Tissue Engineering

Vesicoureteral Reflux

Vesicoureteral reflux is a congenital abnormality of the ureterovesical junction of the urinary bladder. In normal people, the ureterovesical junction allows urine to enter the bladder from the kidneys and prevents retrograde flow of urine through the ureter to the kidneys, thus protecting the kidneys from both the pressures in the bladder and possible bacterial infection. The junction is not fully developed at birth in 1 to 3% of children, in whom backward flow of urine to the kidneys can occur, particularly during voiding.

The severity of reflux, corresponding to the degree of abnormality at the ureterovesical junction, is graded from 1 to 5, with high numbers associated with more severe cases and higher rates of renal injury. Open surgery is typically suggested for patients with severe reflux (grades 4 and 5). Grade 3 reflux may be treated either surgically or with antibiotics. Grades 1 and 2 are treated initially with antibiotics and may be treated with surgery when medical management has failed.

The risks involved with use of long-term antibiotic treatment and with open corrective surgery have led to attempts to develop a minimally invasive technique to correct reflux. An attractive alternative therapy is the endoscopic injection of a suitable bulking agent to the ureteral submucosa, resulting in compression of the ureteric roof that prevents retrograde flow of urine.

Endoscopic correction (bulking) of reflux has been performed using poly(tetrafluoroethylene) paste (Teflon) since 1981, and the correction rate after one or two treatments is reported to be 83%. Nonetheless, Teflon has not been widely used in the United States because of safety concerns associated with the possible migration of Teflon microparticles. Similarly, glutaraldehyde-crosslinked bovine dermal collagen has been used for this purpose since 1991 but has not found great acceptance because of resorption and subsequent loss of efficacy, as well as because of its immunogenic potential.

It is predicted that the use of bulking therapy to treat these patients would become widespread if a material could be developed that was neither migratory, resorbable, nor immunogenic. Chondrogel is a tissue-engineered bulking agent that is currently being investigated for its safety and efficacy in the treatment of vesicoureteral reflux.

Bovine cells and a degradable matrix material have been successfully used in recent years to produce small implants, 1 to 3 ml in volume, in immunodeficient animals. Robert Langer of MIT and Joseph P. Vacanti of the Children's Hospital in Boston showed that bovine articular chondrocytes combined with poly(glycolic acid) remain viable for up to six months after surgical implantation into the subcutaneous space of athymic mice. Furthermore, the cells proliferate and eventually produce their own natural extracellular matrix. Over time, the synthetic matrix resorbs, and the resulting cartilaginous tissue has the histological characteristics of native cartilage.

Charles Vacanti and colleagues at the University of Massachusetts Medical Center obtained similar results after combining cells with biodegradable, injectable hydrogels and delivering the material using minimally invasive endoscopic instrumentation. The scaffold material used in these studies consisted of a poly(ethylene oxide)-poly(propylene oxide) block co-polymer and calcium-crosslinked sodium alginate. Autologous auricular chondrocytes in a calcium-crosslinked sodium alginate matrix have been used successfully by Anthony Atala and associates, also at the Children's Hospital in Boston, to cure pigs in which vesicoureteral reflux had been surgically created.

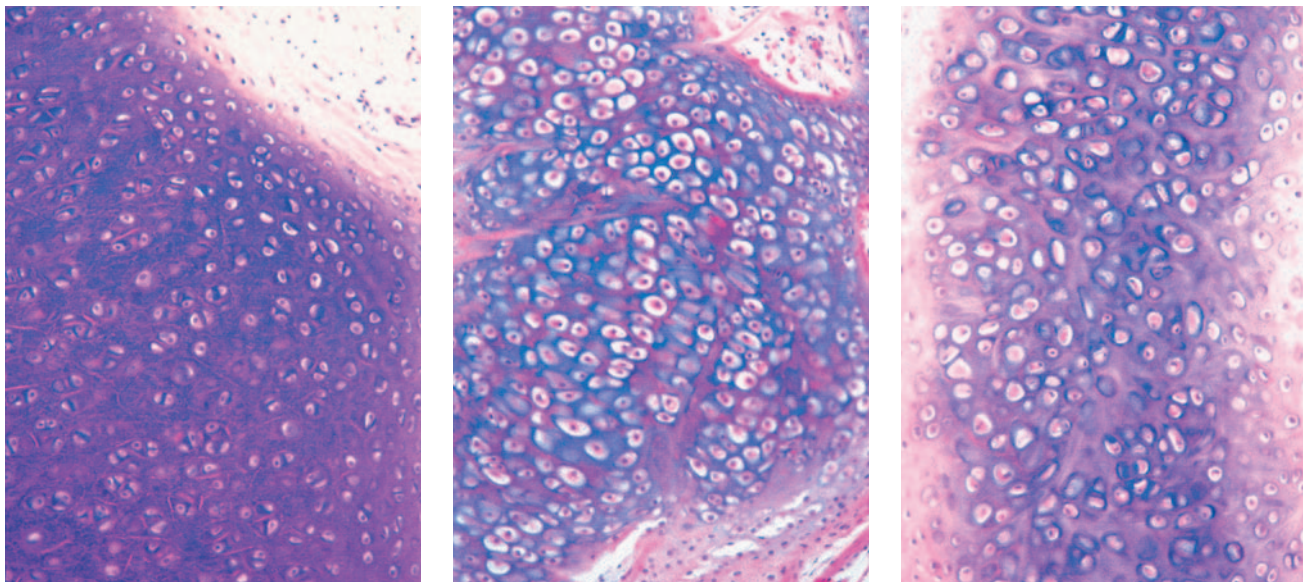
The preclinical development of a tissue-engineered product to treat reflux has been based upon these findings and has focused on (1) identifying a suitable injectable hydrogel system that would facilitate the generation of a cartilaginous "neotissue" in vivo, and (2) isolating and expanding human auricular chondrocytes.

Initial studies used sodium alginate crosslinked with a large stoichiometric excess of Ca^{++} obtained from calcium sulfate. Alginates are a family of co-polymers consisting of α-L-guluronic and β-D-mannuronic acid monomeric moieties. In an aqueous environment and in the presence of divalent cations, guluronic acid blocks can form stable crosslinks, yielding a hydrogel.

Further work led to a formulation containing 20 to 30 million chondrocytes per ml at an alginate concentration of 1.5%, crosslinked with $CaSO_4$ to yield a stable hydrogel that could be injected through a 22-gauge cystoscopic needle. Studies were done using both human cells in SCID mice and autologous cells in sheep and rabbits.

The studies in SCID mice using 0.25 ml subcutaneous implants showed that implants containing chondrocytes maintained their vol-

"Tissue Engineering" is edited by Jeffrey R. Morgan and Martin L. Yarmush of the Center for Engineering in Medicine, Massachusetts General Hospital and Harvard Medical School.



Formation of new cartilage in sheep (subcutaneous implant, *middle panel*) and rabbit (intramuscular implant, *right panel*) at three months compared to native adult sheep auricular cartilage (*left panel*). Hematoxylin and eosin stain, X 20.

ume compared to acellular alginate implants at 25 weeks post implantation. Moreover, the average compressive modulus of the cell-containing implants at 25 weeks was much higher than that of the acellular implants. Histology of the cell-containing implants was consistent with new cartilage formation. These studies indicate that chondrocytes isolated from human adults can form new tissue in this animal model.

Sheep and rabbits were used to demonstrate new tissue formation in immunocompetent large animal models with results similar to those seen in SCID mice.

The feasibility of the tissue engineering approach to vesicoureteral reflux was initially investigated in a small clinical trial in normal adult volunteers. An auricular cartilage biopsy consisting of 125 to 150 mg of tissue was obtained from each volunteer. Chondrocytes were recovered by enzymatic digestion with collagenase and were seeded into tissue culture flasks, where they were allowed to attach and replicate.

Cell numbers were expanded over five to seven weeks, first in primary cultures (T-flasks) and then in secondary cultures (roller bottles), with about 10 cell doublings taking place. Chondrocytes were harvested from roller bottles using trypsin, formulated with alginate, and injected into SCID mice. These studies showed that adult human auricular chondrocytes could be safely harvested and expanded to sufficient numbers to form implants.

David Diamond at Children's Hospital, Boston, and Anthony Caldamone at Hasbro Children's Hospital, Providence, have initiated clinical trials to investigate the safety and efficacy of autologous chondrocytes in alginate hydrogel to treat reflux in pediatric patients. In the initial study, patients having grades 2 to 4 reflux were anesthetized for diagnostic cystoscopy and auricular cartilage biopsy. Chondrocytes from the biopsy were isolated, expanded in vitro, formulated in alginate, and crosslinked. The cell-containing hydrogel was injected endoscopically into the bladder submucosa at the vesicoureteral junction until a nipple with a flattened ureteral orifice on top was visualized in an inverted crescent shape. An average volume of 0.91 ml of Chondrogel was injected per ureter.

Patients were evaluated at one, three, and 12 months after treatment and continue to be followed annually through 36 months. Renal ultrasonograms are scheduled during follow-up visits at one, 12, 24, and 36 months to assess for hydronephrosis, obstruction, or other adverse events. Voiding cystourethrograms were performed at three and 12 months after treatment to determine the success of the treatment.

Clinical benefit was defined as improvement in reflux grade, determined by either the absence of reflux or avoidance of surgery (correction to grade 1). If reflux was not resolved by the three-month follow-up visit, an additional treatment was allowed, providing that autologous cryopreserved cells were available for expansion and formulation.

Preliminary results of this trial have been published. Reflux was eliminated in 28 of 46 ureters (61%), and 24 of 29 patients (83%) were successfully treated at three months. A phase III study is currently underway.

FRANK T. GENTILE

Reprogenesis, Inc.