Tissue Engineering Applications in the Genitourinary Tract System

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--- Abstract ---

The concept of cell transplantation using tissue engineering techniques has provided numerous possibilities in the area of urologic tissue reconstruction. Tissue engineering applications in the genitourinary tract system have been investigated in almost every tissue in order to improve, restore and replace existing tissue function. Although most reconstructive efforts still remain in the experimental stage, several technologies have been transferred to the bedside with satisfactory outcome. In this article, we describe tissue engineering approaches attempted in the genitourinary system for reconstruction.

Key Words: Tissue engineering, reconstruction, urinary tract

INTRODUCTION

Reconstructive procedures in the genitourinary tract system are usually performed in conditions, such as congenital abnormalities, trauma, inflammation, infection and cancer. The goal of urologic tissue reconstruction is to replace, restore or improve existing tissue function. Surgical reconstruction is best achieved by using existing native urologic tissues, however, this is not possible in numerous instances due to the limited autologous tissue available. Whenever there is a lack of native urologic tissue, reconstruction is usually performed with native non-urologic tissue, such as bowel, skin, muscle or mucosa from various body sites. Other alternate sources, such as allogeneic, xenogeneic or synthetic biomaterials have also been used. However, the use of these tissue substitutes often creates problems, such as metabolic abnormalities, infection, perforation, urolithiasis and malignancy. In addition, these complications often necessitate additional procedures, including ablation, repair or reconstruction.

The initial tissue engineering attempts in the genitourinary tract system was somewhat limited due to the difficulty of growing urologic cells in large quantities. In the past, it was believed that urothelial cells had a natural senescence. Normal urothelial cells could be grown in the laboratory setting, but with limited expansion. Urothelial cell harvest, culture and harvest system was developed in our laboratory which does not require any enzymes or serum and has a large expansion potential. Using this method of culture system, it is possible to expand a urothelial strain from a single 1 cm² tissue specimen to a surface area of 4,204 m² within 2 months. Development of urologic cell culture system indicated the feasibility of collecting autologous urothelial cells from patients, grow them in culture, and return them to the same patient in sufficient quantities for reconstruction. Cells from bladder, ureter and renal pelvis can be equally harvested, cultured and expanded in a similar fashion. We have shown that normal human bladder epithelial and muscle cells can be efficiently harvested, expanded in culture. Their differentiation characteristics, growth requirements and other biological properties were extensively studied.

The success of using cell transplantation strategies depends on the ability to use donor tissue efficiently and to provide the right conditions for long term survival, differentiation and growth. We have achieved an approach to tissue regeneration by patching isolated cells to a support structure, which would have suitable surface chemistry for guiding the reorganization and growth of the cells. The supporting matrix is composed of biodegradable artificial or
natural matrices which can allow cell survival by diffusion of nutrients across short distances once the cell-support matrix is implanted. The cell-support matrix becomes vascularized in concert with expansion of the cell mass following implantation.

Studies have shown that cells or tissue components may not be implanted in large volumes due to the limited diffusion distance. If cells were implanted in volumes greater than 3 mm³, only the cells on the surface would survive, due to a lack of vascularity. Thus, a cell-support matrix is necessary to allow diffusion of nutrients across the entire implant. A variety of biomaterials were examined in order to determine ideal support structures for the regeneration of urologic tissue. Biomaterials, such as poly-lactic and glycolic acid polymers, and collagen scaffolds have been used due to their porous configuration, thus allowing for capillary infiltration to the interstitial spaces after implantation in vivo. In this manner, large numbers of cells can be implanted with maximal survival, or adjacent cells can migrate onto the matrix with its appropriate vascularity. Our laboratory has also used natural materials, such as processed collagen derived from allogeneic donor bladder submucosa and intestinal submucosa as cell delivery vehicles. These matrices have many desirable features; they are biocompatible and easily processable. These matrices, both natural and synthetic, can be readily modified, depending on their intended application, into a variety of shapes and structures.

The initial experiments were designed in order to explore the possibility of engineering urologic tissue components in an in vivo animal model. Normal urothelial and muscle cells were expanded in vitro, seeded onto PGA polymer scaffolds either separately or together, and allowed to attach and form sheets of cells in vitro. Several parameters, regarding specific cell attachment properties on the polymers scaffolds, were evaluated over time. A series of in vivo cell-polymer scaffold experiments were performed. Normal primary bladder urothelial and muscle cells were expanded, seeded onto biodegradable polymer scaffolds, and implanted in the subcutaneous space of athymic mice.

Histologic analysis of normal primary urothelial, bladder muscle, and composite urothelial and bladder muscle-polymer scaffolds, implanted in athymic mice and retrieved at different time points, indicated that viable cells were evident in all three experimental groups. Implanted cells oriented themselves spatially along the polymer surfaces. The cell populations appeared to expand from one layer to several layers of thickness with progressive cell organization with extended implantation times. Polymers alone or with cells, evoked an aggressive angiogenic response by 5 days, which increased with time. Polymer fiber degradation was evident. Cell-polymer composite implants of urothelial cells alone, retrieved at extended time (50 days), showed extensive formation of multilayered sheet-like structures. Polymers seeded with both muscle and urothelial cells and manipulated into a tubular configuration showed layers of muscle cells lining the multilayered epithelial sheets. Cell polymers implanted with human bladder muscle cells alone showed almost complete replacement of the polymer scaffold with sheets of smooth muscle 50 days after implantation.

These experiments demonstrated that newly isolated human bladder urothelial and muscle cells would attach to artificial and natural matrices in vitro and that, when implanted into animals, these constructs could survive, proliferate and reorganize into newly formed multilayered structures which exhibit spatial orientation and a normal histomorphology in vivo. These experiments also demonstrated that composite tissue engineered structures could be created de novo.

BLADDER AND URETER

Experiments were performed in an animal model of ureteral replacement in order to determine the effects of implanting engineered tissues in continuity with the urinary tract. In one study conducted in dogs, urothelial and smooth muscle cells were harvested, expanded in vitro and seeded onto biodegradable matrix scaffolds. These structures where tubularized and used to replace ureteral segments in each animal. The malleability of the synthetic matrix allowed for the creation of cell-matrix implants manipulated into pre-formed tubular configurations. The combination of both smooth muscle and urothelial cell-matrix scaffolds is able to provide a template wherein functional ureteral tissue may be created de novo. In the studies performed, if an entire segment was replaced, cells were needed in order to prevent graft resorption and strictures. However, if the area replaced was small in at least one of its
dimensions, i.e. an onlay graft, the cells were not essential for adequate healing.

Experimental studies involving the bladder tissue were initially performed using animal models of augmentation.7 Partial cystectomies were performed in dogs. Both urothelial and smooth muscle cells were harvested and expanded separately. A collagen matrix obtained from processed allogenic bladder submucosa was seeded with muscle cells on one side and urothelial cells on the opposite side. All dogs underwent cruciate cystotomies on the bladder dome. Augmentation cystoplasty was performed with either cell seeded or unseeded matrices. Bladders augmented with the cell seeded-matrix scaffold showed a 99% increase in capacity compared to bladders augmented with the unseeded matrix, which showed only a 30% increase in capacity, wherein graft contraction and shrinkage occurred. Histologically, the retrieved engineered bladders contained a cellular organization consisting of a urothelial lined lumen surrounded by submucosal tissue and smooth muscle. However, the muscular layer was markedly more prominent in the cell reconstituted scaffold.7

Most of the unseeded matrices utilized for bladder replacement in the past have been able to show adequate histology in terms of a well developed urothelial layer, however they have been associated with an abnormal muscular layer which varies in terms of its full development.7 It has been well established that the bladder is able to regenerate generously over free grafts. Urothelium is associated with a high regenerative capacity.20 Bladder muscle tissue is less likely to regenerate in a normal fashion. Both the urothelial and muscle ingrowth are believed to be initiated from the edges of the normal bladder towards the region of the free graft.20,21 Usually, however, contracture or resorption of the graft has been evident. The inflammatory response towards the matrix may contribute to the resorption of the free graft.

We hypothesized that building a three-dimensional structure construct in vitro, prior to implantation, would facilitate the eventual terminal differentiation of the cells after implantation in vivo, and would minimize the inflammatory response towards the matrix, thus avoiding graft contracture and shrinkage. In the study above, there was a more aggressive inflammatory reaction in the matrices implanted without cells. Of interest, is that the urothelial cell layers appeared normal, even though its underlying matrix was significantly inflamed. We further hypothesized, therefore, that having an adequate urothelial layer from the outset, would limit the amount of urine contact with the matrix, and would therefore decrease the inflammatory response, and that the muscle cells were also necessary for bioengineering, being that native muscle cells are less likely to regenerate over the free grafts. Further studies performed in our laboratory confirmed this hypothesis.19 Thus, the presence of both urothelial and muscle cells, on the matrices we used for bladder replacement, appear to be important for successful tissue bioengineering.

In the bladder augmentation study above, a large portion of the native bladders were preserved.7 When performing studies wherein a large portion of the native bladder is preserved, it is hard to define if the functional parameters seen (urodynamic findings, such as compliance) are derived from the native bladder itself, or the implanted matrix. In order to better address the functional parameters of tissue engineered bladders, an animal model was designed which required a subtotal cystectomy (bladder removal) with subsequent replacement with either a cell-seeded or unseeded polymer scaffold.10

Fourteen beagle dogs underwent a trigone-sparing cystectomy. The animals were randomly assigned to one of three groups. Group A (n=2) underwent closure of the trigone without a reconstructive procedure. Group B (n=6) underwent reconstruction with an unseeded 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 months postoperatively. Gross, histological and immunocytochemical analyses were performed.10

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 pre-cystectomy volume was achieved in the tissue-engineered bladder replacements (Fig. 1). The subtotal cystectomy reservoirs which were not reconstructed and polymer only reconstructed bladders showed a marked decrease in bladder compliance (10% and 42%).
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 tri-layer of urothelium, submucosa and muscle. Immunocytochemical analyses for desmin, alpha 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 engineer anatomically and functionally normal bladders using cell-seeded matrices. However, unseeded matrices, without cells, are not adequate for the formation of functionally adequate bladder reservoirs.

**URETHRA**

In situations where there is limited urethral mucosa for adequate reconstruction, tissue from other sources have been used, such as genital and extragenital skin flaps or grafts, mucosal grafts from the bladder or buccal regions, tunica vaginalis and peritoneal grafts.\textsuperscript{22-24} However, complications such as hair growth, graft shrinkage, stricture, stone formation and diverticuli have been associated with skin grafts.\textsuperscript{25-29} Although several innovative tissues have been proposed as possible free grafts for urethral repair, it is evident that all have specific advantages and disadvantages. The use of these tissues may be associated with additional procedures for graft retrieval, prolonged hospitalization and donor site morbidity.

Our initial experiment involving urethral tissue engineering was directed toward creating urethral tissue, composed of urologic cells. Autologous bladder urothelial and muscle cells were harvested, grown and expanded in culture. The cells were seeded onto non-woven meshes of polyglycolic acid.\textsuperscript{30} Partial urethrectomies were performed in rabbits and a segment of the polymer mesh with the appropriate diameter was interposed to form the neourethra in each animal. There was no evidence of voiding difficulties or any other complications. Retrograde urethrograms showed
no evidence of stricture formation. Histologic examination of the neourethras demonstrated complete-re-epithelialization of the polymer mesh implanted sites by day 14 and continued for the entire duration of the study. Polymer fiber degradation was evident.

In a subsequent study we investigated whether a naturally derived acellular collagen matrix from bladder would be suitable for urethral reconstruction. A ventral urethral defect measuring approximately 1/2 of the urethral circumference was created in male rabbits. The acellular collagen matrix was trimmed and used to replace the urethral defect in an onlay fashion. Serial urethrogram confirmed the maintenance of a wide urethral caliber without any signs of strictures. Gross examination at retrieval showed normally appearing tissue without any evidence of fibrosis. Histologically, the implanted matrices contained host cell infiltration and generous angiogenesis by 2 weeks after surgery. There was no evidence of fibrosis or scarring in the urethras at any of the retrieval time periods. The presence of a complete transitional cell layer over the graft was confirmed 2 weeks after the repair, and this was consistent throughout the study. The urothelial cell layers stained positively with the broadly reacting antipancytokeratins AE1/AE3 in all implants. Normally appearing organized muscle fiber bundles were evident six months after implantation. These results demonstrated that the acellular collagen matrix could be a useful material for urethral repair.

Following our experimental study with the collagen based acellular matrix, we used the material clinically for urethral reconstruction. Four patients with a history of prior hypospadias surgery underwent reparative repair using the collagen based matrix for urethral reconstruction. The collagen matrix, obtained from donor cadaver bladder, was processed and trimmed to size as needed for each individual patient. The neourethras were created by anastomosing the matrix in an onlay fashion to the urethral plate. The size of the created neourethra ranged from five centimeters to fifteen centimeters. After a twenty-two month follow-up, three of the four patients had a successful outcome in regards to their cosmetic appearance and function. One patient who had a 15 cm neourethra created developed a subglanular fistula. These results show that the use of a collagen based acellular matrix appears to be beneficial for patients with prior hypospadias repair who may lack sufficient genital skin for reconstruction.

In a subsequent study 26 patients with a diagnosis of urethral stricture underwent reconstructive surgery using a collagen-based inert matrix for urethral reconstruction. The inert collagen matrix was trimmed to size as needed for each patient and the neourethras were created by anastomosing the matrix in an onlay fashion to the urethral plate. The size of the created neourethra ranged from 1.5 to 16 cm. Urethrogram was performed routinely 4 months post-operatively. Cystoscopic studies with urethral biopsies were also performed. After an 18 month follow-up, 23 of the 26 patients had a successful outcome in regards to their function. Two patients had a recurrent anastomotic stricture and one patient developed a subcoronal fistula which closed spontaneously after one year of repair. Cystoscopic studies showed adequate caliber conduits and normally appearing urethral tissues. Histologic examination of the biopsy specimens showed the typical urethral stratified epithelium.

Other treatment modalities for urethral strictures, such as ablation and the placement of synthetic stents, are suboptimal, and recurrence rates are high. The use of a natural urethral stent made of autologous tissue would be advantageous due to its biocompatibility. In another study we investigated the feasibility of engineering tubular cartilaginous stents in vitro which could be used as permanent urethral stents in stricture disease. Thirty cylindrical tubes were fabricated from poly-L-lactic acid-coated, polyglycolic acid polymer scaffolds. Chondrocytes, harvested and isolated from the articular surface of calf shoulder, were seeded onto the tubular scaffolds at a density of $60 \times 10^6$ cells/ml. Fifteen stents were placed in a stirring bioreactor in culture. The remaining 15 stents were implanted in the subcutaneous space of athymic mice for comparison. Gross and histological analyses, and biomechanical studies were performed at 4 and 10 weeks after cell seeding. Scanning electron microscopy showed adequate chondrocyte attachment to the matrix. Gross examination of the stents engineered in vivo or in vitro showed the presence of well-formed, milky white tubular cartilage structures without any evidence of tissue ingrowth into the lumen. Histological analyses confirmed the presence of mature cartilage and the deposition of collagen and GAG in both groups. Biomechanical studies demonstrated that the engineered stents, formed in both conditions, were readily
elastic and could withstand high degrees of pressure. This study demonstrates the feasibility of engineering cartilaginous stents in vitro using a bioreactor. The ex vivo engineered cartilage stents composed of autologous chondrocytes may be useful clinically.

KIDNEY

End stage renal failure is a devastating disease, which involves multiple organs in affected individuals. Although currently available treatment modalities, including dialysis and transplantation, can prolong survival for many patients, problems, such as donor shortage, complications and graft failure remain a continued concern. Numerous investigative efforts have been attempted in order to improve, restore or replace renal function. Tissue engineering approaches using cell therapies have been attempted in order to achieve functional kidney support.

Components required to achieve partial or total renal function are renal cells, three-dimensional scaffolds and an in vivo environment. The challenge associated with renal cell culture is due to their unique structural and cellular heterogeneity present within the kidney. The system of nephrons and collecting ducts is composed of multiple functionally and phenotypically distinct segments. For this reason, appropriate conditions need to be provided for long-term survival, differentiation and growth of the cells. Extensive research has been performed in order to determine optimal growth conditions for renal cell enrichment.\textsuperscript{33-38} Based on the literature and our experience, we were able to obtain optimal growth conditions for a stable cell culture system.

Although isolated renal cells retain their phenotype and function in culture, transplantation of these cells in vivo may not result in structural remodeling. In addition, cell or tissue components may not be implanted in large volumes due to the limited diffusion.\textsuperscript{11} Thus, a cell-support matrix is necessary to allow diffusion of nutrients across the entire implant. A variety of synthetic materials were examined in order to determine ideal support structures for the regeneration of urologic tissue.\textsuperscript{39,40} Biodegradable materials, such as poly-lactic and glycolic acid polymers, and collagen scaffolds have been preferred due to their biocompatibility and easy processing.\textsuperscript{41-45}

Our initial study involved investigating the feasibility of achieving renal cell growth, expansion and in vivo reconstitution using tissue engineering techniques.\textsuperscript{39} Rabbit kidney tissue was harvested to yield three separate purified suspensions of distal tubules, glomeruli, and proximal tubules. The cells were plated separately in vitro and seeded onto biodegradable polyglycolic acid scaffolds. Polymer scaffolds were implanted subcutaneously into athymic mice. This included implants of proximal tubular cells, glomeruli, distal tubular cells, and a mixture of allthree cell types. Animals were sacrificed at one week, two weeks, and one month after implantation and the retrieved scaffolds were analyzed. Histologic examination demonstrated progressive formation and organization of the nephron segments within the polymer fibers with time. Renal cell proliferation in the cell-polymer scaffolds was detected by in vivo labeling of replicating cells with the thymidine analog bromodeoxyuridine (BrdU). BrdU incorporation into renal cell DNA was identified immunocytochemically with monoclonal anti-BrdU antibodies. These results demonstrated that renal specific cells can be successfully harvested, survive in culture and attach to artificial biodegradable polymers. The renal cell-polymer scaffolds can be implanted into host animals where the cells replicate and organize into nephron segments, as the polymer, which acts as a cell delivery vehicle, undergoes biodegradation.

The initial experiments demonstrated that implanted cell-polymer scaffolds gave rise to renal tubular structures. However, it was unclear whether the tubular structures reconstituted de novo from dispersed renal elements, or they merely represented fragments of donor tubules, which survived intact. Further investigation was conducted in order to examine the tubular reconstitution process.\textsuperscript{46} Mice renal cells were harvested, grown and expanded in culture. Subsequently, single isolated cells were seeded on biodegradable polymers and implanted into syngeneic hosts. Renal epithelial cells were observed to reconstitute into tubular structures in vivo. Sequential analyses of the retrieved implants over time demonstrated that renal epithelial cells first organized into a cord-like structure with a solid center. Subsequent canalization into a hollow tube could be seen by two weeks. Histologic examination with nephron segment specific lactins showed successful reconstitution of proximal tubules, distal tubules, loop of Henle, collecting tubules and collecting ducts.
These results showed that single suspended cells are capable of reconstituting into tubular structures, with homogeneous cell types within each tubule.

The kidneys are critical to body homeostasis because of their excretory, regulatory and endocrinologic functions. The excretory function is initiated by filtration of blood at the glomerulus, and the regulatory function is provided by the tubular segments. Although our prior studies demonstrated that renal cells seeded on biodegradable polymer scaffolds are able to reconstitute into renal structures in vivo, complete renal function could not be achieved due to the type and structural configuration of polymers used. In our subsequent study we explored the feasibility of creating a functional artificial renal unit, wherein urine production could be achieved. Mouse renal cells were harvested and expanded in culture. The cells were seeded onto a tubular device constructed from polycarbonate (4 micron pore size), connected at one end with a silastic catheter which terminated into a reservoir. The device was implanted in the subcutaneous space of athymic mice. Fluid was collected from inside the implant, and uric acid and creatinine levels were determined.

Histological examination of the implanted device revealed extensive vascularization, formation of glomeruli and highly organized tubule-like structures. Immunocytochemical studies using osteopontin, alkaline phosphatase and fibronectin antibodies identified renal structures. The fluid collected from the reservoir was yellow and contained 66 mg/dl uric acid (as compared to 2 mg/dl in plasma) suggesting that these tubules are capable of unidirectional secretion and concentration of uric acid. The creatinine assay performed on the collected fluid showed an 8.2 fold increase in concentration, as compared to serum. These results demonstrated that single cells form multicellular structures and become organized into functional renal units that are able to excrete high levels of solutes through a urine-like fluid.

In our previous study, we showed that renal cells seeded on synthetic renal devices with a collecting system are able to form functional renal structures with urine-like fluid excretion. However, a naturally derived tissue matrix with an existing three-dimensional kidney architecture would be preferable, in that it would allow for transplantation of a large number of cells for the creation of greater renal tissue volumes. We developed an acellular collagen-based kidney matrix, which is identical to the native renal architecture. In a subsequent study we investigated whether the collagen-based matrices could accommodate large volumes of renal cells which could proliferate and form kidney structures in vivo.

Acellular collagen matrices, derived from porcine kidneys, were obtained. Mice renal cells were harvested, grown and seeded on 80 collagen matrices. Forty cell-matrix constructs grown in vitro and the remaining 40 cell-matrices were implanted in the subcutaneous space of 20 athymic mice. Scanning electron microscopy and histologic examination confirmed the acellularity of the processed matrix. RT-PCR performed on the kidney matrices demonstrated the absence of any RNA residues. Renal cells seeded on the matrix adhered to the inner surface and proliferated to confluency 7 days after seeding, as demonstrated by SEM. Histochemical and immunocytochemical analyses performed using H&E, periodic acid schiff, alkaline phosphatase, anti-osteopontin and anti-CD-31 identified stromal, endothelial and tubular epithelial cell phenotypes within the matrix. Renal tubular and glomeruli-like structures were observed 8 weeks after implantation. MTT proliferation and radioactive thymidine incorporation assays performed 6 weeks after cell seeding demonstrated a cell population increase of 116% and 92%, respectively, as compared to the 2 week time points. This study demonstrates that renal cells are able to adhere and proliferate on the collagen-based kidney matrices. The renal cells reconstitute renal tubular and glomeruli-like structures. The collagen based kidney matrix system seeded with renal cells may be useful in the future for augmenting renal function.

Many challenges remain in engineering functional renal tissue. The kidney mass needs to be large enough for functional replacement while allowing for adequate vascularization. In addition efficient systems for urine excretion need to be optimized. Current work in our laboratory is aimed at addressing these challenges.

**INJECTABLE THERAPIES**

Both urinary incontinence and vesicoureteral reflux are common conditions affecting the genitourinary system, wherein injectable bulking agents can be used for treatment. We conducted long-term studies to...
determine the effect of injectable chondrocytes in vivo as a potential bulking agent. We initially determined that alginate, a liquid solution of glutonic and mannuronic acid, embedded with chondrocytes, could serve as a synthetic substrate for the injectable delivery and maintenance of cartilage architecture in vivo. 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 of 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. 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.

Six mini-swine underwent bilateral creation of reflux. All six were found to have bilateral reflux without evidence of obstruction at three months following the procedure. Chondrocytes were harvested from the left auricular surface of each mini-swine and expanded with a final concentration of $50-150 \times 10^6$ viable cells per animal. The animals underwent endoscopically repair of reflux with the injectable autologous chondrocyte solution on the right side only.

Serial 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 showed evidence of normal cartilage formation. The polymer gels were progressively replaced by cartilage with increasing time. Aldehyde fuschin-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. These 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.

Fig. 2. Cystograms of pre- and post-endoscopic treatment of reflux in a patient. A chondrocyte-alginate suspension was injected endoscopically in the subureteral region. Preoperative fluoroscopic cystogram (left) shows bilateral reflux. Postoperative radionuclide cystogram (right) shows resolution of reflux.
Using the same line of reasoning as with the chondrocyte technology, our group investigated the possibility of using autologous muscle cells. In vivo experiments were conducted in mini-pigs and reflux was successfully corrected. 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.

Recently, the first human application of cell-based tissue engineering technology for urologic applications has occurred with the injection of chondrocytes for the correction of vesicoureteral reflux in children and for urinary incontinence in adults. The preliminary outcome of the clinical trials using autologous chondrocytes to correct vesicoureteral reflux in children is promising. A total of 29 children (46 ureters) with grades II to IV vesicoureteral reflux were treated. Each child underwent cystoscopy and ear cartilage biopsy at the initial setting. Chondrocytes, grown and expanded in culture, were injected endoscopically into the bladder trigone to correct reflux. Ultrasonography and radionuclide cystography were performed postoperatively to confirm reflux resolution (Fig. 2). Overall reflux was corrected in 38 of the 46 ureters (83%) and in 24 of the 29 patients (83%). There were no significant complications. This trial demonstrated that endoscopic injection of autologous chondrocytes for the treatment of vesicoureteral reflux in children appears to be an effective and safe technique.

GENITAL AND REPRODUCTIVE TRACT TISSUES

Testis

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 consists of periodic IM injections of chemically modified testosterone, or more recently, of skin patch applications. However, long term non-pulsatile testosterone therapy is not optimal and can cause multiple problems, including erythropoiesis and bone density changes.

A system was designed wherein leydig cells were microencapsulated for controlled testosterone replacement. Microencapsulated leydig cells 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 were isolated, characterized, suspended in an alginate solution and extruded through an air jet nozzle into a 1.5% CaCl2 solution they gelled; and were further coated with 0.1% poly-L-lysine. The encapsulated cells were pulsed with HCG every 24 hr. The medium was sampled at different time points after HCG stimulation and analyzed for testosterone production. Cell viability was confirmed daily. The encapsulated leydig cells were injected in castrated animals and serum testosterone was measured serially. The castrated animals receiving the microencapsulated cells were able to maintain testosterone levels long term. These studies suggest that microencapsulated leydig cells may be able to replace or supplement testosterone in situations were anorchia or testicular failure is present. A similar system is currently being applied for estrogen.

Testicular prosthesis

Due to concerns associated with the use of solid silicone testicular prostheses, American Urological Association imposed moratorium on their use has been in effect since 1992. Although saline filled silicone prostheses are currently in clinical trials, biocompatible prostheses composed of vascularized autologous tissues may be preferable. A feasibility study was performed using engineered cartilage, with its associated elastic properties, for the creation of testicular prostheses.

Chondrocytes, harvested and isolated from the articular surface of calf shoulder, were seeded onto pre-formed testicular shaped poly-L-lactic acid coated polyglycolic acid polymer scaffolds. Polymer scaffolds with and without cells were implanted in the scrotal space of athymic mice. Gross examination at retrieval showed the presence of well formed milky white cartilage structures within the scrotum by 1 month. Histological analyses demonstrated the presence of mature chondrocytes in the retrieved implants. There was no evidence of cartilage formation in the control group. SEM of the cell-polymer scaffolds demonstrated uniform cell attachment on the polymer fibers. The retrieved prosthesis showed the presence of...
extracellular matrices in the interfibrillar spaces at 1 month, which progressively replaced the degrading polymer fibers. Bio-mechanical studies performed on the engineered cartilage prostheses demonstrated a compression module, which is comparable to that of native cartilage, along with its elastic properties. Cartilaginous testicular prostheses can be engineered using chondrocytes seeded on pre-formed biodegradable polymer scaffolds. The use of an entirely autologous system would preclude an immunological and foreign body reaction. This novel technology appears to be useful clinically for the creation of autologous testicular prostheses.

Corporal tissue reconstitution
Abnormal male genitalia due to congenital anomalies or acquired conditions require surgical correction. Due to the shortage of autologous penile tissue, multiple staged surgeries using nongenital tissues and silicone prosthesis have been the mainstay in phallic reconstruction. However, graft failure and prosthesis-related complications remain a problem.\textsuperscript{53-60} Creation of penile structures composed of autologous tissue would be a preferable treatment approach for these patients.

Our initial effort in engineering autologous penile tissue was focused in the formation of corporal tissue, since corpus cavernosum is one of the major tissue components of the phallus.\textsuperscript{61} Human corporal smooth muscle cells were isolated, grown and expanded in culture. The cells were seeded on biodegradable polyglycolic acid polymers for implantation. Multi-layers of corporal smooth muscle cells were identified grossly and histologically. Smooth muscle phenotype was confirmed immunocytochemically and by Western blot analyses. This study provided the evidence that cultured human corporal smooth muscle cells could be used in conjunction with biodegradable polymers to create cavernosal smooth muscle tissue in vivo.

The main cellular components of corporal tissue consist of cavernosal smooth muscle and endothelial cells. In a subsequent study we investigated the possibility of developing corporal tissue by combining smooth muscle and endothelial cells. Normal human cavernosal smooth muscle cells and ECV 304 human endothelial cells were seeded on biodegradable polymers for implantation.\textsuperscript{62} ECV 304 endothelial cells were used in the study in order to identify the implanted cells from the host endothelial cells. The retrieved structures showed formation of distinct tissue structures, consisting of organized smooth muscle tissue adjacent to endothelial cells. Presence of vascular structures was evident. Each cell type was confirmed using various assessment methods. This study showed that human corporal smooth muscle and endothelial cells seeded on biodegradable polymer scaffolds are able to form vascularized cavernosal tissue when implanted in vivo. Endothelial cells can act in concert with the native vasculature. These results suggest that the creation of well-vascularized autologous corpus-like tissue consisting of smooth muscle and endothelial cells may be possible.

Although we were able to form tissue consisting of corporal smooth muscle and endothelial cells in vivo, three dimensional corporal structures could not be achieved due to the type of polymer matrices used. We developed a naturally derived collagen matrix, which is structurally similar to the native corporal architecture.\textsuperscript{63} Acellular collagen matrices, derived from rabbit corpora, were obtained using cell lysis techniques. Primary human cavernosal smooth muscle and endothelial cells were seeded in a stepwise fashion. Cavernosal smooth muscle cells were initially seeded on the collagen matrices at a concentration of \(30 \times 10^6\) cells/ml. Endothelial cells were then seeded at a concentration of \(3 \times 10^6\) cells/ml. Cell-matrices seeded with corporal cells were implanted in vivo. The implanted cell matrices showed neovascularity into the sinusoidal spaces by 1 week after implantation. Increased organization of smooth muscle and endothelial cells lining the sinusoidal walls was observed at 2 weeks and continued with time. The matrices were covered with the appropriate cell architecture 4 weeks after implantation.\textsuperscript{63,64} This study demonstrates that human cavernosal smooth muscle and endothelial cells seeded on three-dimensional acellular collagen matrices derived from donor corpora are able to form a well vascularized corporal architecture in vivo.

Penile prostheses for reconstruction
Penile reconstruction was attempted several decades ago using rib cartilage as a stiffener. However, unsatisfactory functional and cosmetic results, due to curvature, discouraged its use.\textsuperscript{57,65} Silicone prostheses were popularized due to their unique mechanical properties. Although silicone is an acceptable biomaterial, biocompatibility is a concern for selected pa-
The use of a natural prosthesis composed of autologous cells may be beneficial. Of the tissues existing in the human body, cartilage would serve as an ideal prosthesis for penile reconstruction, due to its biomechanical properties. Initial studies performed in our laboratory showed that chondrocytes suspended in biocompatible polymers form cartilage structures when implanted in vivo. A feasibility study of engineering natural penile prostheses made of cartilage was attempted. Chondrocytes, harvested from bovine articular cartilage tissue, were grown, expanded and seeded onto pre-formed cylindrical polyglycolic acid polymer rods for implantation in vivo. Chondrocytes were seeded onto pre-formed cylindrical polyglycolic acid polymer rods at a concentration of $50 \times 10^6$ chondrocytes/cm$^3$. The cell-polymer rods were implanted in vivo.

The retrieved implants formed milky white rod shaped cartilaginous structures, maintaining their preimplantation size and shape (Fig. 3). Biomechanical properties of the engineered cartilage rods, including compression, tension and bending, showed that the cartilage tissues were readily elastic and could withstand high degrees of pressure. These results indicate that the engineered cartilage rods possessed the mechanical properties required to maintain penile rigidity. Histomorphologic analyses confirmed the presence of mature and well formed cartilage in all the cell seeded implants.

In a subsequent study using an autologous system, the feasibility of applying the engineered cartilage rods in-situ was investigated. Autologous chondrocytes were harvested, grown and expanded until sufficient cell quantities were available. Chondrocytes were seeded onto pre-formed poly-L-lactic acid coated polyglycolic acid polymer rods at a concentration of $50 \times 10^6$ chondrocytes/cm$^3$. The Chondrocyte-polymer scaffolds were implanted in the corporal spaces of rabbits. Bilateral intra-corporal implantation of the cell-polymer scaffolds were performed. The implants were retrieved and analyzed grossly and histologically 1, 2, 3 and 6 months after surgery.

Gross examination at retrieval showed the presence of well formed milky white cartilage structures within the corpora at 1 month. There was no evidence of erosion or infection in any of the implant sites. Histological analyses demonstrated the presence of mature and well formed chondrocytes in the retrieved implants. Autologous chondrocytes seeded on pre-formed biodegradable polymer-structures are able to form cartilage structures within the rabbit corpus cavernosum. This technology appears to be useful for the creation of autologous penile prostheses.

FETAL TISSUE ENGINEERING

Due to the advance in prenatal diagnostic tools, the detection of fetal abnormalities is now more prevalent. Prenatal ultrasonography allows for a thorough survey of fetal anatomy. For example, 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 extrophy. 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 urologic conditions, such as extrophy, may be beneficial under certain circumstances. There is now a renewed interest in performing a single-stage reconstruction in some patients with bladder extrophy. 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.

Several strategies may be pursued using today’s technological and scientific advances. Having a ready supply of urologic associated tissue for surgical reconstruction at birth may be advantageous. Theoretically,
once the diagnosis of bladder extrophy 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. Bladder extrophy was created surgically in ten 90–95 day 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. 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 extrophy closure was performed using only the native bladder. The engineered bladders were more compliant and had a higher capacity than the native bladder closure group. Histologic analysis of the engineered tissue showed a normal histological pattern, indistinguishable from native bladder at 2 months. Similar prenatal studies were performed in lambs, engineering skin for reconstruction at birth. Other fetal 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.

GENE THERAPY

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. 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-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.

This technology is applicable throughout the spectrum of diseases which 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.

CONCLUSION

Most tissue engineering efforts in the genitourinary system has occurred within the last decade. Although many applications still remain in the experimental stage, several technologies have been tried clinically. The first human application of cell based tissue engineering technology for urologic applications has occurred at our institution with the injection of autologous cells for the correction of vesicoureteral reflux in children. These clinical trials are currently ongoing. The same technology has been recently expanded to treat adult patients with urinary incon-
tinence. Furthermore, trials involving urethral tissue replacement using processed collagen matrices are in progress and bladder replacement using tissue engineering techniques are currently being conducted with satisfactory results. An increasing number of tissue engineering applications is being proposed, designed and planned. However, confirmation at each and every stage of the experimental studies must be demonstrated prior to clinical trials.

REFERENCES

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