Alveolar regeneration of the post-extraction site with cortical deficiency using the Lamina Socket Sealing technique: Retrospective study with histomorphometric analysis of regenerated bone and evaluation of soft tissue changes. Part 2/2

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Abstract: Purpose: This retrospective observational study evaluated the histomorphometric and soft tissue outcome of a new alveolar ridge preservation (ARP) technique, "Lamina Socket Sealing" (LSS) technique, using a porcine barrier. **Methods:** Patients with maxillary premolars to be extracted and extensive alveolar wall defects were enrolled and treated. Porcine-derived collagenic barriers and mesenchymal membrane were used to seal the extraction socket with alveolar particulate graft. An optical scan of the dental arches was performed with an intraoral scanner (Trios 3, 3Shape) at baseline and at a 4-month follow-up. At the time of implant placement, a bone sample was taken at the implant site with a trephine drill (Hager & Meisinger) and subjected to histomorphometric analysis. **Results:** 36 subjects (21 females and 15 males) were treated. At 4 months, histomorphometric analysis of the bone samples revealed that the percentage of vital bone was 42.87%, 8.75% of residual granules, 30.76% of soft tissue. Linear comparison of the width of the keratinized gingiva showed an increase (mean + SE) of 3.16 ± 0.35 mm. The net volumetric change of soft tissue was (mean + SE) +28.41 ± 19.52 mm^3. (*Am J Dent* 2024;37:9A-12A).

Clinical significance: This alveolar ridge preservation technique (Lamina Socket Sealing) using a resorbable heterologous cortical lamina with a flapless approach has proven effective in maintaining adequate soft tissue and grafting of particulate bone and lamina, with a high percentage of viable bone.

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Introduction

A spontaneously healed socket may show a reduction of 29-63% in width and 11-22% in height at 6 months after extraction, with an average bone loss of 3.87 mm in crestal buccolingual width and 1.67 mm in height on the buccal side.[1]

Horizontal buccal bone resorption was significantly greater in the presence of dehiscence or deficiency with an increased risk of gingival recession for final implant-prosthetic treatment.[2]

Alveolar ridge preservation (ARP) techniques may include the placement of different grafting materials, with or without the use of membranes, to preserve the volume of both hard and soft tissue for future dental implant placement.[3] It has been observed that this technique cannot completely and predictably prevent alveolar bone resorption, with results varying substantially among extraction sites receiving the same therapy.[4,5]

The effect of raising a flap on the healing process of the socket after tooth extraction is still controversial. Results from experimental models report less pronounced bone remodeling of the alveolar ridge after tooth extraction with a flapless approach.[6]

A statistically significant shrinkage in keratinized gingiva width was noted with flapped ridge preservation compared with flapless ridge preservation.[7]

Considering that there is also volumetric reduction of soft tissue and keratinized mucosa,[2] it is important to evaluate and reduce these changes for adequate maintenance of future implant-prosthetic rehabilitations.[8]

Several methods have been introduced to quantify changes in oral tissue volume over time, but the most recent techniques are based on intraoral optical scanning (IOS) systems.[9,10]

This study evaluated the histomorphometric results of regenerated bone and soft tissue changes of a new technique for ARP, "Lamina Socket Sealing" (LSS), which uses a porcine cortical barrier and a resorbable mesenchymal membrane with a particulate socket graft.

Materials and Methods

This study, in terms of setting, population, inclusion and exclusion criteria, and clinical and surgical procedures, is like Part 1 study on the LSS technique previously reported[11] and conducted on subjects treated between February 2019 and October 2022. The Ethics Committee of the Agostino Gemelli University Hospital Foundation IRCCS approved this study (Protocol number 0004468).

The linear width of keratinized tissue was measured from the mid-buccal gingival margin to the basal mucogingival junction at baseline and from the mid-buccal top from the post-extraction ridge to the mucogingival junction after 4 months,[12] having as reference the same horizontal distance from at least one adjacent tooth.

Volumetric analysis - For the volumetric analysis, an optical scan of the dental arches was performed with an intraoral

scanner (Trios 3[a]) at baseline and at the 4-month follow-up for each patient included in the study, according to the Morelli et al study.[10] The data obtained were exported as STL files and imported into an open-source software (Slicer 4.11[b]) for their processing.

For each subject, the digital model obtained at baseline and that obtained at follow-up were superimposed to evaluate the linear and volumetric changes. Through the semi-automatic registration module "surface registration", the models were superimposed. The model obtained from the preoperative scan (T1) was used as the reference model, while the model obtained at follow-up (T2) was the moving model. Before registration, the same reference points were marked on both models (adjacent dental elements, palate) to provide input to the program.

Once the models were superimposed, a manual check was carried out to ensure a perfect match and to correct any discrepancies.

To perform the volumetric measurement, the region on the preoperative model was identified in the mesiodistal between two repeatable retrievals and apical-coronal from the most apical point of the alveolus visible on the model to the free gingival margin. The overlapping models were segmented accordingly.

Once isolation of the region of interest was achieved, the follow-up image was subtracted from the preoperative image to obtain an image showing the net change in ridge volume.

Finally, the segment obtained was analyzed using the "Segment Statistics" module to calculate its volume, thus obtaining a value representing the volumetric difference in soft tissue in the region of interest between the preoperative and postoperative models at 4 months.

During each step of the process, the segments obtained were carefully compared with the original models for case verification. In the final step, they were superimposed on the phantom of the preoperative model for visual analysis of the soft tissue change.

The outcomes of interest were:

1. The volumetric variation measured on models generated by intraoral optical scanning.
2. The linear change in the keratinized gingiva of the extraction site.

Histomorphometric analysis - At the time of implant placement, a 10 mm-deep bone sample was taken at the implant site with a 3 mm inner diameter trephine drill[c] and underwent histological analysis. Histomorphometric analysis was performed by an independent investigator. Bone specimens were fixed in 10% phosphate-buffered formalin, then decalcified in a hydrochloric acid/formic acid solution (4/5%). After decalcification, the specimens were dehydrated in a sequence of alcohol-soaking baths and then incorporated in paraffin. Histological slices of 5 µm thickness were then prepared and stained with hematoxylin-eosin. The slices were digitally scanned at different magnifications, and images of each area were examined with ImageJ[d] image analysis software (public domain software) and LOCI[e] (Laboratory for Optical and Computational Instrumentation). The percentage of newly formed bone, residual graft, and other tissue constituents (bone marrow and/or connective tissue) in each sample was calculated.

Statistical analysis - Qualitative variables were shown as absolute and relative frequencies, whereas continuous data were shown as mean ± standard deviation (standard error for differences).

The alveolus was used as the statistical unit in statistical analysis, and variables were compared between baseline and 4 months later. The significance threshold for Wilcoxon's paired-sample signed ranks test was set at $P< 0.05$, and it was utilized for within-group comparison due to the non-normal distribution (Shapiro-Wilk test) and small sample size. R statistical software[f] was used for the statistical study.

Results

Thirty-six subjects were recruited (age range 39-68 years); 21 females and 15 males, 36 histological bone samples were taken at 4 months.

Of the 36 specimens, histomorphometric analysis was performed on 34 (formalin fixation of two specimens was not congruous) and revealed the representation of different tissues within the histological sections. The percentage of vital bone was 42.87% ± 19.88%, 8.75% ± 6.53% of residual granules, and 30.76% ± 24.93% of soft tissue.

Linear comparison of the width of the keratinized gingiva showed an increase (mean + SE) of 3.16 ± 0.35mm (+142%). Digital models obtained at baseline and the 4-month follow-up of the 36 subjects were analyzed. In each patient, the region of interest of the follow-up model (T2) was subtracted from that of the baseline model (T1) to obtain the net volumetric change, that was (mean + SE) +28.41 ± 19.52 mm^3.

Discussion

The results obtained in this study corroborate those obtained in Part 1 of this study[11] analyzing radiographically the amount of bone preserved and regenerated in the damaged socket. Histomorphometric analysis revealed the quality of regenerated bone in the socket, while soft tissue evaluation demonstrated increased volumes and keratinized gingiva, which are necessary for subsequent implant-prosthetic rehabilitation.

Soft tissue and keratinized tissues - For soft tissues, an increase in keratinized tissues, related to healing by second intention, was noted; the benefits of not having a flap include keeping the mucogingival junction constant and raising the quantity of keratinized gingiva.

The increase found in our study was 142%, measured in the mid-buccal area, in agreement with the results of Barone et al[12] in which there was a linear increase of about 88.5%, correlated to soft tissue healing by second intention.[12] This result is important considering that an adequate amount of keratinized tissue is related to a better esthetic result and maintenance of peri-implant health.[13]

The volumetric change detected in our study was +28.41mm^3. This finding should be interpreted with caution because many factors may influence this value, including possible initial edema affecting alveoli with baseline defects and the influence of underlying hard tissues at 4 months from

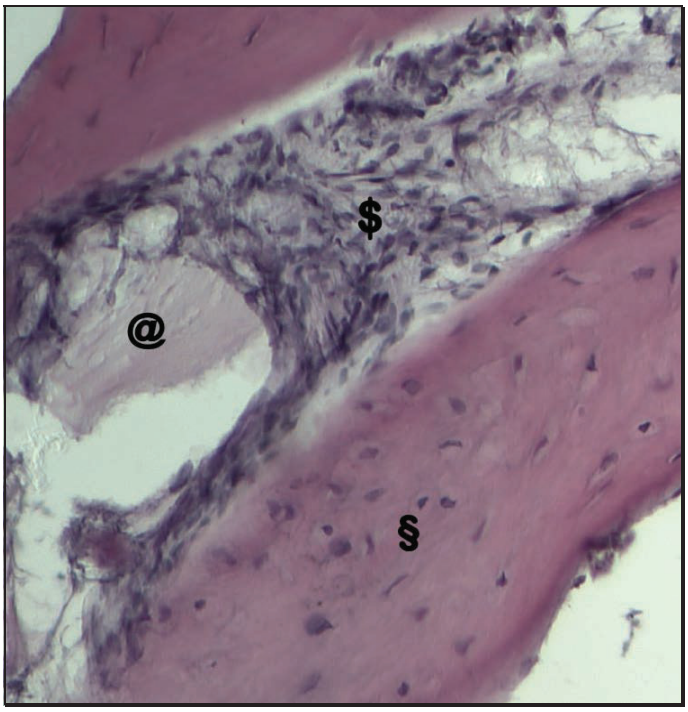

Figure. Histological section of the regenerated bone sample taken at implant insertion. @ residual graft; $ soft tissue; § vital bone; (10×).

the ARP. In addition, the influence of periodontal phenotype has not been investigated.

In the study by Morelli et al[10] comparing two ARP procedures with particulate bovine bone covered with autologous or heterologous collagen, soft tissue volume was decreased (between 68.6 and 87.6 mm^3), but still linearly proportional to hard tissue resorption.

In the cases treated in this study, the LA was covered with a porcine collagen membrane of mesenchymal tissue (OsteoBiol Evolution[g]), although, as suggested by the manufacturer, it could also be used exposed in the oral cavity. This, however, increased the regenerative potential of even the outermost portion of the LA.

The mesenchymal membrane, due to its dense collagen matrix, which lasts at least 8 weeks, protects the graft from infection, allowing healing by second intention.[14] The deliberate exposure of the resorbable membrane did not harm regeneration,[15] but even the rougher outer layer with large pores also allowed the overlying soft tissue to expand.[16]

In addition, the LSS technique does not involve lifting a flap, which, in agreement with the systematic review by Lee et al,[7] appears to preserve bone height, bone width, keratinized gingiva width and lower surgical morbidity.

Histomorphometric analysis - Histomorphometric analysis revealed the representation of different tissues within the histological sections. The percentage of vital bone was 42.87% ± 19.88%, 8.75% ± 6.53% of residual granules, 30.76% ± 24.93% of soft tissue (Figure).

The histomorphometric data in the present study for vital bone (42.87%) are slightly higher than those reported in the review by Corbella et al[17] for porcine bone (range 22.5%-39.6%).

The possible explanation could be related to the greater particle stabilization that can be achieved with the use of OsteoBiol Lamina, as it was not used in the studies analyzed in the review of Corbella et al.[17]

Dried porcine bone OsteoBiol Lamina showed the highest tension values (2.1 MPa) compared to 13 other animal and synthetic membranes.[18] This characteristic together with the slow resorption time (6 months) can guarantee higher stability of the clot from the beginning, like non-resorbable reinforced membranes.

LA is composed of cortical bone of heterologous (porcine) origin produced with a process that avoids the ceramization of hydroxyapatite crystals, thus allowing physiological resorption while maintaining the typical compactness of the bone tissue from which it originates. In addition, this process also preserves collagen, which confers workability in surgical procedures and an osteoconductive property in the healing period. Preservation of collagen appears to increase the proliferation rate of the osteoblasts up to 2/3 time[19] and a strong connection was revealed between pro-osteogenic growth factors expressed on collagen surfaces and bone formation activities within the regeneration area.[20]

Osteoconductive properties of LA were also confirmed by histomorphometric analysis, which revealed the presence of new bone on the porcine bone particle surface. No signs of foreign body reaction were noted, but a well-represented cellular component and a well-vascularized connective, indicated the biocompatibility of OsteoBiol Lamina.

In addition, within the cortical structure of the LA, the vascular vessels of the original bone are maintained, which can be reperfused during integration; therefore, it is preferable to soak it not only with sterile saline but also with blood taken from the surgical site to stimulate reperfusion of the LA.

Cassetta et al[21] compared histologically at 2 months, autologous bone, porcine bone and a 50:50 mixture in sinus augmentation procedures. Histomorphometric analysis revealed comparable results with percentages of newly formed bone of 23.2, 21.6 and 24.5, respectively. Porcine bone, therefore, having a similar response to autologous bone, can replace it, without the need for harvesting.

Within the limitations of this case series, LSS emerges to be a simple procedure that appears to have promising results and LA has properties, confirmed by histological analysis, that should be expected in a regenerative biomaterial: osteoconductivity, induction of neo-angiogenesis, lack of antigenic, teratogenic, or carcinogenic reactions, effective and stable structure, minimized morbidity and complications, hydrophilic properties and simplicity of handling.[22]

These results corroborate the obtained bone volumes after this ARP procedure and comfort for a subsequent appropriate implant-prosthetic rehabilitation. However, they will have to be confirmed in a study, compared with a control group, also considering the possibility of using exclusively LA without heterologous particle grafting.

a. 3Shape, Copenhagen, Denmark.
b. www.slicer.org
c. Hager & Meisinger, Neuss, Germany.
d. NIH-National Institutes of Health, Bethesda, MD, USA.
e. University of Wisconsin, Madison, WI, USA.
f. Foundation for Statistical Computing, Vienna, Austria.
g. Tecnoss, Giaveno, Italy.

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