Tissue Engineering in Urology: Where Are We Going?

Adam R. Metwalli, MD, James R. Colvert III, MD, and Bradley P. Kropp, MD

Introduction

The underlying principles of tissue engineering are to provide functional tissue to repair or replace damaged organs. In the field of urology, these efforts have a broad application from bladder regeneration, urethral repair, pelvic organ prolapse, urinary incontinence, and erectile dysfunction. Two types of tissue engineering technologies exist for urinary reconstruction. The first technology, the unseeded technique, involves the use of a biodegradable scaffold that the host can use to remodel and regenerate functional lower urinary tissue. The second technology, the seeded technique, involves in vitro cell culture techniques of seeding primary cultured epithelium and smooth muscle cells on a biodegradable scaffold. This in vitro-created organ graft is then transplanted back into the host for the continuation of the regeneration process. The major dilemma that the field of tissue engineering faces is to determine whether these technologies can be applied for clinical use.

Another concern with the clinical application of these techniques is whether damaged or abnormal native tissue for seeded grafts simply will result in a dysfunctional or nonfunctional replacement organ. Thus, the quest to provide engineered urologic tissue that is better than the native organ has become two-pronged, with the need to develop reliable scaffolds that promote native tissue regeneration and the search for alternative sources of tissue that will have normal function in vivo. Consequently, the realm of urologic tissue engineering has begun to venture into the fields of gene therapy and stem cell research.

In addition to a review of the recently published literature in urologic tissue engineering, what follows is a presentation of the most recent investigations involving tissue engineering. As a result, some of the references refer to an abstract and the meeting at which the material was presented. The full manuscripts associated with these abstracts should all be published within the next year. Presentation of the data in this fashion best addresses the question of “Where we are going?” with urologic tissue engineering research.

Engineering of Bladder Tissue

Historically and in current urologic practice, augmentation of the urinary bladder has been performed using the stomach, the colon, the small intestine, and the ureter, with concomitant problems of acid-base disruptions, electrolyte abnormalities, excess mucus production, the development of urothelial carcinoma, and stones [1–3]. Furthermore, traditional gastrointestinal bladder augmentation often requires significant labor-intensive maintenance by the patient and the physician. Gastrointestinal augmentation eliminates the intrinsic contractile function of the bladder, thereby forcing the patient to perform life-long intermittent self-catheterization. Consequently, urologic tissue engineering research has focused on developing biodegradable scaffolds that permit regeneration of native tissue with the compliance and contractile properties of a normal bladder. These scaffolds have varied from xenogenic collagen matrices, such as porcine small intestinal submucosa (SIS) or bladder submucosa, to synthetic biodegradable polymers, such as polyglycolic acid (PGA), polylactic acid, and polylactic-coglycolic acid [4–8]. The
Unseeded technology
Small intestinal submucosa is the most thoroughly studied biomaterial used for the unseeded technology. SIS is a non-immunogenic collagen-based material composed of the submucosal layer of porcine small intestine that is mechanically processed to 100-µm thickness.

In vivo implantation of SIS and other collagen matrices derived from bladder submucosa has demonstrated the ability to promote tissue-specific regeneration of functional bladder segments [9–13].

One of the major concerns with the unseeded technology is that shrinkage of the unseeded scaffold may occur; graft calcification has been reported [14]. The reason for the variability of regeneration is unknown; however, it may result from a limited area of ingrowth that can occur from the native bladder. It also may result from the source and processing of the material.

To address the potential material issues related to SIS, a retrospective review of the factors that play a role in the outcome of tissue engineering studies that involve SIS was performed. These factors include the age of the pig in which the intestinal segment is obtained, whether the material is hand-made or machine-made, whether there is a single layer or multiple-layer SIS, and the method of terminal sterilization. Investigation in our laboratory has demonstrated that sow weight material (pig that is older than 3 years of age) is superior to market weight material (pig that is younger than 8 months of age) with regard to bladder regeneration. The laboratory investigation also has demonstrated that machine- and hand-made preparations have similar outcomes. Multiple-ply SIS did not produce reliable bladder regeneration. With regard to terminal sterilization, it appears that E-beam preparation changes the functionality of its native architecture, that affect cell growth and function and likely are critical to the induction of the regenerative process. Among the components contained in SIS (and normally surround cells in the intact organ) are collagen, glycoproteins, proteoglycans, and functional growth factors [20]. Growth factors that have been identified include fibroblast growth factor-2, transforming growth factor-β, and vascular endothelial growth factor [21,22]. Preliminary work has demonstrated that these factors, which are inherent to the SIS material, are instrumental in promoting in vivo and in vitro cell growth and differentiation [21,23]. It is well known that cells are affected by their interaction with other cells, matrix proteins, and growth factors. Although SIS may provide a unique in vitro environment that is advantageous over other materials for the initial

Cell-seeded technology
Several studies have demonstrated that the cell-seeded technology may be superior to unseeded technology in promoting bladder regeneration and preventing the formation of scars. Biomaterials such as PGA and acellular bladder submucosa are able to induce bladder regeneration with less inflammatory reactions and fibroblast ingrowth when these scaffolds are seeded, compared with the same material unseeded [15–18]. These seeded grafts also have shown a significant increase in bladder volume with functional success. Clinical trials for human bladder augmentation using this cell-seeded technology are underway at the Boston Children’s Hospital. Results of these clinical trials are anticipated greatly, but are currently pending.

Initial investigations with cell-seeded SIS demonstrated that SIS supports the three-dimensional growth of bladder cells in vitro. SIS is a unique substrate that provides an in vitro environment for bladder urothelial and smooth muscle cells to interact with each other and with the surrounding matrix in a manner that is similar to what is observed in vivo during the early events in bladder regeneration. Seeding of bladder cells on SIS with different co-culture techniques and subsequent placement of the cell-matrix construct into the host for continued regeneration may enhance our abilities to provide a patient with new bladder tissue for replacement therapy [10]. Other investigations into cell-seeded SIS demonstrated multilayered bladder regeneration in a nude mouse model [19].

Small intestinal submucosa may prove to be advantageous over synthetic biodegradable polymers for several reasons. SIS is derived from natural tissue; therefore, it provides a more conducive environment for tissue regeneration than is found with synthetic scaffolds. SIS contains numerous components or factors, which are independent of its native architecture, that affect cell growth and function and likely are critical to the induction of the regenerative process. Among the components contained in SIS (and normally surround cells in the intact organ) are collagen, glycoproteins, proteoglycans, and functional growth factors [20]. Growth factors that have been identified include fibroblast growth factor-2, transforming growth factor-β, and vascular endothelial growth factor [21,22]. Preliminary work has demonstrated that these factors, which are inherent to the SIS material, are instrumental in promoting in vivo and in vitro cell growth and differentiation [21,23]. It is well known that cells are affected by their interaction with other cells, matrix proteins, and growth factors. Although SIS may provide a unique in vitro environment that is advantageous over other materials for the initial