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Evaluation of the Zone of Keratinized Tissue Using Exposed Acellular Dermal Matrix Over Tooth Extraction Sites: A Randomized Controlled Clinical Trial

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Alveolar ridge resorption is a consequence of tooth loss and makes it difficult to rehabilitate a patient.[1,2] Resorbable membranes have been used to promote bone regeneration.[3,4] However, there are many problems associated with this procedure, such as premature exposure of the membranes to the oral cavity[5] leading to contamination and infection of the surrounding tissues,[6] resulting in less bone formation.[7]

The acellular dermal matrix (ADM) is a lyophilized allograft of human dermis.[8,9] This material has been successfully used as a free graft to increase the width of attached gingiva around teeth and implants,[10,11] for root coverage,[9,12] in the management of soft-tissue ridge deformities[13] and associated with immediate implants.[14] Therefore, the unlimited supply of this membrane and its low

Background: The presence of an adequate zone of keratinized tissue has been associated with implant health. This study evaluated the zone of keratinized tissue using exposed acellular dermal matrix (ADM) over extraction sites.

Material and Methods: Fifteen sites received ADM, and fifteen control sites received no biomaterial. All sites were sutured with no attempt to achieve primary closure. Initial measurements of buccal and lingual keratinized tissue were taken from the mucogingival line (MGL) to the most coronal gingival margins. Final measurements were taken from the buccal MGL to the lingual MGL 90 days after surgery. Gingival biopsies were taken before implant placement.

Results: Test and control groups exhibited a mean value of 4.40 ± 1.45 mm and 1.40 ± 1.40 mm, respectively. The newly formed tissue revealed similar histological aspect of normal keratinized tissue.

Conclusion: Exposed ADM used over tooth extraction sockets can predictably be used to increase the zone of keratinized tissue. (Implant Dent 2015;24:180–184)

Key Words: acellular dermal matrix, guided bone regeneration, keratinized tissue

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degradation, when exposed to the oral environment, make this material a good choice for guided bone regeneration (GBR).[15–18]

Rather than the need of sufficient amount of bone to implants placement, the presence of keratinized tissue is also very important. An adequate zone of keratinized tissue has been associated with improved implant esthetics.[19,20] A recent study showed that patients presenting thin periodontal phenotype had 4.5 times greater probability to present periimplant disease.[21] In addition, a lack of keratinized mucosa surrounding an implant was associated with more plaque accumulation, tissue inflammation, mucosal recession, and loss of attachment.[22]

The aim of this study was to evaluate the increase of the zone of keratinized tissue using exposed ADM over extraction sites.

MATERIALS AND METHODS

This study was performed in compliance with the principles outlined in the Declaration of Helsinki concerning experimentation involving human subjects. Quality assessment was carried out based on the randomized controlled trial (RCT) checklist of the CONSORT statements.[23] All procedures and materials in this study were approved

through the relevant independent committee on the Ethics of Human Research of Fluminense Federal University (CEP/HUAP #068/06), and the volunteer subjects were informed about the study protocol and required to sign a consent form. Thirty patients participated in this RCT, which took place in the Dental Clinical Research Center at Fluminense Federal University, Rio de Janeiro, Brazil.

The control group presented 13 women and 2 men with age ranging from 20 to 45 years (39.53 ± 9.33). The test group presented 9 women and 6 men with ages ranging from 27 to 48 years (40.4 ± 10.75). All patients were in good general health, presenting 30 mandibular posterior teeth with indications for extraction due to root fracture, perforation or periapical lesions, and presenting adjacent teeth. The exclusion criteria were debilitating or systemic diseases, smokers, pregnancy, poor oral hygiene, chronic treatment with any medication known to affect oral status, and bone turnover or any contraindications for surgical treatment.

Patients were given periodontal therapy and oral hygiene instructions to reduce the risk of infection and postsurgical complications.

Surgical Procedures

The volunteer subjects were randomly assigned to the test or control groups using an envelope system distribution provided by the heading investigator. In the test group, 15 extraction sites received ADM (Alloderm LifeCell Corp., Branchburg, NJ). The control group (15 extraction sites) received no biomaterial. The surgical protocol and measurements were the same for both groups. The same surgeon performed all surgeries.

Before tooth extractions, initial measurements (T0) were taken in both test and control groups. A customized acrylic template containing vertical grooves, at the midfacial and midlingual, was used as a fixed reference guide to allow reproducible measurements (Fig. 1). From these reference points, the measurements of the buccal and the lingual keratinized tissues were taken from the MGL to the most

central point of gingival margins, using a periodontal probe (UNC-15; Hu-Friedy Mfg. Inc., Chicago, IL). To evaluate the reliability of this measurement method, before this study, the operator recorded the distance from the MGL to the most coronal gingival margin on 2 different days. These measurements were compared, and the agreement was significant at the level of 0.01.

Tooth extractions were performed under local anesthesia. An intrasulcular incision was made around the teeth to be extracted, extending to the adjacent teeth. Mini full-thickness flaps were reflected, exposing 2 to 3 mm of the adjacent bone (Fig. 2). No vertical releasing incisions were made to keep MGL on its original position. Tooth extractions were performed with care to cause minimal trauma to the remaining bone. If necessary, teeth were sectioned within the socket to preserve the bone walls. All granulation tissues were carefully curetted. The randomization envelope was opened, and the assigned treatment test (ADM) or control (no biomaterial) was revealed to the surgeon.

The matrix was hydrated in saline for 5 minutes on a container and then for more 5 minutes in a second container, as recommended by the manufacturer. The ADM was trimmed and placed over the extraction socket with the epithelial basal lamina facing out and secured under the buccal and palatal flaps. Its biggest extension (20 mm) was placed in the buccal-lingual direction and the smallest extension (10 mm) in the mesial-distal direction. The matrix was kept in position over the extraction site, with its midportion intentionally exposed (Fig. 3). In both control and test groups, sutures were performed with no attempt to achieve primary closure.

Medication and Postoperative Care

Patients were prescribed systemic antibiotic (amoxicillin 500 mg 3 times a day for 7 days after surgery) and analgesics (acetaminophen 750 mg: 6/6 hours for 3 days). Patients were instructed to rinse twice daily with 0.12% chlorhexidine digluconate solution (Periogard; Colgate-Palmolive

Industry and Trade Ltda, São Bernardo do Campo, São Paulo, Brazil) until membrane removal. It strictly prohibited the use of fluoride toothpaste until the end of the treatment, as recommended by the manufacturer. Sutures were removed 1 week after surgery.

Patients were weekly followed up until ADM was clinically indistinguishable from adjacent tissues. Aspects such as color, texture, and presence or absence of signs of inflammation or infection were also observed.

In the test and control groups, 90 days after tooth extractions, patients were reevaluated and final measurements (T1) were taken with the aid of a very thin milimetric ruler placed from the buccal MGL to the lingual MGL, using the reference point. The amount of keratinized tissue after the healing period (90 days) was achieved by the difference between T1 (final measurement) and T0 (initial measurement).

A 5% level of significance and a 95% confidence interval were set for all statistical procedures. The statistical software SPSS for Windows (SPSS 13.0, Chicago, IL) was used throughout. Biopsies (3 mm in diameter) were taken from representative cases at the most central portion of the test areas, before implant placement, for histological analysis.

RESULTS

None of the patients involved in this study reported any unusual pain, halitosis, discomfort, or allergic reactions during the treatment. In the first week, ADM presented with an intense white color (Fig. 4, A). At this time, no attempt to test the stability of the ADM was made. This aspect has changed from the first to the second week (Fig. 4, B). In all cases, between the third and sixth weeks, the exposed portion of the ADM gradually integrated to the host tissues, being no longer identifiable (Fig. 4, C and D).

Although plaque accumulation was observed on surfaces of the intentionally exposed ADM, no signs of tissue inflammation or exudate were
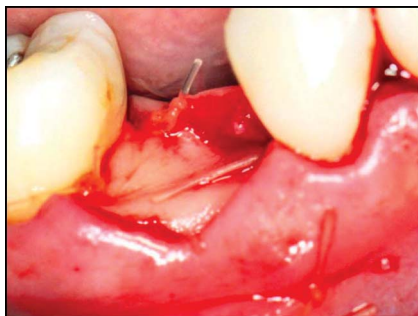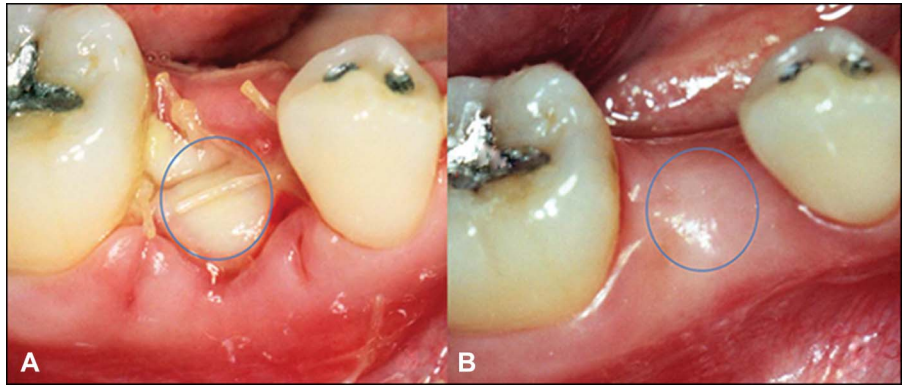
**Fig. 1.** An acrylic template was used as a fixed reference guide to allow reproducible measurements.
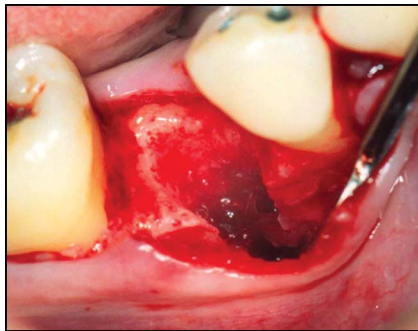
Fig. 2. Extraction socket. Note that no vertical releasing incisions were performed to keep MGL on its original place.

**Fig. 3.** ADM in place. The matrix was kept in position intentionally exposed.

Fig. 5. Ninety days after ADM placement. Clinical signs of normal keratinized tissue can be seen (**B**) in area of previously exposed ADM (**A**).
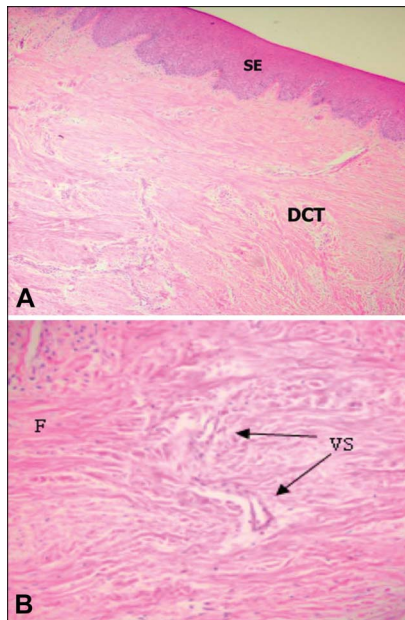

Fig. 6. Histologic Analysis. **A**, Stratified epithelium (SE); dense connective tissue (DCT)—HE ×10 magnification. **B**, Fibers (F); Blood Vessels (VS)—HE ×40 magnification.

detected along the healing period, and no ADM was lost. In all test sites, at the time of the final evaluation (T1), a soft tissue presenting clinical signs of normal keratinized tissue such as color, consistency, and texture was observed (Fig. 5, A and B). Histological analysis of the area where the ADM was left exposed revealed similar aspect of normal keratinized tissue presenting stratified epithelium and dense connective tissue (Fig. 6, A and B).

The amount of keratinized tissue is shown in Figure 7. The negative value presented by the control group shows the loss of keratinized tissue in 1 outlier patient (#22) of control group. Test and control groups had a mean value of 4.40 ± 1.45 mm and 1.40 ± 1.40 mm, respectively. The groups presented normal distribution (Shapiro-Wilk test: Test Group: $P = 0.409$; Control Group $P = 0.68$) and significant difference (*t*-test $P < 0.05$).
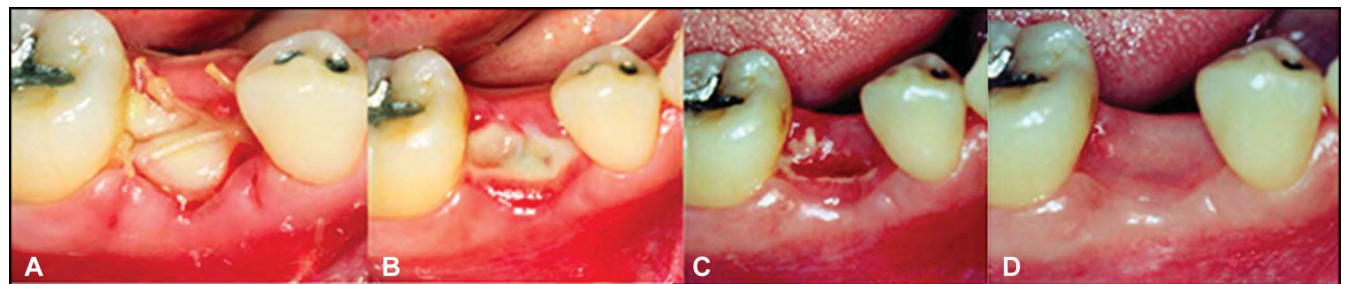
Fig. 4. Follow-up of clinical aspect of ADM. **A**, 1 week; **B**, 2 weeks; **C**, 3 weeks; **D**, 4 weeks. Note the exposed portion of the ADM gradually integrated to the host tissues, being no longer identifiable.
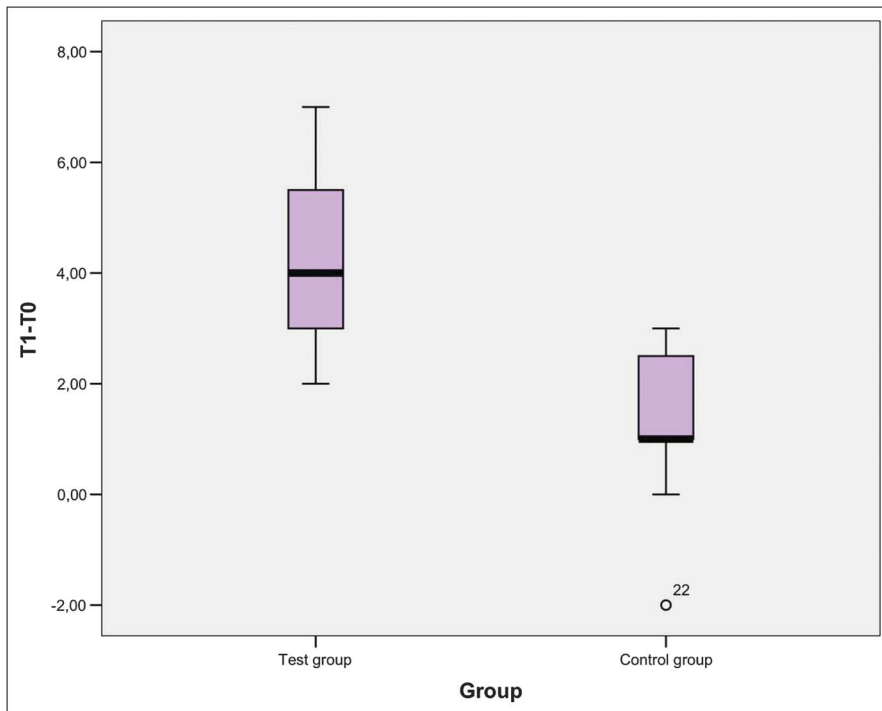
Fig. 7. Boxplot presenting the increase of keratinized tissue (T1-T0) after 90 days in test and control groups (in millimeters).

DISCUSSION

This randomized controlled clinical study evaluated the increase of the zone of keratinized tissue using exposed ADM over extraction sites.

Resorbable and nonresorbable membranes have been used in GBR. However, some problems are associated with this procedure, such as premature exposure of the membranes, bacterial colonization, and consequent contamination, which result in infection and soft-tissue complications.[24,25] Thus, to achieve satisfactory results, these membranes must be completed covered by soft tissue. In an attempt to prevent membrane exposure, vertical and periosteal releasing incisions and large flaps have been recommended. These procedures result in changes of original ridge contours and mucogingival line (MGL) position leading to less keratinized tissue.[26,27] In the present study, no problems related to contamination or infection was observed, similarly to other studies utilizing ADM.[28,29] In addition, the use of ADM without vertical releasing incisions to attempt primary closure, not only preserved the MGL on its original position, but also led to an increase in the zone of keratinized tissue. The adjacent connective and epithelial tissues might act, as sources for new cells to repopulate the intentionally exposed ADM.[28] The teeth selected for this study were mandibular single molars and bicuspids. In these cases the MGL is well defined on both buccal and lingual aspects, so that the MGL could be appropriately measured.

In several case reports on periodontal surgeries, it has also been observed that ADM consistently integrates into the host tissue.[18] It maintains the structural integrity of the tissue and vascularizes through preserved vascular channels.[8,28] In this study, ADM seemed to be incorporated into the host tissues around the fifth week. At this time, in only 2 patients, ADM traces could still be observed with great reduction of exposure. After 6 weeks, ADM were no longer identifiable. These findings are in accordance with studies, in which approximately 4 weeks after surgery, the exposed portion of the ADM was completely covered by soft tissue.[15,30] Another study made by Cummings et al[31] did a histologic evaluation after 6 months of healing and reported that ADM was well incorporated within the recipient tissues, which was also demonstrated in our histologic evaluation 90 days after the surgical procedure.

In this study, data analysis showed that the soft-tissue enhancement was higher in the test group; with a mean 4.40 ± 1.45 mm, while in the control group the mean was 1.40 ± 1.40 mm. The results of this study are in agreement with previous studies that demonstrated that the ADM was able to preserve the ridge thickness with concomitant increase of the zone of keratinized tissue.[28,29] Increasing the zone of keratinized mucosa in preparation for implant placement is a relevant subject for long-term implant health. Randomized controlled clinical trials should further be conducted to also evaluate the changes of hard tissues using exposed ADM over extraction sockets.

CONCLUSION

Exposed ADM used over tooth extraction sockets can predictably be used to increase the zone of keratinized tissue.

DISCLOSURE

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

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