Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Tissue-engineered buccal mucosa using silk fibroin matrices for urethral reconstruction in a canine model

Minkai Xie, MD,[a] *Yuemin Xu, MD, PhD,*[a,*] *Lujie Song, MD, PhD,*[a] *Jihong Wang, MD,*[a] *Xiangguo Lv, MD,*[a] *and Yaopeng Zhang, PhD*[b,**]

[a] *Department of Urology, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai, People's Republic of China*

[b] *State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai, People's Republic of China*

ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form
26 October 2013
Accepted 20 November 2013
Available online 6 December 2013

Keywords:
Bioengineering
Tissue engineering
Keratinocytes
Fibroblasts
Silk fibroin
Biomaterials
Buccal mucosa
Urethral reconstruction
Urethroplasty
Canine

ABSTRACT

Background: To investigate the feasibility of urethral reconstruction using tissue-engineered buccal mucosa (TEBM) with silk fibroin (SF) matrices in a canine model.

Materials and methods: Autologous oral keratinocytes and autologous fibroblasts were isolated, expanded, and seeded onto SF matrices to obtain TEBM. The TEBM was assessed using hematoxylin and eosin staining and scanning electron microscopy. A 5-cm urethral mucosal defect was created in 10 female canines. Urethroplasty was performed using TEBM in five canines in the experimental group and with SF matrices without cells in the five canines in the comparison group. Retrograde urethrography was performed after 6 mo of grafting. The urethral grafts were analyzed grossly and histologically.

Results: The oral keratinocytes and fibroblasts exhibited good biocompatibility with the SF matrices. TEBM could be constructed using SF matrices. The canines implanted with the tissue-engineered mucosa voided without difficulty. The retrograde urethrography revealed no sign of stricture. The histologic staining showed that epithelial cells developed gradually and exhibited stratified epithelial layers at 6 mo. In the comparison group, the canines had difficulty voiding, and the retrograde urethrography showed urethra stricture. The histologic staining showed that one to two layers of epithelial cells developed.

Conclusions: The TEBM using SF matrices could be a potential material for urethra reconstruction.

1. Introduction

Long strictures or those in a distal location along the penile shaft frequently require urethra reconstruction that incorporates substitute material to augment the stenotic segment [1]. Various tissues, such as bladder mucosa [2], genital and extragenital skin flaps [3], colonic mucosa [4,5], lingual mucosa [6], and buccal mucosa (BM) [7], have been proposed for urethroplasty, and BM has been the preferred tissue for use as a urethral substitute in recent decades [8].

* *Corresponding author*. Department of Urology, Shanghai Jiaotong University Affiliated Sixth People's Hospital, No 600, Yishan Road, Shanghai, People's Republic of China 200233. Tel.: +86 18930177466; fax: +86 021 64083783.

** *Corresponding author*. State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai, People's Republic of China. Tel.: +86 21 67792954; fax: +86 21 67792855.

E-mail addresses: yueminxu@hotmail.com (Y. Xu), zyp@dhu.edu.cn (Y. Zhang).

0022-4804/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.11.1102

Harvesting BM is associated with donor site morbidity and prolongs the length of surgery. The amount of BM tissue that can be safely obtained is limited. Tissue engineering technology is progressing rapidly to provide a possible solution to avoid limitations on the use of BM tissue. Li *et al.* combined bladder acellular matrix grafts with keratinocytes to construct tissue-engineered buccal mucosa (TEBM) in a rabbit model for urethral reconstruction [9]. Although the effect is ideal within a short-term observation, the collagen fibers are markedly more disordered than normal urethral submucosa tissue [10]. The development of a TEBM based on de-epidermized dermis (DED) [11] in a polylactide-co-glycolide [12] scaffold with autologous BM keratinocytes and fibroblasts has been reported. In 2008, Bhargava *et al.* [11] repaired a urethral stricture secondary to lichen sclerosus in human patients with DED *in vitro* cultured keratinocytes and fibroblasts; fibrosis and contraction occurred in two of the five patients. One of the reasons could be that DED is not the ideal scaffold for reconstructing tissue-engineered tissue. Acellular tissue matrices such as DED, bladder acellular matrix graft, and small-intestinal submucosa inevitably have heterologous genetic substances that cause inflammatory reactions, and these materials present the potential danger of disease transmission. The degradation products of the most synthetic polymeric materials, such as polylactide-co-glycolide, are acidic, which is not conducive to the growth of the surrounding cells [13].

Previous studies have shown that silk fibroin (SF) has excellent biocompatibility and low inflammatory potential; it is a protein that comprised up to 90% of the amino acids glycine, alanine, and serine [14]. As an effective biomaterial, SF has been investigated in research on cartilage [15], bones [16], skin [17], and blood vessels [18]. In this study, we introduced electrospun SF matrices as scaffolds for TEBM that could comprise a potential scaffold for urethral reconstruction.

We developed TEBM based on electrospun SF matrices with autologous keratinocytes and fibroblasts and evaluated the application of TEBM as a potential graft for urethra-tissue engineering in a canine model.

2. Methods and materials

This study was conducted with the approval of the Institutional Animal Care and Use Committee of our institute.

2.1. Preparation of the electrospun silk protein matrices

The electrospinning procedure was described in previous studies [19]. Briefly, natural cocoons of *Bombyx mori* (Tongxiang, China) were degummed twice with a 0.5 wt % Na_2CO_3 aqueous solution at 100°C for 30 min and rinsed with deionized water to remove the sericin. The degummed silk was dissolved in a 9.0 M LiBr aqueous solution. After dilution, the resultant regenerated SF solution was dialyzed against deionized water at 10°C with a cellulose semipermeable membrane (the molecular weight cutoff was 14,000). The SF aqueous solution was concentrated to a 33% wt. The prepared SF aqueous solution was transferred to a 2.5 mL syringe capped with a 6-G needle (Inner diameter = 0.6 mm) as a spinneret. The electrospinning was performed using a voltage of 25 kV, a flow rate of 0.3 mL/h, and grounded aluminum foil placed at a distance of 9 cm to collect the random fibers.

To improve the mechanical properties, the electrospun matrices were mechanically stretched in a 90% ethanol aqueous solution at a stretched rate of 0.1 mm/s and a stretched ratio of 1.4×. The matrices were immersed in an identical solution for 30 min with a fixed length. The morphology of the electrospun SF fibers was observed using a JSM-5600LV (JEOL Co, Tokyo, Japan) scanning electron microscope (SEM).

2.2. Oral keratinocyte culture and characterization

The canines were anesthetized under general anesthesia with an intravenous injection of pentobarbital, and 0.5 × 0.5 cm^2 specimens of canine BM were taken. The specimens were washed with phosphate-buffered saline (100 IU/mL of penicillin and 100 μg/mL of streptomycin). The BM was digested by dispase II enzyme (Roche) at 4°C overnight. The oral keratinocytes were isolated from the epidermis after trypsinization. The dermis samples were retained to isolate the fibroblasts, and 5 × $10^5/cm^2$ i3T3 cells were preseeded for 24 h. The keratinocytes were seeded at a density of 2 × $10^6/cm^2$ in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, New York) and defined-KSFM (Gibco) medium in a 1:1 ratio, supplemented with 10% fetal bovine serum (Gibco). The oral keratinocytes were refed every 2 d until they were 80%–90% confluent. Before the cell passaging, the residual fibroblasts were removed with a dispase II enzyme (Roche, Roche Mannheim Germany), and the keratinocytes were identified by the AE1/AE3 antibody (Abcam, Cambridge). The generation 2–3 cells were used for the experiments.

2.3. Fibroblasts culture and characterization

The epidermis was removed from the BM for the keratinocyte culture, and the dermis was retained for culturing the fibroblasts. The dermis was cut into pieces (of approximately 1 mm^3) and placed in DMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco) in a humidified 5% CO_2 incubator. After 3–4 d, spindle cells appeared around the dermic tissue. The cells could be passaged until they were 50%–60% of the confluent. The fibroblasts were identified with vimentin. For the expansion, the fibroblasts were cultured and refed every 3 d, and the generation 3–5 cells were used for the experiments.

2.4. Oral keratinocytes and fibroblasts were seeded onto the SF matrices

Before use, the matrices were sterilized with 75% ethanol for 2 h, washed three times in sterilized phosphate-buffered saline, and filled in 5 mL DMEM (Gibco) overnight at 37°C.

The oral fibroblasts were seeded on one side of the scaffolds at 1 × 10^5 cells/cm^2. At 3 d, the scaffolds were reversed, and the keratinocytes were seeded on to the other surface of the scaffold at 3 × 10^6 cells/cm^2, and the coculture was cultured for an additional 7 d. The compound graft was cultured at an air-fluid level for the final 3 d. The culture medium was refreshed every