Long-term study of male rabbit urethral mucosa reconstruction using epidermal cell

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Abstract

Aim: To investigate the transformation of characteristics of epidermal cells from foreskin which were used to reconstruct male rabbit anterior urethra in combination with acellular collagen matrices. Methods: In three rabbits, autologous foreskin epidermal cells were isolated, expanded in vitro, and seeded (inoculated) onto a tubular acellular collagen matrix, acquired from allogeneic rabbit bladder submucosa. A urethral mucosal defect was created, and urethral reconstruction was performed with the tubular acellular collagen matrix seeded with epidermal cells. Results: On gross examination at 12 months following the procedure, the mucosa of the urethral grafts appeared lubricous and smooth. Urethrography showed that a wide urethral caliber had been maintained without any sign of strictures. Histological examination showed a transitional cell layer in the graft without evidence of a margin between the graft and the host tissue at 12 months postoperatively. Conclusion: Epidermal cells seeded onto acellular collagen matrices can be successfully used to reconstruct urethras that have defects and are transformed to transitional epithelial cells.

Keywords: urethral stricture; tissue engineering; foreskin; epidermal

1 Introduction

Tissue engineering is one of the exciting areas in biotechnology. It combines the principles and methods of the life sciences with those of engineering to elucidate fundamental understanding of structure-function relationships of normal and diseased tissue to facilitate the development of materials and methods to repair damaged diseased tissue and create entire tissue replacement [1–3]. In our previous study, foreskin epidermal cells successfully replaced urethral epithelium cells in urethra reconstruction [4]. Now we examine over the long-term transformation of the characteristics of epidermal cells obtained from foreskin combined with acellular collagen matrices to reconstruct male rabbit anterior urethra.

2 Materials and methods

Acellular collagen matrix was prepared, sterilized, conserved and identified according to a previously described method [4]. Foreskin incisions were performed...
in three male white rabbits. A 0.8 cm × 0.5 cm incision specimen was excised from the foreskin. Microdissection under loop magnification was used to carefully remove the seromuscular layer of the foreskin tissue. The specimen and epidermal cells were dissociated, and the cells were collected and then isolated in keratinocyte serum-free medium (KSFM) solution (Gibco Invitrogen, USA). Then the cells were plated onto 25-mL culture plates [4]. The cells were expanded to a density of 1–2 × 10⁶ cells/cm² for seeding onto urethral grafts [5]. The cells were dissociated from the culture plates with trypsin and gathered subsequently. The scaffolds were sequentially seeded with epidermal cells on the surface with KSFM solution. The seeded grafts were incubated for 7 days and then 5-bromo-2'-deoxy-uridine (BrdU) was instilled into the seeded grafts as a marker for cell proliferation [6]. Random samples of the seeded grafts were obtained for histological evaluation and electron microscopy examination.

The rabbits were anesthetized with 15 mg/kg ketamine. A 1.5-cm length urethral mucosa defect was created in the anterior urethra. The urethral graft was implanted and anatomized according to a previously described technique [4]. Retrograde urethrograms were obtained and histologic examination was performed at 12 months following the surgical procedure.

The animals were killed using air intravenous injection 12 months postoperatively. The evaluation included urethrography, organ studies, histologic and immunocytochemical analyses (H&E stain, anti-pancytokeratins AE1/AE3 and P63 stain), and an immunofluorescence marker for cell proliferation. Details of these methods are described in our previous study [4].

3 Results

All urethral catheters were removed within 2 weeks after the surgical procedure. Gross examination of the cell-seeded grafts at 12 months after the surgical procedure revealed smooth mucosa, the same as that observed in specimens after 2 and 6 months. Urethrography performed at 12 months postoperatively confirmed maintenance of a wide urethral caliber without any sign of strictures (Figure 1). H&E staining of the specimens revealed transitional cell characteristics within the graft urethral mucosa at 12 months postoperatively with more protuberances, which is different from the graft mucosa observed at 2 and 6 months postoperatively (Figure 2).

There was no evidence of a margin between the urethral graft and the host tissue. The epithelial cells of the urethral graft mucosa at 12 months after the surgical procedure showed positive staining with broadly reacting antipancytokeratins AE1/AE3 (Figure 3) and the basement cells of the urethral graft showed positive staining with P63 (Figure 4). With regard to the immunofluorescence marker, the histologic examination revealed multiple layers of epithelial cells without positive signs of BrdU staining (Figure 5).

4 Discussion

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Epidermal cells are chosen as graft cells because they are abundant, can be obtained and incubated easily. Acquiring epidermal cells is a less invasive method than the traditional method of bladder or urethral biopsy followed by dissection of transitional cells [7]. Although the characteristics of graft urethral mucosal cells are maintained as multilayered epithelial cells similar to the epidermal cells observed during a shorter postoperative period (1, 2 and 6 months), it was shown that the epithelium had more of a papillary structure, the same as transitional epithelium, and there was no evidence of a margin between the graft and host tissue at the longer time interval (12 months postoperatively). It may be that the urethral environment contributes to the transformation of epidermal stem cells to transitional epithelial cells. This contributes to the reconstruction of urethra mucosa with different types of epithelium.

P63 is believed to have a unique role in morphogenesis and epithelial regeneration, because it has been identified as a keratinocyte stem cell marker [8]. At 6 and 12 months postoperatively, we found positive staining of basement cells of the graft urethral mucosa, which proves that stem cells functioning in the regeneration and differentiation, are present.

It is of great interest to identify graft cells, whether these are epidermal cells incubated or originating from surrounding transitional cells. Studies have rarely touched on this issue. We chose BrdU as a marker of cell proliferation and used a confocal microscope to identify the graft cells postoperatively. BrdU labeling has been successfully used to identify slow cycling or mitotically quiescent label-retaining stem cells [9–11]. BrdU can be incorporated into DNA during the S phase in all mitotic cells, including stem cells and transient amplifying cells. Once the cells were labeled, label should be retained for slow cycling cells, while other more mitotically active cells will lose the label through multiple mitoses.

In our study, epidermal cells were incubated and marked with BrdU, and then seeded onto a collagen scaffold. Observations of the graft with regard to staining with BrdU were carried out at different time points postoperatively to evaluate the presence of graft cells. It was demonstrated that BrdU-stained seeded epidermal
cells were present in the graft at 1 month postoperatively and remained in the graft at 2 months postoperatively. Observations did not show positive stain in the graft at 6 months postoperatively. We speculate that this resulted from labeled epidermal cells differentiating and multiplying. This proves that epithelial cells of the graft originated from those that were implanted and then simultaneously differentiated and multiplied postoperatively.

In a previous study, the control group included rabbits with urethral mucosa defect just treated with catheter placement [4]. Unfortunately, we did not obtain the expected result. In addition, we also applied acellular collagen matrix without cells in tubularized urethral replacement. The results of urethrography and morphology showed urethral stricture [4]. Aiming to investigate the morphology alterations of transitional epithelium cell in urethra, we studied, in the present study, only the morphology alterations of transitional epithelium cells in reconstructed urethra, and, therefore, do not show control study data.

The results of our study demonstrate that acellular collagen matrices seeded with epidermal cells can be used for tubularized urethral replacement and that the grafted urethral mucosa maintains the same type of epidermal cells for a short time period. However, at long-term follow-up, the cells were found to transform into transitional cells. It was also demonstrated that the tissue environment can affect the way that graft cells change to the type of host cells.

References