Tomographic evaluation of palatal mucosa thickness in donor areas of connective tissue grafts filled with collagen matrix: a randomized split-mouth clinical study

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Abstract

Aim: The study objective was to evaluate the effect of a collagen matrix (CM) in repairing and maintaining tissue thickness in palatal donor areas.

Materials and methods: Ten patients requiring multiple root coverage were selected and underwent preoperative cone beam computed tomography (CBCT) (t0) to measure the palatal thickness of premolars. Connective tissue grafting (CTG) was obtained bilaterally from this site, using linear incision. The area was filled with collagen matrix (Lyostypt®) (test group) or natural blood clot (control group) and sutured. Another CBCT (t1) was performed 12 weeks later. The t0 and t1 images were overlapped to measure the palatal mucosa thickness, using the distances of 3, 6 and 9mm of the gingival margin as the reference.

Results: The test group presented a smaller difference in palatal thickness between t0 and t1 at 3 and 6mm than the control group ($p<0.05$). In the test group, the differences were similar among the three distances measured ($p>0.05$), whereas the distances of 3 and 6mm in the control group presented a thickness smaller than 9mm ($p=0.02$).

Conclusion: Use of CM in donor areas of palatal connective tissue grafts prevented loss of mucosal thickness, especially in the most critical zones (3 and 6mm).

Keywords: Gingival graft; root coverage; cone beam computed tomography; biomaterial; connective tissue.

Introduction

Subepithelial connective tissue grafting (CTG) is widely used in oral surgeries to maintain the alveolar contour, modify the peri-implant biotype (Akcali *et al*., 2015; Zeltner *et al*., 2017) in peri-implant soft tissue dehiscence (Zuiderveld*et al*., 2018; Frizzera *et al*., 2019), and perform corrective treatment of dyschromia (Elerati *et al*., 2012). However, the most common indication for CTG is root coverage with or without repositioning of flaps for a single tooth or for multiple teeth (Pini-Prato *et al*., 2014; Cairo, 2017; Zucchelli *et al*.,2018). The main advantages of CTG are related to the predictability of the outcomes, especially regarding the soft tissue volume gain, the range of keratinized tissue, and the tissue color compatibility with the surrounding tissues, while risking minimal sequelae to the donor site (Del Pizzo *et al*., 2012; Zuhr *et al*., 2014; Cairo *et al*., 2016; Tonetti *et al*., 2018; Zucchelli *et al*., 2020).

Because more than one access to the same area is usually required, depending on tissue demand, certain strategic maneuvers can be used to gain or preserve the tissue volume in the donor area, with the objective of facilitating future approaches. In the case of insufficient tissue volume, surgical thickening of the palatal mucosa

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has been proposed using interposed lyophilized bovine collagen matrix between the periosteum and the connective tissue, with predictable outcomes (Carnio and Hallmon, 2005, Rocha *et al*., 2012; Carnio and Koutouzis, 2014, Bednarz *et al*., 2016). However, the local effect of these matrices within the palatal flap following excision of connective tissue has been poorly documented in the literature (Yen *et al*. 2016).

Cone beam computed tomography (CBCT) has been proposed as a suitable method for intraoral soft tissue measurement. Shorter exposure and reduced radiation doses have made this technique a safer alternative than conventional computed tomography (CT) scans (Barraviera *et al*., 2007). CBCT studies involving palatal mucosal measurements have provided an accurate assessment of tissue thickness, with values similar to those described in previous reports for conventional measuring methods (Barraviera *et al*., 2007; Yilmaz *et al*., 2015; Gupta *et al*., 2015). These findings have validated CBCT imaging for presurgical planning, especially after the advent of small-volume and high-resolution tomography scanners, which have significantly increased image resolution. This study was devised to address the scarcity of information regarding the use of collagen matrices (CM) to manipulate palatal tissue thickness after CTG surgical procedures, and aims at evaluating the effect of using CM to maintain or gain tissue volume in palatal donor areas using CBCT.

Materials and methods

This study is a single-blind, controlled, randomized, split-mouth clinical trial, and is based on a protocol designed in accordance with the CONSORT checklist. A flowchart of patient screening and inclusion is shown in Figure 1. The study was approved by the Research Ethics Committee (protocol no. 54729116.4.0000.5374/1.791.963), and conducted in accordance with the Helsinki Declaration, revised in 2013. The trial was registered at the Brazilian Clinical Trials Registry under number RBR-9s7wzm. The patients were recruited from two locations, a private dental clinic, and the clinic of the Brazilian Dental Association (ABO), at Mato Grosso. A written consent form was completed for each patient after the participants were given ample explanation of the research. A total of 10 patients were included in the study, and all completed the clinical and tomographic evaluation. The primary outcome of this investigation was the difference in palatal thickness, and the measurement of this thickness between the baseline and 12 weeks later, using CBCT. The sample size was based on a previous study that used similar methodology (Rocha *et al*., 2012).

The selected individuals took an imaging exam using a small-volume CBCT scan (PreXion 3D tomograph, Yoshida Dental, Tokyo, Japan) prior to the surgical procedures. This allowed the donor areas of the palate in the first and second premolars, and the graft-receiving areas, to be registered and measured bilaterally in millimeters. The raw data (DICOM) of the records were exported, reconstructed and saved in the PreXion 3D Viewer software program, thereby creating an initial parameter file entitled initial tomographic record (t0).

The surgical procedures were performed by two surgeons (TBS and MMH) with experience in CTG techniques. A single incision was made to harvest the connective tissue from the palate, needed to obtain the graft, as proposed by Hurzeler and Weng (1999). The surgical technique used to remove the CTG is described in Figure 2. The periosteum of each bone was attached to the segment of graft removed, and thus removed together with the graft. The dimensions of the connective tissue graft (height, length, thickness) were similar on both the left and right side, and among the individuals.

Because this was a split-mouth study, the same patient received both treatment approaches (collagen and blot clot) simultaneously. The side of the palate to receive the control or test treatment was determined randomly by a random list generated using a computer application (Randomizer, Darshan Institute of Engineering & Technology, Rajkot, Gujarat, India), produced before the study began. The randomization was revealed to the surgeons by an external researcher only during the surgical procedure, after the CTG was removed from both sides of the palate.

On the test side, the donor area (where the CTG had been harvested) was filled with CM (Lyostypt®, B. Braun Surgical, Rubí, Spain). The standard dimensions of the CM were approximately twice the volume of the graft removed. On the control side, the donor area was naturally filled with the individual's own blood clot stabilized from local bleeding. The palate was sutured on both sides using a modified anchored mattress suture technique with 5.0 polypropylene thread, as described in Figure 2.

Patients were instructed to use mild mouthwashes of 0.12% chlorhexidine digluconate, and to abstain from mechanical methods of plaque control in the surgical areas during the initial postoperative period (21 days). A pain reliever (sodium dipyrone 500 mg every 6 hours) was prescribed in combination with a non-steroidal anti-inflammatory (ibuprofen 600 mg every 12 hours) for 3 days. The palatal suture was removed after 2 weeks, and the patients were clinically revisited at 4, 8 and 12 weeks.

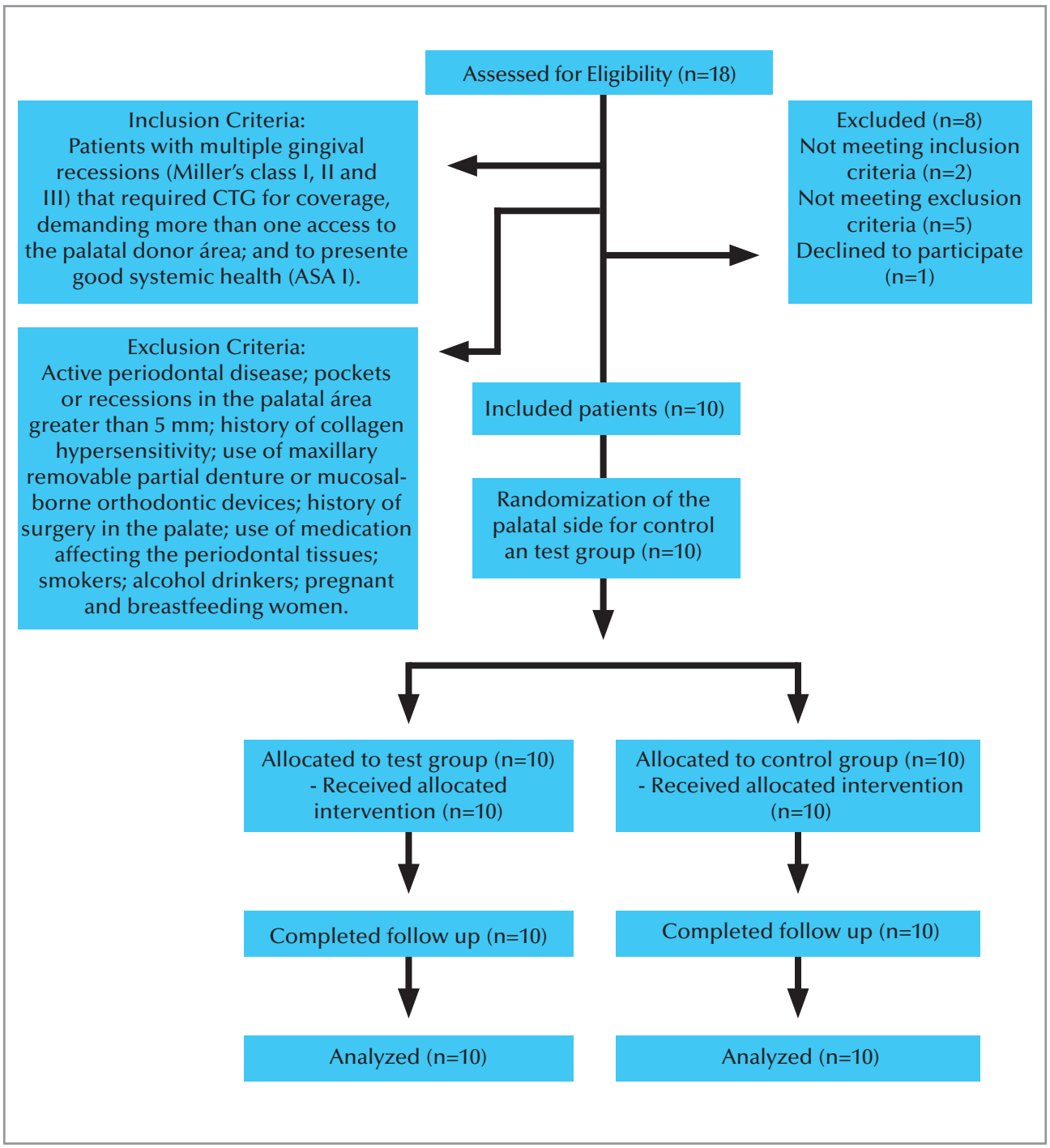

Figure 1. Flowchart of patient screening and inclusion in the study.

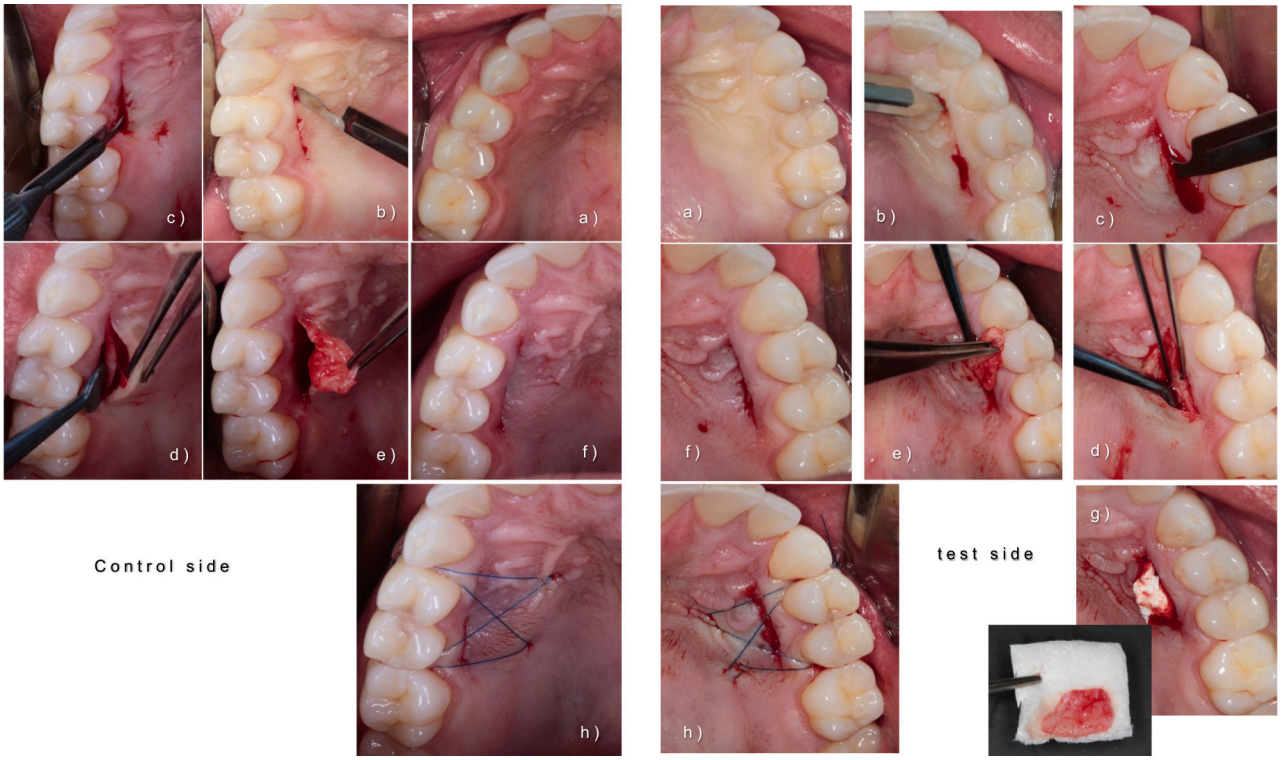

Figure 2. Technical sequence for obtaining the connective tissue graft using the linear incision technique, applied bilaterally, followed by interposition of a collagen matrix on the test side. A) infiltrative anesthesia; B) full horizontal linear incision; C) division of the palatal flap; d) release of connective tissue grafting (CTG) with complementary incisions; D) removal of the CTG; F) clinical appearance after removal of the CTG; G) outline of collagen matrix (CM) dimensions, and placement of CM at the donor site; H) final clinical aspect after suture stabilization.

After 12 weeks postoperatively, the patients were submitted to another CBCT exam following the same parameters of the t0 registration, namely, the raw files of the final tomographic record (t1) were exported (DICOM), rebuilt and saved on an image viewing software program (PreXion 3D Viewer, PreXion, San Mateo, CA, USA).

The t0 and t1 files were merged using a process known as stitching, performed by an image viewing software program. The files of the initial and final images were colored differently, and the registered images were superimposed by making fine overlap adjustments based on coincident maxillary bone anatomical points (Figure 3). The register of the overlap was distinguished by making the t0 image in shades of gray, and the t1 images in colored tones. The PreXion 3D Viewer distance measuring tool was used to determine the linear distance in millimeters of thickness of the palatal mucosa from the edge of the bone to the margin of the mucosa, perpendicular to the cortical bone of the palatal vault in the first and second upper premolars bilaterally. The process entailed demarcating the 3-, 6- and 9-mm points from the palatal gingival margin on both sides at t0, exactly at the center of the first and second premolars (Figure 4). The overlapping images caused the measurements to coincide at the same region in both t0 and t1. This overlap revealed possible differences in tissue volume in the donor areas of the palate, both on the test side and on the control side. Thus, the volume measurements of gain or loss were performed and recorded case by case. The overlaps and measurements were performed by a single, duly trained and calibrated radiologist. The radiologist did not have access to the information regarding which treatment was performed on each side, thus characterizing a single-blind study.

Two-way analysis of variance (ANOVA) for randomized blocks was used to investigate whether the difference in thickness was affected by the collagen matrix, the distance to the gingival margin, and/or the interaction between both variables. The Tukey test was used to perform multiple comparisons. Statistical calculations were carried out on SPSS 23 (SPSS, Chicago, IL, USA), at a significance level of 5%.

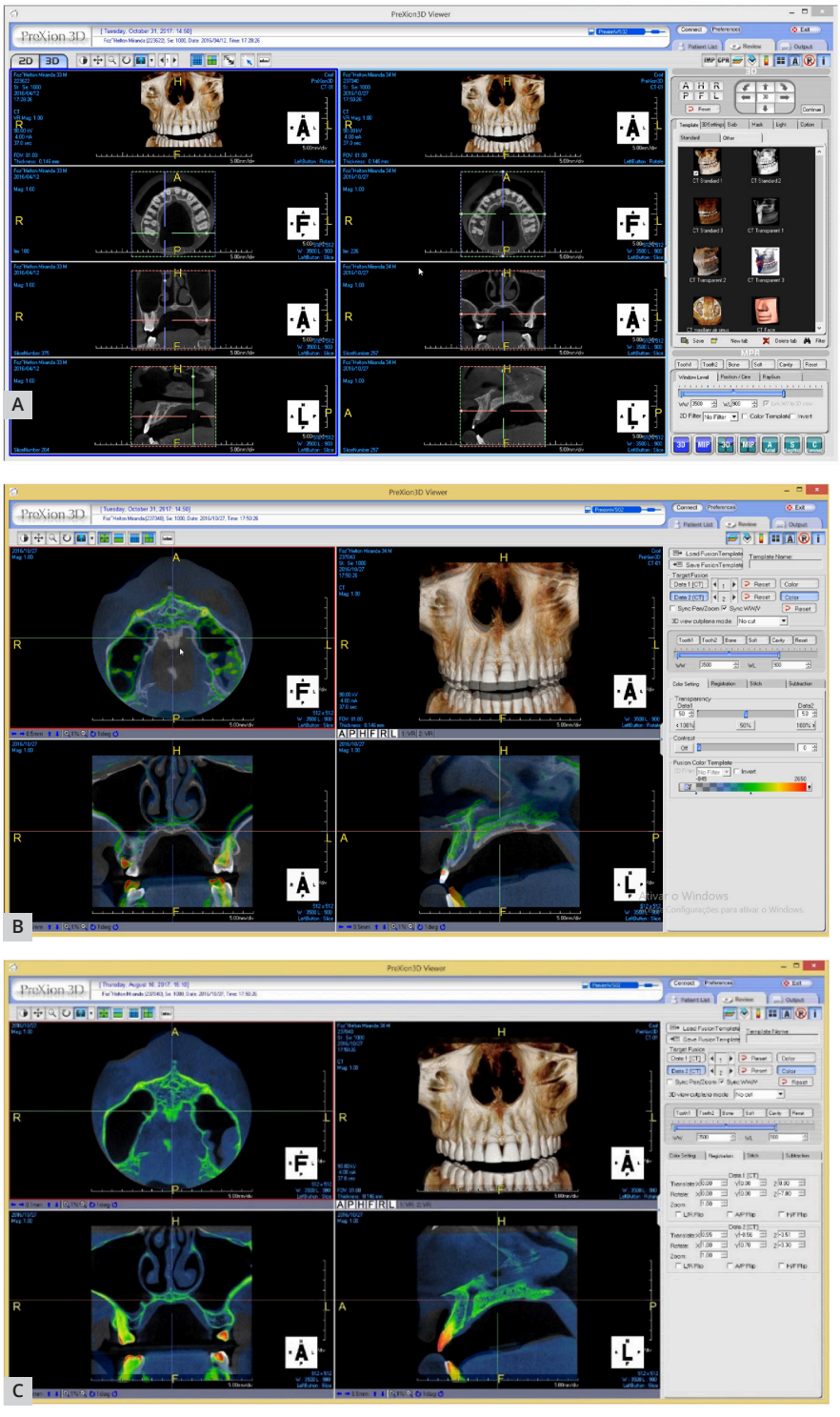

Figure 3. Sequence of overlap of initial and final tomographic images using the PreXion 3D Viewer. A) t0 and t1 side by side on the PreXion 3D Viewer; B) initial stitch of t0 (gray) and t1 (colored) prior to image alignment; C) perfect alignment of the overlap between the initial and final tomographic images.

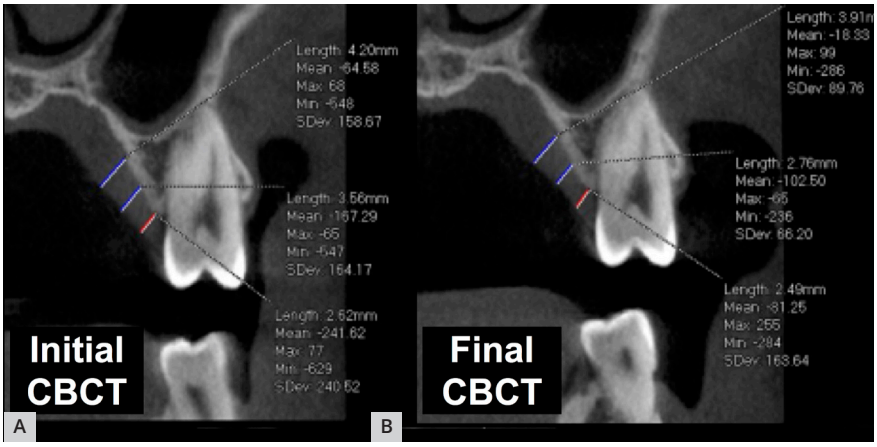

Figure 4. Measurement of palatal mucosa thickness at 3, 6 and 9 mm of the gingival margin using the overlapped CBCT images in the second premolar region after the overlapping performed with PreXion 3D Viewer software. A) Initial CBCT image, B) final CBCT image

Results

Ten patients (7 females and 3 males) aged between 31 and 57 years (mean 41.7 ± 9.47 years) were recruited. The clinical measurements of the palatal mucosa are shown in Table 1. At baseline, no difference was observed in the thickness of the palatal mucosa, between control and test groups, in any of the distances measured ($p>0.05$). The thickness of the palatal mucosa in both groups increased the farther the measurement was taken from the gingival margin. Thus, the mucosal thickness at 9 mm was greater than at 6 mm, and the smallest thickness was observed at 3 mm ($p<0.05$).

From a clinical point of view, the use of a CM ensured easier handling of the palatal wound, promoted hemostatic action, and facilitated the suturing by providing greater stability and support to the surrounding tissues, compared with the control side. The volumetric difference between the sides in the immediate postoperative period was markedly noticeable, with a significant advantage (greater volume) on the test side (Figure 2).

Considering the postoperative clinical evaluations, the CM did not influence the physiological repair process positively or negatively, such as shorter time for complete closure of the palate wound, or normal coloring obtained more quickly than that obtained by the control side (blood clot).

Only 1 patient presented superficial necrosis at the donor area on the control side. After 8 weeks, the surgical scar was nearly normal in terms of coloration and contour patterns. Clinically, at this stage, it was almost impossible to distinguish between the test and the control sides (Figure 5). The two-way ANOVA model for randomized blocks revealed significant interaction between the CM and the distance to the gingival margin ($p=0.02$, with a test power of 91.1%). This model was used to evaluate the interdependent relationship among the measurements performed on the same patients (3 different distances from the margin). The sponge did not always yield better/worse results than the control, and occasionally was even similar to the control, depending on the distance. The interaction was broken down using the Tukey test, and showed that the difference in thickness between t0 and t1 at 3 and 6 mm of the gingival margin was significantly lower ($p<0.05$) when CM was used, and that the palatal mucosa was thicker at 3 mm and 6 mm than at the control side (Table 1). A significantly greater tissue volume was observed in the test group at 3 mm and 6 mm of the gingival margin. On the other hand, no significant volume difference was found between the groups at 9 mm ($p>0.05$).

When CM was used to maintain the palatal mucosa thickness, the difference in thickness between t0 and t1 was similar across the three distances to the gingival margin ($p>0.05$). In contrast, this difference in thickness in the control group was significantly higher at 3 mm and 6 mm than at 9 mm ($p<0.05$). This indicates that a higher volume of tissue was maintained in the region farthest from the gingival margin, and that no significant difference was detected when comparing the tissue thickness at 3 mm and 6 mm.

Table 1. Mean values and standard deviation of the baseline measurements of palatal thickness (t0), and the difference between initial and final thickness (Δ (t0-t1)), in millimeters, for the test and control groups, according to the distance to the gingival margin.

Distance to the gingival margin	Test		Control	
	t0	Δ (t0-t1)	t0	Δ (t0-t1)
3 mm	2.67 (0.83) c	0.39 (0.56) Aa	2.69 (0.78) c	0.56 (0.32) Ab
6 mm	3.75 (0.71) b	0.33 (0.18) Aa	3.77 (0.67) b	0.53 (0.20) Ab
9 mm	4.54 (1.04) a	0.30 (0.31) Aa	4.54 (1.03) a	0.27 (0.28) Ba

Means followed by different uppercase letters indicate a statistically significant difference between the groups, for each distance. Means followed by different lowercase letters indicate a statistically significant difference between distances to the gingival margin, for each group.

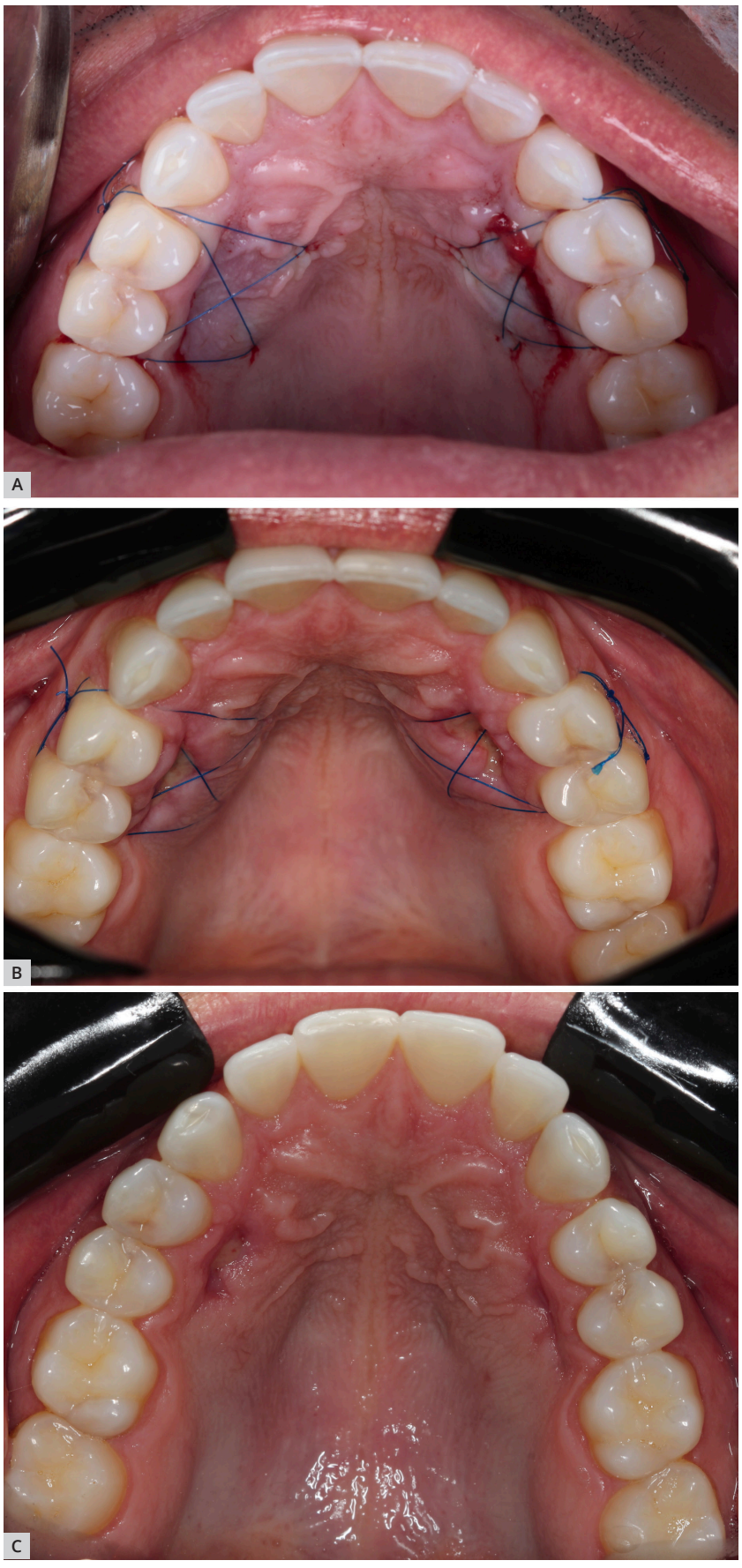

Figure 5. Occlusal view of the test side (left) and control side (right) postoperatively. A) 1 week; B) 4 weeks; C) 8 weeks

Discussion

Regarding gingival recession coverage techniques, recent findings have significantly, repeatedly and definitively reinforced the importance of using CTG for this treatment modality, because of its broad applicability (Tonetti *et al*., 2018). These findings highlight the status quo of CTG as the gold standard treatment for the management of gingival recession, and reinforce the importance of understanding the repair process of the donor areas, especially regarding volume maintenance and tissue recovery time before initiating any future interventions.

In most mucogingival periodontal surgeries, the amount of masticatory palatal mucosa can dictate the treatment plan and affect the surgical outcome. CBCT is a widely accessible method, and allows the palatal thickness to be measured previously, precisely, noninvasively, comfortably, and with safe doses of radiation (Barraviera *et al*., 2009). Therefore, evaluation of palatal thickness is essential to both predicting the maximum graft thickness that can be obtained, and determining the feasibility of obtaining this graft using the linear technique, considering that a minimum thickness of connective tissue must remain under the epithelium to avoid necrosis of the palatal flap. Donor areas with a thickness smaller than 2.5 mm should preferably use other techniques. Seeking to address the CBCT concerns of safety and accuracy suitably, the authors decided to perform another take as the most convenient way of comparing the thickness after repairing the donor areas. Nevertheless, the amount of radiation to which patients are exposed when subjected to a CBCT scan still remains a concern. (Pauwels, 2015).

In this context, studies on CM mostly regard its use to augment soft tissue volume, widths of keratinized mucosa/gingiva and root coverage. Its applications in regard to the donor site are reported less often. CM is known to be effective in cell conduction and angiogenesis, in addition to functioning as a space filler to prevent tissue collapse and favor tissue repair. It provides more comfort and improves the healing process by stimulating collagen neoformation in donor areas after free gingival graft (FGG) (Tavelli *et al*., 2018) . However, the possible benefits of using these matrices in palatal donor areas after CTG are poorly described in the literature, and have mostly been restricted to the report by Yen *et al*. (2007).

In a recent review, Malpartida-Carrillo (2021) evaluated the measurements related to postoperative palatal wound healing after CTG procedures. Most studies on palate wound repair focus on studying varieties of tissue protectors, which speed up the healing process and reduce prolonged pain and bleeding. Several different materials, such as hemostatic agents, growth factors and medicinal plant extracts have been used to investigate the benefits of these materials on the palatal donor site. Studies aimed at dimensional stability are scarce.

The thickness of the palatal mucosa ranges according to different regions within the palate. The initial measurements obtained in the premolar regions at 3 mm, 6 mm and 9 mm of the gingival margin were 2.68 ± 0.81 mm, 3.77 ± 0.66 mm, and 4.56 ± 0.99 mm, respectively. These measurements are comparable to those reported in other CT scan-based studies (Barraviera *et al*., 2007; Yilmaz *et al*., 2015; Gupta *et al*., 2015), as well as studies that used different methods to evaluate gingival thicknesses (Muller *et al*., 2000; Wara-aswapati *et al*., 2001; Yu *et al*., 2014)

In the present study, the volumetric variations observed bilaterally had a constricting healing nature, thus indicating that the matrix was not capable of maintaining or increasing the initial volume after 12 weeks, unlike appositional use of the matrix prior to graft harvesting. The final mean contraction (0.34 mm ± 0.03 mm) was similar to that observed by Yen *et al*. (2007) (0.4 mm). However, when the results between the test and control groups were compared, a significant reduction in thickness loss was observed in the group in which CM was used, mainly at 3 mm and 6 mm from the gingival margin.

Nevertheless, from a statistical standpoint, the lack of difference between the initial (t0) and the postoperative (t1) time, along with the three distances of the gingival margin observed in the test group, clearly suggests that the CM provided a better outcome in tissue volume than the blood clot alone. From the clinical point of view, when the results are analyzed in absolute numbers, the difference between the test and the control sides does not seem very relevant.

The 9th millimeter from the gingival margin was less sensitive to volumetric change in both groups, pointing out that there was no significant difference between the test and the control sides. The probable explanation for the different behavior between the 9th mm and the other distances, regardless of CM use, seems to be related to three circumstances. The first and most obvious is the greater thickness of this region (4.56 mm ± 0.99, versus 2.68 mm ± 0.81 of the 3rd mm and 3.77 mm ± 0.66 of the 6th mm), which guarantees a larger tissue remnant, hence greater cellularity and vascularization. The second is based on the findings of Bertl *et al*. (2015), who showed that the apical portion (6 to 10 mm) of the gingival margin has a fourfold greater percentage of vascular tissue than the marginal portion (from 2 to 6 mm) on the anterior region of the palate (up to the second premolar). Lastly, the third explanation regards the farther distance from the incision margin, which guarantees less mobility and greater tissue stability. Therefore, the triad of cellularity, vascularization, and stability is

a fundamental condition for greater repair potential. Furthermore, based on this knowledge, it can be inferred that the best results obtained at 3 and 6 mm were a consequence of the physical filling of the wound with CM, which provided tissue support with reduced dead space, hence less mobility of the palatal tissue remnants nearest the incision. This control over tissue mobility was clinically visible after performing the suture in all the cases operated on in this study. Another relevant argument is that clot stabilization by CM functions as a scaffold for cell migration, and a source of production of extracellular matrix and connective tissue formation from fibroblasts of higher density, as observed in the histological studies by Yen *et al*. (2007) and Rocha *et al*. (2012). However, it should be borne in mind that this biological process is speculative and requires further investigation using experimental evidence.

Limitations in the construction of precise stitches between the initial and final images, especially regarding the time required to perform this image analysis, and the need to have an experienced radiologist, hindered the large-scale reproducibility of this measurement model. However, systematic clinical evaluations, such as that described in the present study, with objective clinical guidance, are relevant and highly appliable to clinicians. Therefore, the results obtained from this study support the indication of using a CM in palatal donor sites as a beneficial approach for areas with low mucosal thickness, or in areas that require multiple CTG interventions.

Conclusions

The use of collagen matrices as a filling material in palatal connective graft donor areas submitted to a linear incision technique in the premolar region resulted in a beneficial effect for controlling volume loss, especially in the most critical zone, namely at 3 mm and 6 mm of the gingival margin.

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