Journal of Pediatric Surgery (2013) **48**, 1557–1564

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Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Histological and immunohistochemical changes in the rat oral mucosa used as an autologous urethral graft

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Received 3 March 2012; revised 25 January 2013; accepted 26 January 2013

Key words:
Experimental Surgery;
Oral Mucosa graft;
Urethroplasty;
Transdifferentiation

Abstract

Purpose: The purpose of this study was to determine the histological and functional (immunohistochemical) changes that take place in oral mucosa grafts implanted in the rat urethra.

Methods: Urethroplasty was performed in 26 male Wistar rats weighing 250 g. All animals received autologous oral mucosa urethra grafting under general anesthesia. Samples were analyzed 10, 20, 30, 40, 50, 60, 90, and 120 days after surgery using light and scanning electron microscopy and immunofluorescence for the determination of the expression of epithelial markers (pancytokeratin, cytokeratin 1, 4, 13, and filaggrin).

Results: Grafted oral mucosa tissues were subjected to significant histological changes from the beginning with the formation of a well-developed epithelium whose structure was comparable to the native urethra from day 60 of the surgical implant. The immunofluorescence analysis demonstrated that the cytokeratin expression profile tended to mimic the pattern of the native urethra. These data suggest that the oral mucosa is able to efficiently transdifferentiate to the urethral environment.

Conclusions: The efficient transdifferentiation process of the grafted oral mucosa at both the histological and immunofluorescence levels, and the absence of local complications confirm the clinical usefulness of this type of tissues for the repair of the urethra.

Finding an appropriate donor site for urethroplasty procedures has always been a challenge. Currently, one of the tissues that is most currently used as a urethral graft is the oral mucosa [1–5]. Different experimental studies in animals [6] demonstrated that the use of oral mucosa grafts was associated with low complication rates. However, the structural and functional differences between the epithelium of the graft and the epithelium of the tissue where the graft is implanted [7] could lead to complications and functional impairment.

In this context, several reports have identified important structural differences between the oral mucosa and the urethra, especially regarding the number of cell layers and the epithelial organization. Adding to this, recent studies describe the existence of histological changes in patients

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http://dx.doi.org/10.1016/j.jpedsurg.2013.01.056

undergoing oral mucosal grafts for urethral reconstruction in two stages (Bracka surgery) [8]. Finally, a published work in which oral mucosa was used during the urethroplasty procedure in rabbits [9] showed that there was a complete integration of the grafted oral squamous epithelium with the recipient urethral epithelium, and the histological characteristics of the graft did not significantly change.

To our knowledge, most of the previously published studies focused on the description of the major macroscopic and microscopic changes that take place in the implanted graft and on the assessment of the functionality of different urethroplasty techniques on the urinary flow [10]. However, none of them describes the key immunohistochemical protein expression changes that occur during the biointegration process and the histological and immunohistochemical changes that take place in grafts and recipient urethral tissues. Particularly, the study of specific oral mucosa markers such as cytokeratins and filaggrin (a cytokeratin-associated cytoskeleton fiber) has not been carried out to this date. Since the expression of these epithelial differentiation markers is tissue-specific and may vary among different epithelia, a time-course sequential expression study is in need to determine if protein expression tends to mimic that of the grafted urethra.

In this work, we have analyzed the histological and immunohistochemical protein expression changes that take place after urethral reconstructions using oral mucosa grafts in Wistar rats in order to identify the structural, histological and functional changes that may happen in the urethra at increasing follow-up periods to determine if these changes vary with time.

1. Materials and methods

1.1. Animals

We used a total of 29 (26 urethroplasties included in the study) male Wistar rats weighing 250 g provided by Harlan® laboratories and maintained in the Experimental Unit of the University Hospital Virgen de las Nieves (FIBAO).

In this study, two animals were used as controls. In these cases, the urethra was ventrally incised (incision of 0.4 cm of length from about 3 mm) and primarily repaired using 7/0 Safil quick® suture stitches without implanting any graft tissue. The rest of the rats were subjected to surgical intervention as described below. After a follow-up period of 10, 20, 30, 40, 50, 60, 90 or 120 days post surgery, one group of animals (3 rats per group) was euthanatized under general anesthesia for histological analysis. Both control animals were euthanatized after 30 and 90 days.

This study and its procedures were approved by the Institutional research and ethics committees, and the animal care and use committee. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) regarding the care and use of animals for experimental procedures.

1.2. Surgical procedure for urethral reconstruction

Urethral reconstruction using oral mucosa grafts was performed under general anesthesia by induced by intraperitoneal injection of a mixture of 100 mg/kg ketamine, 0.05 mg/kg atropine and 1 mg/kg acepromazine.

The surgical technique was carried out as follows (Fig. 1): 1) removal of an oral mucosa graft of approximately 0.4 × 0.3 cm size from the animal's lower lip; 2) repair of the mucosa defect using 7/0 Safil quick® suture; 3) urethral catheterization with a 3F venous catheter and fixation to the glans; 4) penis skin circular incision approx. 2 mm below the coronal sulcus, sliding the skin to the base of the penis to expose the urethra; 5) longitudinal ventral urethral incision of 0.4 cm in length from about 3 mm below the sulcus, in a proximal direction; 6) onlay implant of the oral mucosa graft patch on the ventral urethral face defect using 7/0 Safil quick® suture stitches on both sides of the urethral incision; the grafted area was labeled using non-absorbable stitches to facilitate the identification of the grafted tissue after the follow-up period for histological study; 7) repositioning the penis skin to the coronal sulcus to cover the urethra and the graft, and reparation of the skin using the same suture material. The skin suture was placed distally to the graft so that no extra suture material was placed on the graft; 8) the catheter was removed from the urethra.

The same surgical technique was carried out in the two control animals, but no oral mucosa graft was implanted. In addition, the urethral tissue allocated proximal and distally to the operated area of each animal was also used as controls. The oral mucosa tissue excised from these sham animals was used as control tissue for the histological and immunohistochemical analyses.

1.3. Evaluation by light and electron microscopy

Immediately after euthanasia, the rat penis was dissected and tissue samples corresponding to native urethra (controls) and grafted tissue were fixed in 4% buffered formaldehyde for histological analysis. The grafted area of the urethra was identified by the presence of the marker stitches proximally, distally and at the lateral margins of the graph and a biopsy was taken of this area to include the graft and the native urethra.

Samples were dehydrated and placed in paraffin. Tissue sections of 4 μm thickness were stained with haematoxylin and eosin for histological examination using a light microscope. In each case, the structure of the epithelial and stromal layers was analyzed, and the following parameters were recorded: integrity of the epithelium, number of cell layers, type of epithelium, presence of cell apoptosis or