

Chapter 25

History and Development of Regenerative Medicine and Tissue Engineering in Urology

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Over the course of the past half-century, the field of urology has born witness to the translation of a multitude of tissue engineering and regenerative medicine technologies from the laboratory to the bedside. While early advancements involved the use of synthetic materials for structural replacement along the genitourinary tract, further development of cell and tissue culturing techniques, along with breakthroughs in the disciplines of cellular and molecular biology, gave rise to the field of tissue engineering, which combined this newfound understanding of cell and tissue growth with material science and engineering. Along with the biotechnology of cell transplantation and nuclear transfer, the field of tissue engineering sought to regenerate living tissues and organs, hence giving birth to the concept of regenerative medicine. Major discoveries in the field of genitourinary regenerative medicine over the past 30 years have progressed from injectable biomaterials to synthetic polymer and naturally-derived scaffolds seeded with cellular material and subsequently implanted. Over time, these scaffolds have advanced from simple onlay grafts to tubularized structures, hollow organs, and organs with highly complex stromal and vascular architectures.

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Injectable Therapies for Vesicoureteral Reflux and Urinary Incontinence

Vesicoureteral reflux is most commonly observed in the pediatric patient population, with the goal of contemporary first-line therapies, namely observation or antibiotic prophylaxis, to prevent sequelae such as reflux nephropathy and subsequent renal scarring. Prior to the development of injectable materials and the advent of endoscopic technology for their delivery, the earliest surgical interventions for these patients were highly invasive and involved either intravesical or extravesical approaches for reimplantation of the distal ureter into the bladder mucosa [1, 2]. Urinary incontinence is more prevalent in women and increases in incidence directly with age, but also occurs in males, most commonly in a post-radical pelvic surgery setting [3]. For female patients with stress urinary incontinence, endoscopic urethral bulking is a viable option following failure of first-line therapies. In the setting of both reflux and urinary incontinence, the properties of ideal injectable materials have been identified throughout the literature as those which are non-migratory, non-tumorigenic, non-antigenic, biocompatible, and will conserve their volume long-term following injection in order to maintain functionality. In an attempt to identify materials that demonstrate these properties, a spectrum of novel agents have been evaluated in the laboratory and clinical settings over the past century, ranging from non-autologous, synthetic materials and evolving over time towards patient-derived autologous therapies and stem cell-derived therapies.

Non-autologous, Synthetic Biomaterials

The earliest materials evaluated for use as injectable therapies date back to the early twentieth century when Gersuny performed urethral injections with paraffin as a sclerosing agent to treat urinary incontinence [4]. Over the next 70 years a variety of injectable sclerosing agents were evaluated clinically, but were unable to demonstrate long-term efficacy and were discredited by reports of local tissue sloughing and pulmonary emboli, eventually resulting in their abandonment as therapeutic options for incontinence [5–7]. The next generation of biomaterials to be used as injectable bulking therapies was ushered in by Berg, who in 1973 first utilized polytetrafluoroethylene (Teflon) paste for the treatment of urinary incontinence [8]. Teflon injections for periurethral bulking became more widespread in the mid-1970s, as Politano reported good functional outcomes in treating urinary incontinence, and became rapidly adopted to treat reflux as an endoscopically-delivered subureteral bulking agent, as first reported by Matouschek in 1981 [9, 10]. Throughout the 1970s and 1980s, Teflon was the primary injectable bulking agent utilized to treat thousands of ureters for reflux, yielding resolution at rates of upwards of 76–82% following a single injection in patients with long-term follow-up [11, 12]. However, the use of Teflon was mostly discontinued, as several reports surfaced over concerns of

particle migration and systemic granuloma formation, with an 80 μm particle size proposed as the threshold to prevent the migration of injectables [13, 14]. As this major disadvantage to Teflon usage became realized, the search for a safer, but equally efficacious, bulking agent as a therapy for both reflux and urinary incontinence ensued. In the early 1990s, a variety of new injectable biomaterials were developed and evaluated in animal models, including polyvinyl alcohol, silicone microparticles in hydrogel, and injectable bioglass [15–17]. Unfortunately, these biomaterials were plagued by complications, with reports of distant particle migration, potential tumorigenesis, and diminished implant volume over time. As a result, these biomaterials did not gain substantial traction within the urologic community.

From the initial reports of its clinical application for the treatment of urinary incontinence in 1989 and reflux in 1991, glutaraldehyde cross-linked bovine collagen (GAX35; bovine collagen, containing at least 95% type I collagen and up to 5% type III collagen, cross-linked with glutaraldehyde at 35 mg/mL) has been widely utilized as an endoscopic injectable therapy [18, 19]. Intermediate-term outcomes for treatment of urinary incontinence have varied widely, with reported rates of symptom improvement as low as 57% and as high as 94% at 2-years of follow-up [20, 21]. Moreover, a relatively poor durability of cure of urinary incontinence upon completion of the last injection therapy was observed, with only a 45% cure rate at 3 years [22]. Although GAX collagen is cross-linked to prevent degradation by collagenases, several studies evaluating its use as an injectable therapy have reported decreasing implant volume over time and high rates of failure following a single injection therapy necessitating retreatment [23–25]. Up until its production was discontinued in 2010, GAX collagen was the most commonly used and extensively studied injectable biomaterial for these applications.

Additional biomaterials have been clinically evaluated as injectable therapies and are still currently in use today for the treatment of reflux and urinary incontinence. Dextranomer/hyaluronic acid (HA) microspheres (Deflux) were first described for the treatment of reflux in 1995, with reported success rates for correction of reflux between 72 and 86% following a single treatment with up to 1-year follow-up [26–28]. Use of dextranomer/HA for treatment of urinary incontinence has also been reported with an initial improvement of incontinence symptoms of 85% and a sustained response of 69% at 5-years follow-up [29]. Although a 23% loss of implant volume at 3 months following treatment has been reported, dextranomer/HA is touted for its ease of delivery and generally favorable durability; it is widely regarded as the contemporary standard for endoscopic injectable treatment of reflux [26].

Polydimethylsiloxane (Macroplastique) was first described for the treatment of reflux, but has been primarily used in the setting of urinary incontinence, with a systematic review and meta-analysis reporting a long-term (>18 months) cure rate of 36%, symptom improvement rate of 64%, and a 30% median reinjection rate to achieve these long-term outcomes [30–32]. Moreover, a large meta-analysis compiling data on over 8000 renal units that received endoscopic injectable therapy for reflux reported reflux resolution rates after a single course of treatment as 76.5% for polydimethylsiloxane, 68.7% for dextranomer/HA, and 56.9% for bovine cross-linked collagen [33].

Carbon-coated zirconium beads (Durasphere) were first described for treatment of urinary incontinence in a multi-center, double-blind trial, where patients were randomized to receive endoscopic injections of either Durasphere or cross-linked bovine collagen [34]. At 1-year of follow-up, a higher rate of patients receiving Durasphere reported at least one grade of continence improvement versus patients receiving bovine collagen (80.3% vs. 69.1%, respectively, $p = 0.16$). Moreover, the required injection volume for patients receiving Durasphere was significantly lower than the reported injection volumes for bovine collagen (4.83 vs. 6.23 mL, respectively, $p < 0.001$). Of note, this investigation experienced a 45% loss to follow-up from initial enrollment to the 1-year time point. The same group later reported their long-term follow-up experience with Durasphere as an injectable therapy for urinary incontinence in 2004, where patients treated with Durasphere were age-matched to patients treated with bovine collagen [35]. At 2- and 3-years follow-up, Durasphere produced cure rates of 33% and 21%, while bovine collagen produced cure rates of 19% and 9%, respectively. The authors note neither Durasphere nor bovine collagen provided patients with durable improvement in incontinence. To date, no investigations have demonstrated favorability of outcomes for Durasphere over bovine collagen.

Calcium hydroxyapatite (Coaptite) was first described for the treatment of reflux in 2006 in a 2-year, multi-center trial in which 155 ureters received endoscopic injections [36]. Resolution of reflux was achieved in 46% of ureters at 1-year and 40% of ureters at 2-years follow-up, with good safety and durability of treatment reported. Additionally, calcium hydroxyapatite was described for the endoscopic treatment of urinary incontinence in 2007 in a 1-year, prospective, randomized trial versus glutaraldehyde cross-linked collagen, where patients could receive no more than five treatments in the first 6 months of the injectable therapy to which they were randomized [37]. At 1-year, although there was not a significant difference in improvement of patient-reported Stamey grade, patients receiving calcium hydroxyapatite injections were more likely to receive only a single therapy with a lower average injected volume when compared to bovine collagen.

Tissue and Cell-Based Therapies

Recognizing the limitations of the synthetic biomaterials, several groups sought to develop and investigate new injectable therapies for reflux and urinary incontinence with improved safety, efficacy, and durability profiles. The use of autologous adipose tissue was first reported for the treatment of urinary incontinence in 1989 with 15 women and 5 men undergoing abdominal wall liposuction and endoscopic periurethral injection, yet only 23% of patients reported improvement of symptoms [38]. Intermediate-term outcomes (median 18 months follow-up) for autologous adipose as an injectable therapy for urinary incontinence were reported in 21 patients who underwent a similar abdominal wall harvest and periurethral injection, but improvement of symptoms in a subset of female patients were only achieved after one to four injections (mean 2.4 injections) [39]. After a randomized, controlled, double-blind trial comparing periurethral injections of autologous adipose

to saline demonstrated poor rates of improvement at 3 months of 22.2% and 20.7%, respectively, and no difference in treating urinary incontinence compared to placebo, autologous adipose was abandoned as an injectable therapy [40].

The first use of tissue derived cells as an injectable therapy was proposed in the early 1990s with the concept of suspending chondrocytes in a biodegradable alginate polymer. Subcutaneous injections in a mouse model demonstrated several important characteristics, namely the suspensions were non-migratory, conserved their implant volume, were non-immunogenic, and enabled progressive replacement of polymer gels with cartilage [41]. Further studies demonstrated the feasibility of endoscopic subureteral injections of chondrocyte-alginate gel suspensions to resolve reflux in a porcine model of reflux via replacement of the polymer gel with growth of cartilage [42]. This technology found its way to the bedside in 1999 when 29 children with reflux underwent ear cartilage biopsy, expansion of cells in culture, and endoscopic subureteral injection of chondrocytes [43]. Overall, reflux was corrected in 83% of treated ureters following one or two treatments at the 3-month time point. At 1-year follow-up for this cohort of patients, resolution of reflux was maintained in 70% of ureters [44]. Autologous chondrocyte-alginate gel suspensions were also used for periurethral bulking in the setting of urinary incontinence, with 81.3% of patients reporting improved continence following a single treatment and patients reporting significant improvements in quality of life scores [45].

In a similar fashion to chondrocytes, use of smooth muscle cells suspended in a biodegradable alginate polymer as an injectable therapy was proven feasible by demonstrating progressive replacement of the implanted polymer gel with muscle cells in a mouse model [46]. Follow-up studies for this technology were also performed using a porcine model of reflux, where muscle cells were harvested, expanded in culture, complexed with the alginate polymer, and injected for subureteral bulking of a refluxing ureter [47]. Reflux was corrected in all ureters receiving injections and histologic examination of the injection site demonstrated the implants were biocompatible, non-migratory, and non-immunogenic.

Other muscle cell therapies have also been evaluated as an injectable therapy. First described in 2000 using a rat model, cultured cells from a myoblast cell line were injected periurethrally and resulted in formation of myotubes and myofibers on histologic evaluation 3–4 days following injection [48]. The same group advanced this technology by harvesting and injecting autologous muscle-derived cells into the urethral and bladder wall of a rat model, reporting cell survival and gene transfer in this setting [49]. When injected periurethrally in a stress urinary incontinence rat model, muscle-derived cells were superior in improving urethral contraction and leak point pressures (LPP), without causing urinary retention, when compared to fibroblasts [50].

Injectable autologous myoblasts and fibroblasts were eventually evaluated in patients with stress urinary incontinence in a randomized, controlled clinical trial, where patients were assigned to receive either transurethral injections of autologous myoblasts and fibroblasts or injections of collagen [51]. Although the rates of continence were promising for patients receiving injections of autologous cells, concerns over irregularities in conducting the trial ultimately led to retraction of the manuscript. In the pediatric setting, injectable autologous myoblasts were evaluated in a series of patients with bladder exstrophy and urinary incontinence [52]. Following staged repairs and bladder neck reconstruction, patients underwent a

regimen of pelvic floor electrical stimulation and pelvic floor exercises for 1 year prior to injection therapy, resulting in 88% of patients socially continent, defined as dryness during the daytime for more than 3 h. In another clinical trial, eight women received transurethral injections of muscle-derived stem cells (MDSC) for stress urinary incontinence [53]. Investigators reported an improvement of symptoms in five of eight patients, with the onset of symptom improvement between 3 and 8 months and a duration of improvement for a median 10 months following therapy.

Functional electrical stimulation has also been used post-operatively following ultrasound-guided transurethral injection of autologous myoblasts in an attempt to accelerate myoblast integration and promote early tissue functionality [54]. Patients continued electrical stimulation at home for 5 weeks post-operatively. At 6-month follow-up, 24% of patients considered their urinary incontinence cured, while 53% of patients reported improvement of symptoms. In an attempt to determine the appropriate concentration of MDSCs to yield optimal improvement of urinary incontinence, 1-year outcomes of two pooled phase II trials were reported [55]. Following harvesting biopsies and preparation in culture, patients received injections ranging in concentration from 10 to 200×10^6 autologous MDSCs. Those receiving higher concentrations of autologous MDSCs were more likely to have reported at least a 50% reduction in stress leaks and pad weights, while all patients reported significantly improved UDI-6 and IIQ-7 scores at 1-year follow-up. Other groups have sought to circumvent the necessity to prepare cells in culture, with the objective of decreasing time and cost, by harvesting and mincing autologous striated muscle at the time of injection [56]. At 1-year follow-up, 25% and 63% of patients with uncomplicated stress urinary incontinence, along with 7% and 57% of patients with complicated stress urinary incontinence, reported cure and improvement of symptoms, respectively.

Adipose-derived stem cells (ADSC) have been suggested as an alternative source of tissue engineered smooth muscle for the lower genitourinary tract. Lipoaspirate was harvested from female patients, processed in culture to induce pluripotency, and injected into the urethra and bladder wall of both a rat and mouse model [57]. As early as 8 weeks following injection, the processed lipoaspirate (PLA) demonstrated markers of smooth muscle differentiation; up to 12 weeks following implantation, the injected PLA maintained viability *in vivo*. In another investigation, ADSCs were harvested from rats, induced towards myoblastic differentiation in culture, and injected into the urethra and bladder neck of a rat model of stress urinary incontinence [58]. Urodynamics and histological analyses performed at 1- and 3-months post-injection revealed significant increases in LPP and increased number of myoblasts and large longitudinal muscle bundles compared to controls, respectively. This technology was further developed with a report of combining autologous ADSCs with nerve growth factor (NGF) encapsulated in PLGA microspheres for injection into a rat model of stress urinary incontinence, allowing for controlled-release of NGF and resulting in improved ADSC viability [59].

In the first therapeutic application of ADSCs for urinary incontinence in humans, three male patients with stress urinary incontinence secondary to prior prostate surgery underwent harvesting of abdominal adipose, isolation of ADSCs, and transurethral injection [60]. Continence outcomes were reported to have improved as early as 2 weeks following injections, with patients experiencing decreased frequency

and volume of incontinence at 6 months. Functionally, increases in both functional profile length and maximum urethral closing pressure were identified on urodynamics studies. In another clinical investigation, five female patients with stress urinary incontinence underwent harvesting of subcutaneous adipose, expansion of cells in culture, and transurethral injection of a mixture of ADSCs and bovine collagen gel [61]. Investigators identified the cough test as the primary outcome for this study, and at 1-year follow-up, three patients had a negative test.

Biomaterials Scaffolds for Urethral Tissue Regeneration

A variety of clinically challenging urologic conditions may necessitate reconstruction of the urethra to restore functionality and ideally improve a patient's overall quality of life. Most commonly, urethral stricture disease, urogenital trauma, congenital abnormalities, such as hypospadias in the pediatric population, or genitourinary malignancies are the conditions requiring such a complex reconstructive procedure. Historically, when the urethral defect is substantial or the quantity or quality of urethral tissue available is inadequate, making an excision and reanastomosis urethroplasty unfeasible, reconstruction with autologous tissues has been the clinical standard. Typically, buccal mucosa, bladder epithelium, or epidermal grafts have been the principle source of autologous tissue for urethral repair; however, use of these tissues not only requires patients undergo an additional harvesting procedure, but has also been associated with complications, primarily bladder mucosal glandular protrusion and diverticula, hair growth, and stricture formation with skin grafts [62–64]. Autologous tissue grafts must also be adequately vascularized and received by the native tissue bed. In seeking potential substitutes for autologous tissues, several challenges in designing scaffolds must be overcome; specifically, vascularization of new tissue, promotion of cellular localization, adhesion, and interaction, and creation of a biomaterial that mimics the physical properties of surrounding natural tissues [65].

Either naturally derived or synthetic acellular scaffolds have long been used in regenerative medicine, having become popularized for their biodegradability. Acellular polyglactin (PGA) fiber mesh tubes coated with polyhydroxybutyric acid were used to repair urethral defects in a canine model [66]. PGA was bioabsorbable and at 12 months following grafting demonstrated substantial urothelial regeneration and viable surrounding tissues without evidence of stricture. Investigators also have explored the feasibility of collagen-based, xenogenic tissues for urethral regeneration. Segments of harvested acellular porcine small intestinal submucosa (SIS), initially used experimentally for vascular grafts in the 1980s, were used as a graft for urethral tissue regeneration in an animal model [67]. The acellular patch grafts proved successful in promoting tissue neovascularization, along with urothelial and smooth muscle regeneration while remaining non-immunogenic. The first use of acellular matrices in patients for organ regeneration in the field of regenerative medicine occurred in 1996 through the use of acellular bladder submucosal collagen matrices for urethral patch grafting for failed previous hypospadias repairs or for urethral stricture disease, showing good results long term [68, 69].

Additional studies were performed, and when randomized to either buccal mucosal or acellular bladder collagen matrix grafts for patch graft repair of urethral defects, patients with healthy, uncomplicated native tissue beds demonstrated patent urethras over the length of follow-up regardless of the source of their graft, whereas patients with either unhealthy native tissue beds or multiple prior interventions who received acellular bladder submucosa matrix grafts demonstrated higher failure rates compared to those who received buccal mucosal grafts [70]. From this trial arose a juxtaposition between these two grafting options for urethral regeneration: while buccal mucosa provides superior reconstructive outcomes, it carries several risks, including prolonged operative times and increased morbidity associated with tissue harvesting, unrelated to the use of acellular collagen matrices.

Other clinical experiences with acellular matrices have been reported. Acellular tubularized SIS was evaluated for repair of bulbar and membranous urethral strictures in human patients, but the investigation was terminated early due to high rates of post-graft stricture development [71]. Clinical evaluation of acellular SIS grafts for patch bulbar urethroplasty was initially reported in 2007, with results after a median follow-up of 71 months detailing a 76% overall success rate, but 100% failure rate for urethral defects greater than 4.0 cm in length [72, 73].

Cell-Seeded Engineered Urethral Tissues

Prior investigations into synthetic and naturally-derived matrices had only shown efficacy as patch grafts and acellular scaffolds. Challenged by the need of tubularized urethral segments, the concept of engineering urethral tissue with scaffolds seeded with expanded urothelium and smooth muscle cells became an area of inquiry. Additional challenges surrounded maintaining urethral patency without the development of strictures, diverticula, or other complications. Reports of successful tubularized urethral replacement were first demonstrated in a rabbit model in 2002, where investigators seeded tubularized, acellular bladder submucosa with autologous bladder urothelium that had been previously harvested from the animal and expanded in culture [74]. Subsequent studies seeded biodegradable poly L-poly(lactide-co-glycolide) stents with rabbit urothelial cells, allowed them to expand in culture, and repaired urethral strictures in a rabbit model with the seeded scaffolds, which demonstrated complete regeneration of urothelium at 24 weeks [75]. While regeneration was eventually demonstrated, one of the limitations cited for these synthetic polymer scaffolds was the prolonged time required for complete urothelialization along the length of the graft.

The first use of an engineered urethra in patients, constructed with a scaffold and cells, was in 2005. Polyglycolic acid:poly(lactide-co-glycolide acid) scaffolds were utilized clinically in human patients to repair urethral defects of traumatic origin. Urothelium and bladder-derived smooth muscle cells were harvested from five male pediatric patients and scaffolds were co-cultured with these autologous cells; specifically, the luminal surface was seeded with patient-derived urothelial cells, while the outer surface was seeded with patient-derived bladder smooth muscle cells [76]. After expansion in culture, seeded scaffolds were used to repair urethral defects.

Serial post-operative biopsies demonstrated organized layers of smooth muscle and urothelium starting at 3 months following scaffold placement. Functionally, urethras were patent and without strictures or diverticula, and uroflowmetry studies demonstrated adequate flow rates over a median follow-up time of 71 months (Fig. 25.1).

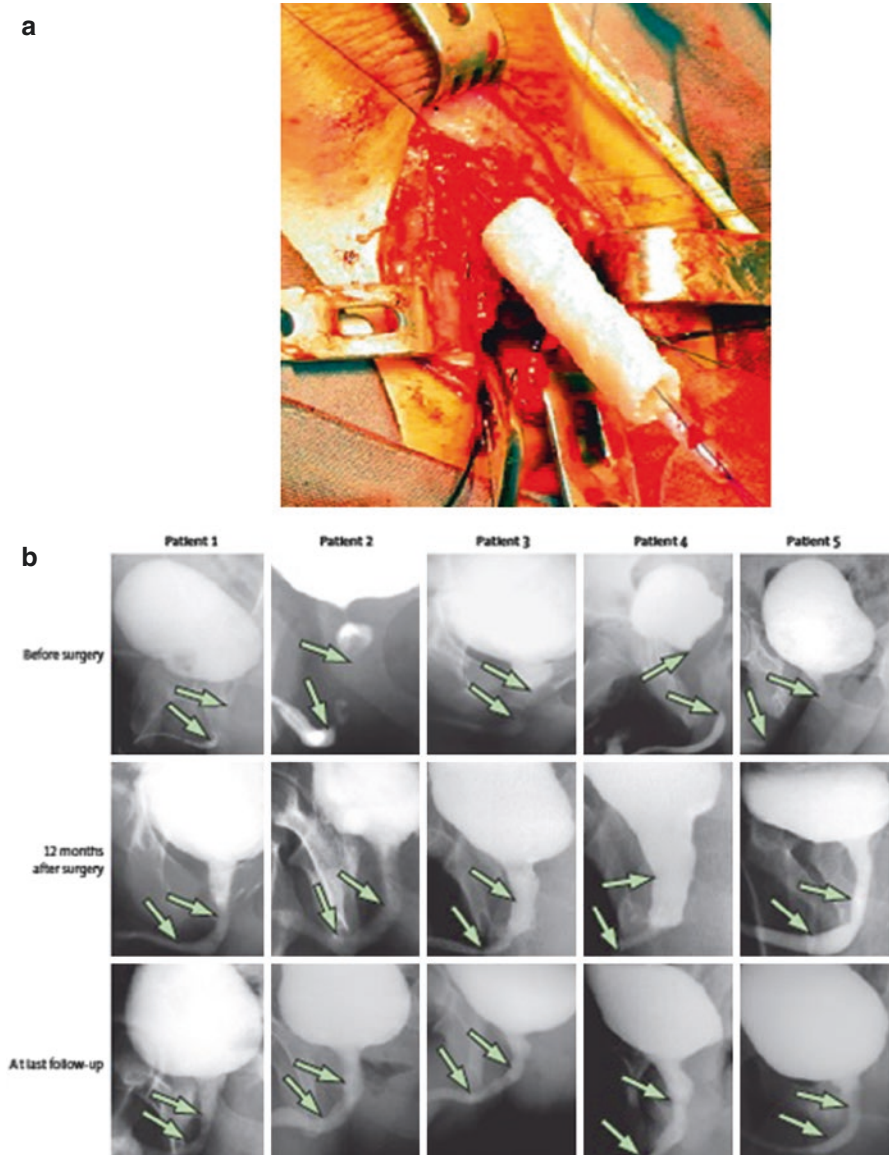


Fig. 25.1 Neo-urethra implantation and clinical outcome. **(a)** A cell-seeded graft sutured to the normal urethral margins from the first patient. **(b)** Voiding cystourethrograms of all five patients before surgery (*arrows* show the abnormal margins), 12 months after surgery (*arrows* show margins of tissue engineered urethras), and at last follow-up (*arrows* show margins of tissue engineered urethras)

Recognizing the unique advantages for each of the aforementioned biomaterials, studies investigating the utility of scaffolds comprised of both synthetic polymers and natural collagen-based materials, so-called hybrid scaffolds, as potential acellular or seeded grafts, are currently under evaluation in animal models [77, 78].

Tissue Engineering of Urinary Bladders

Surgical removal or replacement of the urinary bladder may be indicated for a variety of etiologies, including congenital abnormalities and subsequent end-stage bladder disease, pelvic trauma, or genitourinary malignancy. The oldest, and still today, most commonly utilized source of tissue for bladder repair or replacement are segments of bowel. However, reconstruction of the genitourinary tract with gastrointestinal tissues is associated with substantial morbidity, including excessive mucous production, development of nephrolithiasis, an increased risk of neoplasia, and the onset of metabolic abnormalities owing to the absorptive nature of gastrointestinal mucosa [79, 80].

Acellular Grafts

The earliest known surgical replacement of the bladder was reported in 1917 by Neuhoff, who used a graft of fascia for bladder augmentation in a canine model [81]. Since then, a variety of sources for tissue grafts, including skin, bladder submucosa, and small intestinal submucosa, along with multiple synthetic materials, such as plastic molds, polyvinyl sponge, tetrafluoroethylene, and collagen matrices have been evaluated for bladder replacement [82]. These substrates ultimately proved to be inadequate, as they were unable to demonstrate the mechanical, functional, and/or biocompatibility properties needed for bladder reconstruction. More recently, tissue engineering and regenerative medicine strategies have been applied towards the bladder, including the use of biomaterials derived from collagen matrices and synthetic polymers as scaffolds for regeneration.

One of the earliest reports of the use of collagen as a bladder substitution material consisted of a collagen/vicryl composite membrane to repair defects [83]. SIS was also used for augmentation cystoplasty in rat and canine models [84, 85]. The investigators reported histologic evidence of regeneration of urothelium, smooth muscle, and serosa along the implanted scaffold. Canine bladders demonstrated similar functionality to control animals on urodynamics studies. In 1997, Probst and colleagues used a rat model to perform partial cystectomy and grafting with homologous acellular bladder matrix, demonstrating neovascularization of the graft and regeneration of bladder urothelium and smooth muscle cells at 8 weeks [86].

The ability to successfully regenerate both the luminal urothelial and smooth muscle layers of the bladder on an acellular naturally-derived scaffold is somewhat

variable in the literature. Investigations as recent as 2008 report successful regeneration of bladder urothelium, but incomplete or absent regeneration of the smooth muscle layer when using natural collagen-based scaffolds [87, 88]. Moreover, limitations in maximum scaffold size have been suggested, as investigators who have used both acellular and seeded SIS to replace the bladder in a canine model that has undergone subtotal cystectomy reported severe graft shrinkage, minimal regeneration of urothelium, and inflammatory infiltrate and smooth muscle hypertrophy on histological examination [89]. In a clinical investigation, five exstrophic pediatric patients presenting with poor bladder function underwent augmentation cystoplasty with an acellular SIS scaffold [90]. Although functional parameters, such as bladder capacity and compliance, increased by upwards of 30% compared to the pre-operative state, this did not translate to a clinically meaningful improvement in the duration of dry intervals reported by patients. Moreover, histological analysis of grafts following implantation revealed diminished presence of bladder smooth muscle. Due to the challenges encountered with acellular matrices, several modifications to enhance their preparation have been proposed, including incubating scaffolds in bioreactors that simulate physiological stretch of the bladder wall or exposing three-dimensional urothelial cultures to cyclical increases and rapid decreases of pressure to simulate physiological bladder filling and rapid emptying [91, 92].

Tissue Engineered Bladders

Harvesting autologous urothelium, expanding cells in culture, and then seeding those cells onto a biodegradable polymer scaffold for eventual replacement of genitourinary tissues was first described in 1992 [93]. In this proof of concept study, culture-expanded rabbit urothelial cells were seeded onto nonwoven PGA mesh and implanted into mice. This model was further expanded on by demonstrating the ability of harvested human urothelium and bladder smooth muscle cells to be expanded *in vitro*, seeded onto biodegradable PGA scaffolds, and implanted *in vivo* to create urological structures consisting of both cell types [94]. Subsequent studies in the canine model demonstrated improved regenerative capacity of allogenic acellular bladder matrix scaffolds when grafts were seeded with autologous cells prior to implantation. Specifically, investigators harvested bladder urothelium and smooth muscle cells, isolated each cell type and expanded them separately in culture, and seeded the luminal surface of the scaffolds with urothelium and the outer surface with smooth muscle [95]. Additionally, when compared to animals receiving unseeded acellular matrices, augmentation with seeded matrices resulted in a significantly greater increase in bladder capacity (99% vs. 30% for acellular matrices). Seeded matrices also retained their size compared to acellular matrices, which demonstrated graft contraction and shrinkage. In another study, culture-expanded autologous urothelial and smooth muscle cells were seeded on the luminal and exterior surfaces, respectively, of biodegradable PGA scaffolds molded into the shape of a bladder [96]. These scaffolds were then implanted into a canine model after animals

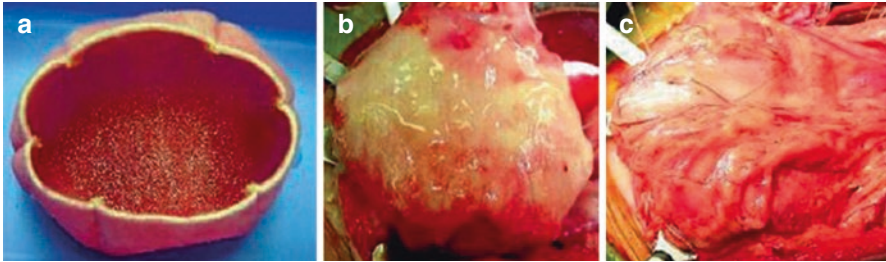


Fig. 25.2 Construction of engineered bladder. Scaffold seeded with cells (a) and engineered bladder anastomosed to native bladder with running 4–0 polyglycolic sutures (b). Implant covered with fibrin glue and omentum (c)

underwent trigone sparing cystectomy. At 11 months following implantation, organs demonstrated filling capacities of upwards of 95% of the baseline, pre-cystectomy bladder volume, physiological properties of elasticity, and normal bladder histology consisting of organized urothelium, muscle fiber, and submucosal layers, making this the first report of a successfully tissue-engineered autologous hollow organ.

This technology was eventually translated into the clinical setting when seven patients with myelomeningocele were identified to undergo cystoplasty with tissue-engineered autologous bladders [97]. Autologous bladder urothelium and smooth muscle were harvested and individually expanded *in vitro*. Scaffolds fashioned from a combination of collagen and PGA were molded into the shape of a bladder; the luminal surface and outer surface were seeded with patient-derived urothelium and smooth muscle cells, respectively. Constructs used for reconstruction cystoplasty with the addition of an omental wrap resulted in increased bladder volume and compliance, along with decreased LPP at an average of 46 months post-operative follow-up (Fig. 25.2). Moreover, the cellular phenotype and structural architecture of the implanted constructs resembled that of histologically normal bladder tissues. The most frequently encountered complications of cystectomy, including mucous production, metabolic abnormalities, neoplasia, or nephrolithiasis, were absent in this cohort, demonstrating the safety and feasibility of using tissue engineering technologies for the structural and functional regeneration of human bladders. Although phase II trials, which utilized cell expansion and manufacturing scale-up methods, did not show functional differences long term, the phase I patients continue to do well and Phase III trials are currently in preparation with a modified scale-up protocol [98, 99].

Tissue-Engineered Urinary Conduits

Patients who undergo cystectomy and urinary diversion face exceptionally high rates of perioperative complications (50–70%), 90-day hospital readmission (25%), intensive care unit admission (20%), and perioperative mortality (5%) [100]. To this

end, tissue engineered urinary conduits (TEUC) have been proposed with the goal of obviating the need for elective surgical manipulation of the gastrointestinal tract, arguably one of the most morbid aspects of this surgery. One of the earliest descriptions of TEUCs was made in 2007 by Drewa, who constructed a conduit in a rat model using SIS seeded with culture-expanded fibroblasts [101]. In another pre-clinical study using a porcine model, conduits were created from tubularized constructs of type I bovine collagen and a synthetic polymer mesh and subsequently seeded with porcine urothelial cells [102]. While implanted conduits demonstrated growth of luminal urothelium and neovascularization on histological analysis, the rates of animal survival and creation of a functional urostomy was only 80% and 50%, respectively. Additional work using a porcine model was performed by seeding biodegradable polymer scaffolds with autologous adipose- and peripheral blood-derived smooth muscle cells [103]. Histological analysis of tubular conduits following their implantation revealed *de novo* regeneration of “urinary-like neotissue” with similar morphologic appearance to native bladder.

TEUCs have also been evaluated in a phase I open label clinical trial in nine patients with bladder cancer undergoing radical cystectomy and urinary diversion. Biodegradable PGA polymer mesh constructs coated with a PLGA copolymer were tubularized to create a 20 cm long conduit, which was subsequently seeded with autologous adipose-derived smooth muscle cells and implanted at the time of surgery [104]. Investigators utilized the omental pedicle for vascularization of the TEUC and optimized surgical techniques for ureteral implantation and stoma creation. Histologically, the implanted conduits demonstrated regeneration of urothelium and smooth muscle along the tract. Long-term follow-up detailing the patency and structural integrity of these TEUCs, along with rates of complications, are pending.

Tissue Engineered Female Genital and Reproductive Tissues

A variety of conditions may result in either absence or loss of female genital and reproductive tissues. Congenital disorders, such as Mayer-Rokitansky-Kuster-Hauser syndrome (MRKHS), cloacal malformations, or intersex disorders, may result in vaginal aplasia, while acquired disorders, such as malignancies or trauma, may lead to substantial structural and/or functional organ damage. Patients requiring vaginal reconstruction commonly undergo McIndoe vaginoplasty, where a pelvic canal is created from the potential space posterior to the urethra and urinary bladder and anterior to the rectum. In creating this neovagina, a tissue substitute is needed to line the wall of this cavity and aid in its functionality. Multiple sources of tissue have been previously evaluated, including skin grafts, buccal mucosa, vaginal epithelium, acellular dermal matrix, and acellular porcine SIS [105–109]. While some of these tissues have demonstrated satisfactory take to native tissue beds, they have typically only resulted in epithelial regeneration with an absence of an adequate muscle layer resulting in eventual graft stenosis or contracture and need for

dilation. Reconstruction of vaginal structures has also been attempted using segments of bowel; however, similar to what has been previously reported in the setting of urinary diversions or bladder reconstruction, use of intestine to reconstruct female genital tissues has also been associated with complications, such as excessive mucous production, poor hygiene, and risk for onset of neoplasia [110, 111]. To attempt to overcome these challenges, tissue engineering strategies have been applied towards the regeneration of vaginal organs, with the objective to provide patients with both structural and functional organ replacement and an improved quality of life over currently available options.

The first report of successfully engineering vaginal tissues with cells was published in 2003. In this proof of concept study, vaginal epithelial and smooth muscle cells were harvested from female rabbits and individually expanded in culture [112]. These cells were then seeded onto biodegradable PGA scaffolds and constructs were implanted into female mice. At 4 weeks post-implantation, grafted cell seeded scaffolds demonstrated neovascularization and tissue-level organization of vaginal epithelium and smooth muscle; additionally, regenerated vaginal tissues demonstrated functional contractile properties similar to that of control tissues when subjected to electrical stimulation. In a follow up study, the feasibility of engineering a functional autologous vagina was demonstrated using a rabbit model [113]. Harvested and culture-expanded vaginal epithelial cells and smooth muscle cells were seeded onto the inner surface and outer surface, respectively, of PGA scaffolds constructed to resemble a vaginal canal. At 6 months following implantation, histological examination of constructs demonstrated neovascularization and organized epithelial and smooth muscle. Moreover, vaginal canals remained patent without the development of strictures and tissue functionality testing demonstrated appropriate physiological responses to either stimulation with electrical currents or an adrenergic agonist.

This technology was further developed with the first report of successful tissue engineered autologous vaginal organs in human patients in 2014. Four patients with vaginal aplasia secondary to MRKHS underwent vulvar tissue biopsy, followed by isolation of epithelial and smooth muscle cells and expansion in culture [114]. SIS scaffolds were molded to the unique pelvic anatomy for each patient and subsequently seeded with autologous epithelial cells on the inner surface of the vaginal canal and autologous smooth muscle cells on the outer surface. Following growth and maturation of these constructs in an incubator, tissue engineered neovaginas were surgically implanted into patients. At 8 years follow up, annual vaginal biopsies consistently demonstrated organized vaginal histology consisting of epithelial, submucosal, and smooth muscle layers. Before and after pelvic cross-sectional imaging demonstrated the severity of vaginal aplasia pre-operatively and the durability of regenerated tissues post-operatively (Fig. 25.3). Moreover, adequate functional outcomes were reported based on the Female Sexual Function Index questionnaire. Certainly, the use of seeded constructs for female genital organ regeneration is promising and necessitates further investigation.

Investigations directed at engineering uterine tissue have also been performed [115]. In a similar fashion as described above, uterine epithelial cells and smooth muscle were harvested from female rabbits, individually expanded in culture, and

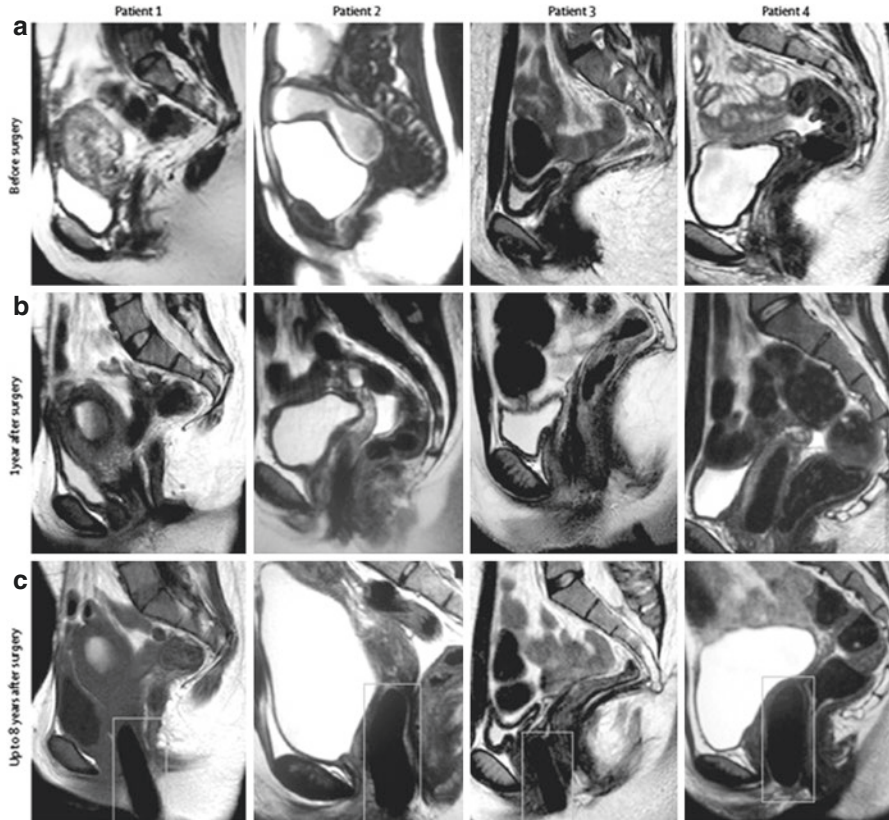


Fig. 25.3 Preoperative and Postoperative MRI images. (a) Preoperative MRI images show absence of vaginal organs. (b) MRIs 1 year after surgery show engineered vaginal organs. (c) Latest MRI images up to 8 years after surgery (boxes within the MRIs show engineered vaginal organs)

seeded onto biodegradable polymer scaffolds which were constructed to resemble a uterine cavity. Seeded scaffolds were implanted into corresponding autologous animals. Histologic, molecular, and biomechanical studies at 6 months following implantation demonstrated the presence of normal uterine tissue components and normal uterine functionality.

Tissue Engineering of Renal Structures

Perhaps one of the greatest medical achievements over the past half-century has been the development of surgical techniques that have made renal transplantation feasible. First reported in 1955, renal transplantation is the gold standard and only

curative therapy available for end-stage renal disease [116]. However, a severe donor organ shortage for renal transplantation exists and, although substantial advancements have been made, immunosuppressive pharmacology regimens are still associated with significant morbidity and mortality. Transplant recipients are also at risk of rejection or loss of graft function over time. Currently, patients with renal failure are most commonly treated with dialysis as a renal replacement therapy, but, this too, is highly morbid. Given its highly complex architecture and cellular heterogeneity, the kidney is arguably the most difficult organ to regenerate in the genitourinary system. Efforts in renal tissue engineering have sought to create tissues or whole organs capable of regenerating diminished renal function, with the ultimate objective of providing an alternative therapy to dialysis or transplantation. Towards this goal, investigators have proposed several concepts for renal tissue regeneration, ranging from therapies derived from stem cells and embryologic precursors to total functional renal replacement with decellularized naturally-derived scaffolds.

Cell-Based Therapies

The complexities of renal stromal architecture and the heterogeneous cell types which comprise the kidney make developing cellular therapies for renal disease inherently challenging. Many investigations have revolved around mesenchymal stem cells (MSC) for tissue engineering with the objective of harnessing their capacity to differentiate into one of many different cell types as a therapeutic option. The restorative capabilities of MSCs are often studied in the setting of acute kidney injury (AKI) or chronic kidney disease (CKD), where the number of primary renal cells remaining may be inadequate to achieve proper renal function.

Bone marrow-derived MSCs have been demonstrated to be capable of differentiating into, and ultimately regenerating, several cell lineages, including glomerular endothelial cells in the setting of significant damage [117]. Bone marrow-derived MSCs also have a role in renal development, specifically in nephron formation, when stimulated by a variety of nephrogenic signals following injection into an embryologic rat metanephros [118]. However, this report generated controversy as to whether the primary cell type responsible for kidney regeneration in the setting of AKI is intrarenal versus extrarenal. Additional experimentation using a renal ischemia-reperfusion injury mouse model demonstrated a majority of regenerated cells arose from renal tubular epithelial cells originating from the host [119].

Multiple animal models have been utilized to determine the renoprotective capabilities of systemically administered bone marrow-derived MSCs in the setting of renal injury [120, 121]. In a mouse model for renal injury induced by cisplatin administration, injected MSCs honed to the damaged kidney and differentiated into tubular epithelial cells [122]. Evidence of MSC induced restoration of renal structure and function were observed with enhanced rates of renal tubular proliferation and significant reductions in serum urea following MSC administration. In a rat model for ischemia-reperfusion AKI, early administration of fluorescently labeled

MSCs following injury resulted in their localization to the basement membranes of glomeruli, as identified by *in vivo* two-photon laser confocal microscopy [123]. Several renoprotective effects, including recovery of renal function, high rates of proliferation, and low rates of apoptosis, were reported following MSC administration. Human cord blood MSCs have also been delivered to a mouse model of cisplatin-induced renal injury, resulting in production of pro-regenerative growth factors and inhibition of inflammatory mediators [124].

Mesenchymal stem cells as a renoprotective therapeutic have been translated into the clinic for evaluation in a variety of settings. The immunomodulatory effects of MSCs were first evaluated in a clinical trial of eight patients with steroid-resistant graft versus host disease (GVHD), resulting in resolution of disease in six patients and significantly improved survival compared to a matched cohort not receiving MSCs [125]. A subsequent multi-center, phase II clinical trial evaluating MSCs for treatment of steroid-refractory, acute GVHD treated 55 patients with culture expanded MSCs [126]. Overall, a complete response was achieved in 30 patients and nine additional patients demonstrated clinical improvement; there were no reported side effects or toxicities associated with infusion of MSCs. In a randomized, controlled clinical trial, living-related kidney transplant recipients who received pre-transplant induction therapy with autologous MSCs had a significantly lower rate of acute graft rejection, a significantly higher rate of recovery of renal function (eGFR) at 1 month post-operatively, and a significantly lower rate of opportunistic infection within the first year post-operatively compared to patients who underwent induction therapy with standard anti-IL2 receptor antibodies [127].

Other investigations have sought to isolate renal cell types of certain functionality as a targeted therapeutic towards a specific aspect of renal dysfunction. Whereas currently available therapies for anemia secondary to end-stage renal disease (ESRD) necessitate regular administration of recombinant erythropoietin (Epo), cell-derived therapies could potentially be used to treat anemia in this setting. Investigators first isolated and expanded in culture renal cells from mice that stably expressed Epo and later demonstrated these cells were capable of regulating expression of Epo in response to their environmental oxygen tension [128, 129]. This concept was further developed using a model for chronic kidney injury and human primary kidney cells enriched with renal cells expressing Epo [130]. These cells were introduced following injury, resulting in significant improvement of renal function and reduction of renal injury markers, such as urinary albumin, urinary kidney injury molecule-1 (a tubular injury marker), and 8-hydroxy-deoxyguanosine (an oxidative DNA marker). As a mechanism for delivery of cell-based therapies, a three-dimensional collagen-based culture system was developed to enable *in vitro* generation of renal structures comprised of primary renal cells [131]. Glomeruli and renal tubules, identified by positive Tamm-Horsfall protein staining, developed as early as 1 week in culture. Further studies involved expanding isolated human primary renal cells in culture and constructing *in vitro* three-dimensional renal cell cultures [132]. These functional three-dimensional cultures were subsequently implanted in a rat kidney model, surviving *in vivo* for up to 6-weeks post-implantation.

Transplantation of Metanephroi

Another potential mechanism for regeneration of renal function is through transplantation of embryological precursors, such as the metanephros. Metanephroi were first transplanted into cortical tunnels in a mouse model by Woolf et al. in 1990 and subsequent investigations demonstrated development of new nephrons, neovascularization, and glomerular and tubular cytodifferentiation when metanephroi were implanted subcapsularly in a mature rat host [133, 134]. In another investigation, embryologic day 15 (E15) metanephroi were transplanted either subcapsularly or into the omentum of adult rats, demonstrating renal differentiation, cortical and medullary architecture, neovascularization, and production of urine [135]. The same group later reported implanted metanephroi into rat omentum are viable for upwards of 32 weeks [136]. Further studies transplanted E15 metanephroi from rats into an adult rat omentum, with investigators performing unilateral nephrectomy at the time of the initial implantation and contralateral nephrectomy 20 weeks later to create a model of end-stage renal disease [137]. Animals that received transplanted metanephroi and underwent ureteroureterostomy to create a continuous urinary tract demonstrated significantly prolonged survival. This was the first report to illustrate transplanted metanephroi were capable of prolonging survival.

Eventually, human embryologic metanephroi were utilized as a renal precursor and transplanted into kidneys of a mouse model [138]. These implants differentiated into functional nephrons that produced dilute urine, but did not generate ureters. Further work in this area involved *in vitro* culturing of isolated metanephric mesenchyme and ureteric bud tissues derived from rat metanephroi [139]. When cultured individually, both mesenchymal- and ureteral-derived tissues were successfully propagated and grew to the approximate size of their progenitors; moreover, combining propagated ureteric buds with fresh-harvested mesenchymal tissues *in vitro* generated a contiguous neokidney with identical morphology to the whole rat kidney rudiment. This same group later developed a stepwise technique for inducing budding of an epithelial tubule and then combining it with mesenchymal tissues *in vitro* [140]. When implanted into an adult rat model, the recombined tissue demonstrated evidence of early neovascularization and development of glomeruli. These studies have proven the feasibility of developing a metanephros-like structure by culturing ureteral buds and mesenchymal tissues, indicating the potential for propagation of the metanephros under these conditions as a technique for engineering renal tissues for substitution.

As techniques for developing and culturing renal precursors advanced, investigators sought to further elucidate the functional benefits which could potentially be acquired through transplantation of metanephroi. In 2012, a group from Japan published multiple reports describing the effect of metanephroi transplantation in a rat models. Specifically, transplanted metanephroi expressed increased levels of renin, increased plasma renin activity, and maintained mean arterial blood pressures in a rat hypotension model [141]; expressed increased levels of Epo in a rat

anemia model [142]; and prevented progression of vascular calcification in a rat chronic renal failure model [143]. Cumulatively, these experiments indicate the potential for transplanted renal precursors to not only promote neovascularization and nephrogenesis in renal tissues, but to also regenerate lost renal function in a diseased kidney.

In Situ Development of Renal Units

In vitro renal units have also been proposed as a potential renal replacement therapy. These scaffolds could ideally be seeded with autologous cells to prevent complications associated with immune system rejection or immunosuppression seen in transplant recipients. The realization of tissue engineered renal units was not only dependent on the development of techniques for growth and expansion of renal cells in culture, but also the development of biomaterials scaffolds to serve as adequate vehicles for the eventual delivery of these regenerative therapies. Early advancements in this area were made by successfully culturing individual populations of renal cells harvested from rabbits, including proximal tubules, glomeruli, and distal tubules [144]. Cells were expanded in culture, seeded both individually and as mixed cultures on biodegradable PGA scaffolds, and subsequently implanted into a mouse model. Seeded cells were able to successfully attach to the polymer scaffold and histologic examination demonstrated progressive organization of nephrons. However, it could not be concluded whether the observed tubular structures within polymer fibers regenerated *de novo* from previously dissociated renal elements or whether they were remnants of intact tubular structures that survived harvesting and expansion in culture. To this end, renal cells were harvested from mice, culture-expanded, and individually isolated cells were seeded on biodegradable polymer scaffolds for implantation into immune-competent syngenic hosts [145]. Histological analyses of the implants demonstrated renal epithelial cells developed into tubules over time by first generating a solid, cord-like structure and then canalizing to create a hollowed core. Further examination of cell types confirmed the ability of these individually isolated cells to reconstitute renal tubular structures comprised of proximal tubules, distal tubules, loop of Henle, collecting tubules, and collecting ducts.

In another investigation, a tubular polycarbonate device was used as a scaffold for renal cells which had been harvested from mice and culture-expanded [146]. The device was subcutaneously implanted in a mouse model, while the other end was fed into a Silastic catheter that entered into a reservoir. When the device was evaluated histologically, well-organized glomeruli and tubular structures, including proximal and distal tubular cells and loop of Henle, were observed in the setting of widespread neovascularization. Osteopontin and fibronectin were identified on immunohistochemistry staining of tubular cells and regions of extracellular matrix, respectively. Analysis of fluid collected from the reservoir revealed significantly elevated mean concentrations of uric acid (66 mg/dL, vs. 2 mg/dL in serum) and creatinine (27.91 mg/dL, vs. 4.49 mg/dL in serum) compared to those found in

serum. Overall, results from this investigation proved single renal cells are able to form complex, multicellular structures and functional renal units, which demonstrate unidirectional secretion of solutes and concentration of uric acid in the form of a urine-like fluid.

An extracorporeal renal tubular assist device (RAD) has also been developed which functions in a complementary fashion to hemodialysis units. The RAD is comprised of a multi-fiber bioreactor in which a confluent monolayer of proximal tubule cells are seeded. In the setting of hemodialysis, blood is first processed through a conventional hemofilter and is then directed towards the RAD for filtration across the fibers of the device as a means to provide supplemental cellular metabolic functionality to hemofiltration. Using porcine renal tubular cells in a canine model in which acute uremia was induced, the RAD resulted in increases in both ammonia excretion and plasma 1,25-dihydroxyvitamin D₃ [147]. A RAD comprised of monolayers of human-derived proximal tubule cells was evaluated clinically in a phase I/II trial in ten patients admitted to the ICU with AKI [148]. Although these patient's likelihood of pre-treatment survival was low secondary to comorbidities and multiple organ failures in an intensive care setting, 60% of the patients who used the RAD survived past 30 days. A follow-up randomized, controlled phase III clinical trial was performed comparing RAD with hemofiltration ($n = 40$) versus hemofiltration alone ($n = 18$) for patients admitted to the ICU with AKI [149]. Those who were randomized to treatment with the RAD and hemofiltration had significantly improved survival at 180 days and demonstrated earlier recovery of renal function, although this outcome was non-significant.

Renal Tissue Regeneration Through Therapeutic Cloning

While prior strategies for renal tissue regeneration have relied on stem cells, embryologic precursors, or allogenic renal cells as a biologic starting material, somatic cell nuclear transfer has also been evaluated as a potential cell source for regenerative therapies. Nuclear material was extracted from harvested bovine fibroblasts and subsequently transferred into unfertilized bovine oocytes that had undergone enucleation [150]. From these cloned embryos, renal cells were isolated, culture-expanded, and seeded onto three-dimensional biodegradable scaffolds, which were then implanted into the exact animal from which the original cells were harvested. Twelve weeks following implantation, these functioning renal units were capable of secreting solutes unidirectionally, concentrating urea nitrogen and creatinine, and producing urine (Fig. 25.4). Additionally, glomerular architecture and tubular structures were identified on histological examination and the tissues regenerated in this setting were genetically identical to the animal from which the cells were originally harvested. Results from this investigation were the first to demonstrate therapeutic cloning techniques can produce viable cells for *in vitro* expansion and subsequent seeding onto biodegradable scaffolds for implantation and regeneration of tissues *in vivo*.

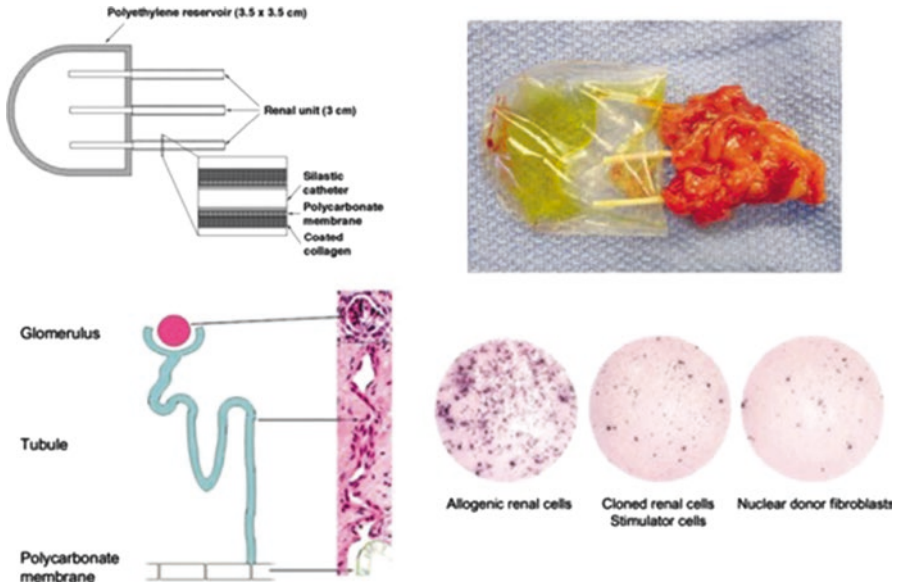


Fig. 25.4 Illustration of tissue engineered renal unit (*top left*); Unit seeded with cloned cells and retrieved 3 months after implantation demonstrating accumulation of urine-like fluid (*top left*); Renal explants demonstrated a clear unidirectional continuity between the mature glomeruli, their tubules, and the polycarbonate membrane (*bottom left*); and ELISpot analyses of the frequencies of T cells that secrete IFN-gamma after primary and secondary stimulation with allogenic renal cells, cloned renal cells, or nuclear donor fibroblasts (*bottom right*)

Total Renal Function Replacement

Development of natural collagen-derived acellular kidney matrices would allow for transplantation of large volumes of renal cells. Such grafts are created through extensive whole organ decellularization protocols, which utilize specific combinations of detergents and enzymes for removal of renal cellular material and maintenance of vascular and stromal architecture for subsequent recellularization. Advancements in the area of total renal function replacement began with the development of an acellular collagen-based matrix mimicking renal stromal architecture [151]. The surface of such scaffolds was capable of receiving a large quantity of cells, allowing for adhesion, proliferation, and differentiation into renal tubular and glomerular structures as early as 8-weeks post-implantation. The technique of organ decellularization arose from the necessity to deliver larger volumes of renal tissue within structures that closely resemble the highly complex stromal and vascular architecture of the kidney. One of the earliest reports of this technology involved decellularization of rat kidneys and subsequent seeding with pluripotent murine embryonic stem cells, which eventually proliferated and differentiated into various renal cell types under the suggested guidance of the extracellular matrix [152]. Further studies using this model demonstrated evidence of the aforementioned

hypothesis, as signaling from 'matrix-to-cell' produced endothelialization of vascular cells and basement membrane remodeling [153].

Decellularization methods were applied to rhesus monkey kidneys, which served as adequate scaffolds for cell adhesion and migration [154]. Acellular renal extracellular matrix scaffolds have also been generated from porcine kidneys, which investigators subsequently seeded and re-implanted in pigs [155]. While explanted scaffolds revealed maintenance of renal ultrastructure, further pathologic analysis revealed pericapsular inflammatory infiltrate and widespread vascular thrombosis. Additional experimentation with the porcine kidney scaffold sought to determine optimal conditions through the design of a high-throughput system for rapid decellularization [156]. Investigators concluded a 0.5% sodium dodecyl sulfate solution produced the most effective removal of porcine renal cells, while maintaining the vascular and stromal architecture of the scaffold and enabling subsequent seeding with human primary renal cells. Further optimization of methods have been proposed involving use of non-ionic detergents to accelerate the decellularization process, physiological infusion of stem cells through the renal artery, and pressure-controlled perfusion to promote seeding and differentiation towards vascular and glomerular cell types [157].

In another study, rat, porcine, and human kidneys underwent decellularization to generate an acellular stromal scaffold with a collecting system and ureters [158]. While incubated in a bioreactor, epithelial and endothelial cell seeded rat scaffolds were perfused through their native vasculature and demonstrated the ability to generate rudimentary urine. Moreover, orthotopic transplantation of these scaffolds in rats yielded graft urine production *in vivo*. Additional developments in cell culture and seeding methods have been reported, which enable culture-expansion of porcine primary renal cells with preserved phenotype and efficient repopulation of scaffolds, respectively [159]. Functionally, these renal proximal tubular cells reabsorbed electrolytes, demonstrated hydrolase activity, and produced erythropoietin.

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