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Tissue Engineering for the Replacement of Urologic Organs

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INTRODUCTION

Numerous urologic tissue substitutes have been attempted with both synthetic and organic materials (1). The first application of a free-tissue graft for bladder replacement was reported by Neuhoﬀ in 1917, when fascia was used to augment bladders in dogs (2). Since that first report, multiple other free-graft materials have been used experimentally and clinically, including skin, bladder submucosa, omentum, dura, peritoneum, placenta, sero-muscular grafts, and small intestinal submucosa (3–8). Synthetic materials, which have been tried previously in experimental and clinical settings, include polyvinyl sponge, tetrafluoroethylene (Teflon), gelatin sponge, collagen matrices, vicryl matrices, resin-sprayed paper, and silicone (9–15). Some of the aforementioned attempts have not gained clinical acceptance owing to either mechanical, structural, functional, or biocompatibility problems. Permanent synthetic materials have been associated with mechanical failure and calculus formation. Natural

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materials usually resorb with time and have been associated with marked graft contracture.

Some of the free grafts utilized for bladder replacement have been able to show a trilayered histologic distribution in terms of a urothelial layer, a midlayer composed of connective tissue, and a muscular layer, all of which have varied in terms of their full development. It has been well established for decades that the bladder is able to regenerate generously over free grafts (16,17). Urothelium is associated with a high replicative capacity. However, the muscle layers are less likely to regenerate in a normal fashion. Both urothelial and muscle ingrowth are believed to be initiated from the edges of the normal bladder toward the region of the free graft (18). Usually, however, contracture or resorption of the graft has been evident. We have hypothesized that the inflammatory response toward the matrix, and the paucity of normal muscle regeneration may contribute to resorption of the free graft when used alone.

TISSUE-ENGINEERING STRATEGIES

The overall failure in the strategies attempted for genitourinary tissue replacement in the past led us to apply the principles of cell transplantation, materials science, and engineering toward the development of a biological substitute that would restore and maintain normal function. Cell transplantation has been proposed for the replacement of a variety of tissues, including skin, pancreas, and liver. However, the concept of urothelial-associated cell transplantation had not been formerly approached in the laboratory setting until earlier this decade because of the inherent difficulties encountered in growing urothelial cells in large quantities. Our laboratory was successful in culturing and greatly expanding urothelial cells from small biopsy specimens. Using our methods of cell culture, we estimate that it would be theoretically possible to expand a urothelial strain from a single specimen that initially covers a surface area of 1 cm² to one covering a surface area of more than 4000 m² within 8 wk (19–21). This would result in a cell yield that would be sufficient to cover an entire football field. Bladder, ureter, renal pelvis, and corporal cavernosal muscle cells can be equally harvested, cultured, and expanded easily. Based on these observations, we proposed an approach to tissue regeneration by patching isolated cells to support structures that would have a suitable surface chemistry for guiding cell reorganization and growth.

CELL DELIVERY MATRICES

It is known from previous studies that artificial permanent support structures are lithogenic (Teflon, silicone) (1). Investigators have tried

permanent homograft or heterograft support structures such as dura, however, these contract with time and are problematic in a clinical setting. Natural permanent support structures, such as denuded bowel, retain their inherent properties and mucosal regrowth invariably occurs with time. A variety of synthetic polymers, both degradable and nondegradable, have been utilized to fabricate tissue engineering matrices (22). Bladder submucosa was proposed as a matrix for tissue regeneration in 1961, and there has been a recent resurgence of interest in this material for bladder replacement (3,4). Intestinal submucosa has also been proposed as a scaffold for the regeneration of urologic tissue (8). All of the materials used until recently in the urinary tract, both synthetic and natural, were applied without the use of cells. A common finding with these materials was usually an adequate histological result, but with a paucity of muscle tissue and subsequent graft contraction and shrinkage (23,24).

Synthetic polymers can be manufactured reproducibly and can be designed to exhibit the necessary mechanical properties (22). Among synthetic materials, resorbable polymers are preferable because permanent polymers carry the risk of infection, calcification, and unfavorable connective tissue response. Polymers of lactic and glycolic acid have been extensively utilized to fabricate tissue engineering matrices (22). These polymers have many desirable features; they are biocompatible, processable, and biodegradable. Degradation occurs by hydrolysis and the time sequence can be varied from weeks to over a year by manipulating the ratio of monomers and by varying the processing conditions. These polymers can be readily formed into a variety of structures, including small diameter fibers and porous films.

The porosity, pore size distribution, and continuity dictate the interaction of the biomaterials and transplanted cells with the host tissue. Fibrovascular tissue will invade a device if the pores are larger than approx 10 μm , and the rate of invasion will increase with the pore size and total porosity of a device (25,26). This process results in the formation of a capillary network in the developing tissue (26). Vascularization of the engineered tissue may be required to meet the metabolic requirements of the tissue and to integrate it with the surrounding host. In urologic applications, it may also be desirable to have a nonporous luminal surface (e.g., to prevent leakage of urine from the tissue).

The direction that we have followed to engineer urologic tissue involves the use of both synthetic (polyglycolic and/or poly-lactic acid and alginate) and natural (bladder submucosa, intestinal submucosa, peritoneum, and reconstituted collagen) biodegradable materials with and without cells.

TISSUE ENGINEERING OF UROLOGIC STRUCTURES

Ureter and Urethra

Urothelial and muscle cells can be expanded *in vitro*, seeded onto the matrix, and allowed to attach and form sheets of cells. The cell-matrix scaffold can then be implanted *in vivo*. We have performed a series of *in vivo* urologic associated cell-matrix experiments. Histologic analysis of human urothelial, bladder muscle, and composite urothelial and bladder muscle-matrix scaffolds, implanted in athymic mice and retrieved at different time-points, indicated that viable cells were evident in all three experimental groups (27,28).

Implanted cells oriented themselves spatially along the matrix surfaces. The cell populations appeared to expand from one layer to several layers of thickness with progressive cell organization with extended implantation times. The matrix alone evoked an angiogenic response by 5 d, which increased with time. Matrix degradation was evident after 20 d. An inflammatory response was also evident at 5 d, and its resolution correlated with the biodegradation sequence. Cell-matrix composite implants of urothelial and muscle cells retrieved at extended times (50 d) showed extensive formation of multilayered sheet-like structures and well-defined muscle layers. Matrices seeded with cells and manipulated into a tubular configuration showed layers of muscle cells lining the multilayered epithelial sheets. Cellular debris appeared reproducibly in the luminal spaces, suggesting that epithelial cells lining the lumina are sloughed into the luminal space. Cell matrices implanted with human bladder muscle cells alone showed almost complete replacement of the polymer with sheets of smooth muscle at 50 d. This experiment demonstrated, for the first time, that composite tissue engineered structures could be created *de novo*. Prior to this study, only single-cell-type tissue engineered structures had been created. The malleability of the synthetic matrix allowed for the creation of cell-matrix implants manipulated into preformed tubular configurations. The combination of both smooth muscle and urothelial cell-matrix scaffolds is able to provide a template wherein a functional ureter or urethra may be created *de novo*.

In the studies performed for tubularized structures, such as ureters and urethras, if an entire segment was replaced, cells were needed in order to prevent contracture. However, if the area replaced was small in at least one of its dimensions, i.e., an onlay graft for urethral replacement, the cells were not essential for adequate healing (29–31). A collagen-based matrix has been used successfully for urethroplasty in patients requiring re-do hypospadias repair (*see* Fig. 1) (31).



Fig. 1. Radiographic urethrogram of a patient with a reconstructed urethra using a collagen matrix shows maintenance of a wide caliber without any evidence of stricture.

Bladder Engineering

In other sets of experiments, bladder tissue was engineered and used for augmentation using similar techniques as aforementioned (32). Partial cystectomies were performed in beagles. Both urothelial and smooth muscle cells were harvested and expanded separately. Allogenic bladder submucosa obtained from sacrificed animals was seeded with muscle cells on one side and urothelial cells on the opposite side. All beagles underwent cruciate cystotomies on the bladder dome. Augmentation cystoplasty was performed with the allogenic bladder submucosa seeded with cells, and with the allogenic bladder submucosa without cells. Bladders augmented with the allogenic bladder submucosa seeded with cells showed a 99% increase in capacity compared to bladders augmented with the cell-free allogenic bladder submucosa, which showed only a 30% increase in capacity.

In all of the studies performed at our laboratory, whenever entire segments of tissue were replaced, there was a difference evident between matrices used with autologous cells and those used only as a regenerating scaffold. Matrix-cell composites retained most of their implanted diameter, as opposed to matrix only, wherein graft contraction and shrinkage occurred (33).

The results of all our prior studies showed that the creation of artificial bladders may be achieved *in vivo*, however, much work remained to be done in terms of the functional parameters of these implants. In order to address the functional parameters of tissue-engineered bladders, an animal model was designed that required a subtotal cystectomy with subsequent replacement with a tissue-engineered organ (34).

A total of 18 beagle dogs underwent a trigone-sparing cystectomy. The animals were randomly assigned to one of three groups. Group A ($n=6$) underwent closure of the trigone without a reconstructive procedure. Group B ($n=6$) underwent reconstruction with a cell-free bladder-shaped biodegradable polymer. Group C ($n=6$) underwent reconstruction using a bladder shaped biodegradable polymer that delivered autologous urothelial cells and smooth muscle cells. The cell populations had been separately expanded from a previously harvested autologous bladder biopsy. Preoperative and postoperative urodynamic and radiographic studies were performed serially. Animals were sacrificed at 1, 2, 3, 4, 6, and 11 mo postoperatively. Gross, histological, and immunocytochemical analyses were performed (34).

The cystectomy-only controls and polymer-only grafts maintained average capacities of 24% and 46% of preoperative values, respectively. An average bladder capacity of 95% of the original precystectomy volume was achieved in the tissue-engineered bladder replacements. The subtotal cystectomy reservoirs that were not reconstructed and polymer-only reconstructed bladders showed a marked decrease in bladder compliance (10% and 42%). The compliance of the tissue engineered bladders showed almost no difference from preoperative values that were measured when the native bladder was present (106%). Histologically, the polymer-only bladders presented a pattern of normal urothelial cells with a thickened fibrotic submucosa and a thin layer of muscle fibers. The retrieved tissue-engineered bladders showed a normal cellular organization, consisting of a trilayer of urothelium, submucosa, and muscle (Fig. 2). Immunocytochemical analyses for desmin, α -actin, cytokeratin 7, pancytokeratins AE1/AE3 and uroplakin III confirmed the muscle and urothelial phenotype. S-100 staining indicated the presence of neural structures. The results from this study showed that it is possible to tissue engineer bladders that are anatomically and functionally normal.

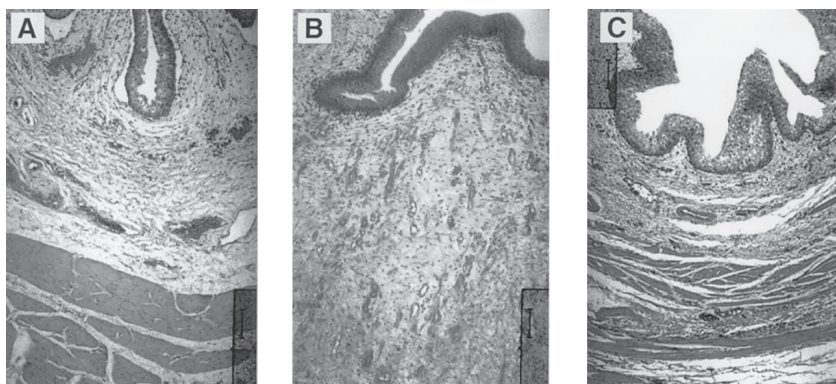


Fig. 2. Histological analysis of canine bladders 6 mo after surgery. Hematoxylin and eosin histological results (orig. magnif. X100) are shown for (A) bladder control; (B) the bladder dome of the cell-free polymer reconstructed bladder showing extensive fibrosis, and (C) the bladder dome of the cell-seeded polymer reconstructed bladder showing a normal architecture.

Genital Tissues

PENILE AND CLITORAL CORPORA CAVERNOSA

A large number of congenital and acquired abnormalities of the genitourinary system, including ambiguous external genitalia, the extrophy-epispadias complex and impotence, would benefit from the availability of transplantable, autologous corpus cavernosum tissue for use in reconstructive procedures. Given the major structural and functional importance of corpora cavernosal tissue, it is clear that the availability of autologous corporal smooth muscle tissue for use in reconstructive procedures would be of great clinical utility, facilitating enhanced cosmetic result, while providing the possibility of *de novo*, functional erectile tissue.

Experiments performed in our laboratory were designed to determine the feasibility of using cultured human corporal smooth muscle cells seeded onto biodegradable matrix scaffolds for the formation of corpus cavernosum muscle *in vivo*. Primary cultures of human corpus cavernosum smooth muscle cells were derived from operative biopsies obtained during penile prosthesis implantation and vaginal resection. Cells were maintained in continuous multilayered cultures, seeded onto polymers of nonwoven polyglycolic acid, and implanted subcutaneously in athymic mice. Animals were sacrificed at various time-points after surgery and the implants were examined via histology, immunocytochemistry, and Western blot analyses (35).

Corporal smooth muscle tissue was identified grossly and histologically at the time of sacrifice. Intact smooth muscle cell multilayers were observed growing along the surface of the polymers throughout all retrieved time-points. There was evidence of early vascular in-growth at the periphery of the implants by 7 d. By 24 d, there was evidence of polymer degradation. Smooth muscle phenotype was confirmed immunocytochemically and by Western blot analyses with antibodies to α -smooth muscle actin.

Further studies were performed wherein corpora cavernosal muscle cells were co-cultured with endothelial cells. The co-cultured cells were seeded on polymers and implanted in vivo. At retrieval, by 42 d, there was tissue organization similar to normal corpora (36). These studies provided the first evidence that cultured human corporal smooth muscle cells could be used in conjunction with biodegradable polymer scaffolds to create corpus cavernosum tissue *de novo*.

In the future, it could be foreseen that corpora cavernosal tissue could be safely and easily obtained under local anesthesia in a percutaneous, office-based procedure. Once harvested, this tissue could be used to establish explant cultures of autologous human corporal smooth muscle cells, fibroblasts, and endothelial cells. These cells, after expansion in vitro, could be seeded onto biodegradable polyglycolic acid polymer scaffolds where they would attach and multiply. Once delivered to the in vivo environment as an autograft in a reconstructive procedure, they might reorganize and resume their highly specialized physiologic function.

PENILE PROSTHESES

Currently, the principal method of reconstructing a phallus when insufficient tissue is present, is to utilize silicone rigid prostheses. Although silicone penile prostheses has been an accepted treatment modality since the 1970s, issues with biocompatibility remain (37). Creation of a natural penile prostheses composed of vascularized autologous tissue may be advantageous. We had previously demonstrated that autologous chondrocytes suspended in biodegradable polymers would form cartilage structures when implanted in vivo (38,39). We recently investigated the possibility of creating a natural phallic prosthesis consisting of autologous chondrocytes, which, if biocompatible and elastic, could be used in patients who require genital reconstruction.

Cartilage was harvested from the articular surface of calf shoulders. Chondrocytes were isolated, grown, and expanded in vitro. The cells were seeded onto preformed cylindrical polyglycolic acid polymer rods at a concentration of 50×10^6 chondrocytes/cm³. Cell-polymer scaffolds were implanted in vivo. Each mouse had two implantation

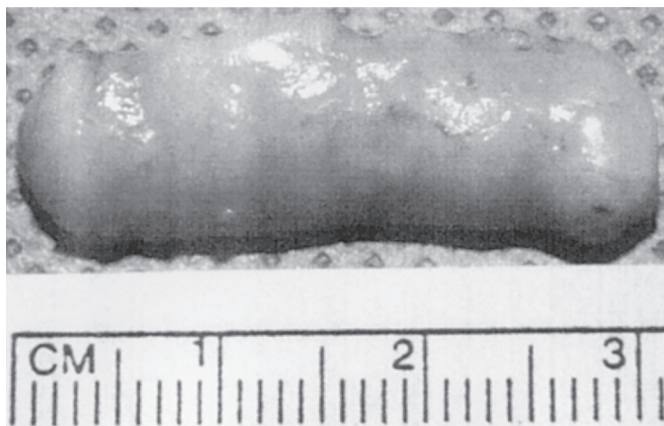


Fig. 3. Penile rod made from autologous cartilage cells.

sites consisting of a polymer scaffold seeded with chondrocytes and a control (polymer alone). The engineered rods were retrieved at 1, 2, 4, and 6 mo after implantation. Stress-relaxation studies to measure biomechanical properties, including compression, tension, and bending, were performed on the retrieved structures. Histological analyses were performed with hematoxylin and eosin, aldehyde fuschin-alcian blue, and toluidine blue staining (40).

Gross examination showed the presence of well-formed milky-white rod-shaped solid cartilage structures which were approximately the same size as the initial implant (*see* Fig. 3). A series of stress-relaxation tests were performed in order to determine whether the engineered cartilage rods possessed the mechanical properties required to maintain penile rigidity. Biomechanical analyses of all specimens demonstrated similar patterns. The compression studies showed that the retrieved cartilage rods were able to withstand high degrees of pressure. A ramp compression speed of 200 $\mu\text{m/s}$, applied to each cartilage rod up to 2000 μm in distance, resulted in 3.8 kg of resistance. The tension relaxation studies demonstrated that the retrieved cartilage rods were able to withstand stress and were able to return to their initial state while maintaining their biomechanical properties. A ramp-tension speed of 200 $\mu\text{m/s}$ applied to each cartilage rod created a tensile strength of 2.2 kg, which physically lengthened the rods an average of 0.48 cm. Relaxation of tension at the same speed resulted in retraction of the cartilage rods to their initial state. The five cycles of bending studies performed at two different speeds showed that the engineered cartilage rods were durable, malleable, and were able to retain their mechanical properties. None of the rods was ruptured during the biomechanical stress relaxation studies, which

showed that the cartilage structures were readily elastic and could withstand high degrees of pressure. Histochemical analyses with hematoxylin and eosin, aldehyde fuchsin-alcian blue, and toluidine blue staining demonstrated the presence of mature and well-formed chondrocytes in all the implants. There was no evidence of cartilage formation in the controls.

In a subsequent study, autologous cartilage seeded rods were implanted into rabbit corporas. The scaffolds were able to form cartilage rods in vivo, in the corpora. The engineered penile prostheses were stable, without any evidence of infection or erosion (41).

These preliminary studies indicate that creation of a penile prosthesis composed of chondrocytes can be achieved using biodegradable polymer scaffolds as a cell-delivery vehicle. The engineered tissue forms a cartilaginous structure that resists high pressures. The use of an autologous system would preclude an immunologic reaction. This technology could be useful in the future for the creation of a biocompatible malleable prosthesis for patients undergoing penile reconstruction.

Formation of Renal Structures

End-stage renal failure is a devastating disease that involves multiple organs in affected individuals. Although dialysis can prolong survival for many patients with end-stage renal disease, only renal transplantation can currently restore normal function. Renal transplantation is severely limited by a critical donor shortage. Augmentation of either isolated or total renal function with kidney cell expansion in vitro and subsequent autologous transplantation may be a feasible solution. However, kidney reconstitution using tissue-engineering techniques is a challenging task. The kidney is responsible not only for urine excretion, but for several other important metabolic functions in which critical kidney byproducts, such as renin, erythropoietin, and vitamin D, play a large role. We explored the possibility of harvesting and expanding renal cells in vitro and implanting them in vivo in a three-dimensional organization in order to achieve a functional artificial renal unit wherein urine production could be achieved (42,43). Studies demonstrated that renal cells can be successfully harvested, expanded in culture, and transplanted in vivo where the single suspended cells form and organize into functional renal structures that are able to excrete high levels of uric acid and creatinine through a yellow urine-like fluid. These findings suggest that this system may be able to replace transplantation in patients with end-stage failure.

Other approaches have also been pursued for renal functional replacement. Polysulphone hollow fibers have been prelined with various extra-

cellular matrix (ECM) components and seeded with mammalian renal tubular and endothelial cells (44). Permselective convective fluid transfer and active transport of salt and water were demonstrated. Using this approach, prototypic biohybrid constructs have been developed that are able to replicate the renal excretory functions. In addition, this system is able to facilitate gene and cell therapies by modifying the cells prior to seeding.

Injectable Therapies

URINARY INCONTINENCE AND VESICoureTERAL

Both urinary incontinence and vesicoureteral reflux are common conditions affecting the genitourinary system, wherein injectable bulk-ing agents can be used for treatment. There are definite advantages in treating urinary incontinence and vesicoureteral reflux endoscopically. The method is simple and can be completed in less than 15 min, has a low morbidity, and can be performed in an outpatient basis.

The goal of several investigators has been to find an ideal implant material for the endoscopic treatment of reflux and incontinence. The ideal substance should be injectable, nonantigenic, nonmigratory, volume stable, and safe for human use. Toward this goal, we had previously conducted long-term studies to determine the effect of injectable chondrocytes *in vivo* (38). We initially determined that alginate, a liquid solution of gluronic and mannuronic acid, embedded with chondrocytes, could serve as a synthetic substrate for the injectable delivery and maintenance of cartilage architecture *in vivo*. Alginate undergoes hydrolytic biodegradation and its degradation time can be varied depending on the concentration of each of the polysaccharides. The use of autologous cartilage for the treatment of vesicoureteral reflux in humans would satisfy all the requirements for an ideal injectable substance. A biopsy of the ear could be easily and quickly performed, followed by chondrocyte processing, and endoscopic injection of the autologous chondrocyte suspension for the treatment reflux.

Chondrocytes can be readily grown and expanded in culture. Neocartilage formation can be achieved *in vitro* and *in vivo* using chondrocytes cultured on synthetic biodegradable polymers (38). In our experiments, the cartilage matrix replaced the alginate as the polysaccharide polymer underwent biodegradation. We then adapted the system for the treatment of vesicoureteral reflux in a porcine model (39).

Six miniswine underwent bilateral creation of reflux. All six were found to have bilateral reflux without evidence of obstruction at 3 mo following the procedure. Chondrocytes were harvested from the left auricular surface of each miniswine and expanded with a final concen-

tration of $50\text{--}150 \times 10^6$ viable cells/animal. The animals then underwent endoscopic repair of reflux with the injectable autologous chondrocyte solution on the right side only.

Cystoscopic and radiographic examinations were performed at 2, 4, and 6 mo after treatment. Cystoscopic examinations showed a smooth bladder wall. Cystograms showed no evidence of reflux on the treated side and persistent reflux in the uncorrected control ureter in all animals. All animals had a successful cure of reflux in the repaired ureter without evidence of hydronephrosis on excretory urography. The harvested ears had evidence of cartilage regrowth within one month of chondrocyte retrieval.

At the time of sacrifice, gross examination of the bladder injection site showed a well-defined rubbery-to-hard cartilage structure in the subureteral region. Histologic examination of these specimens using hematoxylin and eosin stains showed evidence of normal cartilage formation. The polymer gels were progressively replaced by cartilage with increasing time. Aldehyde fuchsin-alcian blue staining suggested the presence of chondroitin sulfate. Microscopic analyses of the tissues surrounding the injection site showed no inflammation. Tissue sections from the bladder, ureters, lymph nodes, kidneys, lungs, liver, and spleen showed no evidence of chondrocyte or alginate migration, or granuloma formation.

Our studies showed that chondrocytes can be easily harvested and combined with alginate in vitro, the suspension can be easily injected cystoscopically and the elastic cartilage tissue formed is able to correct vesicoureteral reflux without any evidence of obstruction (39).

Using the same line of reasoning as with the chondrocyte technology, our group investigated the possibility of using autologous muscle cells (45). In vivo experiments were conducted in minipigs and reflux was successfully corrected.

The chondrocyte technology is currently being used in FDA-sanctioned studies for in-patients with reflux and incontinence (*see* Fig. 4) (46). In addition to its use for the endoscopic treatment of reflux and urinary incontinence, the system of injectable autologous cells may also be applicable for the treatment of other medical conditions, such as rectal incontinence, dysphonia, plastic reconstruction, and wherever an injectable permanent biocompatible material is needed.

TESTICULAR FUNCTIONAL REPLACEMENT

Leydig cells are the major source of testosterone production in males. Patients with testicular dysfunction require androgen replacement for somatic development. Conventional treatment for testicular dysfunction

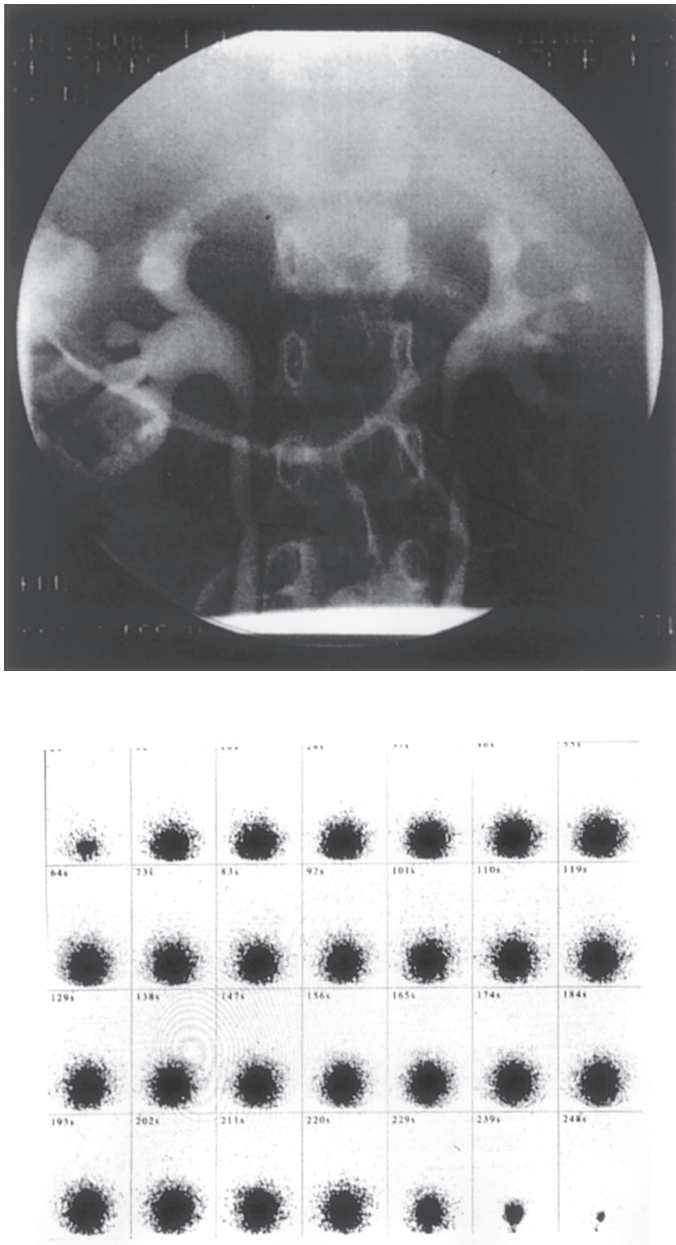


Fig. 4. (Top) Preoperative VCUG in a patient shows bilateral vesicoureteral reflux. (Bottom) Postoperative radionuclide cystogram after endoscopic treatment with autologous engineered chondrocytes shows resolution of reflux bilaterally.

consists of periodic intramuscular (im) injections of chemically modified testosterone, or more recently, of skin patch applications. However, long-term nonpulsatile testosterone therapy is not optimal and can cause multiple problems, including erythropoiesis and bone density changes. The possibility of using leydig cell microencapsulation for controlled testosterone replacement was evaluated (47). Microencapsulated leydig cells may offer several advantages, such as serving as a semipermeable barrier between the transplanted cells and the host's immune system, as well as allowing for the long-term physiological release of testosterone.

Purified leydig cells from rat testes were prepared on a Percoll gradient. Cell viability and identification was performed by Trypan blue and 3 β -hydroxysteroid dehydrogenase (3 β -HSD), respectively. Leydig cells were suspended in 1.2% sodium alginate solution and extruded through an air-jet nozzle into a 1.5% CaCl₂ solution where they gelled, and were further coated with 0.1% poly-L-lysine. The encapsulated cells were pulsed with human chorionic gonadotropin (hCG) every 24 h. The medium was sampled at different time-points after hCG stimulation and analyzed for testosterone production. MTT assay was performed every day to ensure cell viability. Control experiments were performed using nonencapsulated purified leydig cells under the same conditions (47).

More than 90% of the cells recovered from the Percoll gradient stained positively for 3 β -HSD. Both Trypan blue exclusion and MTT assays showed that 95% of the cells were viable. Biochemical measurements, which were performed every 4 h, showed that the microencapsulated leydig cells produced testosterone. Testosterone levels in the presence of hCG ranged between 35–60 ng/dL/10(6)/24 h. Testosterone levels measured from nonencapsulated leydig cells ranged between 45–50 ng/dL/10(6)/24 h.

Microencapsulated leydig cells are viable and are able to produce high levels of testosterone. The microencapsulation system renders the cells nonimmunogenic. These studies suggest that microencapsulated leydig cells may be able to replace or supplement testosterone in situations where anorchia or testicular failure is present.

FETAL TISSUE ENGINEERING

The prenatal diagnosis of patients with bladder disease is now more prevalent. Prenatal ultrasonography allows for a thorough survey of fetal anatomy. The absence of bladder filling, a mass of echogenic tissue on the lower abdominal wall, or a low set umbilicus during prenatal sonographic examination may suggest the diagnosis of bladder exstrophy. These findings and the presence of intraluminal intestinal calcifications suggest the presence of a cloacal malformation.

The natural consequence of the evolution in prenatal diagnosis led to the use of intervention before birth to reverse potentially life-threatening processes. However, the concept of prenatal intervention itself is not limited to this narrow group of indications. A prenatal, rather than a postnatal, diagnosis of exstrophy may be beneficial under certain circumstances. There is now a renewed interest in performing a single-stage reconstruction in some patients with bladder exstrophy. Limiting factors for following a single- or multiple-stage approach may include the findings of a small, fibrotic bladder patch without either elasticity or contractility, or a hypoplastic bladder.

There are several strategies that may be pursued, using today's technological and scientific advances, which may facilitate the future prenatal management of patients with bladder disease. Having a ready supply of urologic-associated tissue for surgical reconstruction at birth may be advantageous. Theoretically, once the diagnosis of bladder exstrophy is confirmed prenatally, a small bladder and skin biopsy could be obtained via ultrasound guidance. These biopsy materials could then be processed and the different cell types expanded in vitro. Using tissue-engineering techniques developed at our center and described previously, reconstituted bladder and skin structures in vitro could then be readily available at the time of birth for a one-stage reconstruction, allowing for an adequate anatomic and functional closure.

Toward this end, we conducted a series of experiments using fetal lambs. (48,49). Bladder exstrophy was created surgically in 10 90–95-d gestation fetal lambs. The lambs were randomly divided into two groups of five. In Group I, a small fetal bladder specimen was harvested via fetoscopy. The bladder specimen was separated and muscle and urothelial cells were harvested and expanded separately under sterile conditions in a humidified 5% CO₂ chamber, as previously described. Seven to ten days prior to delivery, the expanded bladder muscle cells were seeded on one side and the urothelial cells on the opposite side of a 20-cm² biodegradable polyglycolic acid polymer scaffold. After delivery, all lambs in Group I had surgical closure of their bladder using the tissue engineered bladder tissue. No fetal bladder harvest was performed in the Group II lambs, and bladder exstrophy closure was performed using only the native bladder. Cystograms were performed 3 and 8 wk after surgery. The engineered bladders were more compliant ($p=0.01$) and had a higher capacity ($p=0.02$) than the native bladder closure group. Histologic analysis of the engineered tissue showed a normal histological pattern, indistinguishable from native bladder at 2 mo (42). Similar prenatal studies were performed in lambs, engineering skin for reconstruction at birth (49).

These studies show that the potential for replicating this technology in humans is possible. Other tissues, such as cartilage, corpora cavernosa, and skeletal muscle can also be harvested and expanded in the same manner. Similar studies addressing these tissues are now in progress in our laboratory.

In addition to being able to manage the bladder exstrophy complex in utero with tissue-engineering techniques, one could also manage patients after birth in a similar manner, whenever a prenatal diagnosis is not assured. In these instances, bladder tissue biopsies could be obtained at the time of the initial surgery. Different tissues could be harvested and stored for future reconstruction, if necessary. A tissue bank for exstrophy complex patients could preserve different cell types indefinitely.

In addition to having an exstrophy complex tissue bank serve as a repository of cells for future tissue reconstitution, it could also serve as a resource to elucidate the cellular, molecular, and genetic mechanisms required for the development and future prevention of these anomalies.

GENE THERAPY AND TISSUE ENGINEERING

Based on the feasibility of tissue-engineering techniques in which cells seeded on biodegradable polymer scaffolds form tissue when implanted in vivo, the possibility was explored of developing a neo-organ system for in vivo gene therapy (50).

In a series of studies conducted in our laboratory, human urothelial cells were harvested, expanded in vitro, and seeded on biodegradable polymer scaffolds. The cell-polymer complex was then transfected with PGL3-luc, pCMV-luc, and pCMV β -gal promoter-reporter gene constructs. The transfected cell-polymer scaffolds were then implanted in vivo and the engineered tissues were retrieved at different time-points after implantation. Results indicated that successful gene transfer could be achieved using biodegradable polymer scaffolds as a urothelial cell-delivery vehicle. The transfected cell/polymer scaffold formed organ-like structures with functional expression of the transfected genes (50).

This technology is applicable throughout the spectrum of diseases that may be manageable with tissue engineering. For example, one can envision the use of effecting in vivo gene delivery through the ex vivo transfection of tissue engineered cell/polymer scaffolds for the genetic modification of diseased corporal smooth muscle cells harvested from impotent patients. Studies of human corpus cavernosum smooth muscle cells have suggested that cellular overproduction of the cytokine, transforming growth factor-1 (TGF-1) may lead to the synthesis and accumulation of excess collagen in patients with arterial insufficiency resulting in corporal fibrosis. Prostaglandin E1 (PGE1) was shown to suppress

this effect in vitro. Theoretically, the in vitro genetic modification of corporal smooth muscle cells harvested from an impotent patient, resulting in either a reduction in the expression of the TGF-1 gene, or the overexpression of genes responsible for PGE1 production, could lead to the resumption of erectile functionality once these cells were used to repopulate the diseased corporal bodies.

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