Journal of
PERIODONTAL RESEARCH

J Periodont Res 2012; 47: 758–765

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2012.01491.x

Clinical and histological evaluation of subepithelial connective tissue after collagen sponge implantation in the human palate

A. L. Rocha[1], B. K. Shirasu[1], R. M. Hayacibara[2], O. Magro-Filho[3], J. N. Zanoni[4], M. G. Araújo[2]

[1]Private practice, Maringá, PR, Brazil, [2]Department of Dentistry, State University of Maringá, Maringá, PR, Brazil, [3]Department of Surgery, UNESP – São Paulo State University, Araçatuba, SP, Brazil and [4]Department of Morphological Science, State University of Maringá, Maringá, PR, Brazil

Rocha AL, Shirasu BK, Hayacibara RM, Magro-Filho O, Zanoni JN, Araújo MG. Clinical and histological evaluation of subepithelial connective tissue after collagen sponge implantation in the human palate. J Periodont Res 2012; 47: 758–765.

Background and Objective: Successful root-coverage treatment depends on the thickness of the donor tissue. This study aimed to evaluate the thickness of donor tissue after augmentation of the connective tissue in the palatal area by implantation of lyophilized collagen sponge (Hemospon®).

Material and Methods: Ten patients with an indication for root coverage, whose palate was deficient in adequate connective tissue, were recruited. The procedure was carried out in two stages. In the first stage, the palatal thickness in the donor site was measured at three standardized points (points 1, 2 and 3), from the distal of the canine to the distal of the first molar, and the lyophilized collagen sponge was inserted. In the second stage, the palatal thickness over the implant was measured (at points 1, 2 and 3), two biopsies of the palatal mucosa were collected – one over the implant (experimental sample) and the other on the contralateral side (control sample) – and then root-coverage treatment was performed. Analyses consisted of clinical assessment of the palatal measurements before and after sponge implantation, and histological assessment of the experimental and control biopsy samples. Data were analyzed using the Wilcoxon test.

Results: Both analyses showed a significant increase in mean thickness, of 1.08 mm of neoformed tissue in the clinical analysis (the tissue at point 2 was the thickest of the three points) and of 0.53 mm in the histological analysis.

Conclusion: The insertion of lyophilized collagen sponge induced a significant increase in the thickness of palatal connective tissue.

Ana Luiza Rocha, DDS, Av. Dona Maria Gaspar Pedrosa Moleirinho, 18, Jardim Novo Horizonte, CEP 87010-120, Maringá-PR, Brasil
Tel: +44 3028 4083
e-mail: ana_mga19@hotmail.com

Key words: lyophilized collagen sponge; marginal gingival recession; root coverage; subepithelial connective tissue

Accepted for publication March 29, 2012

Dentistry plays its role in the search for contemporary esthetic values – harmonious face, lips, gum and teeth arrangement are the essence of a pleasing smile (1), in itself a fundamental component of beauty. Gingival recession treatment has become a frequent procedure owing to increasing requests by patients for cosmetic procedures (2–5). However, gingival recession is not only a cosmetic matter, as an exposed root surface is associated with discomfort, sensitivity and, consequently, poor oral hygiene (4,6,7).

Although several surgical root-coverage techniques have been described (7–11), one of the most popular, owing to its effectiveness and good predictability (12,13), concerns the use of the subepithelial connective tissue graft (STG), as introduced by Langer & Langer (14). Among some advantages over other procedures, STGs provide a similar color to the adjacent structures, good alignment of gingival margins and a palatal donor site with minimal postoperative discomfort (8,14). However, despite the excellent esthetic results, the STG technique requires donor surgery (10). The present study describes a tissue-augmentation method aimed toward increasing the chances of success of STG procedures.

Traditionally, the hard palate has been the primary source of donor tissue for root-coverage treatment (8–10,13–21). The region between the first molar and the canine seems to be the most suitable donor site (13,19,21), given that the volume of tissue in the posterior palate is limited by anatomic structures (9,17,21–23). In addition, the thickness of the connective tissue may vary as a result of palatal anatomy or show insufficient thickness of the investing soft tissue (10,15,19,24).

Even if the palatal mucosa does not show optimal thickness, a graft could be obtained if it is harvested as a full-thickness free gingival graft with the epithelium, which is later removed. Such a procedure would also influence the indication of the described augmentation technique. It has been reported that a free gingival harvest is accompanied by greater morbidity in the patient (9,25), although this was not found in another study (26). Procedures that do not require connective tissue grafts have been described as nonautogenous substitutes for palatal tissue (15). However, the long-term prognosis of some of these techniques has been questioned (11,20), whereas STGs have shown long-term stability (11,27).

The present augmentation technique is applied if palatal mucosal thickness is insufficient, but it is important to bear in mind that the thickness-measurement technique may influence the indication of the procedure. The literature describes thickness-measurement techniques that yield results with high variations – the lowest thicknesses are typically measured using blunt periodontal probes and ultrasonic devices (18,19,28–30), whereas the highest thicknesses are measured using sharp cannulas (31).

Carnio & Hallmon (22) described a technique for augmenting the palatal connective tissue of donor sites using sterile lyophilized bovine collagen sponge. Yen *et al.* (32) reported maintaining donor-site thickness using platelet concentrate (PC) and collagen sponge in multiple root-coverage procedures. However, to the best of our knowledge, neither of these studies (22,32) nor any other has quantitatively assessed the amount of neoformed tissue after the use of lyophilized sponge. The purpose of the present study was to make a clinical and histological quantitative and qualitative evaluation of tissue neoformation in the palatal donor area after implantation of the collagen sponge Hemospon® (Technew, Rio Janeiro, Brazil). Hemospon® is a porous gelatine sponge made with lyophilized hydrolyzed porcine collagen and is fully absorbed within 15 d. It is widely used in oral surgery because of its high hemostatic and healing capacity; it is inserted into the cavity where hemostasis is required and may or may not be withdrawn after a period of time.

Material and methods

Study population

Ten participants (one male subject and nine female subjects; 20–39 years of age) were recruited and referred to the Dentistry Department of the State University of Maringá and Maringá Dentistry Association. Approval was obtained from the University Ethics Committee, and patients gave their written informed consent to participate in the study. Subjects' selection criteria were: (i) good systemic health; and (ii) the presence of vestibular gingival recessions. Exclusion criteria were: (i) periodontal disease; (ii) a history of palatal surgery; (iii) use of medication that might affect periodontal health; (iv) pregnancy or lactation; (v) any use of tobacco; and (vi) use of a partial denture or an orthodontic retainer or any removable device in the upper arch.

Presurgical procedures

Initial therapy consisted of oral hygiene instruction; scaling and root planing; polishing; and, if needed, occlusal adjustment. Alginate impressions (Jeltrade; Dentsply International Inc., Milford, DE, USA) of the maxilla were taken and immediately poured in stone. Acrylic resin stents were produced (Fig. 1A), with a relief (wax 07), from the distal region of the canine to the distal region of the first molar. Three points were identified in the

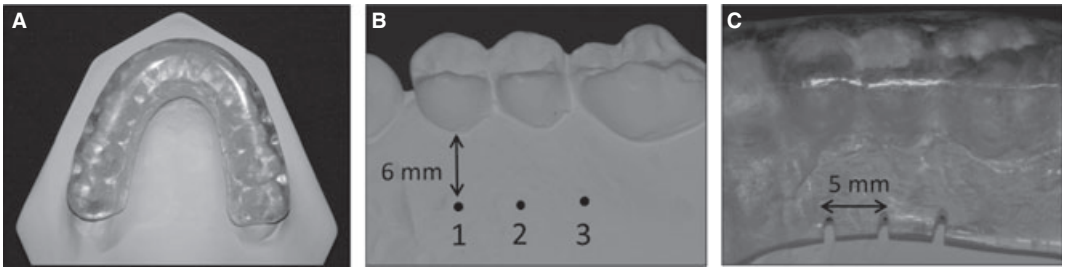

Fig. 1. Manufacture of acrylic stent (A); measurement points defined and marked on the study model (B); and measurement points drilled on the stent (C).

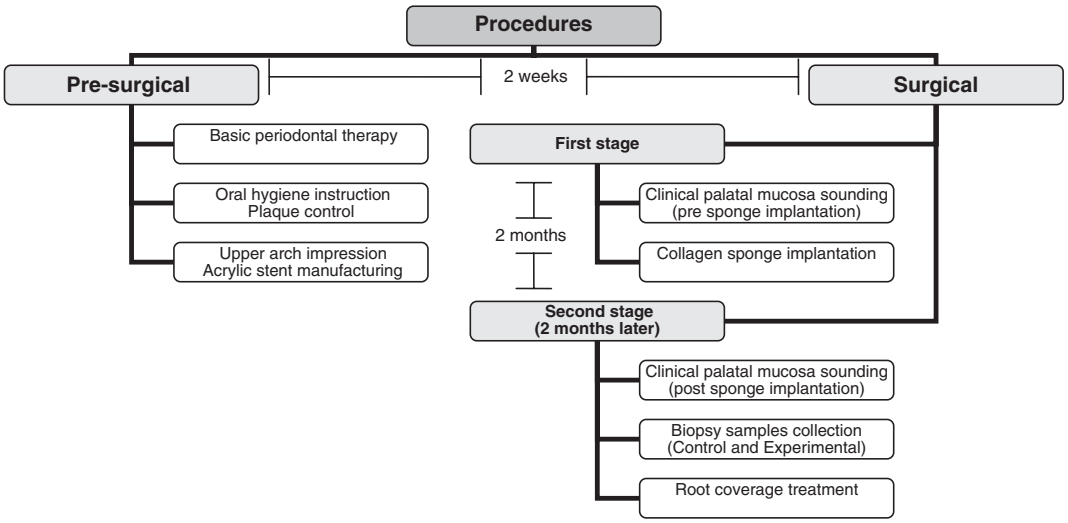

Fig. 2. Chronological sequence of presurgical and surgical procedures.

study models (numbers 1, 2 and 3 in Fig. 1B), located 6 mm from the gingival margin. Point 1 was marked perpendicularly to the center of the first premolar, with the three points being 5 mm equidistant from one another. The resin stents were diamond-drilled over the three points, to provide standardized stents with three perforations through which the palatal donor site could be measured in the surgical stages (Fig. 1C).

Surgical procedures

The experimental period, which took 60 d for each patient and was performed by a single operator, was carried out in two stages. The first stage consisted of measurements of the palatal donor site and implantation of the collagen sponge. In the second stage, 8 wk after the first, palatal measurements were repeated, two biopsy samples were collected (one from each side) from the palate and the patient was referred for root-coverage surgery. The sequence of procedures is detailed in Fig. 2 and the procedures are explained in the following sections.

First stage

Palatal mucosa measurements and implantation of the collagen sponge Bone probing (PCP-UNC 15; Hu-Friedy, Chicago, IL, USA) was the evaluation method used to measure tissue thickness in the hard palate (19,22,29); it is a relatively safe technique with a margin of error of 0.2 mm (14,19,29). A 20-min wait after greater palatine foramen block (2% mepivacaine, epinephrine 1 : 100,000; DFL, Rio de Janeiro, RJ, Brazil) was followed by acrylic stent positioning and measurement of the thickness of the palatal donor sites (Fig. 3).

For sponge implantation, an incision perpendicular to the bone surface was made 3 mm from the gingival margin, 15 mm mesio-distally from the distal region of the canine. A full-thickness periosteal STG was raised and a 13-cm^2 collagen sponge (Fig. 4A), previously hydrated in sterile saline solution (Fig. 4B), was inserted between the connective tissue and the bone (Fig. 4C). The area was sutured (4-0 Silk, Ethicon; Johnson & Johnson, São José dos Campos, SP, Brazil) (Fig. 4D), and a thin, protective isobutyl cyanoacrylate layer was applied to cover the wound surface.

Second stage

Palatal mucosa measurements and removal of biopsy samples The thickness of the palatal tissue was measured for the second time, following the same procedures of the first stage. In addition, two biopsy samples were obtained from the region of the first premolars (point 1) with the use of a 4-mm-diameter punch positioned perpendicularly to the palatal bone. The collagen-implanted mucosa provided the experimental sample, whereas the control sample was removed from the contralateral side of the palate, without the implantation. The surgical areas were sutured in an 'X' with No. 4-0 silk, and the samples were placed in Bouin's solution for 6 h before analysis using routine histological techniques.

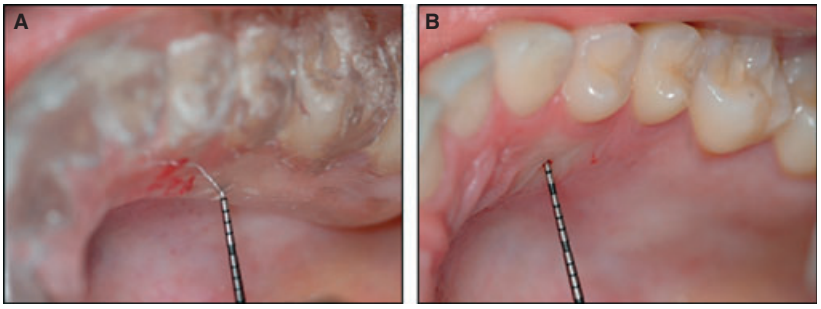

Fig. 3. Marking probing point with the acrylic stent (A), and measuring palatal depth after stent removal (B).

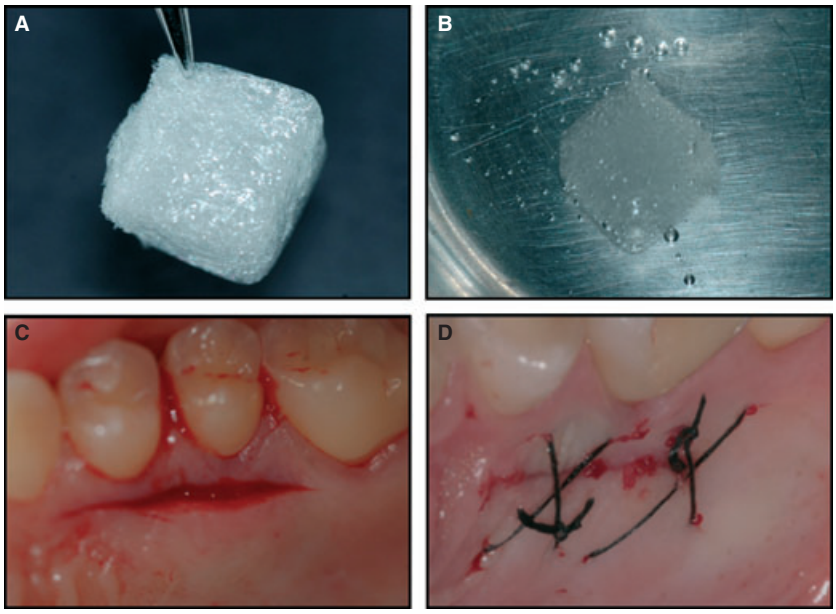

Fig. 4. Lyophilized collagen sponge (Hemospon®) (A), sponge hydration (B), sponge implanted in the palate (C) and sutures (D).

STG removal and root covering

A 5-mm incision from the gingival margin was made for the removal of the STG, with the mesiodistal length determined by the graft size needed. The partial-thickness flap was dissected; a small periosteal elevator released the graft, which was placed in the previously prepared recipient area.

Postsurgical care

Ibuprofen (600 mg, twice a day for 4 d) and sodium dipyrone (500 mg, 35 drops every 6 h, as long as needed) were prescribed for pain and inflammation. Patients were placed on 0.12% chlorhexidine gluconate mouthrinse twice a day for 1 wk, and the sutures were removed after 1 wk. No postoperative complication was observed.

Biopsy samples – preparation and analysis of histological specimens

All steps in sample preparation were performed by a single observer, who used an Olympus BX41 microscope (Olympus, Wendenstrasse, Hamburg, Germany) (2 × objective for measuring tissue thickness, and 40 × objective for quantifying blood vessels, fibroblasts and inflammatory cells), coupled to a high-resolution camera (Olympus Q-Color 3; Olympus, Wendenstrasse, Hamburg, Germany) and transferred to a computer with the use of QCapture Pro software (Media Cybernetics, Silver Spring, MD, USA). Images were analyzed using the program Image-Pro Plus 4 (Media Cybernetics).

Two procedures were carried out in the histological analysis of the biopsy samples: (i) measurement of the palatal tissue; and (ii) quantification of blood vessels, fibroblasts and inflammatory cells. To evaluate the palatal mucosa thickness, the biopsy samples were cut into sections of 7 μm thickness and the conjunctive epithelium specimens were stained with hematoxylin and eosin. Five images were obtained for each sample, and five measurements were taken from each image.

For the quantification of blood vessels, fibroblasts and inflammatory cells, 10 histological sections were used for each sample. Images were standardized so that the selected 0.89-mm^2 area was located adjacent to the oral epithelium.

Statistical analysis

All statistical analyses were performed using SPSS version 17 (SPSS, Chicago, IL, USA) with significance level set at $p \leq 0.05$. The results were expressed as mean ± standard deviation, except for percentages, and were analyzed using the Wilcoxon signed-rank test for paired samples.

For the clinical measurements of palatal thickness, the three points in the acrylic stent were evaluated in both pre- and postcollagen implantation stages. In each stage, 10 samples for individual points (each point × 10 patients) and 30 samples for the three points in total (the three points × 10 patients) were analyzed.

For histological analysis of the biopsy samples, measurement of the palatal tissue resulted in analysis of 250 samples for each experimental and control sample (10 tokens × five images × five measures for each image) and in 100 measures for each sample in the quantification of blood vessels, fibroblasts and inflammatory cells (10 tokens × 10 images).

Results

Clinical analysis

In the control sample, the mean palatal thickness did not differ significantly between the two assessments – point 1 remained as 2.60 ± 516 mm in both estimations, point 2 as 2.85 ± 241 mm and point 3 as 2.70 ± 0.349 mm. For comparison of the mean values of the three points in total, again the differences were not significant – from 2.71 ± 0.386 mm in the first evaluation to 2.68 ± 0.404 mm in the second assessment.

In the experimental sample, the mean thicknesses of points 1, 2 and 3, analyzed individually before collagen implantation, were 2.40 ± 0.516 mm, 2.85 ± 0.241 mm and 2.80 ± 0.586 mm, respectively, and after implantation they were 3.80 ± 0.537 mm, 3.95 ± 0.497 mm and 3.60 ± 0.516 mm, respectively, with differences being statistically significant only in point 2 ($p = 0.004$). Taking the means of the three points into consideration (Fig. 5), the palatal thickness in the donor area increased from 2.68 ± 0.499 mm to 3.76 ± 0.537 mm, a significant difference of 1.08 mm ($p = 0.000$).

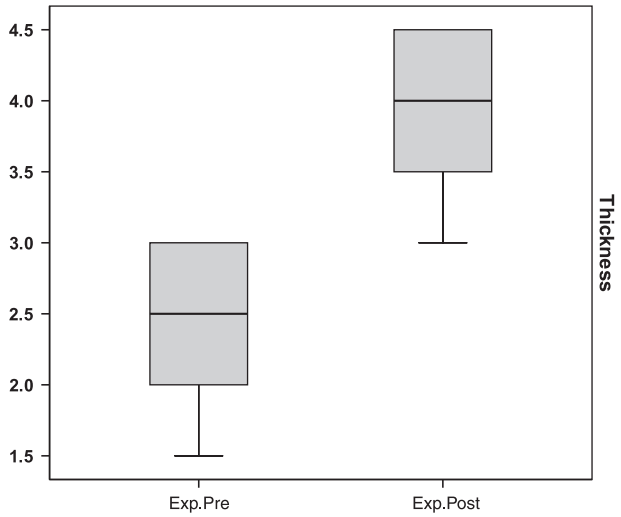

Fig. 5. Clinical analysis of the experimental sample. The mean thickness (in mm) of the three palatal points, pre and post sponge implantation, are presented. $p < 0.05$; $n = 30$.

Histological analysis

Histological analysis of the palatal thickness revealed a significant difference ($p = 0.000$) of 0.53 mm between the control sample (2.15 ± 0.582 mm) and the experimental sample (2.68 ± 0.777 mm), interpreted as a 24% increase in tissue neoformation (Figs 6, 7A and 7B).

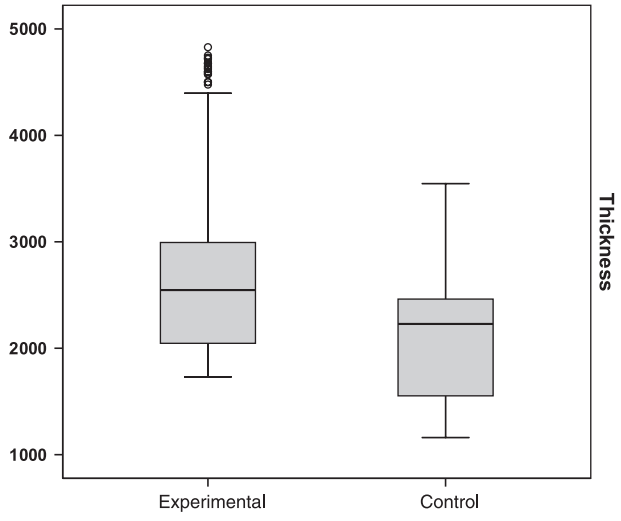

Fig. 6. Histological analysis of the palatal thickness (in mm) of the control and the experimental biopsy samples. $p < 0.05$; $n = 250$.

Table 1. Density of fibroblasts, quantity of inflammatory cells and density of blood vessels in 0.89-mm^2 sections from the control and experimental biopsy samples

Biopsy sample	Fibroblasts	Inflammatory cells	Blood vessels
Control	804.9 ± 273.4	473.9 ± 16.8	25.50 ± 2.2
Experimental	978.7 ± 439.1	642.2 ± 24.7	49.80 ± 3.0

Values are given as mean ± standard deviation. $p < 0.05$; $n = 100$.

For the quantification analyses in 0.89-mm^2 section (Table 1), the mean density of fibroblasts was 804.9 in the control sample and 978.7 in the experimental sample. The mean densities of inflammatory cells in the control and experimental samples were 473.9 and 642.2, respectively. With regard to the density of blood vessels, the mean values were 25.50 for the control sample and 49.80 for the experimental sample, the difference being statistically significant ($p < 0.001$) in all analyses (Fig. 7C and 7D).

Discussion

In root-coverage treatment, more than one surgical procedure may be needed if the amount of tissue graft is limited (2,10). Therefore, a conservative and successful treatment depends on the evaluation of the donor site before any surgical intervention is carried out (14,22). STG combined with coronally advanced flaps is one of the most commonly used techniques in root-coverage treatment because of its highly successful prognosis (14,27). For ensuring both safety and clinical predictability of this technique, the palatal mucosa requires an adequate thickness.

Several authors (3,6,22), consider a thicker graft, of 1.5–2 mm, as more appropriate for root-coverage treatment. Nonetheless, because of its anatomical limitations, the hard palate does not necessarily offer the ideal condition for obtaining a graft of appropriate size (7,24). The present study evaluated, both clinically and histologically, the palatal thickness after the implantation of lyophilized collagen sponge, an area subsequently used as the connective tissue donor site.

Collagen sponges have long been used in dentistry, mainly for their biocompatibility and haemostatic properties. They are physiologically metabolized, fully reabsorbed by the host system, do not present an untoward reaction, have a favorable effect on wound healing and blood coagulation, and facilitate wound maturation and stability by enhancing initial clot and fibrin linkage formation (22). They have also been used as a vehicle for

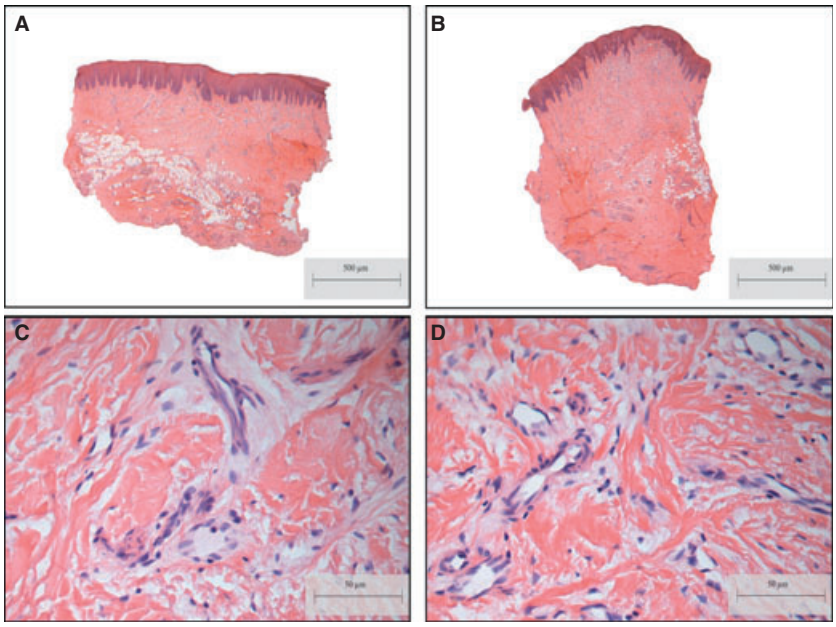

Fig. 7. Microphotographs of tissue specimens stained with hematoxylin and eosin. Control sample (A) and experimental sample (B) viewed at 2 × magnification and 500 μm calibration. Control sample (C) and experimental sample (D) viewed at 40 × magnification and 50 μm calibration.

substances in periodontal surgery, with no harmful inflammatory response (33,34).

Carnio & Hallmon (22) examined a method for augmenting the palatal connective tissue of the donor sites with the use of lyophilized bovine collagen sponge. They found that the experimental area was three times thicker than the control area. Although the present study showed significant tissue neoformation in the implanted area, the results were not as notable as those of Carnio & Hallmon. The differences might have been the result of distinct methodologies, number of samples, or the composition of the sponges used in the two studies.

Yen *et al.* (32) compared palatal tissue neoformation using two methods – placement of a PC and collagen sponge (Collacote®; Zimmer dental, Carlsbad, NM, USA) in the experimental area, and placement of collagen sponge only in the control area. The results showed thickness gain in the experimental area (0.7 mm) and tissue loss in the control area (0.4 mm). Although the findings of Yen *et al.* and those of the present study are similar, they also seem to suggest that the effectiveness in tissue neoformation may vary depending upon the type of collagen sponge used.

Hemospon®, used in the present study, shows a resilient nature – after implantation it tends to regain its original form, providing a framework for the cells involved in the healing process and stability for blood clotting. As a consequence, any space occupied by the material is replaced with connective tissue, which increases the thickness of the palate.

Regarding clinical measurements, the response to sponge implantation varied among the three palatal points, with point 2 being the only one showing a significant increase. The finding that point 3 did not show statistical differences between pre and post sponge implantation was an expected result, given that the mucosa adjacent to the palatal root of the first molar shows lower thickness (18,19,29). Indeed, previous studies (15,18,19,29) showed that the area adjacent to the first molars (point 3) is considered a limited region for the removal of connective tissue graft. The lack of significance in tissue neoformation in point 1 is possibly a result of its location in the palate – an area of marked anterior–posterior concavity and near the transverse palatine folds. These folds are formed by fibrous tissue, strongly adherent to the subjacent bone (35), which might have prevented the release of soft tissue and limited its augmentation as a result of possible compression of the implanted collagen sponge. Point 2, however, is flatter and posteriorly located, in an area that might have suffered less stress and allowed for greater tissue neoformation.

Quantitative and qualitative histological analyses of biopsy samples were innovations in the present study, and different from the qualitative interpretations carried out by Carnio & Hallmon (22) and by Yen *et al.* (32). The histological analyses carried out by Carnio & Hallmon (22) showed that the augmented area had a normal structure, no evidence of inflammation or notable residual sponge material and no visible adipose tissue. Eight weeks after placement, the collagen sponge had been completely absorbed and replaced with connective tissue. Similarly to the results of Carnio & Hallmon (22), there was no residual sponge in the present study, but in contrast to their results, both adipose cells and inflammatory infiltrate were observed in the neoformed tissue.

Inflammation is not related to the composition of the sponge, but to its physical presence in the body, which leads to a delay in healing. Studies that examined the biocompatibility of porcine sponge (36–38) showed that sponge implantation caused a delay in healing, evidenced by traces of sponge and inflammatory cells in the surgical site, which, more importantly, suggests that the material had caused a foreign-body reaction. In the present study, after 60 d no remnants of collagen sponge were observed, although some inflammatory cells were still present, suggesting that the inflammation was not completely resolved. In addition, no foreign-body giant cells were observed, given that the period of sample collection was already in a more advanced stage in the healing process (60 d). However, Cegielski *et al.* and Gabrielli *et al.* reported the presence of giant cells by days 45 and

30, respectively, after surgery, which suggests their association with the degradation and resorption of sponges.

In the present study, the significant difference in the numbers of inflammatory cells and fibroblasts, and in the density of blood vessels, before and after sponge placement, is interpreted as a positive phenomenon, as inflammation is a fundamental source of nutrients and oxygen, factors that contribute to the healing process (39,40). Given their low level of replication, fibroblasts are considered as stable structures. Facing an inflammatory reaction, such as the implantation of a collagen sponge, they react by replicating and synthesizing larger amounts of collagen fibers (39,40).

The histological analysis of Yen *et al*. (32) showed less infiltration of inflammatory cells in the experimental samples (PC and collagen sponge) than in the control samples (collagen sponge only). As a result of stronger staining in the experimental samples than in the control samples, immunohistochemical analysis revealed that PC up-regulated type I collagen synthesis, whereas the control samples showed more staining of type III collagen. Yen *et al*. (32) concluded that this difference was the result of distinct concentrations of growth factors, which were higher in the PC plasma present only in the experimental samples. The present study used a different collagen sponge from that of Yen *et al*. and found a higher proportion of type III collagen than of type I collagen. It was expected that immature type III collagen, the first to be synthesized, would later be replaced with mature type I collagen (41).

To conclude, the findings demonstrated that implantation of lyophilized collagen sponge in the palate augments the thickness of connective tissue and that the technique may be a simple, alternative procedure for tissue neoformation. This tissue-augmentation technique can be used for procedures that require more than one donor site, multiple root-coverage procedures or when the palatal mucosa does not provide an appropriate thickness for graft harvesting. The technique can guarantee that the minimum thickness required for the safe harvesting of STGs – in fact, a final graft of at least 1.5 mm thick and nearly twice its original length – can be obtained from augmented palatal tissue (7).

References

1. Charruel S, Perez C, Foti B, Camps J, Monnet-Corti V. Gingival contour assessment: clinical parameters useful for esthetic diagnosis and treatment. *J Periodontol* 2008;**79:**795–801.
2. Zuchelli G, Sanctis M. Treatment of multiple recession–type defects in patients with esthetics demands. *J Periodontol* 2000;**71:** 1506–1514.
3. Wang HL, Bunyaratavej P, Labadie M, Shyr Y, Macneil RL. Comparison of 2 clinical techniques for treatment of gingival recession. *J Periodontol* 2001;**72:**1301–1311.
4. Zucchelli G, Amore C, Sforza NM, Montebugnoli L, De Sanctis M. Bilaminar techniques for the treatment of recession–type defects. A comparative clinical study. *J Clin Periodontol* 2003;**30:**862–870.
5. Hirsch A, Goldstein M, Goultschin J, Boyan BD, Schwartz Z. A 2-year follow-up of root coverage using subpedicle acellular dermal matrix allografts and subepithelial connective tissue autografts. *J Periodontol* 2005;**76:**1323–1328.
6. Nelson SW. The subpedicle connective tissue graft. A bilaminar reconstructive procedure for the coverage of denuded root surfaces. *J Periodontol* 1987;**58:**95–102.
7. Ribeiro FS, Zandim DL, Pontes AE, Mantovani RV, Sampaio JEC, Junior EM. Tunnel technique with a surgical maneuver to increase the graft extension: case report with a 3-year follow-up. *J Periodontol* 2008;**79:**753–758.
8. Guiha R, el Khodeiry S, Mota L, Caffesse R. Histological evaluation of healing and revascularization of the subepithelial connective tissue graft. *J Periodontol* 2001;**72:** 470–478.
9. Del Pizzo M, Modica F, Bethaz N, Priotto P, Romagnoli R. The connective tissue graft: a comparative clinical evaluation of wound healing at the palatal donor site. A preliminary study. *J Clin Periodontol* 2002;**29:**848–854.
10. Çetiner D, Bodur A, Uraz A. Expanded mesh connective tissue graft for the treatment of multiple gingival recessions. *J Periodontol* 2004;**75:**1167–1172.
11. Harris RJ. A short-term and long-term comparison of root coverage with an acellular dermal matrix and a subepithelial graft. *J Periodontol* 2004;**75:**734–743.
12. Nemcovsky CE, Artzi Z, Tal H, Kozlovsky A, Moses O. A multicenter comparative study of two root coverage procedures: coronally advanced flap with addition of enamel matrix proteins and subpedicle connective tissue graft. *J Periodontol* 2004;**75:**600–607.
13. Cairo F, Pagliaro U, Nieri M. Soft tissue management at implant sites. *J Clin Periodontol* 2008;**35:**163–167.
14. Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J Periodontol* 1985;**56:**715–720.
15. Cummings LC, Kaldahl WB, Allen EP. Histologic evaluation of autogenous connective tissue and acellular dermal matrix grafts in humans. *J Periodontol* 2005;**76:** 178–186.
16. Miller PD Jr. Root coverage with the free gingival graft factors associated with incomplete coverage. *J Periodontol* 1987;**58:** 674–681.
17. Reiser GM, Bruno JF, Mahan PE, Larkin LH. The subepithelial connective tissue graft palatal donor site: anatomic considerations for surgeons. *Int J Periodontics Restorative Dent* 1996;**16:**130–137.
18. Müller HP, Schaller N, Eger T, Heinecke A. Thickness of masticatory mucosa. *J Clin Periodontol* 2000;**27:**431–436.
19. Wara-aswapati N, Pitiphat W, Chandrapho N, Rattanayatikul C, Karimbux N. Thickness of palatal masticatory mucosa associated with age. *J Periodontol* 2001;**72:**1407–1412.
20. Harris RJ, Miller LH, Harris CR, Miller RJ. A comparison of three techniques to obtain root coverage on mandibular incisors. *J Periodontol* 2005;**76:**1758–1767.
21. Monnet-Corti V, Santini A, Glise J *et al*. Connective tissue graft for gingival recession treatment: assessment of the maximum graft dimensions at the palatal vault as a donor site. *J Periodontol* 2006;**77:**899–902.
22. Carnio J, Hallmon WW. A technique for augmenting the palatal connective tissue donor site: clinical case report and histologic evaluation. *Int J Periodontics Restorative Dent* 2005;**25:**257–263.
23. Klosek SK, Rungruang T. Anatomical study of the greater palatine artery and related structures of the palatal vault: considerations for palate as the subepithelial connective tissue graft donor site. *Surg Radiol Anat* 2009;**31:**245–250.
24. Sullivan HC, Atkins JH. Free autogenous gingival grafts. I. Principles of successful grafting. *Periodontics* 1968;**6:**5–13.
25. Wessel JR, Tatakis DN. Patient outcomes following subepithelial connective tissue graft and free gingival graft procedures. *J Periodontol* 2008;**79:**425–430.
26. Zucchelli G, Mele M, Stefanini M *et al*. Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: a comparative randomized-controlled clinical trial. *J Clin Periodontol* 2010;**37:**728–738.

27. McGuire MK, Scheyer ET. Comparison of recombinant human platelet-derived growth factor-BB plus beta tricalcium phosphate and collagen membrane to subepithelial connective tissue grafting for the treatment of recession defects: a case series. *Int J Periodontics Restorative Dent* 2006;**26:**127–133.
28. Kydd WL, Daly CH, Wheeler JB. The thickness measurement of masticatory mucosa *in vivo*. *Int Dental Journal* 1971;**21:** 430–441.
29. Studer SP, Allen EP, Rees TC, Kouba A. The thickness of masticatory mucosa in the human hard palate and tuberosity as potential donor sites for ridge augmentation procedures. *J Periodontol* 1997;**68:** 145–151.
30. Song JE, Um YJ, Kim CS *et al*. Thickness of posterior palatal masticatory mucosa: the use of computerized tomography. *J Periodontol* 2008;**79:**406–412.
31. Schacher B, Bürklin T, Horodko M, Raetzke P, Ratka-Krüger P, Eickholz P. Direct thickness measurements of the hard palate mucosa. *Quintessence Int* 2010;**41:** e149–e156.
32. Yen CA, Griffin TJ, Cheung WS, Chen J. Effects of platelet concentrate on palatal wound healing after connective tissue graft harvesting. *J Periodontol* 2007;**78:** 601–610.
33. Boyne PJ, Marx RE, Nevins M *et al*. A feasibility study evaluating rhBMP-2/ absorbable collagen sponge for maxillary sinus floor augmentation. *Int J Periodontics Restorative Dent* 1997;**17:**11–25.
34. Howell TH, Fioreline J, Jones A *et al*. A feasibility study evaluating rhBMP-2/ absorbable collagen sponge device for local alveolar ridge preservation or augmentation. *Int J Periodontics Restorative Dent* 1997;**17:**125–139.
35. Katchburian E, Arana-Chaves VE. Periodonto. In: Katchburian E, Arana-Chaves VE, eds. *Histologia e Embriologia Oral*. Rio de Janeiro: Guanabara Koogan, 2004:275–326.
36. Cegielski M, Izykowska I, Podhorska-Okolow M, Zabel M, Dziegiel P. Development of foreign body giant cells in response to implantation of spongostan® as a scaffold for cartilage tissue engineering. *In vivo* 2008;**22:**203–206.
37. Gabrielli MAC, Hochuli-Vieira E, Paleari AG, Cerri PS, Klüppel LE. Histologic evaluation of hemostatic agents in the rabbit mandible. *Rev Cir Traumatol Buco-Maxilo-fac* 2009;**9:**97–106.
38. Alpaslan C, Alpaslan GH, Oygur T. Tissue reaction to three subcutaneously implanted local hemostatic agents. *Br J Oral Maxillofac Surg* 1997;**35:**129–132.
39. Cotran RS, Kumar V, Collins T. Tissue repair: cellular growth, fibrosis, and wound healing. In: Cotran RS, Kumar V, Collins T, eds. *Pathologic Basis of Disease*. Philadelphia, PA: WB Saunders, 1999:89–112.
40. Abbas AK, Lichtman AH, Pober JS. Antigen processing and presentation to T lymphocytes. In: Abbas SK, Lichtman AH, Pillai S, eds. *Cellular and Molecular Immunology*, 4th edition. Philadelphia: W.B. Saunders Company, 2000: 79–101.
41. Carlson MA, Longaker MT. The fibroblast-populated collagen matrix as a model of wound healing: a review of the evidence. *Wound Rep Reg* 2004;**12:**134–147.