Review

Platform technologies for tubular organ regeneration

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As a result of recent successes in regenerative medicine approaches to engineering multiple disparate tubular organs, methodology commonalities are emerging. Principal themes include the importance of a biodegradable scaffold seeded with a population of smooth muscle cells. Such composites trigger a regenerative response following *in vivo* implantation, resulting in *de novo* organogenesis. In this review, we examine bladder regeneration as a foundational platform technology to highlight key principles applicable to the regeneration of any tubular organ, and illustrate how these general concepts underlie current strategies to regenerate components of gastrointestinal, vascular, pulmonary and genitourinary systems. We focus on identifying the elements of this platform that have facilitated the transition of tubular organ regeneration from academic proof-of-concept to commercial viability.

Introduction

Two recent efforts have been significant in bringing the promise of *de novo* regeneration of tubular organs in humans to clinical reality. First, the bladders of seven pediatric patients were enlarged by implanting hollow biodegradable scaffolds seeded with autologous urothelial cells (UCs) and bladder smooth muscle cells (SMCs). Such implants initiated regeneration of a full-thickness bladder wall with laminarly organized architecture in animals [1] and concomitant urologic functionality in patients [2]. Second, a functional human trachea was engineered using a scaffold of decellularized cadaveric tracheal segment seeded with respiratory epithelial cells and chondrocytes generated by directed differentiation of autologous bone marrow cells [3,4]. Both efforts used biomaterials 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, together with other recent reports of tubular structure regeneration, provide perspective on overlapping technology platforms and insight into key methodological differences that might impact the commercial feasibility of tubular organ regeneration.

A foundational platform for tubular organ regeneration

Tubular organ regeneration involves a specific temporal sequence of cellular infiltration, vasculogenesis and neurogenesis, with concomitant differentiation of mucosal, stromal and parenchymal laminar tissue architecture (Box 1). Strategies for organ and tissue regeneration must therefore achieve the dual objectives of triggering a true regenerative response and ameliorating any tendency towards fibrotic repair. The strategy first pioneered for bladder regeneration used committed bladder-derived UCs and SMCs complexed with a biodegradable synthetic scaffold to trigger *de novo* organogenesis [1]. This strategy might serve as a foundational platform for the regeneration of any tubular organ, including penis, lung, vagina, small intestine, stomach and vessels. The methodology is illustrated in Figure 1 (center panel) for implantation of a Tengion Neo-Bladder Augment™ (NBA) in a human subject presenting with neurogenic bladder. The NBA is composed of a biodegradable scaffold seeded with autologous bladder-derived UCs and SMCs. The NBA is wrapped with omentum (center panel, right) to facilitate vascularization of the developing neo-organ.

A more detailed examination of the stages of the regenerative process as catalyzed by this foundational platform technology is shown in Figure 2, which illustrates regeneration using the Tengion Neo-Bladder Conduit™ (NUC), a tubular bladder derivative currently undergoing Phase I clinical trials. Analysis of the mature construct at the cellular level highlights interaction of committed SMCs with the polymer fibers of the biodegradable scaffold (Figure 2b). The mature construct (Figure 2c) is implanted and wrapped with omentum or peritoneum to facilitate vascularization. Over time (~3 months), a laminarly organized neo-organ develops (Figure 2d). SEM and immuno-fluorescence images of SMC-seeded poly(glycolic acid) (PGA) scaffolds show the development of cell–biomaterial interactions resulting in the formation of a true composite (Figure 2e-h). Variations of this approach have recently been described and a review of the key methodological elements in reports demonstrating *in vivo* regeneration of neo-bladder or neo-bladder-related tissue is presented in Table 1.

The studies in Table 1 were selected because they highlight application of committed SMCs [derived directly or through inductive differentiation of mesenchymal stem cells (MSCs)] seeded onto synthetic, biodegradable polymeric scaffolds to trigger regeneration of urinary-like neotissue within an *in vivo* model. Two of the four studies only documented expression of smooth-muscle-associated markers from subcutaneously implanted cell-seeded scaffolds [5,6]. Short-term (12 weeks) bladder functionality and incomplete bladder smooth muscle regeneration were observed in a small-animal cystectomy model [7]. However, long-term (24 months) clinically relevant neo-bladder function in a large-animal cystectomy model, together with histological evidence of complete urothelial and

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Box 1. Mechanism of action for *de novo* organ regeneration

The lineage of cell populations that populate a regenerated neo-organ remains unclear. Do the seeded cells expand and multiply, or do cells from nearby tissues or via the circulation (e.g. immune response) migrate into the developing neo-organ? For example, omentum and peritoneum have been used to wrap neo-organs to provide water-tightness and a vascular source; they may also be a source for other cells (Figure 1, center panel) [8,9]. Data addressing this question have been provided in one study on regenerated vaginal replacement in which both the epithelial cell and SMC populations used to seed the neo-vagina were labeled independently with fluorochromes and tracked to three months post-surgery [35]. Labeled cells of both types comprised over 85% of the regenerated neo-organ. However, when a neo-vessel scaffold seeded with human bone marrow-derived cells was implanted into immunodeficient mice, loss of all seeded human cells was observed within days of implant and repopulation occurred first with mouse monocytes, followed by mouse SMCs and endothelial cells recruited via the monocyte chemoattractant protein MCP-1 [31]. 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 stem and multilineage progenitor cells are not required to trigger neo-organ formation. SMCs and other committed cell types seem to be capable of initiating cellular scaffold repopulation and catalyzing a host-specific regenerative response that might include mediation of paracrine signaling pathways to modulate recruitment of resident stem and progenitor cell populations and regulation of immune responses, apoptosis and fibrosis [36]. The ability to engineer neo-organs without a requirement for the isolation, expansion and manipulation of stem cells has greatly facilitated the commercial viability of the organ regeneration platform technology, and will continue to do so in the future.

Role of extracellular matrix (ECM) in regeneration

Analysis of the bladder submucosal matrix generated by bladder SMCs revealed multiple key paracrine signaling factors, including VEGF, bone morphogenetic protein 4, PDGF-ββ, keratinocyte growth factor, TGF-β1, insulin-like growth factor, basic FGF, epidermal growth factor and TGF-α, as resident components [37]. It might be possible to replace living cells with a combination of paracrine signaling factors capable of triggering a regenerative healing response. Such an approach could decrease costs by eliminating the need to source and/or expand cells for neo-organ production. From a process development perspective, a synthetic scaffold could be matured with a committed cell population merely to facilitate deposition of ECM. The mature scaffold would then be decellularized and implanted in the recipient to induce organ regeneration.

Alternatively, it might be possible to define a set of synthetic ECM components or growth factors that could be introduced into the scaffold and recapitulate key aspects of organogenesis following implantation [38]. However, in all studies to date, ECM alone in the context of, for example, an acellular matrix, such as small intestinal submucosa or bladder mucosa, has been insufficient to induce organogenesis *in vivo*. We speculate that the presence of living cells might be required to modulate a more sustained, physiologically relevant regenerative response.

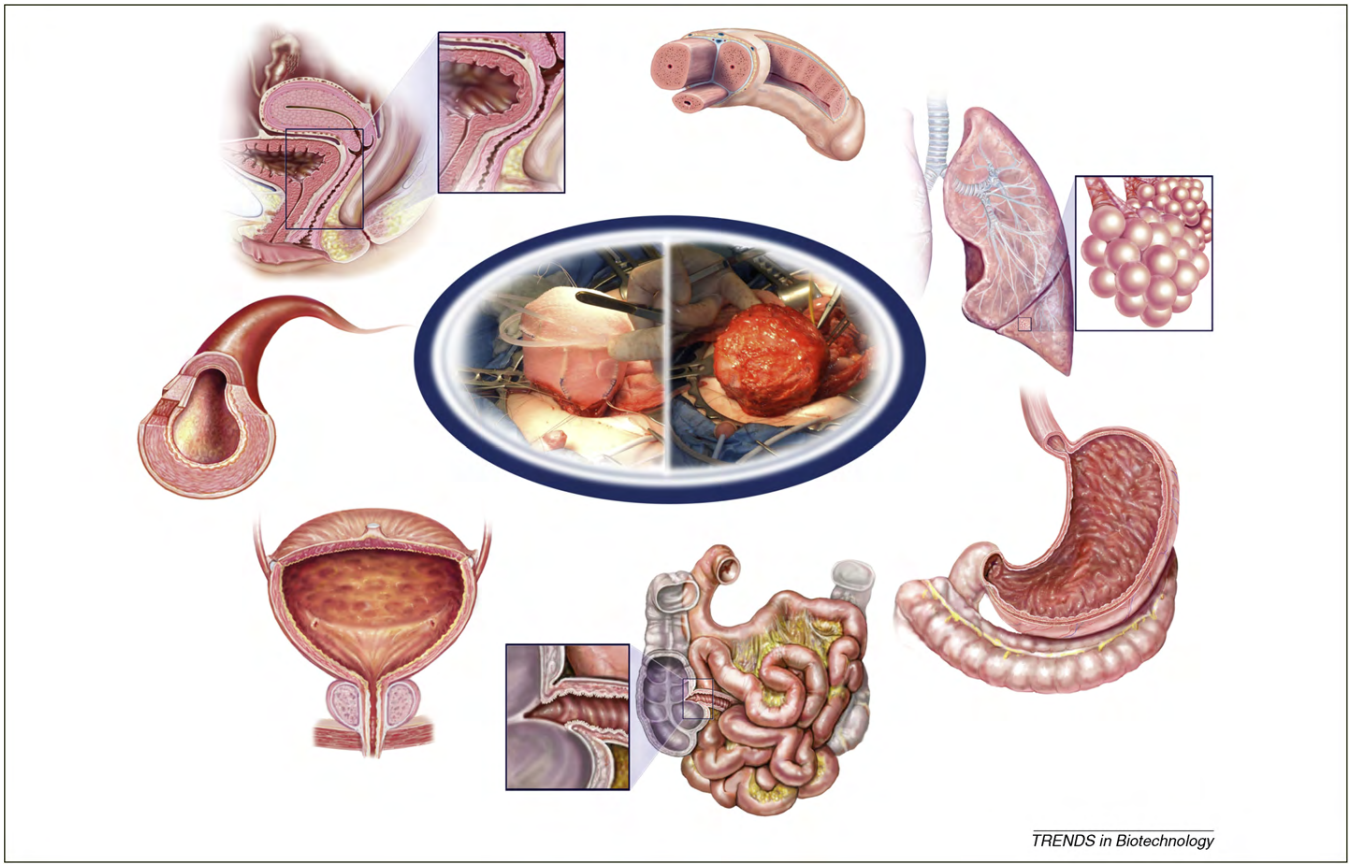

Figure 1. The bladder is a foundational platform for tubular organ regeneration. Center, left: implantation of the Tengion Neo-Bladder Augment™ (NBA) at the dome of a native bladder during augmentation cystoplasty in a patient presenting with neurogenic bladder secondary to spina bifida. Center, right: wrapping of omentum around NBA for vascularization. Examples of laminarly organized tubular organs that can be regenerated using the foundational platform technology demonstrated for the bladder. Top, clockwise: penis, lung, esophagus and stomach, small intestine, bladder, vessel, vagina.

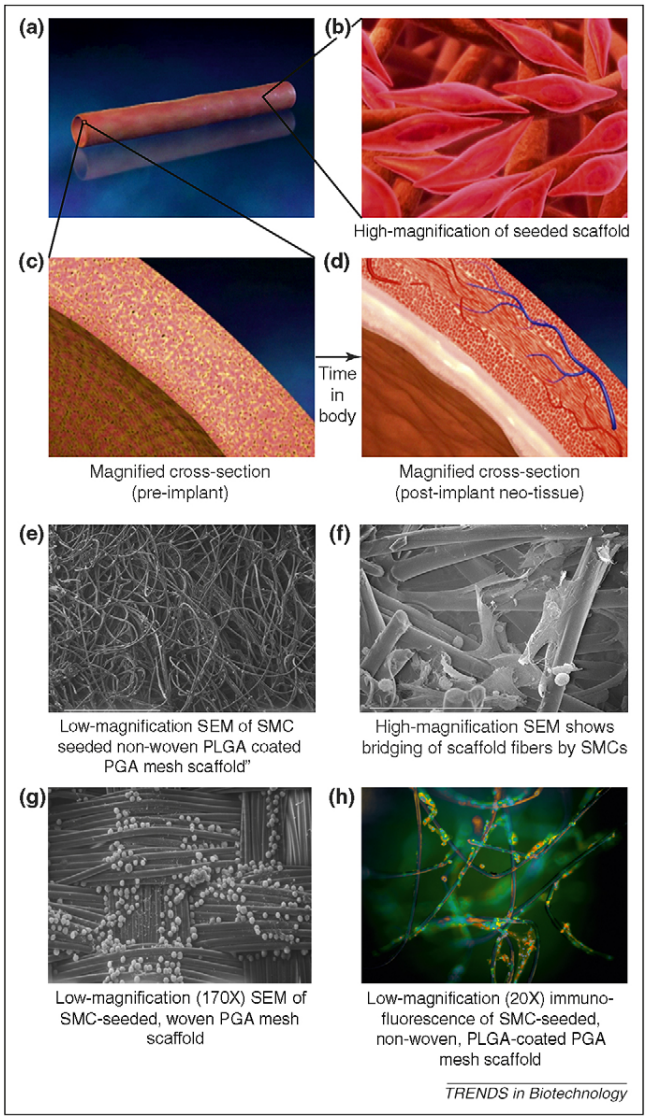

Figure 2. Stages in regeneration of a tubular organ, as exemplified by the Tengion Neo-Urinary Conduit™ (NUC), panels (**a**–**d**); cell/biomaterial interactions during maturation of seeded scaffolds, panels (**e**–**h**). (**a**) A tubular degradable biopolymeric scaffold is seeded with smooth muscle cells, as depicted by cartoon images in (**b**) and (**c**). (**d**) On implantation *in vivo*, the composite undergoes a systematic sequence of cellular infiltration, vascularization and neurogenesis, resulting in *de novo* regeneration of urinary-like tissue. Images were extracted from a video describing the regeneration of a Tengion Neo-Urinary Conduit™ available at www.tengion.com/news/video1-small.cfm. (**e**,**f**) Low- (scale bar = 1 mm) and high-magnification (scale bar = 0.1 mm) SEM images of a PGA felt scaffold seeded with SMCs, showing the interaction of SMCs with scaffold fibers. (**g**) SEM image of woven mesh PGA scaffold seeded with SMCs. Such cell/biomaterial composites may be used to catalyze regeneration in hollow organs beyond bladder. (**h**) Immuno fluorescence image of SMCs seeded onto non-woven, PLGA-coated PGA mesh scaffold, showing actin (orange) and DNA (green).

tri-laminar smooth muscle regeneration, has only been documented for canine models of augmentation cystoplasty [8,9]. Additional examples of the regeneration of bladder wall histoarchitecture following implantation of SMC-seeded NUCs in a swine cystectomy model are available on the Tengion website (www.tengion.com/pdfs/ISSCR-poster-final.pdf). None of the approaches highlighted in Table 1 are unique to the bladder and, as discussed in detail later in this review, iterations of these methodologies have been successfully applied towards regeneration of a broad spectrum of laminarly organized tubular organs (Figure 1).

Analysis of the dynamics of neo-bladder regeneration in subtotal cystectomized canines serves to illustrate the dichotomy in outcomes between implantation of acellular and cellularized scaffolds. This distinction is a central tenet of most reports describing the regeneration of tubular organs [8–10]. In one study, bladder-shaped scaffolds composed of non-woven PGA felt and poly(lactic-*co*-glycolic acid) (PLGA) seeded with autologous bladder-derived UCs and SMCs facilitated a regenerative response within one month post-implantation, as characterized by induction of an extensively vascularized, smooth muscle-like parenchyma [8]. By contrast, acellular PGA–PLGA scaffolds triggered a principally fibrotic, reparative outcome featuring disorganized collagen fibers with minimal vascularization. Baseline urodynamics were reconstituted within 4 months after implantation with a cell-seeded scaffold, whereas urodynamic profiles of animals implanted with acellular scaffolds remained abnormal throughout the nine-month study [8]. In a related cystectomized canine study, native-like tri-laminar bladder-wall tissue architecture was observed at three months after implantation with a bladder shaped non-woven PGA–PLGA scaffold seeded with autologous bladder-derived UCs and SMCs (1.5×10^8 cells each); normal compliance characteristics of a urinary bladder developed by 12 months [9]. Regenerated bladders in animals receiving these cell-seeded scaffolds have shown functional and structural stability for up to two years post-implantation [9]. Importantly, although the volume of the cell-seeded scaffold was held constant in this case, implantation into dogs of different sizes that had gained varying amounts of weight over the course of the study yielded organs that, as measured by the ratio of bladder capacity to body weight, adapted to the individual recipient animal's size, demonstrating that the neo-organ was capable of responding to homeostatic mechanisms regulating organ volume [9].

Although the implants used in the aforementioned canine studies were seeded with bladder-derived UCs and SMCs, subsequent studies have demonstrated that UCs are not required for bladder regeneration, thereby simplifying process development and commercialization of the cellular components for such products [7–10]. Nevertheless, the use of bladder-derived SMCs is problematic in patients presenting with bladder-related malignancies; therefore, a number of alternative SMC sources are in various stages of preclinical and clinical testing [5–7,11]. Such alternative cell sources might have broad application in the regeneration of additional laminarly organized tubular organs. Directed differentiation of MSCs with recombinant, inductive cytokines [e.g. transforming growth factor (TGF)-β] is one possible alternative source of SMCs. MSCs have been procured from bone marrow or adipose tissue and differentiated into SMCs in *ex vivo* culture (Table 1) [5–7,11]. In addition, cells with a committed SMC phenotype have been isolated from omentum [12], subcutaneous adipose tissue [11,12] and the mononuclear fraction of peripheral blood [13].

The use of MSCs or other stem cell populations (adult-derived, embryonic or induced pluripotent) for organ regeneration is informative as a proof of concept. However, from a process development perspective focused on eventual

Box 2. Key factors facilitating the commercial viability of tubular organ regeneration platforms

1. A biodegradable synthetic scaffold based on well-characterized materials (e.g. PGA, PLGA) that can be manufactured reproducibly with defined characteristics is desirable.
2. A population of committed cells (typically SMCs) that is easily isolatable and expandable is required for scaffold seeding to trigger regeneration *in vivo*.
3. A purified population of defined stem cells is neither needed nor desirable. Costs increase significantly with requirements to monitor stem cell potential and to control directed differentiation.
4. Engineering of 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 innate regenerative response in the mammalian body, resulting in *de novo* organogenesis *in vivo* (including in humans).

product manufacturing and release, the requirements to monitor and control stem cell potential and directed differentiation towards a smooth muscle lineage substantially complicates efforts to streamline and standardize procedures and assure quality; additionally, it can lead to significant increases in costs. Directly sourced committed SMCs represent a phenotypically uniform cell population that does not require multi-step directed differentiation regimens and associated ancillary testing and monitoring protocols. From a product development and commercialization perspective, SMCs can be reliably isolated and expanded from patient candidates with minimal donor-dependent variability. The generation of smooth-muscle-like cells from MSCs, however, is contingent on successful isolation, characterization and expansion of phenotypically variable MSCs, as well as maintenance and continued analysis of the stem cell potential of the MSC population. In addition, considerable expense and time are associated with directed MSC differentiation using recombinant cytokines, and few if any data are available on the long-term clinical outcomes for MSC treatment using inductive agents that might impact cell cycle regulation. Box 2 lists factors facilitating the commercial viability of tubular organ regeneration platforms.

A more detailed summary of the potential technical and regulatory considerations that could affect the commercial utility of stem cells relative to committed SMCs for applications in tubular organ regeneration is presented in Table 2, using MSCs as an example. Issues similar to those outlined for MSCs in Table 2 arise for isolation, maintenance, expansion and differentiation of embryonic and induced pluripotent stem cells. To simplify bioprocessing procedures, enhance product consistency, reduce the risk of rejection and ensure a robust supply of cellular raw material, we focus here on developing classes of committed SMCs for tubular organ regeneration.

Extending the platform from bladder to other tubular organs

Iterations of the foundational technology platform outlined above have been successfully applied for regeneration of multiple additional tubular organs besides the bladder, including trachea, gastrointestinal (GI) tract, genitourinary system, lung and blood vessels (Figure 1). Below, we examine these variations in more detail and highlight their principal commonalities and their potential for successful commercial process development.

Trachea

Tissue-engineered trachea is an iteration of the foundational bladder-based platform for tubular organ regeneration. A 7-cm decellularized tracheal segment from a 51-year-old cadaveric donor was used as a scaffold [4]. Autologous epithelial cells were seeded at a density of 1×10^6 cells/mL in diluent at the luminal surface within a specialized bioreactor. Autologous MSCs derived from the recipient's bone marrow were differentiated towards a chondrocytic lineage with recombinant cytokines prior to seeding at similar densities onto the abluminal

Table 1. Example strategies for bladder regeneration *in vivo*

Cell source	SMC differentiation strategy[a]	Scaffold composition	Regenerative outcome	Refs.
Autologous bladder-derived UCs and SMCs (canine)	None applied	PGA/PLGA	Durable organ regeneration with complete urothelial coverage of lumen and tri-laminar bladder musculature in canine subtotal (≥70%) cystectomy model. Structure and function were evident at three months and improved with bioresponsiveness of organ capacity throughout study (24 months).	[9]
Xenogenic adipose SVF-derived MSCs (human to athymic rats)	100 U/mL heparin for 6 weeks	PLGA (bilayer composite)	Short-term (12 weeks) regeneration of functionality (capacity and compliance) in an athymic rat (50%) cystectomy model observed in cell-seeded and non-implanted groups. Muscle bundles, but no tri-laminar muscular architecture, as reported at 12 weeks.	[7]
Xenogenic BMSCs (human to nude mice)	5 ng/mL PDGF-ββ+2.5 ng/mL TGF-β for 7–14 days; static or 3D culture matrices were porcine bladder ECM or collagen type IV	PLLA	Bladder tissue regeneration not assessed. At 30 days after subcutaneous implantation in nude mice, myogenic markers were observed by protein and gene expression assays preferentially with BMSC-seeded scaffolds.	[5]
Xenogenic BMSCs directed to UC and SMC phenotypes (human to nude mice)	Co-culture with SMCs or SMC-conditioned medium	PLLA	Bladder tissue regeneration not assessed. At 30 days post-subcutaneous implantation in nude mice, myogenic markers were observed by protein and gene expression assays, and contractility by organ bath preferentially with BMSC-seeded scaffolds.	[6]

[a]Culture conditions used to induce directed differentiation to SMC phenotype.
Abbreviations: BMSC, bone-marrow-derived mesenchymal stem cell; PDGF-ββ, platelet-derived growth factor (ββ form); SVF, stromal vascular fraction.

Table 2. Comparison of MSCs and committed SMCs for application in commercially viable organ regeneration platforms

Mesenchymal stem cells	Committed smooth muscle cells
• Considerable initial variability in proliferative capacity and tri-lineage differentiation potential (e.g. donor effects, number of passages in culture) [39–43]	• Autologous sources readily available from multiple tissues using existing minimally invasive procedures; SMC phenotype does not exhibit donor variability [8,9]
• *Ex vivo* MSC expansion leads to loss of differentiation potential	• SMC phenotype remains stable during *ex vivo* expansion ($\leq$8 passages) [8,9]
• Monitoring of tri-lineage differentiation potential during *ex vivo* expansion is lengthy and expensive	• No requirement to monitor multi-lineage potential
• Directed differentiation is an uncontrolled inefficient process; only a subset of cells acquires SMC characteristics	• No requirement to monitor directed differentiation
• Directed differentiation requires defined inductive cytokine addition during *ex vivo* expansion, increasing costs	• No requirement for defined inductive cytokines during *ex vivo* expansion
• Multiple molecular, proteomic and functional tests required to characterize differentiated cellular component; test results can be misleading and unreliable [43]	• SMC phenotype remains stable during *ex vivo* expansion [8,9], facilitating standardization of cell characterization procedures
• Long-term effects of *ex vivo* exposure to inductive cytokines (e.g. TGF-β) are unknown and potential for generating transformed cells exists	• Long-term experience with media used for *ex vivo* SMC expansion exists as well as defined procedures for approved dilution of residual levels of media components
• Differentiation protocol significantly increases process times for SMC generation (e.g. up to 6 weeks for differentiation in 1% FBS-based media) [7]; cells not expandable during differentiation	• No requirement for directed differentiation
• Limited *in vivo* preclinical and clinical testing with clinically relevant models to date	• SMC-based organ regeneration platforms have been tested *in vivo* across multiple organ systems and species using clinically-relevant preclinical models and Phase I and II clinical trials

surface. Although the patient showed reconstitution of pulmonary function within 4 months, detailed histological examination of the regenerated organ was not possible [4]. However, in a swine model of this tissue-engineered trachea, both chondrocytes derived from differentiated MSCs and epithelial cells were needed for host survival [14].

Although this methodology is valuable as a proof of concept, the possibility of scale up to clinical production remains an open question. One significant drawback is the need for cadaveric donors as a scaffold source. Although it might be possible to create donor banks of cadaveric trachea for tissue engineering applications within specialized medical centers, broader application might require synthetic biodegradable scaffold materials similar to the PGA–PLGA polymers used for bladder regeneration [8–10] to facilitate reproducible, large-scale manufacture of scaffolds with defined chemical and physical properties. Furthermore, as outlined in Table 2, application of directed differentiation strategies to cells destined for patient implantation might involve additional regulatory hurdles, further complicating commercialization efforts. To this end, chondrocytes derived directly from autologous tracheal explants might be applicable to *de novo* regeneration of trachea [15,16].

GI tract

Individual components of the GI tract, including the oral cavity, esophagus, stomach, small intestine and colon, represent locally specialized iterative variations of essentially the same laminarly organized tubular histologic architecture as the bladder. The small intestine (SI) represents the most pressing unmet clinical need by far. For example, small bowel transplantation is an unsatisfactory current standard of care for pediatric small bowel syndrome owing to technical difficulties inherent in surgery, the requirement for extended immunosuppression and high rates of graft rejection [17].

Small intestine

Intestinal epithelium is highly regenerative in adult mammals; therefore, it might reasonably be expected to be amenable to tissue engineering or regenerative medicine methodologies. Discrete multipotent intestinal stem cells positive for the Lgr5 marker typically reside at the base of intestinal villi and are capable of self-organizing *in vitro* into organoid structures that recapitulate the intestinal lumen, crypt and villi domains [18]. Autologous organoid units, composed of incompletely dissociated clusters of epithelial and mesenchymal cells, were generated by partial digestion of intestinal epithelium (presumably containing resident intestinal stem cells) and used to seed poly(L-lactic acid) (PLLA) scaffold tubes, which were subsequently matured within the peritoneal cavity of pigs. At 7 weeks post-implantation, the retrieved implants contained tissue segments that recapitulated the gross overall laminar organization of native SI [19]. Importantly, acellular scaffolds similarly implanted into the peritoneum did not yield SI tissue segments. However, grafting such tissue-engineered SI segments to host SI in large mammals remains to be demonstrated. Furthermore, this approach required harvesting of up to 10 cm of autologous SI to derive organoids for implant; whether *ex vivo* expansion could reduce the amount of autologous SI required is an open question. Other concerns include whether organoid units capable of seeding a scaffold structure can be isolated from diseased human intestine and how much autologous tissue is required to generate clinically relevant implants. Finally, using the peritoneal cavity as a bioreactor might limit application of this approach.

A foundational tubular organ regeneration platform involving seeding of biopolymeric scaffolds with SMCs might be applicable for SI regeneration. To this end, collagen sponge scaffolds with (+) or without (−) stomach-derived SMCs were each grafted onto two defects of 1 cm $\times$ 1 cm in surgically isolated ileal loops in dogs [20]. SMC+ sponges were seeded at a density of 5×10^6

cells/cm^3. The two loops were converted into bioreactors by constructing a double ileostomy on the anterior abdominal wall. At 8 weeks post-ileostomy, one of the loops was re-anastamosed to native intestine in four animals (two dogs received the SMC+ loop, two received the SMC– loop) for an additional four weeks. Macroscopic analysis of tissue at the SMC+ implant sites demonstrated regeneration of native-like neo-mucosa. However, tissue at the SMC– implant sites remained ulcerated. Significantly enhanced vascularization, epithelialization and circular muscle organization were also observed at SMC+ compared to SMC– implant sites [20]. An increase in the number of SMCs seeded onto the scaffold resulted in a greater area of regenerated SI tissue; however, no concomitant increase in the thickness of the smooth muscle layer was observed, indicating that there might not be a simple linear relationship between the initial scaffold seeding density and the final regenerative outcome [21]. Nevertheless, these data suggest that the foundational organ regeneration platform involving biodegradable scaffolds seeded with SMCs might be adequate for facilitating SI regeneration. Although this approach still needs to be tested by anastomosing a length of tubular scaffold into SI, if successful, the methodology could represent a straightforward, clinically relevant and commercially viable strategy for SI regeneration.

Esophagus

The tubular organ regeneration platform might also be applicable to esophagus regeneration. In one study, defects created in the abdominal esophagus of 27 female rats were patched with cell-free scaffolds generated from gastric acellular matrix [22]. Of the 24 survivors, none showed evidence of lamina muscularis mucosa regeneration, even at 18 months post-implantation. By contrast, in a canine model of esophageal resection and replacement, PGA tubes seeded with a mixture of keratinocytes and fibroblasts triggered regeneration of smooth muscle laminar organization similar to native esophagus within three weeks post-implantation, whereas acellular PGA tubes formed esophageal strictures and led to near-complete obstruction within 2–3 weeks [23].

In another canine study, cervical esophageal defects were patched with either SI submucosa (SIS) alone or with SIS (5 cm $\times$ 2.5 cm) seeded with 1×10^7 autologous oral mucosal epithelial cells. After four weeks, dogs implanted with cell-seeded SIS showed near-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 [24]. Attempts to introduce an acellular SIS tubular construct into the cervical esophagus of piglets were also unsuccessful, demonstrating scarification and a minimal regenerative response [25].

Stomach

Stomach-derived organoid units (analogous to the SI organoids used to tissue engineer the SI) seeded on a biopolymeric scaffold composed of PGA tubes coated with PLLA triggered reconstitution of gastric and muscularis mucosa within the peritoneal cavities of swine at seven weeks post-implantation [19]. Immunohistological evidence confirmed regeneration of all differentiated epithelial cell types (enterocytes, enteroendocrine cells, goblet cells), as well as putative intestinal stem cells and elements of the intestinal nervous system. In an analogous approach in a canine model, circular defects were created in the stomach of seven animals and a composite comprised of layers of PGA, collagen and poly(D,L-lactide-*co*-ε-caprolactone) (PDLCL) soaked in either autologous peripheral blood or bone marrow aspirate, was sutured over the defect as a patch [26]. By 16 weeks post-implantation, the patch site exhibited evidence of healing, with re-epithelialization, villus formation and vascularization, with fibrosis in the submucosal layer. However, minimal regeneration of the smooth muscle layer was evident. The neo-tissue expressed smooth muscle α-actin, but not calponin, a marker consistent with a mature SMC phenotype [26].

Neo-vessels

Neo-blood vessels represent a significant commercial opportunity for application of a tubular organ platform technology to patients requiring bypass surgery. In a recently published clinical trial, vascular grafts composed of autologous bone marrow aspirate seeded onto a PGA/PDLCL scaffold were implanted into a cohort of 25 patients presenting with single ventricle physiology [27]. All patients were asymptomatic 30 days post-implantation, and 24 out of 25 patients were alive one year post-implantation. Efforts to seed tubular PGA neo-vessel scaffolds with SMCs derived from directed differentiation of bone marrow- or adipose-derived MSCs with TGF-β have also been described [28,29]. The requirement for directed MSC differentiation using TGF-β or related agents, as well as a prolonged maturation period under pulsatile conditions needed to achieve a mature SMC phenotype, probably mean that this approach is impractical for commercial application. Identification of a readily isolatable source of committed SMCs (e.g. the vascular fraction of adipose tissue or omentum) might represent a more commercially feasible platform for reasons elaborated on in Table 2. Direct luminal seeding of neo-vessels with endothelial cells (ECs) has been used as a strategy to promote neo-vessel endothelialization [30]. However, endothelialization of neo-vessel lumen occurs *in vivo* without prior nucleation by ECs, suggesting that seeded ECs might not be essential to mediate the formation of a functional endothelium *in vivo* [31].

Lung

The lung is a highly specialized tubular organ that might be amenable to regeneration using the platform approach discussed in this review. A study investigating the regenerative potential of PGA felts seeded with adipose-derived stromal cells (ADSCs) in stimulating pulmonary regeneration in a rat lung lobectomy model has provided evidence of the ability of the lung to regenerate [32]. PGA sheets (1 cm$\times$0.5 cm) seeded with up to 5×10^7 ADSCs were sealed onto the remaining lung lobe. Alveolar and vascular regeneration was observed within one week of implantation, with concomitant recovery of pulmonary functionality. The

authors hypothesized that factors secreted by the seeded cells, including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and fibroblast growth factor (FGF), elicited paracrine activity 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 ($5\times5\times2$ mm^3) at a density of 6×10^4–6×10^5 cells per sponge and implanted into adult rat lung [33]. Alveolar-like structures with apparent vascular networks regenerated within degrading scaffolds by four months post-implantation. Importantly, the formation of these alveolar-like networks was strongly dependent on prior seeding of the scaffold with lung cells. As in the previous study, the authors hypothesized that paracrine activity of factors secreted from seeded lung cells facilitated regenerative ingrowth of lung cells from surrounding native lung tissue as the likely mechanism of action. Most recently, tissue-engineered lungs, created from recellularized scaffolds derived from decellularized lung, have been characterized [44–46].

Genitourinary system

Recent reports of functional regenerated neo-phallus and neo-vagina in a rabbit model illustrate how principles of the tubular organ regeneration platform can be extrapolated to facilitate the regeneration of functionally distinct tubular organs [34,35]. Decellularized corpora cavernosa was used as a scaffold matrix for seeding autologous corporal endothelial cells and SMCs in a rabbit model of penile replacement. Seeding was performed using a multi-step static–dynamic seeding method. Implantation of decellularized scaffold alone led to the formation of a non-functional fibrotic phallus. However, cell-seeded scaffolds regenerated native-like corporal tissue organization within 3–6 months post-implantation. Tissue-engineered phalluses were functional, as demonstrated by the ability of recipient animals to copulate normally, resulting in pregnancy [34].

For the neo-vagina, autologous vaginal epithelial cells and SMCs were seeded at a density of 1×10^7 and 5×10^7 cells/cm^3 onto the luminal and abluminal surfaces, respectively, of PGA tubular scaffolds (6 cm^3) preconfigured to resemble native rabbit vagina. Native vagina was replaced with seeded or unseeded scaffolds in rabbits. Once again, unseeded scaffolds failed to trigger a regenerative response, whereas cell-seeded scaffolds generated stage-specific histogenesis, vascularization, innervation and regeneration of a patent neo-vagina by six months post-implantation, with a defined muscular layer and a luminal invaginated epithelium. Organ functionality was assessed by measuring the contractile response of the neo-tissue to electrical stimulation and compared to that of native tissue [35].

Future research and clinical outlook

The outlook for tubular organ regeneration in the clinic is promising. A foundational organ regeneration platform of a biodegradable scaffold nucleated with a population of committed SMCs, first pioneered for the bladder, has been applied to regenerating various, functionally disparate tubular organs. Proof-of-concept studies in humans have proven successful in regenerating bladder, trachea and vasculature. Tengion has initiated a Phase I clinical trial of the Neo-Urinary Conduit™ that is designed to passively conduct urine from ureters to a skin stoma (http://clinicaltrials.gov/ct2/show/NCT01087697?term=Tengion&rank=3), and was developed using the tubular organ regeneration platform that has been described in this review. These advances notwithstanding, there is considerable room for further research and development efforts. Questions that remain outstanding are listed in Box 3.

Box 3. Outstanding questions

- Can acellular scaffolds be designed to mimic the regenerative response of cell-seeded scaffolds through novel biomaterials that incorporate and release key paracrine factors in a controlled manner? If so, what is the minimal set of tissue- or organ-specific factors required to yield such a response?
- What is the exact mechanism of action by which SMCs seeded on a scaffold regenerate a tubular neo-organ?
- What is the minimal number of SMCs or other committed cell type of cells required to mediate neo-organ regeneration *in vivo*?
- What are the specific contributions of the seeded cell population and host cells towards regeneration of a complete neo-organ?
- How do regenerated neo-organs regulate organ volume relative to host size?

Despite the unanswered questions, we anticipate that the bladder regeneration platform will continue to serve as a paradigm for the development of other tubular neo-organs and advancement through the clinical trial process over the next 5–10 years.

Conflict of interest statement

The authors are listed as inventors on provisional patent applications relating to the bladder regeneration platform technologies discussed in this article.

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