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Dynamic Reciprocity in Cell-Scaffold Interactions

Joshua R. Mauney[1,2] and **Rosalyn M. Adam**[1,2,*]

[1]Department of Urology, Boston Children's Hospital, Boston, MA 02115

[2]Department of Surgery, Harvard Medical School, Boston, MA 02115

Abstract

Tissue engineering in urology has shown considerable promise. However, there is still much to understand, particularly regarding the interactions between scaffolds and their host environment, how these interactions regulate regeneration and how they may be enhanced for optimal tissue repair. In this review, we discuss the concept of dynamic reciprocity as applied to tissue engineering, i.e. how bi-directional signaling between implanted scaffolds and host tissues such as the bladder drives the process of constructive remodeling to ensure successful graft integration and tissue repair. The impact of scaffold content and configuration, the contribution of endogenous and exogenous bioactive factors, the influence of the host immune response and the functional interaction with mechanical stimulation are all considered. In addition, the temporal relationships of host tissue ingrowth, bioactive factor mobilization, scaffold degradation and immune cell infiltration, as well as the reciprocal signaling between discrete cell types and scaffolds are discussed. Improved understanding of these aspects of tissue repair will identify opportunities for optimization of repair that could be exploited to enhance regenerative medicine strategies for urology in future studies.

Keywords

constructive remodeling; acellular matrix; cell-seeded construct; host response

1. Introduction

Tissue engineering is defined as the repair or replacement of damaged tissues or organs with biodegradable 3-D scaffolds, either alone or seeded with exogenous cells, which are capable of promoting the de novo formation of site-specific functional tissues. In the field of urology, the feasibility of acellular and cell-seeded tissue engineered constructs to serve as therapies for defect reconstruction has been explored in a number of urinary tract organs including the bladder [1, 2]. The process by which tissue engineered implants support

*Correspondence: Rosalyn M. Adam, PhD, Enders Research Building, Rm 1061.1, Department of Urology, Boston Children's Hospital, 300 Longwood Avenue, Boston MA 02115, Tel: 001 617 919-2019, rosalyn.adam@childrens.harvard.edu.

functional tissue regeneration has been coined ‘constructive remodeling’ wherein ingrowth of the graft microenvironment by host cells leads to remodeling events that promote biomaterial degradation with subsequent deposition and maturation of functional new tissue [3, 4]. In general, these outcomes are inherently different from the default wound healing response in mammals that frequently results in scar tissue formation coupled with loss of function [4].

Over the course of constructive remodeling events, biomaterials not only act as structural support for defect consolidation, but also serve as substrates for host cell adhesion, migration, proliferation, differentiation and apoptosis. In turn, invading host cell populations play crucial roles in scaffold degradation and production of de novo extracellular matrix (ECM) in order to restore native tissue architecture and function. These bi-directional interactions between tissue-engineered grafts and the host tissue microenvironment are essential components in the constructive remodeling cascade and represent the concept of ‘dynamic reciprocity’ [4–6]. In this article, we review the need and current state of bladder tissue engineering, explore the impact of biomaterial properties on constructive remodeling, address the significance of dynamic reciprocity in cell-scaffold interactions and consider how these processes may be enhanced and optimized for maximum clinical benefit.

2. Current Limitations in Bladder Tissue Reconstruction

Enterocystoplasty is the “gold standard” surgical procedure utilized to reduce urinary storage and voiding pressures and reduce the risk of renal damage and incontinence associated with a variety of congenital and acquired bladder pathologies including neurogenic bladder, bladder exstrophy, and posterior urethral valves [7, 8]. Transposition of gastrointestinal segments into the urinary tract leads to a number of significant complications including chronic urinary tract infection, metabolic abnormalities, secondary malignancies, as well as donor site morbidity [9, 10], all of which can compromise the efficacy of the treatment and adversely affect the patient’s quality of life. Despite extensive research into alternatives to conventional enterocystoplasty, only a limited number of options have translated into clinical practice. Ureterocystoplasty is capable of circumventing complications associated with the absorptive epithelium of gastrointestinal segments; however, the possibility of urothelium-lined augmentation is effectively confined to a few patients with a combination of gross ureteric dilation and an ipsilateral nonfunctioning kidney [11]. Seromuscular enterocystoplasty, where a de-epithelialized colonic segment is combined with native urothelium after detrusorectomy and autoaugmentation, has also been used in patients [12, 13]. Unfortunately, this procedure has limited efficacy and is not well suited to small or trabeculated bladders of the sort commonly encountered in neurogenic bladder and bladder exstrophy [14]. Therefore, tissue engineering approaches for bladder reconstruction have been driven by the need to identify a suitable replacement for the use of autologous gastrointestinal segments for augmentation cystoplasty.

3. Properties and Performance of Tissue Engineered Constructs for Bladder Tissue Repair

Three main classes of biodegradable, biomaterials have been investigated for bladder tissue engineering: synthetic polyester-based grafts, matrices composed of decellularized tissues, and silk fibroin scaffolds. Recent findings from preclinical animal studies and clinical trials will be discussed in order to compare and contrast constructive remodeling outcomes achieved by these diverse biomaterial types for bladder reconstruction.

3.1 Polyesters

Synthetic polyesters are attractive candidates for tissue engineering applications since their structural, mechanical, and degradative properties can be specifically tailored to match the target tissue of interest by manipulating processing methods [15, 16]. In particular, polyester meshes composed of poly-glycolic acid (PGA), poly–dl–lactide–co– glycolide (PLGA), or their co-polymers have been primarily deployed as cell-seeded constructs for urologic tissue repair [17–22]. In a model of trigone-sparing cystectomy in canines, Atala and colleagues first reported the ability of a PGA mesh coated with poly– dl–lactide–co–glycolide 50:50 (PLGA), seeded with ex vivo expanded, primary smooth muscle and urothelial cells to mediate de novo tissue formation and enhance organ capacity and compliance over unseeded controls [17]. This study demonstrated that the ability of polyester grafts to mediate constructive remodeling of the bladder defects is significantly dependent on the presence of scaffold-seeded cell sources.

Based on these initial results, clinical trials of collagen-coated PGA matrices seeded with autologous bladder cell populations were explored for bladder augmentation in children afflicted with myelomeningocele [19]. Phase II studies of this technology failed to show significant improvements in bladder capacity or compliance and the level of serious adverse events was found to surpass an acceptable safety standard [21]. Limitations in the translation efficacy of this approach may be attributed to the abnormal physiology of the diseased, autologous cell sources utilized for scaffold seeding. Indeed, neurogenic bladder cell populations are known to exhibit deficiencies in their proliferative and differentiation potentials [23, 24], which may have ultimately hampered their capacity to participate in constructive remodeling. Recent studies deploying polyester grafts seeded with mesenchymal stem cells (MSC) in animal models of augmentation cystoplasty have shown improvements in bladder smooth muscle regeneration and attenuation of fibrosis in comparison to unseeded implants [22, 25]. The capacities of MSC to differentiate into functional smooth muscle cells [25, 26], undergo urothelial specification [27, 28], as well as release trophic factors encouraging de *novo* innervation [29, 30] and angiogenesis [31, 32] implicate them as promising cell sources for increasing regenerative properties of PGA implants in diseased settings.

3.2 Decellularized matrices

Scaffolds derived from decellularized tissues such has acellular bladder matrix (ABM) and small intestinal submucosa (SIS) provide a complex of functional and structural proteins including collagens, elastin, fibronectin, glycosaminoglycans (GAGs), proteoglycans, and

growth factors [33] which are capable of mediating urologic tissue repair through retention of existing bioactive ligands preserved following tissue processing. These matrices have been utilized as both acellular grafts and cell-seeded constructs for bladder reconstruction in preclinical studies and clinical trials. In non-diseased animal models of augmentation cystoplasty, both unseeded ABM and SIS scaffolds have been demonstrated to promote formation of innervated, vascularized bladder tissue as well as improved urodynamic outcomes such as increased bladder compliance and capacity following partial cystectomy [34–45]. In addition, studies of cell-seeded approaches for bladder augmentation with ABM and SIS have shown that the combination of these matrices with primary bladder smooth muscle and urothelial cells or MSC is capable of leading to higher degrees of smooth muscle regeneration as well as enhanced bladder function in comparison to unseeded counterparts [26, 46–48].

The performance of unseeded ABM and SIS in bladder tissue repair has also been investigated in a number of diseased animal models. Cayan and colleagues showed that ABM grafts were capable of increasing bladder capacity and compliance over non-augmented controls in rat model of chemical cystitis [49]. In addition, urothelial and smooth muscle regeneration was observed throughout the original implantation sites comparable to normal bladder architecture; however nerve regeneration was less developed. In a rat model of neurogenic bladder induced by spinal cord injury, Obara's group indicated that ABM promoted host integration of smooth muscle and urothelial cells throughout the grafted area [50] while studies by Urakami demonstrated that voiding function could be improved following ABM implantation [51]. Recent data from our laboratory has also shown that acellular SIS matrices undergo remodeling and facilitate ingrowth of vascularized smooth muscle as well as urothelial cells throughout implantation sites in the setting of neuropathogenic bladder disease [52].

Despite evidence of de novo tissue formation and improved functional outcomes across a variety of preclinical animal models, a number of deleterious side-effects have been observed with the use of ABM and SIS for bladder augmentation in disease settings. Kropp and colleagues reported that implantation of SIS grafts into inflammatory canine bladders induced by 90% partial cystectomy for 1 month prior to repair led to incomplete biomaterial degradation, organ perforation, matrix contracture, as well as the urinary stone formation [53]. The authors concluded that implantation of SIS scaffolds alone or seeded with primary bladder cell populations does not promote the same quality or quantity of bladder regeneration that is seen in non-inflammatory canine counterparts subjected to 40% partial cystectomy. In addition, the use of ABM for bladder augmentation in a neurogenic rat model has been associated with complications such as graft atrophy, pyuria, implant contracture, urinary tract infections, and urinary calculi [50, 51].

Several recent clinical trials of decellularized matrices for augmentation cystoplasty have also produced suboptimal outcomes. Schaefer's group noted that bladder augmentation with SIS cannot be recommended as a substitute for enterocystoplasty due to insufficient increases in bladder compliance following implantation in children with neurogenic bladder and cloacal exstrophy [54]. In addition, serious adverse events including bladder rupture and stone formation were observed in this patient cohort. A report by Caione demonstrated that

implantation of acellular SIS matrices failed to establish long-term urinary continence and did not support significant increases in bladder capacity and compliance as well as regeneration of normal smooth muscle architecture 3 years post-operatively [55]. These studies demonstrate that further optimization of decellularized grafts is necessary in diseased animal models in order to identify a clinically viable matrix configuration for bladder reconstruction.

3.3 Silk fibroin scaffolds

Biomaterials derived from *Bombyx mori* silk fibroin represent an emerging option for bladder tissue engineering due to their unique set of properties including high structural strength and elasticity [56], diverse processing plasticity [57], and tailorable biodegradability [58]. Similar to polyester matrices, the structural, mechanical, and degradative features of silk fibroin scaffolds can be specifically tuned by manipulating fabrication parameters in order enhance constructive remodeling outcomes in the bladder [59–61]. This processing flexibility is particularly advantageous for optimizing biomaterial characteristics for bladder repair in comparison to SIS and ABM grafts wherein the physical attributes of these scaffolds are often dependent upon the properties of the source tissue and methods required for decellularization [4]. In contrast to the degradation metabolites of polyesters which can evoke strong inflammatory responses [62] contributing to long-term implant failure [63], enzymatic breakdown of silk fibroin results in the release of nontoxic amino acids [64]. Our published data has also demonstrated that surface chemistries of silk fibroin biomaterials can be functionalized with fibronectin coatings [65]. This matrix modification was shown to enhance cellular processes required for host tissue integration such as urothelial and smooth muscle cell attachment and proliferation [65].

Our previous reports in non-diseased rodent models of augmentation cystoplasty have demonstrated that acellular silk fibroin scaffolds display particular advantages in comparison to conventional PGA and SIS scaffolds including improved functional performance, enhanced tissue regeneration, and minimal inflammatory reactions [60, 63]. In addition, these studies also provided evidence that a bi-layer silk fibroin scaffold design, comprised of a porous foam compartment buttressed by a non-permeable layer, was capable of supporting superior functional bladder tissue regeneration in respect to gel spun silk fibroin matrix variants [60]. The distinct compartments of this bi-layer matrix architecture serve specific purposes: the film layer acts as a fluid-tight barrier that allows for organ retention of urine following initial scaffold implantation while the porous compartment supports host bladder tissue ingrowth during defect consolidation [60, 61]. Our results have shown the feasibility of the bi-layer silk fibroin scaffold to support regeneration of innervated, vascularized smooth muscle and urothelial tissues with structural, mechanical, and functional properties comparable to non-augmented controls in a non-diseased porcine model of bladder repair [61]. Recent work from laboratory has also detailed the ability of bi-layer silk fibroin scaffolds to promote de novo bladder tissue formation and mitigation of high intravesical pressures encountered in rat model of neurogenic bladder [52]. Further validation of silk fibroin biomaterials for augmentation cystoplasty in large animal models of bladder disease is necessary before clinical translation is considered.

4. Stages and mechanisms of bladder tissue regeneration

There exists a paucity of information in the literature concerning the exact mechanisms governing the formation of de novo urothelial and smooth muscle tissues at sites of acellular scaffold implantation. Qualitative observations of time-dependent stages of bladder tissue regeneration following SIS and silk fibroin matrix grafting in rodent models of augmentation cystoplasty have shown that repopulation of defect sites with a transitional urothelium is apparent by 2–3 weeks post-operatively, while reconstitution of the smooth muscle layer can occur following 2 months [63, 66]. The adult urothelium is comprised of 3 cell layers: basal, intermediate, and superficial which are normally quiescent, but can regenerate in response to acute damage. Lineage-tracing studies by the Mendelsohn laboratory have shown intermediate cells expressing p63 and uroplakin can self-renew and generate uroplakin-positive, p63-negative, superficial daughter cells in response to cyclophosphamide-induced damage; a process mediated by retinoid pathways [67]. Shh, Wnt, and BMP signal transduction mechanisms have also been implicated as significant drivers of urothelial regeneration in response to bacterial injury or chemical insults via bi-directional signaling events with the underlying stroma [68, 69]. However, it remains to be determined if these processes play a role in urothelial tissue formation following bladder augmentation with acellular biomaterials. In respect to mechanisms governing smooth muscle tissue consolidation at scaffold implantation sites, several processes have been proposed to play a role including mesenchymal-epithelial interactions, transdifferentiation of fibroblasts into phenotypic SMC, as well as dedifferentiation and migration of peripheral SMC from the host bladder wall into the defect site [70–72]. It has also been reported that circulating bone marrow-derived cells harbor the ability to migrate and differentiate into bladder SMC at sites of ABM grafting [73]. Further experimentation is ultimately needed to elucidate the contribution of these individual processes to the overall regenerative response.

5. Scaffold degradation and host cell response in constructive remodeling

Scaffold degradation is essential for integration of matrices into host tissue, and the rate at which this occurs is a major contributor to successful defect repair. Breakdown of natural and synthetic biomaterials is dependent on variety of factors such as configuration, crystallinity, molecular weight, pore size, porosity, biomechanical stresses, and grafting site [64, 74]. Following scaffold implantation, tissues initially display an acute inflammatory response associated with wound healing, which is characterized by infiltration of polymorphonuclear neutrophils and mononuclear cells. As defect consolidation progresses, a feedback mechanism occurs wherein host inflammatory reactions, originally elicited by biomaterial implantation [62, 75, 76], ultimately play a role in scaffold degradation and subsequently tissue remodeling outcomes. The nature of the host response is dependent on the type and composition of biomaterial configurations. For example, the polyesters, PGA and PLGA, undergo random hydrolysis of their ester bonds in vivo which can produce acidic degradation metabolites that stimulate complement pathway activation. Fragments of polyester matrices are phagocytized by macrophages and multi-nucleated giant cells [77] and have the potential to produce foreign body responses culminating in fibrosis [63]. Natural matrices derived from decellularized tissues and silk fibroin are proteolytically degraded with macrophage-dependent processes playing a dominant role in this process [58,

78]. Badylak and colleagues demonstrated the requirement of macrophages in SIS degradation by treating rats implanted with SIS in abdominal wall defects with liposomes containing bisphosphonate clodronate [78], phagocytic uptake of which leads to macrophage apoptosis and depletion from the circulation [79]. Inhibition of macrophage function significantly inhibited scaffold degradation at early time points, leading to the conclusion that adequate access of biomaterials to circulating phagocytic cells is required for successful cell-mediated scaffold degradation.

Alterations in the structural stability of biomaterial structure can also influence the balance between constructive remodeling and scar tissue formation, thus demonstrating a link between scaffold degradation rate and the extent of tissue repair achieved. A report by the Badylak laboratory provided evidence for this concept by showing significant differences in the nature of host cell infiltration following implantation of native or slowly-degrading, chemically cross-linked SIS into rat abdominal wall defects [80]. Whereas both native and cross-linked SIS elicited acute inflammation to a comparable extent within 1–2 weeks following implantation, tissue consolidation with cross-linked SIS displayed a long-term response typical of chronic inflammation, foreign body response and tissue fibrosis. In contrast, host tissue receiving native SIS showed little to no evidence of inflammation at time points up to 4 months and was associated with constructive remodeling [80]. In that study, variations in macrophage functional phenotype were shown to correlate with the downstream remodeling outcomes elicited by the two scaffold variants suggesting a potential mechanism by which host tissue response mediates regenerative outcomes. Macrophages display functional plasticity across a broad spectrum of activation states and are generally classified into two divergent classes, M1 and M2, based upon observations of differential cytokine expression profiles and cell surface markers [81, 82]. M1 macrophages are generally characterized by expression of specific cell surface markers such as CD68, CD80, and CCR7 and are considered to be a pro-inflammatory phenotype via their production of reactive oxygen intermediates and inflammatory cytokines such as IL-1β, IL-6, and TNF-α [83, 84]. In contrast, M2 macrophages express CD68 and CD163, secrete anti-inflammatory cytokines such as IL-10 and TGFβ1, scavenge debris, and promote angiogenesis [80, 82]. Badylak and colleagues observed that M1 macrophages were dominant in the host response to cross-linked SIS, whereas native SIS was associated with a predominant M2 macrophage phenotype [80]. Macrophages subsequently identified as M2 were evident between individual layers of the native SIS graft, in contrast to cells displaying an M1 phenotype that did not enter the cross-linked scaffold [80]. These observations suggest that the activation of specific macrophage phenotypes during scaffold remodeling may be an important control point for dictating the quality of tissue regeneration produced.

To date there has been limited study of the contribution of specific populations of macrophages to urinary tract tissue regeneration and repair. A recent study from the Southgate laboratory has begun to shed light on the signaling events underlying the contribution of host cells to constructive remodeling of scaffolds [85]. Using an ex vivo model comprising porcine ABM interfaced with human urinary tract tissue this study revealed infiltration of human macrophages expressing high levels of the M2 marker CD163 into the ABM. The infiltrating cells also displayed robust nuclear expression of PPARγ, activation of which further expanded the population of CD163-positive M2 macrophages.

These findings suggest potential mechanisms whereby macrophage phenotype can be controlled, potentially through the incorporation of pharmacologic modulators of PPARγ signaling within the scaffold matrix. In a related study, Sharma and colleagues demonstrated the potential of functionalizing SIS matrices with antiinflammatory peptide amphiphiles (AIF-PA) in order to modulate the innate host immune response in a rat model of bladder augmentation [86]. In comparison to control matrices, scaffolds impregnated with AIF-PA were capable of decreasing levels of M1 macrophages, while stabilizing levels of M2 macrophages. This scaffold modulation resulted in increased production of anti-inflammatory cytokines within the target tissue culminating in reductions in fibrosis and enhanced constructive remodeling. The authors conclude that AIF-PA can provide a highly conducive regenerative milieu for bladder tissue engineering applications.

As discussed in the following section, host cell-mediated scaffold degradation by macrophages is also likely to release bioactive factors present in acellular matrices that in turn stimulate recruitment of additional host cells, including endothelial cells and progenitors that contribute to effective tissue repair.

6. Modulation of Scaffold Properties with Trophic Factors

Endogenous ECM promotes effective morphogenesis during development and wound healing through the elaboration of bioactive signaling molecules that mediate communication between cell types. Functional repair of urinary tract structures requires appropriate regeneration of a diverse array of tissue types including epithelium, smooth muscle, nerves and vasculature. Prototypical mitogens and motogens incorporated into scaffolds have been shown to enhance the survival, proliferation, migration and differentiation of some or all of these cell types in the setting of experimental regeneration. For natural scaffolds such as ABM or SIS, tissue processing strategies can be optimized to retain existing bioactive ligands within the matrix structure. These substances are believed to participate in signaling with constituents in the host tissue to promote tissue regeneration and repair. However, tissue repair using unmodified acellular matrices has yielded suboptimal results and has often been associated with poor regeneration, scar formation and graft shrinkage. As a result, a variety of tissue engineering approaches have incorporated bioactive factors into both natural and synthetic scaffolds to enhance tissue remodeling and repair, including adhesion-promoting peptides, growth factors and cytokines, matricellular proteins and proteolytic enzymes. Results from these studies have not only suggested ways in which modifications may improve integration of scaffold biomaterials into host tissue for clinical applications, but have also shed light on the biological basis for endogenous mechanisms of tissue regeneration. The role of selected factors in key aspects of tissue repair will be considered in more detail below.

6.1 Adhesion-promoting peptides and matrices

Among the simplest elements incorporated into biomaterial scaffolds are those that enhance adhesion, proliferation and migration either of ingrowing host cells or of exogenously seeded cells, thereby enhancing consolidation of grafts. These include the integrin-binding peptides such as Arg-Gly-Asp (RGD) or Tyr-Ile-Gly-Ser-Arg (YIGSR) and their derivatives. Several studies have explored the contribution of specific ECM coatings to

ingrowth, survival and proliferation of progenitor or primary cell populations on different scaffold types. Hudson et al., compared collagen types I and IV, laminin and fibronectin for their ability to support attachment and proliferation of primary human urothelial cells and observed some enhancement of DNA synthesis in cells seeded on collagen type IV, laminin or fibronectin, although no significant differences were noted between coatings [87]. A similar comparison was performed by Franck and colleagues who found fibronectin to be superior to collagens for each endpoint assessed, including attachment, proliferation and differentiation of primary urothelial and smooth muscle cells, as well as ESC and iPS cells [65].

Tissues undergoing remodeling are believed to expose non-canonical 'cryptic' ECM-binding epitopes that contribute to altered cell behavior. To test this idea, Herz and colleagues compared the impact of native or denatured collagen on DNA and protein synthesis in primary bladder smooth muscle cells in culture [88] and observed profound increases in SMC proliferation and hypertrophy upon exposure to damaged (denatured) collagen. Furthermore, these alterations were only partially attenuated following re-exposure to native collagen. These findings demonstrate that the specific ECM conformation is a powerful determinant of cell phenotype and are particularly relevant to cases where tissue engineered constructs are introduced into diseased tissues with altered ECM such as the neuropathic bladder or strictured urethra.

6.2 Peptide growth factors

In view of their ability to control discrete aspects of cell behavior relevant to regeneration, a variety of growth factors have been employed in scaffolds, both singly and in 'cocktails' to promote diverse biological endpoints in engineered tissues including proliferation, differentiation, migration of host and donor cells, as well as angiogenesis from the host microenvironment.

The detrusor smooth muscle comprises the bulk of the bladder wall. As a result, many tissue regeneration strategies have relied on incorporation of canonical smooth muscle mitogens such as bFGF/FGF-2, PDGF and IGF-1, in scaffolds to stimulate smooth muscle regeneration. Kanematsu and colleagues provided one of the first demonstrations for enhanced bladder repair following implantation of bladder acellular matrix containing FGF-2 in a rat model of bladder augmentation [89]. In that study, FGF-2 was released from the scaffold matrix in a sustained fashion, and in turn led to elevation of endogenous VEGF levels and a corresponding enhancement of vascularization in the graft. In subsequent studies other investigators have used acellular matrix incorporating a modified exogenous human FGF-2 with a collagen-binding domain from collagenase to enhance release kinetics and bioavailability. This variant was found to be superior to matrix containing native FGF-2 in a bladder repair model, leading to improved performance of the regenerated organ [90]. Similar improvements in graft integration in vivo were observed with use of a fibrin matrix containing a modified IGF-1 fusion protein that enabled retention of the growth factor at the site of injury/wound repair [91]. By comparison with wild type IGF-1, the α2-plasmin inhibitor-modified IGF-1 ($\alpha 2\text{-PI}_{1-8}$-IGF-1) promoted greater proliferation of SMC in a rat model of bladder repair.

Since the totality of signals required for complete regeneration is unlikely to be accomplished through the use of a single stimulus, many groups have explored the use of growth factor 'cocktails' comprising multiple mitogens, motogens and differentiation factors that elicit the range of cell behaviors required for tissue repair. This is particularly important in cases where scaffolds are seeded with pluripotent or multipotent cells such as mesenchymal stem cells that undergo differentiation to desired target cell types. Rigorous control over expansion and tissue-appropriate differentiation of MSCs is essential to minimize adverse effects from uncontrolled proliferation or differentiation into undesired tissue types. Thus, several groups have employed growth factor 'cocktails' that comprise both mitogens and differentiation factors such as TGFβ1, HGF [92, 93] or NGF [94]. Zhou et al., reported significant improvements in regeneration of the rabbit bladder using bladder acellular matrix containing a PDGF-BB/VEGF cocktail [95]. In that study, the histological appearance and contractile activity of bladder tissue from rats receiving the PDGF/VEGF-impregnated scaffold was significantly improved compared to that in rats receiving control scaffold lacking added growth factors [95]. However, since neither growth factor was tested alone the relative contributions of each to subsequent repair could not be assessed in that study. Similar improvements in bladder function were observed in a rat model of spinal cord injury coupled with partial bladder replacement using ABM co-administered with either NGF or VEGF or both growth factors together [94]. Inclusion of NGF, VEGF or the NGF/VEGF cocktail yielded greater improvement in bladder capacity and compliance compared to scaffold alone, although the difference in these parameters between the cocktail and individual growth factors was not statistically significant [94]. Although the mechanistic basis for these observations was not addressed, the findings suggest that administration of either NGF or VEGF may up-regulate endogenous levels of the other trophic factor resulting in the observed regeneration and repair. To date, growth factors incorporated into scaffolds have been selected based on known activities in a variety of organ systems. Ongoing studies by a number of groups are characterizing the molecular events that occur both in regenerating tissue following injury [67, 69, 96] and during development [67] to better understand how this knowledge could be exploited for tissue engineering.

In the examples described above, growth factors were provided in scaffolds in their recombinant form. However, actively remodeling tissues are known to release bioactive factors into the microenvironment through the coordinated actions of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases, TIMPs [97]. MMPs are released by cells within the host tissue and by cells seeded on grafts, and are thought to be essential for constructive remodeling. Levels of several MMPs, including MMP-1, −2, and −9, were found to be increased following implantation of bladder acellular matrix grafts seeded without or with cells in vivo [95, 98–100]. The importance of MMP activity for appropriate regeneration, albeit in vascular structures, was demonstrated in an elegant study by Sung and colleagues, who showed that genetic deficiency of MMP-9 impaired collagen degradation and also attenuated angiogenesis in scaffolds implanted in vivo [101]. Results from this study also demonstrated the importance of MMP-9 activity in cells seeded on the scaffold as well as those in host tissue for appropriate integration of tissue-engineered constructs.

In addition to classical peptide growth factors, additional substances have been tested for their ability to enhance tissue repair in association with acellular matrices. The glycosaminoglycan hyaluronic acid (HA) has been incorporated into acellular matrices including BAM and SIS, based on its known roles in development and wound repair (reviewed in [102]). In vitro, HA cross-linked to ABM was shown to increase contraction of grafts seeded with bladder SMC and urothelial cells, as well as MMP-2/MMP-9 (gelatinase) activity [103]. These observations are consistent with the ability of HA provided in scaffolds to mediate reciprocal signaling with the microenvironment. Incorporation of HA into SIS enhanced neovascularization in regenerated bladder tissue in a canine model of partial cystectomy [104]. A subsequent study by the same group, in which HA was provided in the form of nanoparticles, failed to show significant differences in graft size or cystometric endpoints between unmodified and HA-modified SIS, despite some evidence of enhanced smooth muscle regeneration in the latter group [105]. HA-coated matrices have been further modified by rehydration in solutions containing VEGF. HA-VEGF-modified scaffolds were associated with improved smooth muscle and urothelial regeneration, decreased fibrosis and increased vascularity in a porcine model of bladder augmentation, compared to unmodified or HA-matrices [106], although the difference between HA- and HA-VEGF scaffolds was not statistically significant. HA is known to exert immune modulatory effects during wound healing in a variety of systems [102]. Consistent with this possibility, preliminary evidence suggests HA-VEGF-modified scaffolds were able to modify the immune response elicited by scaffold implantation during bladder regeneration [107]. Transcript levels for a number of pro-inflammatory cytokines and receptors were significantly different between scaffolds without or with HA and VEGF. Expression of TLR4 protein appeared to decline in tissue regenerated with HA-VEGF scaffold compared to that with acellular matrix alone, although no quantitative data were provided.

An essential aspect of effective tissue regeneration is the development and maintenance of an adequate blood supply. As noted above, a number of studies have explored strategies to ensure appropriate neovascularization, either by optimization of processing strategies to retain bioactive factors in acellular scaffolds or by addition of recombinant angiogenic factors such as FGF-2 or VEGF [95, 106, 108]. In addition to covalent linkage of VEGF recombinant proteins, some investigators have incorporated VEGF plasmid into cells by viral transduction prior to seeding on scaffolds [109, 110] to ensure sustained production of protein following engraftment. These types of experiments exemplify dynamic reciprocity in that the scaffold ECM signals to endothelial cells in the host tissue, which in turn responds by undergoing angiogenesis to vascularize the graft and promote integration into the host organ.

6.3 Interaction of growth factors and mechanical factors

Tissues comprising the urinary tract such as the bladder, ureters and urethra are mechanically active, enabling them to fulfill both storage and expulsion functions. In the context of tissue engineering, it has been demonstrated that mechanical conditioning of scaffolds, in particular cell-seeded grafts, can enhance integration into host tissue and subsequent performance (reviewed in [111]). A substantial literature now exists describing transcriptional responses to mechanical stimulation in bladder smooth muscle cells,

fibroblasts and other cell types [112–119], and these reports have begun to delineate the signaling networks that underlie physiologic and pathologic bladder wall remodeling. It is anticipated this knowledge could be exploited in the development of ‘smart’ scaffolds to improve the process of constructive remodeling.

Application of mechanical stimuli, in the form of static or dynamic stretch paradigms has been shown to modulate the phenotype of tissue constructs in vitro. Early studies demonstrated improvements in the contractile phenotype of SMC seeded on scaffolds when exposed to cyclic mechanical strain [120, 121], although subsequent reports yielded conflicting results regarding the impact of physiologic strain parameters on expression of differentiation markers in SMC [122, 123], possibly due to the use of different scaffolds or stimulation parameters. Heise and colleagues showed that mechanical stimuli interact functionally with soluble factors to alter bladder SMC phenotype on SIS grafts [100]. In particular, treatment of SMC-seeded SIS with FGF-2 increased matrix metalloproteinase activity and enhanced penetration of SMC into the matrix. Subsequent exposure to defined mechanical stimulation led to the production of elastin fibers. Additional studies from this group have suggested that increased elastin content in bladder wall ECM is associated with higher tissue compliance [124].

The contribution of mechanical stimulation to tissue regeneration in vivo has also been explored. Integration of SIS and ABM scaffold matrices into the bladder wall in a canine partial cystectomy model under conditions of physiologic filling and emptying was found to be enhanced, compared to scaffolds exposed to minimal mechanical stimulation [125]. In that study, exposure to mechanical stimulation was controlled by either short-term (1 d) or long-term (28 d) catheterization. Early restoration of cyclic filling and emptying, in the short-term catheterization group, was associated with improved tissue remodeling, differentiation into both urothelium and smooth muscle, and robust vascularization. In contrast, tissues in the long-term catheterization group displayed reduced repair and evidence of pathologic remodeling, as a consequence of minimal cycling [125]. Together, these studies imply that mechanical stimulation is an essential component of constructive remodeling, and acts to alter production of growth and differentiation factors, and increase protease production and/or activity.

7. Conclusions

In summary, we have reviewed the current understanding of constructive remodeling in urologic tissue engineering. Dynamic reciprocity between implanted biomaterials and host tissues has emerged as a central regulator of tissue remodeling events which dictate the extent of functional tissue repair achieved. Key factors such as scaffold composition and architecture, ex vivo seeded cell sources, the presence of endogenous and exogenous growth factors, the host immune response as well as mechanical stimuli have all been implicated in bi-directional signaling events which govern successful graft integration and de novo tissue formation at implantation sites. Improved understanding of these processes will identify opportunities for optimization of repair that could be exploited to enhance regenerative medicine strategies for urology in future studies.

Abbreviations

ABM	acellular bladder matrix
ECM	extracellular matrix
ESC	embryonic stem cells
FGF2	fibroblast growth factor-2
HA	hyaluronic acid
HGF	hepatocyte growth factor
IGF-1	insulin-like growth factor-1
iPSC	induced pluripotent stem cells
MMP	matrix metalloproteinase
MSC	mesenchymal stem cells
NGF	nerve growth factor
PDGF-BB	platelet-derived growth factor-BB
PGA	poly-glycolic acid
PLGA	poly–dl–lactide–co–glycolide
PMNL	polymorphonuclear leukocyte
SIS	small intestine submucosa
SMC	smooth muscle cell
TGFβ1	transforming growth factor-beta 1
TIMP	tissue inhibitor of metalloproteinase
TLR	toll-like receptor
UC	urothelial cell
VEGF	vascular endothelial growth factor

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Table 1

Trophic factors in tissue regeneration

Trophic factor	Intrinsic	Extrinsic	Biological activity	Citation
FGF-2/bFGF	Y	Y	Smooth muscle cell proliferation, angiogenesis	[89][90][100] [106][108]
PDGF-BB	Y	Y	Smooth muscle cell proliferation and differentiation	[95][115][117]
VEGF	Y	Y	Angiogenesis	[89][94][95][100] [106][108]
EGF		Y	Urothelial and endothelial cell proliferation	[93]
IGF-1		Y	Smooth muscle cell proliferation	[91]
TGFβ1		Y	Growth inhibition; Smooth muscle cell differentiation	[93]
HA		Y	Immune modulation	[103][104][105] [106][107]
HGF		Y	Smooth muscle cell proliferation and differentiation	[93]
NGF		Y	Neuronal growth and differentiation	[94]
Shh	Y		Induction of Wnt2, Wnt4	[69]
Wnt2, Wnt4	Y		Induction of stromal and epithelial proliferation	[69]
BMPs	Y		Induction of urothelial proliferation and differentiation	[68]
Retinoids	Y		Induction of urothelial specification and regeneration	[67]
Matrix-derived peptides	Y	Y	Smooth muscle cell proliferation; immune modulation	[86][88]