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Mini review

Tissue-engineered constructs for urethral regeneration☆

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ABSTRACT

Those who have urethral injury, long-distance urethral stricture, hypospadias, or epispadias need tissue for urethral repair. Tissue engineering is one of the solutions for urethroplasty. Three components essential for tissue engineering are cells, scaffolds, and bioactive factors. Several animal studies of tissue-engineered urethras have been conducted and progressed to human clinical trials by 1999. These studies have shown that the maximum distance for normal tissue regeneration in tubularized urethral replacement with unseeded matrices is 0.5 cm. Although autologous tissue-engineered tabularized urethras have been successful in clinical trials, this method could be an alternative treatment for urethral reconstruction.

1. Introduction

Those who have urethral injury, long-distance urethral stricture, hypospadias, or epispadias, need tissue for urethral repair. Skin grafts, bullock's urethra, veins, ureters, appendix vermiformis, fascia of the thigh, bladder mucosa, tunica vaginalis, peritoneum, rectal mucosa, buccal mucosa, and the prepuce have been used for this purpose.[1] However, two reasons making the aforementioned tissues unsuitable for urethroplasty are that either the surgeons did not succeed, or the patients suffered from donor-site surgery and even donor-site complications. Tissue engineering is one of the solutions for urethroplasty.

What are tissue engineering and regenerative medicine? The term tissue engineering was introduced to medicine in 1987.[1] Badylak and Nerem provided a vivid description of it in 2010: tissue engineering involves the *ex vivo* creation of replacement tissues intended for subsequent *in vivo* implantation.[2] They also indicated that regenerative medicine emphasizes tissue replacement with *ex vivo* manufactured products that have evolved to include broad strategies to induce both *in vivo* constructive remodeling of cell-based and cell-free scaffold materials and true tissue regeneration.[2]

2. Principles of tissue engineering

Three components essential for tissue engineering are cells, scaffolds, and bioactive factors. A normal study design for tissue engineering is to seed cells onto scaffolds with or without bioactive factors, which are the constructs for the experimental group. In the control group, scaffolds without cells are used for comparison. All of them are implanted in patients for later retrieval and future evaluation.[3] Cells for tissue engineering might be stem cells (e.g., fertilized eggs, or embryonic, parthenogenetic, induced pluripotent or adult stem cells.), progenitor cells (e.g., endothelial progenitor cells), and differentiated cells (e.g., urothelial, smooth muscle, or squamous cells). Scaffolds for tissue engineering can be classified as natural or synthetic, and absorbable or nonabsorbable. Most scaffolds are absorbable. They can be synthetic or natural polymers. The biomaterials for urethral tissue engineering in the past were all absorbable, including synthetic polymers (aliphatic polyesters) and natural collagens (e.g., small-intestinal submucosa, bladder-derived acellular submucosa, acellular urethral submucosa, foreskin acellular matrix, or acellular arterial matrix). Bioactive factors for tissue engineering might be growth factors, drugs, genes, gene products, and bioreactors.

3. Maximum distance for tissue regeneration

What is the maximum distance for normal tissue regeneration? To answer this question, Dorin et al have designed a study using varying lengths (0.5 cm, 1 cm, 2 cm and 3 cm) of tubular scaffolds without cells for up to 4 weeks in an *in vivo* rabbit model.[4] They

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used acellular bladder submucosa as the scaffold. Follow-up urethrograms demonstrated normal urethral calibers in the 0.5-cm group at all time points. Evolution of a stricture was demonstrated in the other longer grafts by 4 weeks. There were ingrowths of urothelial cells from the anastomotic sites in all grafts at 1 week. They concluded that 0.5 cm appeared to be the maximum defect distance using acellular grafts that rely on native cells for tissue regeneration.

4. Onlay versus tubularized replacement of urethras

To regenerate urethras, onlay and tubularized replacements have been used (Fig. 1). Onlay replacement needs a healthy urethral bed that provides healthy cells to migrate into the construct.[5] The crucial point is the width of the construct instead of the length. We foresee successful urethroplasty using scaffolds without cells as long as the width of the construct is < 0.5 cm.[4,6] The width of the construct can be longer than 0.5 cm if scaffolds with cells are used. We normally use tubularized replacement to treat long-distance urethral stricture. It can be successful if the construct is a scaffold with enough cells. If only scaffolds are used without cells, the maximal length for the tubularized replacement is 0.5 cm, as in the aforementioned animal study.[4]

5. Cellular origin for tubularized replacements

Examples of the most common study designs for tissue-engineered urethras can be found in studies by De Filippo et al and Fu et al.[3,7] In the latter study, scaffolds were allogeneic bladder submucosa, and cells were autologous foreskin epidermal cells. They compared tubular grafts at 1 month, 2 months and 6 months using bladder submucosa with or without foreskin epidermal cells in an *in vivo* rabbit model. They have concluded that acellular bladder submucosa seeded with epidermal cells can be used for tubularized urethral replacement without stricture. However, unseeded tubularized bladder submucosa can lead to poor recovery and strictures of the urethra. They have used cell-labeling techniques and have found that bromodeoxyuridine stains foreskin epidermal cells at 1 month and 2 months after grafting, but not at 6 months. Therefore, they have concluded that epithelial cells of the graft originate and subsequently proliferate from implanted epidermal cells, instead of extensions from surrounding transitional cells.

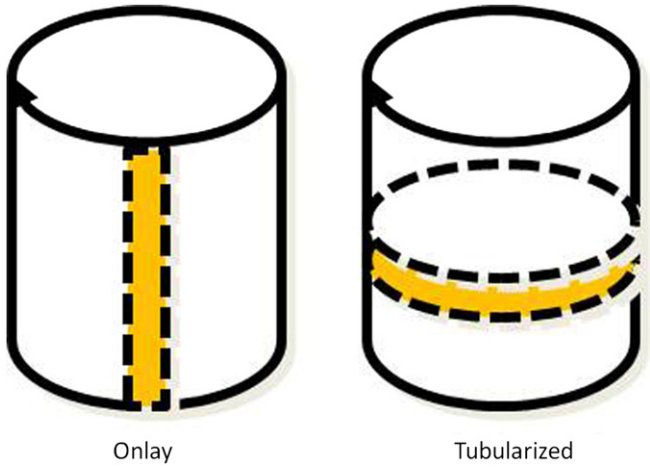

Fig. 1. Onlay and tubularized replacement used in tissue-engineered urethral regeneration.

6. Bioactive factors

Gene therapy with the vascular endothelial growth factor (VEGF) has been used as the bioactive factor for urethral tissue engineering. Guan et al have compared rabbit grafts after subcutaneous implantation into nude mice for 4 weeks using rabbit bladder urothelial cells seeded into a decellularized rabbit artery matrix, with or without VEGF *ex vivo*.[8] Their scaffolds were decellularized rabbit carotid artery matrices, and cells were rabbit bladder urothelial cells that were transfected with a murine stem cell virus–$VEGF_{165}$–green fluorescent protein (GFP) retrovirus in the experimental group and murine stem cell virus–GFP retrovirus in the control group. They found that VEGF-modified cells exhibited significantly enhanced neovascularization and formation of a urethral layer compared to GFP-modified cells. Those results indicate that VEGF gene therapy might increase the blood supply in tissue engineering for treating urethral damage or loss.

7. Human clinical trials

Animal studies of tissue-engineered urethras had progressed to human clinical trials by 1999. Atala et al have reported a clinical trial using unseeded bladder submucosa for onlay replacement in hypospadias patients with a 75% success rate in 1999.[9] Bhargava et al also have presented a clinical trial using tissue-engineered buccal mucosa for onlay replacement in humans with a 60% success rate in 2008.[10] One of the most remarkable clinical trials for urethral regeneration, which has yielded important results and concepts, was a study by Raya-Rivera et al.[11] Synthetic tubularized polyglycolic acid: poly(lactide-coglycolide acid) scaffolds were used. Both autologous bladder smooth muscle and urothelial cells from previous urinary bladder biopsies were harvested and expanded. Urothelial cells were seeded onto the luminal surface and muscle cells onto the outer surface of the tubular scaffolds. Five boys who had urethral defects underwent urethral reconstruction with the tissue-engineered tubularized urethras. They remained functional in a clinical setting for up to 6 years. To the best of our knowledge, that was the first successful clinical trial of tubularized tissue-engineered urethral replacement.

8. Conclusions

In conclusion, the maximum distance for normal tissue regeneration for tubularized urethral replacement with unseeded matrices is 0.5 cm. Although autologous tissue-engineered tubularized urethras have been successful in clinical trials, this method could be an alternative treatment for urethral reconstruction.

Conflicts of interest statement

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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