



The buccal mucosa fenestrated graft for Bracka first stage urethroplasty: experimental study in rabbits

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ABSTRACT

Objective: To histologically evaluate, in an experimental study in rabbits, the integration process of the buccal mucosa fenestrated graft applied in the corpora cavernosa for Bracka first stage urethroplasty.

Materials and Methods: A urethral defect was surgically created in 16 male rabbits of the New Zealand breed through the excision of the penile urethra. The urethral defect was corrected by applying buccal mucosa fenestrated graft through two cruciform incisions in the distal portions of its longitudinal axis. The animals were sacrificed at 2, 4, 8 and 12 weeks post surgery and their genitals were subjected to clinical and histological assessment.

Results: The buccal mucosa fenestrated graft showed complete uptake in all groups, with keratinization squamous metaplasia and mucosal proliferation of the fenestrated areas. The fenestrated graft area represented an increase in length of 25% in length in relation to the original standard graft.

Conclusions: The fenestrated buccal mucosa graft presented total integration to the adjacent epithelia with re-epithelization of the incision areas of the graft (fenestrations) and no significant inflammatory or scarring reactions when compared to other mucosa transplanted areas; therefore its application is viable in cases of extensive urethral defect whenever the donating area might be insufficient.

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INTRODUCTION

Urethral reconstruction surgery has been challenging the skills and creativity of surgeons since antiquity. A wide variety of flaps and grafts have been used, particularly in procedures such as complex hypospadias and urethral stenosis (1). Currently, the vast majority of techniques for hypospadias repair is based on the preservation of the urethral plate, which when intact is well vascularized, has a good nervous supply and is sustained by strong muscle and connective tissue (2,3).

However, in as many as 10% of cases, maintaining the integrity of this urethral plate is practically impossible, requiring its removal for the straightening of the penis (4,5). Once the urethral plate is removed, most authors justify that surgery should be done in two steps (two-stages). Lately we have carried out clinical and experimental protocols in order to better understand the behavior and healing of the tissue regarding these complex reconstructions, and in this context, we have conducted experimental studies in animal model (6).

In patients with previous surgery or severe hypospadias, Bracka described a two-stage repair with a free graft of buccal mucosa, reaching a success rate of approximately 87% and accounting for only 5.7% of fistulas and 7% of stenosis (7). However, when regarding major defects, it is sometimes necessary to use many segments of buccal mucosa or the combination of buccal mucosa and skin to cover the entire surface and reconstruct the urethral plate (8). In these cases, the use of fenestrated grafting simulating mesh grafting, a technique well known in correcting major defects especially by using skin grafting in burn reconstructive plastic surgery, could be an interesting option to increase the area to be covered by grafting in situations where the availability of donor tissue is limited (9). Our proposal was to evaluate the histological integration process of fenestrated buccal mucosal grafting in the corpora cavernosa as the first stage of urethral reconstruction in an experimental model in rabbits.

MATERIALS AND METHODS

After approval by the Research Ethics Committee of the Universidade Federal de São Paulo - Hospital São Paulo, the study included a total of 16 New Zealand male rabbits, aged approximately 8 weeks and weighting 2.0 to 2.5 kg. Before the surgeries, the animals were acclimated in the Department of Experimental Surgery of the Universidade Federal de São Paulo for a period of adjustment of 72 to 96 hours.

The study was performed in 4 months, including the adjustment period, 12 weeks of the longest interval between intervention and sacrifice and finally the period of histological analysis.

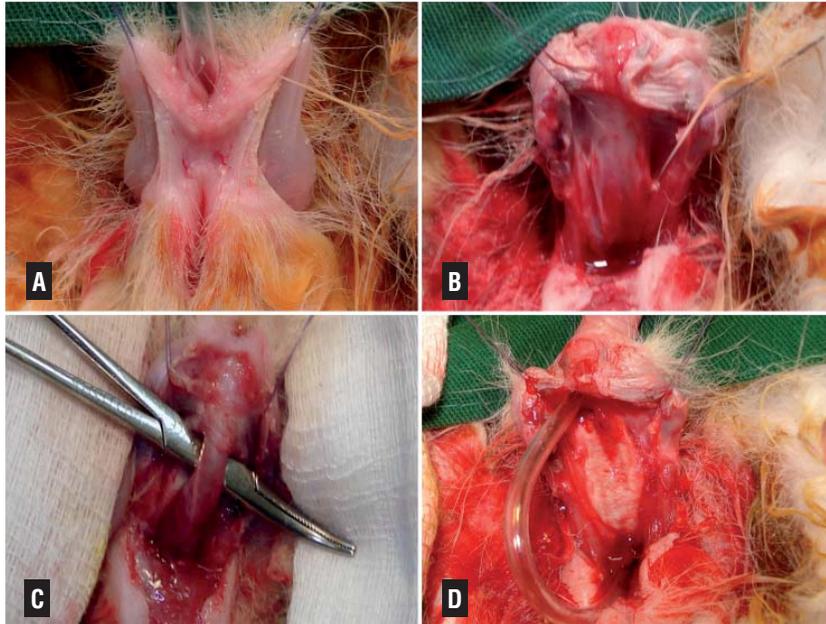
Anesthesia was initiated by the intramuscular administration of a preanesthetic agent (Midazolam - 5mg/kg) and hydration through a peripheral vein. Anesthesia was complemented with an intramuscular injection of an anesthetic solution containing ketamine hydrochloride 30 mg/kg + xylazine 5mg/kg and penile nerve block with bupivacaine 0.25% and lidocaine 1% without vasoconstriction (10,11). The surgical procedure was carried out under sterile conditions and the use of a magnifying glass of 3.5X.

First the urethra was catheterized with a urethral catheter of 8 Fr and the perineal fold between the penis and the anus of the animal was sectionalized, thus allowing access to the urethra (Figures 1A and 1B). Buck's fascia was incised at the junction of the corpus spongiosum and the corpus cavernosum on each side, thus allowing the structures to be isolated (Figure-1C). The corpus spongiosum and urethra were then exposed and completely sectioned with a scalpel, resulting in two stumps and defining a lengthy urethral defect, similar to a proximal hypospadias. Part of the distal urethral stump was excised to obtain complete exposure of the ventral surface of the corpus cavernosum (Figure-1D). To avoid a greater retraction or stenosis, the proximal urethral stump was fixed at the base of the penis and perineum with a 5.0 catgut® suture.

Next, buccal mucosa was extracted from the rabbit's jugal region. The donating site was exposed with interrupted stitches using 5.0 catgut® (Figure-2A). A centesimal solution of lidocaine at 1% with vasoconstrictor (adrenaline 1:200,000) was then injected locally into the submucosa (Figure-2B), thus facilitating the withdrawal of a buccal mucosa fragment 1.0 cm long and 0.4 cm wide (Figure-2C). After review of the hemostasis the donor region was not sutured, the wound healed by itself.

The buccal mucosa graft was harvested and fenestrated through two cruciform incisions created in distal portions of its longitudinal axis, approximately 1 mm from the superior and side edges. These incisions allowed for a macroscopical augmentation of 25% of the graft's longest strip, which went from 10 mm to 12.5 mm (Figures 2C and 2D). The fenestrated buccal mucosa graft was then brought to the urethral surgical area and applied onto the defect created in the ventral region of the penis with six interrupted stitches of polydioxanone suture (PDS® II) 6-0. The submucosa area of the graft was merged with the ventral surface of the exposed corpus cavernosum, thus constituting the neourethra plate. After the main part of urethral surgery, the penile fold was then partially rebuilt with separate stitches of 5.0 catgut® suture, while the patency of the urethroplasty was maintained without the need of a catheter.

Figure 1 – A) The rabbit's urethra catheterized with repairs exposing the fold between the phallus and the anus of the animal; B) Opening of the fold exposing the rabbit's urethra; C) Urethral spongiosum body isolated from the corpora cavernosa; D) After excision of the urethra it is observed the surface of the corpora cavernosa completely exposed.



Experimental animals were divided into 4 equal groups of 4 each and sacrificed 2, 4, 8 and 12 weeks after surgery, respectively. The genital of each animal was examined according to its ventral surface aspect. The penises were sectioned along the base, allowing the withdrawal of surgical parts that were immediately fixed in 10% formalin. These fixed segments were longitudinally sectioned, from the glans to the base of the penis, to allow for a full analysis of the two fenestrated areas in relation to the graft itself. It was compared the integration and histological changes that occurred during the different times of sacrifice. All blocks were cut to produce segments of 5 microns in thickness and stained with hematoxylin-eosin (HE) and Masson's trichrome. The animal group was not revealed to the pathologist for histological evaluation enabling an unbiased analysis.

The inflammatory response was classified as acute, when there was infiltration by polymorphonuclear cells, and chronic, with infiltration by mononuclear cells. We adopted a semi-quantitative assessment of the intensity of inflamma-

tion, graded on a 0 to 4 + scale, as follows: (0) no inflammatory infiltrate, (1+) minimal inflammation, (2+) moderate inflammation, (3+) severe inflammation and (4+) finding of aggregates of leukocytes / lymphocytes with formation of micro-abscesses. The degree of sub-epithelial fibrosis was also analyzed in a semi-quantitative scale, graded from 0 to 3+, as follows: (0) no scarring, (1+) minimal scarring, (2+) moderate scarring and (3+) severe fibrotic scar.

RESULTS

There were no deaths related to the surgical procedure. Moreover, no signs of local or systematic bacterial infection were verified. All animals were sacrificed on the pre-established dates. At the time of sacrifice and emasculation, the location of the graft was macroscopically identifiable, demonstrating its good integration and the fenestration areas were all fulfilled, being impossible to distinguish them from the buccal mucosa grafted (Figure-3).

Figure 2 – A) Exposition of the jugal mucosa with submucosal injection of anesthetic substance with vasoconstrictor; B) Fragment of mucosa that will be transformed into graft; C and D) Demonstrating the increased size of the graft after fenestration at the benchmarking of its length.

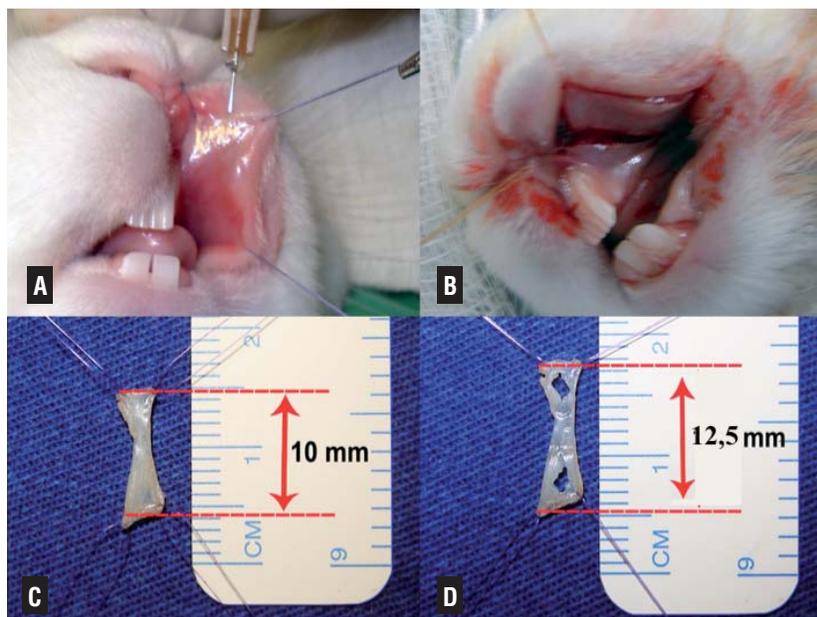


Figure 3 - Aspect of the ventral surface of the penis of the rabbit to be sacrificed after four weeks of surgical procedure. Notice the good integration of graft that is shown in red coloring.



After two weeks of surgery the histological assessment showed an intense inflammatory infiltrate with the presence of neutrophils and scarce eosinophils, featuring a recent inflammatory process. These signs of acute inflammation gradually reduced as the postoperative period increased and within 12 weeks we noticed a minimal inflamma-

tory response with scarce lymphocytes (Figures 4A, 5A and 5B).

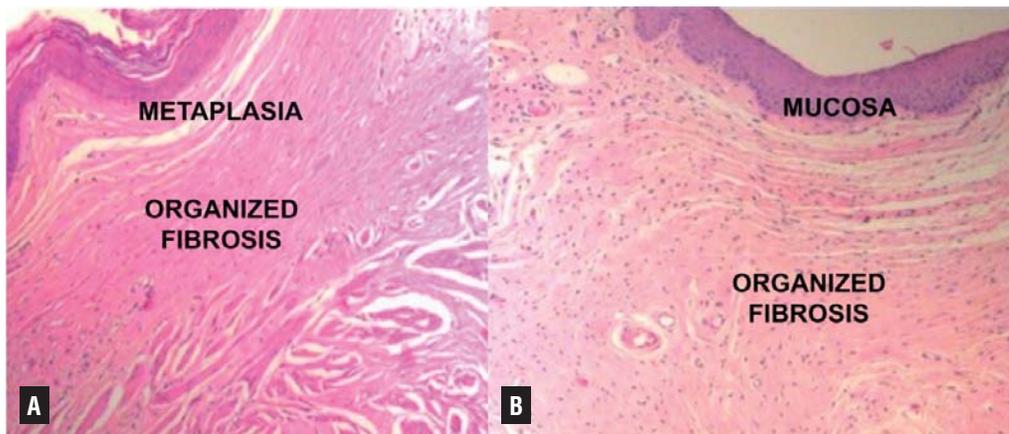
In relation to changes that occurred over time in the epithelial mucosa, we realized that after 2 weeks of surgery the mucosa had thickened, with keratinization of some areas, the presence of a few vessels, areas of urothelium epithelization and recent metaplasia, particularly in cuts where the fenestrated graft was found, from the incisions made in the mucosa. With time, this epithelium had become squamous keratinized and in the 8th week it was separated by small areas of squamous non-keratinized epithelium (Figure-4B). Within 12 weeks of post-operative care there were only small areas of immature squamous epithelium or in keratosis in probable areas of fenestration. It is important to stress the common histology of the buccal mucosa of rabbits, which consists of stratified epithelium with no attachments and no grainy layer, therefore, without any production of keratin.

The healing took place through the formation of a moderate sub-epithelial fibrosis, most

Figure 4 – A) Subgroup of 2 weeks. Mucosa and transition to younger epithelium. Moderate fibrosis (HE 40 x); B) Subgroup of 8 weeks. Metaplasia of the mucosa with moderate organized fibrosis (Masson 40 x).



Figure 5 - A) Subgroup of 12 weeks. Squamous mucosal metaplasia with mature healing of the graft (HE 40 x); B) Subgroup of 12 weeks. Well-integrated mucosa without signs of inflammation and mature scarring of the graft (HE 40 x).



evident on the border of the grafts within two weeks of postoperative and becoming more noticeable in 4 weeks when we could perceive the emergence of young fibroblasts (rounded nuclear), and areas of mature collagen. Within 8 weeks the fibrosis remained moderate, however, with organized fibers and deposition of collagen more evident in some areas. Finally, within 12 weeks accentuated fibrosis was noticed with a disposition of collagen more evident on the borders of the grafts (Figures 5A and 5B). Resume of histological findings are shown in Table-1.

DISCUSSION

The application of grafts or autologous flaps in urethral surgery is technically more feasible with the use of certain perigenital tissues such as foreskin or tunica vaginalis. However, and especially in reoperations, these tissues may no longer be available, making the use of free grafts from other tissues necessary. In these applications, and especially in the correction of serious forms when it is necessary to excise the urethral plate, one of the most studied tissues is the buccal

Table 1 - Resume of histological findings. Inflammatory response: (0) no inflammatory infiltrate, (1+) minimal inflammation, (2+) moderate inflammation, (3+) severe inflammation and (4+) finding of aggregates leukocyte / lymphocyte with formation of microabscesses. Sub-epithelial fibrosis: (0) no scarring, (1+) minimal scarring, (2+) moderate scarring and (3+) severe fibrotic scar.

	2 Weeks	4 Weeks	8 Weeks	12 Weeks
Acuteness of the inflammatory response	2+/3+	2+	1+/2+	0 / 1+
Types of inflammatory cells	Neutrophils and rare Eosinophils	Lymphocytes	Eosinophils and Lymphocytes	Rare Lymphocytes
Level of Subepithelial Fibroses	2+/3+	2+/3+	2+	2+/3+
Modification of the area in 'Mesh'	Urotelial Epithelization and Young Metaplasia	Stratified Epithelium with Keratinization	Squamous Keratinized Epithelium and Squamous non-keratinized	Squamous Keratinized Epithelium and of the immature squamous type
Other Findings	Cysts of Inclusion with micro abscesses	Microfocos of Ulceration	Acanthosis on the margins of the graft	-

mucosa, whose usage has also provided superior results (12-14). Secrest has reviewed indications and techniques of staged urethroplasty and has made a historical survey concluding that the staged technique is the oldest form of urethral reconstruction still in use today (15). The author also credits the best current results of staged urethroplasty to the works of Venn et al and especially to the changes and results of Schreiter et al., who applied urethroplasty techniques by using mesh grafts prepared for staged procedures in great defects (16-18).

Bracka has published excellent results with the use of buccal mucosa for the staged treatment of great urethral defects, especially in reoperations (19,20). The author performed this procedure in 121 patients and obtained a success rate of almost 90% after the second period of surgery. Rosito et al. demonstrated in an experimental study that the tunica vaginalis graft is an alternative tissue for the confection of the neo urethral plate in the first surgical period of a staged urethroplasty, however, broader clinical studies and longer follow-ups are needed until the tunica vaginalis is to be considered an al-

ternative of general use (21). Barroso et al. suggested that the buccal mucosa graft in Bracka's technique could be applied in "U" shape, thus increasing the lateral area of the graft (22-25). This shape allows a more feasible tubularization in the second period, presenting positive results in 10 children and only two complications after the second period of urethroplasty.

Based on the innovations and advantages of Schreiter's and Bracka's techniques, we decided to investigate histological changes that occur in an experimental model of staged correction of urethral defects, adding to the same procedure the innovative precepts postulated by the two authors. We also evaluated the integration of fenestrated buccal mucosa graft in the first stage of this surgical technique. The advantage of fenestrated graft is to cover a larger area of the urethral surface with the same donor area.

The rabbit was the chosen animal for being docile and easy to handle, and also for its penis size, which is compatible with that of a 12 month infant. Moreover, it is known that the embryological development of the phallus of the rabbit is similar to that of men, including the

formation of the foreskin (26). We also considered the fact that the healing process in rabbits has already been extensively studied and that this animal has often been used as a model in penile surgeries (27). No deaths occurred during the surgical procedure or the post-operative period. It is our belief that local anesthesia together with the extraction of the buccal mucosa patch decreased the need for general anesthetics, thus reducing the potential mortality inherent to the act of anesthesia.

Macroscopically, an augmentation of the buccal mucosa fenestrated graft was observed, confirming an increase in length of 25%. It was also observed that the grafts presented no important signs of macroscopic retraction in any of the sacrificed subgroups and that the buccal mucosa grafted area was flat and flawless.

Our histological results were consistent with the findings of previous studies, where the characteristically intense inflammatory reaction and infiltrated polymorphonuclears were gradually reduced into a discrete and chronic response, mainly of lymphocytes (6). These findings show a greater integration of the mucosa and are macroscopically noticeable at the moment of the animals' sacrifice. The histological response of the presence of fenestrated graft in our model was compared to the findings of Mokhless et al., who evaluated 31 patients who underwent the staged reconstruction proposed by Bracka through biopsies and histological analysis of the buccal mucosal grafts at the time of the second period of the reconstruction, six months after the grafting (28).

Mokhless' findings were similar for all patients, showing no statistical difference regarding their age or the specific area where the transplanted buccal mucosa originated. The authors found minor reaction modifications, mild acanthosis, mild epithelial hyperplasia and mild focal keratosis with a discreet lengthening of the lamina propria papillae. There was still a discreet infiltrated lymphocytic in the lamina propria papillae, and all grafts presented perfect vascularization, the same as the original buccal mucosa. The stratified squamous non-keratinized epithelium of our buccal mucosa graft presented as-

pects of immature squamous metaplasia, evident until the last sacrificed subgroup with 12 weeks of transplantation. The fenestrated areas did not alter the integration or vascularization of any of the grafts.

One of the major differences that occurred in our evaluation was the presence of non-keratinized tissue areas, separated by areas of keratosis and keratinized tissue, with a thinner appearance and with less edema, thus presenting a more organized tissue. This suggests that non-keratinized buccal mucosa undergoes squamous metaplasia, covering the surface of the corpus cavernosum including the fenestrated area, stimulating a non-keratinized mucosal proliferation. However, the reaction found does not seem to be definitive and can change even after the second period of a staged correction, as shown by Smith et al. (29).

Subepithelial fibrosis assessment did not show any important changes in the expected behavior of graft reaction, with the classic evolution of the scarring process and without fibrotic reactions that might occur in fenestrated areas, being this effect similar to the clinical outcome of Schreiter (30).

In summary, our findings have enabled us to define that a fenestrated graft has a behavior similar to a healthy graft, without the presence of macroscopic retraction or any evidence of greater macroscopic scarring process. Through histological examination the behavior of epithelium confirmed metaplasia and keratinization with no relevant difference between healthy and fenestrated areas.

CONCLUSIONS

Buccal mucosa fenestrated graft, dorsally applied onto the corpus cavernosum of a rabbit, has shown in this histological evaluation full integration to the adjacent epithelium with re-epithelization of the fenestrated areas of the graft, without presenting an inflammatory reaction or scarring significantly greater than in the different areas of the transplanted mucosa. The epithelium has undergone squamous metaplasia and began to present keratinization.

ABBREVIATIONS

HE = Hematoxylin-Eosin

CONFLICT OF INTEREST

None declared.

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