organs (Grikscheit *et al.*, 2008).

In this approach, organoid units generated by the incomplete digestion of rat splenic tissue were seeded within tubular PGA/PLA (poly-lactic acid) scaffolds, and the resultant

composites implanted within the omentum of the peritoneal cavity of splenectomized rats. This ectopic tissue engineered spleen demonstrated splenic tissue organization as well as providing protection against *Pneumococcal* induced septicemia. Interestingly, spleen slices cultured ectopically within omentum also mediated formation of quasi-spleen like structures capable of providing protection against induced septicemia.

3.7 Central nervous system

Potentially the best defined examples of a solid organ regeneration platform demonstrating evidence of cellular regeneration *in situ* within a damaged organ and catalyzed by implantation of a cell/biomaterial composite at the injury site comes from brain. Tissue engineering of brain and spinal cord typically involves the introduction of gel-based biomaterials within the brain that may be nucleated with neuronal stem cells and may additionally be supplemented with paracrine signaling factors such as vascular endothelial growth factor (VEGF), brain derived neurotrophic factor (BDNF) or components of the extracellular matrix including laminin and fibronectin.

For example, a biomimetic hydrogel incorporating matrix metalloproteinase (MMP) degradation sites, a laminin derived peptide and the neurotrophic factor BDNF was shown to direct the *in vitro* differentiation of seeded mesenchymal stem cells towards a neuronal lineage. Subsequently, this biomimetic hydrogel was introduced into the intrathecal space within a rat model of spinal cord injury. Although no histological evidence for neuronal regeneration was presented, rats treated with the biomimetic hydrogel showed the greatest improvement in tests of locomotion compared to rats treated with non-biomimetic control hydrogels (Park *et al.*, 2009).

In another approach, defined brain defects were created within rat models and subsequently implanted with a gelatin based scaffold impregnated with VEGF. Histological evidence was provided as evidence for migration of endothelial, astroglial and microglial cells within the periphery of the scaffold at 30 days post-implantation (Zhang *et al.*, 2008). This observed regenerative effect was found to be dependant on the presence of VEGF. A demonstration of the application of a non-gel based scaffold for tissue engineering of brain is the use of polylactic-co-glycolic acid (PLGA) micro-particles in the 50-200 μm size range. These neuro-scaffolds were seeded with neural stem cells and introduced into the brain cavity of stroke induced rats by directed injection under magnetic resonance imaging (MRI) guidance. Within 7 days post-implantation, the neural stem cells had dispersed within the scaffold, and presented as a tightly packed mass at the center of the biomaterial, but with a broader distribution resembling a honey-comb like structure towards the periphery. The graft displayed a mixed population of neuronal, astrocytic and stem cell specific markers, together with evidence of inflammation, but little if any vascularization within the body of the biomaterial. No evidence was provided that the tissue engineered brain tissue had any functional significance in terms of impact to the rat stroke model (Bible *et al.*, 2009).

4. Conclusions

When examined together, platform technologies for regeneration of solid organs remain largely as academic proof of concept. Factors needing to be addressed for successful commercialization include the following:

- Synthetic biomaterials, readily manufactured with defined physical and chemical properties, approved by FDA for implantation within the human body.

- Avoidance of decellularized scaffolds that require cadaveric organ sources and may be potentially immunogenic.
- Use of committed cell types, not stem or progenitor cell populations that require extensive monitoring of stem potential and directed differentiation.
- Autologous cell sources where possible facilitate FDA acceptance of introduction of cellular material.
- Emphasis on a platform approach: cell/biomaterial composites that upon delivery, trigger a broad regenerative response within multiple solid organs.

This is in marked contrast to analogous platform systems developed for tubular organs such as bladder and bladder derivatives, which are currently undergoing Phase I/II clinical trials (Basu & Ludlow, 2010). As we have seen, there are few published reports documenting the *in situ* regeneration of a solid organ in response to damage as a function of the implantation of a cell/biomaterial composite. The majority of published reports have focused on tissue engineering approaches towards solid organ regeneration using the peritoneal cavity or the subcutaneous space as a living bioreactor to facilitate the vascularization of the regenerating composite that is a prerequisite for organogenesis. However, it is our position that such a methodology may not be conducive to large scale clinical application, nor does it represent a commercially viable developmental strategy.

In this regard, a comparison with organ regeneration platforms developed for application towards the regeneration of laminarily organized tubular organs may be helpful. The use of committed smooth muscle cells seeded onto a biodegradable scaffold of synthetic polymer such as PGA, PLGA or PCL provides an example of a commercially viable organ regeneration platform that has been successfully applied towards regeneration of bladder and bladder derivatives. The serial stacking and piling of polyester fleeces nucleated with renal primary cell populations (Roessger *et al.*, 2009; Minuth *et al.*, 2008) may represent a viable solid organ regeneration platform amenable to process development, large scale manufacture and industrial quality assurance regimens, provided the caveats discussed earlier are addressed. Similarly, serial stacking of tissue sheets engineered by monolayer formation over temperature responsive PIPAA_m surfaces as has been demonstrated for liver and pancreas may be viable for commercial development beyond proof of concept.

Regenerative platforms that focus on committed cell populations such as hepatocytes, pancreatic islet cells and cells derived from the stromal vascular fraction of adipose *instead* of stem and progenitor cells have the potential to substantially reduce cost of goods by avoiding technical challenges associated with the isolation and expansion of stem cell populations, maintenance and monitoring of stem cell potential, monitoring and characterization of directed differentiation protocols and the costs associated with recombinant cytokines required to drive directed differentiation. Furthermore, introduction of mesenchymal stem cells within the renal parenchyma of rat models of glomerulonephritis has resulted in abnormal differentiation towards glomerular adipocytes, raising the potential for additional regulatory headaches. It remains unclear how the potential for mis-directed differentiation within the solid organ parenchyma may be definitively eliminated (Kunter *et al.*, 2007).

The observation that adipose derived stromal vascular fraction cells are capable of engraftment *in vivo* within liver and present acquisition of hepatic functionality raises the possibility that readily isolatable adipose cells implanted within the context of synthetic biomaterials or as serially stacked tissue sheets may be capable of stimulating the innate

regenerative potential latent within liver, and potentially, additional solid organs. To this end, it has been reported that conditioned media from MSC enhance the survival and functionality of pancreatic islet cells following transplantation in diabetic rats (Park *et al.*, 2010).

There is no evidence to indicate that the stem cell potential of these cell types is directly connected to their secretomic profiles: it is likely that fibroblasts or smooth muscle cells derived from these "MSC"-like populations may function just as effectively for paracrine signaling during the regenerative process. Such a combination of readily isolatable, committed cell types complexed with a biomaterial that may be reliably manufactured, has defined physical and chemical properties, and is already acceptable to FDA and other regulatory agencies for implantation within the human body would represent the ideal platform technology for the commercially viable regeneration of solid organs.

To summarize, factors impacting successful commercialization of solid organ regenerative technologies include:

- Identification of a synthetic scaffold structure that is broadly relevant to solid organ parenchyma, regardless of organ type.
- Committed cell types that may be capable of triggering organ regeneration within the context of an implanted scaffold.
- Alternatively, common approaches for the isolation of organ specific cell populations capable of mediating regeneration within the context of an implanted scaffold. For example, cell populations may be isolated from multiple solid organs by density centrifugation under standard conditions prior to scaffold seeding.
- Standardized methodologies for combining organ derived or organ independent cell populations with biosynthetic scaffolds to create implantable composites, for example, stacking or piling of cell sheets.
- A focus on methodologies that avoid prolonged maturation periods within bioreactors, but rely instead on triggering the body's innate regenerative potential to stimulate neo-organogenesis.
- Identification of common mechanisms of action being leveraged across multiple organ types through common regeneration platforms.

5. References

- Andrade C.F. *et al.* (2007). Cell based tissue engineering for lung regeneration. *Am J Physiol Lung Cell Mol Physiol* 292, L510-518
- Atala A. *et al.* (2006). Tissue engineered bladders for patients needing cystoplasty, *Lancet* 367, 1241-1246
- Araki M. *et al.* (2009). Development of a new tissue engineered sheet for reconstruction of the stomach. *Artificial Organs* 33, 818-826
- Asnaghi M.A. *et al.* (2009). A double-chamber rotating bioreactor for the development of tissue engineered hollow organs: from concept to clinical trial. *Biomaterials* 30, 5260-5269
- Basu J & Ludlow JW (2010). Platform technologies for tubular organ regeneration, *Trends in Biotechnology*, 28(10): 526-533
- Basu J, Genheimer C, O'Reilly DD, *et al.*, (2010). Distribution and analysis of stem and progenitor cell populations in large mammal and human kidneys. *FASEB J* 24: lb35

- Basu *et al.*, (2011a). Expansion of the human adipose-derived stromal vascular fraction yields a population of smooth muscle-like cells with markedly distinct phenotypic and functional properties relative to mesenchymal stem cells. *Tissue Eng Part C* 17(8): 843-860
- Basu *et al.*, (2011b). Functional evaluation of primary renal cell/biomaterial Neo-Kidney Augment prototypes for renal tissue engineering. *Cell Transplantation* doi: 10.3727/096368911X566172
- Basu *et al.*, (2011c). Regeneration of rodent small intestine tissue following implantation of scaffolds seeded with a novel source of smooth muscle cells. *Regen Med*, In Press
- Bible E, Chau DY, Alexander MR, *et al.*, (2009). The support of neural stem cells transplanted into stroke induced brain cavities by PLGA particles. *Biomaterials* 30: 2985-94
- Boudoulas KD and Hatzopoulos AK. (2009). Cardiac repair and regeneration: the Rubik's cube of cell therapy for heart disease. *Dis Model Mech* 2: 344-58
- Cano DA, Rulifson IC, Heiser PW, *et al.*, (2008). Regulated beta-cell regeneration in the adult mouse pancreas. *Diabetes* 57: 958-66
- Caplan AI. (2009). Why are MSCs therapeutic? New data, new insight. *J Pathol* 217: 318-324
- Campbell G.R. *et al.* (2008). The peritoneal cavity as a bioreactor for tissue engineering visceral organs: bladder uterus and vas deferens. *J Tissue Eng Regen Med* 2, 50-60
- Chen K.L. *et al.* (2010). Bioengineered corporal tissue for structural and functional restoration of the penis. *PNAS* 107, 3346-3350
- Chun S.Y. *et al.* (2007). Identification and characterization of bioactive factors in bladder submucosal matrix. *Biomaterials* 28, 4251-4256
- Chung IM, Enemchukwu NO, Khaja SD, *et al.*, (2008). Bioadhesive hydrogel microenvironments to modulate epithelial morphogenesis. *Biomaterials* 2008, 29: 2637-2645
- Cortiella J *et al.*, (2010). Influence of acellular natural lung matrix on murine embryonic stem cell differentiation and tissue formation. *Tissue Eng Part A* 16, 2565-2580
- De Carlo E, Baiguera S, Conconi MT, *et al.*, (2010). Pancreatic acellular matrix supports islet survival and function in a synthetic tubular device: in vitro and in vivo studies. *Int J Mol Med* 25: 195-202
- De Filippo R.E. *et al.* (2008). Tissue engineering a complete vaginal replacement from a small biopsy of autologous tissue. *Transplantation* 86, 208-214
- Doede T. *et al.* (2009). Unsuccessful alloplastic esophageal replacement with porcine small intestinal submucosa. *Artificial Organs* 33, 328-333
- Dorin R.P. *et al.* (2008). Tubularized urethral replacement with unseeded matrices: what is the maximum distance for normal tissue regeneration? *World J Urol* 26, 323-326
- Fiegel HC, Kneser U, Kluth D, *et al.*, (2009). Development of hepatic tissue engineering. *Pediatr Surg Int* 25: 667-673
- Fong PM, Park J, Breuer CK. (2008). Heart Valves. *Principles of Tissue Engineering*, 3rd edition
- Go T. *et al.* (2010). Both epithelial cells and mesenchymal stem cell derived chondrocytes contribute to the survival of tissue-engineered airway transplants in pigs. *J Thorac Cardiovasc Surg* 139, 437-443
- Gong Z. and Niklason, L.E. (2008). Small diameter human vessel wall engineered from bone marrow derived mesenchymal stem cells (hMSCs). *FASEB J* 22, 1635-1648
- Grikscheit TC, Sala FG, Ogilvie J, *et al.*, (2008). Tissue engineered spleen protects against overwhelming pneumococcal sepsis in a rodent model. *J Surg Res* 149: 214-8

- Hashish M. *et al.* (2010). Surgical implantation of a bioengineered internal anal sphincter. *J Ped Surg* 45, 52-58
- Hibino N. *et al.* (2010). Late term results of tissue engineered vascular grafts in humans. *J Thorac Cardiovasc Surg* 139, 431-436
- Hopkins C, Li J, Rae F, *et al.*, (2009). Stem cell options for kidney disease. *J Pathol.* 217: 265-281
- Jack G.S. *et al.* (2009). Urinary bladder smooth muscle engineered from adipose stem cells and a three dimensional synthetic composite. *Biomaterials* 30, 3259-32
- Jayo M, *et al.* (2008) a. Early cellular and stromal responses in regeneration versus repair of a mammalian bladder using autologous cell and biodegradable scaffold technologies. *J Urol* 180, 392-397
- Jayo M, *et al.*, (2008) b. Long term durability, tissue regeneration and neo-organ growth during skeletal maturation with a neo-bladder augmentation construct. *Regen Med* 3, 671-682
- Jopling C, Sleep E, Raya M, *et al.*, (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* 464, 606-611
- Joraku A, Stern KA, Atala A, *et al.*, (2009). In vitro generation of three dimensional renal structures. *Methods* 47: 129-33
- Juhl K, Bonner-Weir S, Sharma A. (2010). Regenerating pancreatic β -cells: plasticity of adult pancreatic cells and the feasibility of in-vivo neogenesis. *Curr Opin Organ Transplant* 15: 79-85
- Kasimir M.T. *et al.* (2006). Decellularization does not eliminate thrombogenicity and inflammatory stimulation in tissue engineered porcine heart valves. *J Heart Valve Dis* 15, 278-286
- Kelley *et al.*, (2010). Tubular cell enriched subpopulation of primary renal cells improves survival and augments kidney function in a rodent model of chronic kidney disease. *Am J Physiol Renal Physiol* 299(5):F1026-39
- Kin T, O'Neil JJ, Pawlick R, *et al.*, (2008). The use of an approved biodegradable polymer scaffold as a solid support system for improvement of islet engraftment. *Artif Organs* 32: 990-3
- Kodama S, Kojima K, Furuta S, *et al.*, (2009). Engineering functional islets from cultured cells. *Tissue Eng Part A* 15: 3321-3329
- Komura M *et al.* (2008). An animal model study for tissue engineered trachea fabricated from a biodegradable scaffold using chondrocytes to augment repair of tracheal stenosis. *J Pediatr Surg* 43, 2141-2146
- Komura M *et al.* (2008). Human tracheal chondrocytes as a cell source for augmenting stenotic tracheal segments: the first feasibility study in an in vivo culture system. *Pediatr Surg Int* 24, 1117-1121
- Kunter U, Rong S, Boor P, *et al.*, (2007). Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. *J Am Soc Nephrol* 18: 1754-64
- Lesman A, Habib M, Caspi O, *et al.*, (2010). Transplantation of a tissue engineered human vascularized cardiac muscle. *Tissue Eng Part A* 16: 115-125
- Lin CC and Anseth KS. (2009). Glucagon like peptide 1 functionalized PEG hydrogels promote survival and function of encapsulated β -cells. *Biomacromolecules* 10: 2460-7

- Lin G. *et al.* (2008). Defining stem and progenitor cells within adipose tissue. *Stem Cells Dev* 17, 1053-1063
- Macchiarini P. *et al.* (2008). Clinical transplantation of a tissue-engineered airway. *Lancet* 372, 2023-2033
- Metharom P. *et al.* (2008). Myeloid lineage of high proliferative potential human smooth muscle outgrowth cells circulating in blood and vasculogenic smooth muscle like cells in vivo. *Atherosclerosis* 198, 29-38
- Minuth WW, Denk L, Castrop H. (2008). Generation of tubular superstructures by piling of renal stem/progenitor cells. *Tissue Engineering Part C*, 14: 3-13
- Mondrinos M *et al.* (2006). Engineering three dimensional pulmonary tissue constructs. *Tissue Eng* 12, 717-728
- Mondrinos M.J. *et al.* (2007). A tissue engineered model of fetal distal lung tissue. *Am J Physiol Lung Cell Mol Physiol* 293, L639-650
- Montzka K. *et al.* (2009). Neural differentiation potential of human bone marrow derived mesenchymal stromal cells: misleading marker expression. *BMC Neurosci* 10:16
- Nahmias Y, Casali M, Barbe L, *et al.*, (2006). Liver endothelial cells promote LDL-R expression and the uptake of HCV-like particles in primary rat and human hepatocytes. *Hepatology* 43: 257-265
- Nakase Y. *et al.* (2006). Tissue engineering of small intestinal tissue using collagen sponge scaffolds seeded with smooth muscle cells. *Tissue Eng* 12, 403-412
- Nakase Y. *et al.* (2007). Endocrine cell and nerve regeneration in autologous in situ tissue engineered small intestine. *J Surg Res* 137, 61-68
- Nakase Y. *et al.* (2008). Intrathoracic esophageal replacement by in situ tissue engineered esophagus. *J Thorac Cardiovasc Surg* 136, 850-859
- Nakayama KH, Batchelder CA, Lee CI, *et al.*, (2010). Decellularized rhesus monkey kidney as a three dimensional scaffold for renal tissue engineering. *Tissue Eng Part A*
- Ohashi K, Yokoyama T, Yamato M, *et al.*, (2007). Engineering functional two and three dimensional liver systems in vivo using hepatic tissue sheets. *Nat Med* 13: 880-885
- Ott HC, Matthiesen TS, Goh SK *et al.*, (2008). Perfusion decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nature Med* 14: 213-221
- Ott HC. *et al.* (2010). Regeneration and orthotopic transplantation of a bioartificial lung. *Nat. Med.* 16, 927-933
- Paliege A, Rosenberger C, Bondke A, *et al.*, (2010). Hypoxia-inducible factor-2 alpha expressing interstitial fibroblasts are the only renal cells that express erythropoietin under hypoxia-inducible factor stabilization. *Kidney Int* 77: 312-8
- Park KS *et al.*, (2010). Trophic molecules derived from human mesenchymal stem cells enhance survival, function and angiogenesis of isolated islets after transplantation". *Transplantation* 89: 509-517
- Petersen T.H. *et al.* (2010). Tissue-engineered lungs for in vivo implantation. *Science* 329, 538-541
- Presnell S *et al.*, (2011). Isolation, characterization and expansion methods for defined primary renal cell populations from rodent, canine and human normal and diseased kidneys. *Tissue Eng Part C* 17(3): 261-73
- Roessger A, Denk L, Minuth WW. (2009). Potential of stem/progenitor cell cultures within polyester fleeces to regenerate renal tubules. *Biomaterials* 30: 3723-3732

- Roh J. *et al.* (2010). Tissue engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. *PNAS* 107, 4669-4674
- Ross EA, Williams MJ, Hamakazi T, *et al.*, (2009). Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. *J Am Soc Nephrol* 20: 2338-2347
- Ruiz JC, Ludlow JW, Sherwood S, *et al.*, (2010). Differentiated human adipose derived stem cells exhibit hepatogenic capability in vitro and in vivo. *J Cell Physiol*
- Sakuma T. *et al.* (2009). Mature, adipocyte derived, dedifferentiated fat cells can differentiate into smooth muscle like cells and contribute to bladder tissue regeneration. *J Urol* 182, 355-365
- Sagrinati C, Ronconi E, Lazzeri E, *et al.*, (2008). Stem-cell approaches for kidney repair: choosing the right cells. *Trends Mol Med.* 14: 277-285
- Sala F.G. *et al.* (2009). Tissue engineered small intestine and stomach form from autologous tissue in a preclinical large animal model. *J Surg Res.* 156, 205-212
- Sahoo S. *et al.* (2010). Growth factor delivery through electrospun nanofibers in scaffolds for tissue engineering applications. *J Biomed Mater Res A* PMID #20014288
- Sambanis A. (2007). Bioartificial pancreas. *Principles of Tissue Engineering*, 3rd edition
- Seif-Naraghi SB, Salvatore MA, Schup-Magoffin PJ, *et al.*, (2010). Design and characterization of an injectable pericardial matrix gel: a potentially autologous scaffold for cardiac tissue engineering. *Tissue Eng Part A* 16:2017-2027
- Shigemura N. *et al.* (2006). Lung tissue engineering technique with adipose stromal cells improves surgical outcome for pulmonary emphysema. *Am J Respir Crit Care Med* 174, 1199-1205
- Shimizu H, Ohashi K, Utoh R, *et al.*, (2009). Bioengineering of a functional sheet of islet cells for treatment of diabetes mellitus. *Biomaterials* 30: 5943-5949
- Somara S. *et al.* (2009). Bioengineered internal anal sphincter derived from isolated human internal and sphincter smooth muscle cells. *Gastroenterology* 137, 53-61
- Su J, Hu BH, Lowe WL, *et al.*, (2010). Anti-inflammatory peptide-functionalized hydrogels for insulin-secreting cell encapsulation. *Biomaterials* 31: 308-314
- Tai BC, Du C, Gao S, *et al.*, (2009). The use of a polyelectrolyte fibrous scaffold to deliver differentiated hMSCs to the liver. *Biomaterials* 31: 48-57
- Takeda M, Yamamoto M, Isoda K, *et al.*, (2005). Availability of bone marrow stromal cells in three dimensional coculture with hepatocytes and transplantation into liver damaged mice. *J. Bioscience and Bioengineering* 100: 77-81
- Talens-Visconti R, Bonara A, Jover R, *et al.*, (2006). Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. *World J Gastroenterol.* 12: 5834-5845
- Tian H *et al.*, (2010) a. Myogenic differentiation of human bone marrow mesenchymal stem cells on a 3D nano fibrous scaffold for bladder tissue engineering. *Biomaterials* 31, 870-872
- Tian H *et al.*, (2010) b. Differentiation of human bone marrow mesenchymal stem cells into bladder cells: potential for urologic tissue engineering. *Tissue Eng Part A* PMID 20020816
- Togel F, Yang Y, Zhang P, *et al.*, (2008). Bioluminescence imaging to monitor the in vivo distribution of administered mesenchymal stem cells in acute kidney injury. *Am J Physiol Renal Physiol* 295: F315-321

- Toyoda M. et al. (2009). Characterization and comparison of adipose tissue derived cells from human subcutaneous and omental adipose tissues. *Cell Biochem Funct* 27, 440-447
- Underhill GH, Khetani SR, Chen AA, et al., 2007. Liver. *Principles of Tissue Engineering*, 3rd edition
- Urita Y. et al. (2007). Regeneration of the esophagus using gastric acellular matrix: an experimental study in a rat model. *Pediatr Surg Int* 23, 21-26
- Vize PD, Woolf AS, Bard JBL. (2003). *The Kidney: from normal development to congenital disease*. Editors, Academic Press
- Wang C. et al. (2010). A small diameter elastic blood vessel wall prepared under pulsatile conditions from polyglycolic acid mesh and smooth muscle cells differentiated from adipose derived stem cells. *Biomaterials* 31, 621-630
- Zaret KS and Grompe M. (2008). Generation and regeneration of cells of the liver and pancreas. *Science* 322: 1490
- Zhang L, Theise N, Chua M et al. (2008). The stem cell niche of human livers: Symmetry between development and regeneration. *Hepatology* 48: 1598-1607
- Zhang H, Kamiya T, Hayashi T, et al., (2008). Gelatin-siloxane hybrid scaffolds with vascular endothelial growth factor induces brain tissue regeneration. *Curr Neurovasc Res* 5: 112-117
- Zhou J et al. (2010). Impact of heart valve decellularization on 3D ultrastructure, immunogenicity and thrombogenicity. *Biomaterials* 31, 2549-2554
- Zhou J, Fritze O, Schleicher M, et al. (2010). Impact of heart valve decellularization on 3D ultrastructure, immunogenicity and thrombogenicity. *Biomaterials* 31, 2549-2554



Advances in Regenerative Medicine

Edited by Dr Sabine Wislet-Gendebien

ISBN 978-953-307-732-1

Hard cover, 404 pages

Publisher InTech

Published online 21, November, 2011

Published in print edition November, 2011

Even if the origins of regenerative medicine can be found in Greek mythology, as attested by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle; many challenges persist in order to successfully regenerate lost cells, tissues or organs and rebuild all connections and functions. In this book, we will cover a few aspects of regenerative medicine highlighting major advances and remaining challenges in cellular therapy and tissue/organ engineering.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Joydeep Basu and John W. Ludlow (2011). Tissue Engineering of Tubular and Solid Organs: An Industry Perspective, *Advances in Regenerative Medicine*, Dr Sabine Wislet-Gendebien (Ed.), ISBN: 978-953-307-732-1, InTech, Available from: <http://www.intechopen.com/books/advances-in-regenerative-medicine/tissue-engineering-of-tubular-and-solid-organs-an-industry-perspective>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.