This study was designed to assess the effect of enamel matrix derivative (Emdogain, Straumann) and alloplastic bone substitute (BoneCeramic, Straumann) on new bone formation in postextraction alveolar sockets. Twenty-one patients requiring anterior single-tooth extractions and subsequent implant placement were recruited and randomly assigned to one of three treatment groups. Postextraction sockets were filled with either an alloplastic bone substitute (BoneCeramic [BC]), BC combined with Emdogain (EMD+BC), or left to heal spontaneously (SO). Histologic and histomorphometric analyses of the results were performed at 6 months postextraction. A significant increase in the percentage of new bone tissue area was found in EMD+BC compared to SO and BC groups. These findings demonstrate that compared with BC or SO, EMD+BC allowed for better formation of new bone in postextraction sockets after 6 months of healing.


As implantology has advanced, there has been a shift in the paradigm of postextraction socket preservation.1,2 Alveolar socket preservation techniques associated with biomaterials have been reported in the literature, aiming to maintain the alveolar ridge volume.3,4 In the present study, a synthetic biocompatible bone filler consisting of 60% hydroxyapatite (HA) and 40% β-tricalcium phosphate (β-TCP) and an enamel matrix protein gel derived from porcine material (90% amelogenin, ameloblastin, 10% amelin) were used. These are proteins derived from the Hertwig epithelial root sheath acting as mediators for periodontal tissue regeneration.5,6 Enamel matrix derivative (EMD) has a significant influence on the behavior of many cell types by mediating cell attachment, spreading, proliferation, and survival, as well as mediating expression of transcription factors, growth factors, cytokines, extracellular matrix constituents, and other molecules involved in the regulation of bone remodeling.7 On the other hand, the combination of insoluble HA with β-TCP is that HA would maintain the space (scaffold function) while the β-TCP would resorb and simultaneously promote bone regeneration. In human controlled trials, BoneCeramic (BC; Straumann) has been found to produce similar amounts of newly formed bone.
formed bone when compared with a bovine xenograft for maxillary sinus grafting and for periodontal regeneration.

Both biomaterials have been frequently applied in periodontal defects but not for postextraction socket preservation.

Results of an animal study suggest that additional benefits may exist when bone filler particles are precoated with EMD, as this combination improved new bone formation at both 4 and 8 weeks postimplantation. These results also support the clinical evidence that using EMD may not be confined to just cementum and periodontal ligament regeneration but can also be applied to bone.

A clinical and histologic evaluation of human gingival recessions treated with a subepithelial connective tissue graft and EMD showed new cementum, evidence of newly formed woven bone, and connective tissue fibers anchored in the new cementum. To date, there has been no report in the literature on the combination of both materials (BC and EMB) in human postextraction sockets assessed histologically. The present work allows the observation of bone tissue remodeling occurring in postextraction sockets, with and without biomaterial placement in humans.

The present study describes and histologically and histomorphometrically compares the nature of the resulting bone tissue after placing BC (BoneCeramic, Straumann) with or without EMD (Emdogain, Straumann).

**Materials and Methods**

Twenty-one patients were selected and agreed to participate in this histologic study. The study was approved by the Ethical Committee of the School of Dentistry, University of Buenos Aires (no. 30/11/2011-7). The applied methodology has already been reported. Simply, patients were randomly assigned to one of three treatment groups (n = 7 patients per group): after an atraumatic extraction, sockets were (1) left to heal spontaneously (SO group); (2) filled with alloplastic bone substitute (BoneCeramic) (BC group); or (3) filled with BoneCeramic and EMD (Emdogain) (EMD+BC group). A lingual or palatal flap was elevated and displaced to achieve primary closure without elevating a vestibular flap.

**Implant Placement and Histologic Sampling**

At 6 months postextraction, the second surgical phase began. The extracted tooth was replaced with the already planned osseointegrated implant.

During implant placement, a bone sample was obtained from each healed socket using a trephine bur of the same diameter and length as the drill used for implant placement, without altering the orientation, diameter, or depth of the preparation needed for implant placement (Fig 1). The collected tissue biopsy sample was processed for the histologic assessment at the Laboratory for the Study of Biomaterials, Department of Oral Pathology, School of Dentistry, University of Buenos Aires. The samples obtained were placed in a 10% buffer formalin solution, radiographed, demineralized with 10% formic acid, and then embedded in paraffin. Subsequently, they were sectioned...
with a cutting microtome, obtaining a sample in the longitudinal direction. Histologic cuts (5 µm thick) were made parallel to the major axis of the sample.

**Histomorphometric Analysis**

Data from the 21 samples were evaluated and divided into the three groups (SO, BC, and EMD+BC). Each group included 7 samples. Each specimen was sectioned along its long axis, and two to four central cuts were provided that were stained with hematoxylin-eosin. In every cut, the areas of trabecular bone and marrow tissue were calculated in percentages. In the BC and EMD+BC specimens, the areas where particulated biomaterial was still present were also evaluated. The percentages of trabecular bone, marrow, and implanted biomaterials were estimated in each section, and an overall percentage for each sample was obtained. Digital photomicrographs were taken with a photomicroscope (Axio Lab.A1, Zeiss) and camera (AxioCam ERc 5s, Zeiss) at ×5/0.12 magnification. Histomorphometric parameters were assessed on these images (fiji/java6-win32 software) and measured with the same magnification.

**Statistical Analysis**

Quantitative data were described as follows: minimum, first quartile (Q1), median, third quartile (Q3), maximum, mean, and standard deviation. In addition, box plots were drawn with the following features: lower and upper ends corresponding to a minimum and a maximum, respectively; the lower and upper borders of the box, corresponding to Q1 and Q3, respectively; and the inner line of the box corresponding to the median and mean values.

The trabecular bone and marrow tissue percentages obtained in the three healing situations assessed (SO, BC, and EMD+BC) were evaluated by applying the Kruskal-Wallis test. A global significant result was found by the test for both percentages. Consequently, post hoc comparisons were made between paired groups. Comparison between the area (%) of particulated biomaterial of BC and EMD+BC groups was done using Wilcoxon Mann-Whitney nonparametric test, as the data did not meet the homogeneity of variance assumption demanded for the parametric tests. The assumption of normality was analyzed through Shapiro-Wilk test with modifications. To analyze the homogeneity of variance assumption, Levene test (three groups) and F test for equality of variances (two groups) were applied. A level of significance of 5% was set for all inference tests. Results were considered significant when $P < .05$.

**Results**

**Histologic Assessment**

The histologic evaluation (Figs 2 to 5) determined the presence of healthy lamellar bone in all the studied groups with osteon formation, and evident lack of inflammatory infiltrate in marrow spaces (Figs 2 and 3). In the BC group (Fig 2b) and EMD+BC group (Fig 2c), negative images of the bone substitute particles were observed. In some areas, there was evidence of bone tissue in close contact with the biomaterial, demonstrating the biocompatibility of the studied substitutes (Figs 4b and 5). Osteoblasts were seen alongside the newly formed bone, and osteoclasts were also present, indicating the remodeling process of the healing socket. It should be noted that in both experimental groups, some samples had particles present at the coronal end, surrounded by fibrogranulomatous tissue with no inflammatory infiltrate (Fig 4a).

**Histomorphometric Analysis**

**Trabecular bone area**

Significant differences in the area (%) of trabecular bone tissue were found. The bone tissue area was greater in the EMD+BC group than the other two treatment modalities ($P < .05$; Table 1).

**Marrow tissue area**

Similar to the trabecular bone area, differences in marrow tissue area (%) were found, with the EMD+BC group depicting significantly less marrow than the other treatments ($P < .05$; Table 2).

**Remaining particulated implant areas**

When the two experimental groups were compared, the mean amount
Fig 2 Representative histologic views of longitudinally sliced tissue samples from healed sockets of the (a) SO, (b) BC, and (c) EMD+BC groups. The presence of healthy bone tissue (triangles) was seen in all groups. Negative images of bone substitute particles (circles) were observed in 2b and 2c. Hematoxylin-eosin stain; original magnification ×5.

Fig 3 Representative histologic view from a tissue sample in the SO group. The evaluation showed the presence of lamellar bone tissue (triangles) and an evident lack of inflammatory infiltrate in bone marrow (diamonds). The arrows identify osteocytes. Hematoxylin-eosin stain; original magnification ×10.
of remaining particulated implant areas (%) showed no significant difference (P > .05; Table 3).

Discussion

The shape and dimensions of the alveolar bone keep a straight relationship with the presence of the teeth. Once a tooth extraction is performed, the alveolar bone is subjected to significant dimensional changes, both vertically and horizontally. The extent of these changes has been studied in animal models\textsuperscript{1,17,18} as well as in humans\textsuperscript{19,20}.

With the aim of preserving the bone resorption process on the vestibular crest and maintaining the original features, alternative treatments have been developed, such as minimally invasive surgical flapless approaches for tooth extraction, immediate implant placement and alveolar preservation techniques using bone fillers, or membrane placement in the postextraction socket. Another proposed method to preserve the original morphology and diminish the bone remodeling of the postextraction sockets is the application of alveolar ridge preservation techniques.

Animal and human studies have been performed to assess the outcomes of ridge preservation after tooth extraction. Araújo et al\textsuperscript{21} and Araújo and Lindhe\textsuperscript{22} conducted experimental studies in dogs; extractions were performed, andsockets were filled with bovine hydroxyapatite. In another study, autologous bone was used\textsuperscript{23}. The xenograft
successfully prevented the buccolingual ridge contraction, while autologous bone did not achieve a significant outcome in preventing dimensional changes of the postextraction socket. Placing biomaterials (including autologous bone grafts, allografts, xenografts, and growth factors) has also been evaluated in human clinical studies.

Although the extensive literature provides strong evidence that EMD enhances periodontal regeneration, the effects on bone formation have primarily been limited to intrabony defects. The specific stimulatory effects of EMD on bone formation in osseous defects have received much less attention. Although EMD has shown some osteo-promotive effects on the early healing phases in rat femur defects, EMD failed to stimulate significant new bone formation in calvaria defects and rabbit tibia defects.

However, a histomorphometric evaluation of the effect of EMD on bone healing achieved with guided bone regeneration (GBR) in the treatment of peri-implant defects in dogs concluded that EMD+GBR may positively influence bone healing, as a greater percentage of new bone area was observed when compared to the control.
A later study suggests that EMD may have the ability to enhance the speed of new bone formation when combined with natural bone mineral particles in rat osseous defects. These findings may provide additional clinical support for the combination of EMD with bone graft for the repair of osseous and periodontal intrabony defects.13

The goal of the present study was to present a descriptive and quantitative histologic evaluation of the area obtained after therapeutic intervention in humans. Histologic evaluation of the samples showed the presence of lamellar-type spongy bone tissue in all groups, with an evident lack of inflammatory infiltrate. When the mean area (%) of bone tissue was compared among SO, BC, and EMD+BC groups, significant differences were found (Kruskal-Wallis test, P < .05). Specifically, the direct comparisons did not detect a significant difference between SO (35.62%) and BC (32.27%) groups (P > .05), but they did show significant differences between SO and EMD+BC groups and between BC and EMD+BC groups (P < .05 for both). The area of regenerated trabecular bone tissue was significantly greater in the EMD+BC group (47.30%).

Conclusions

Within the limits of this randomized, controlled clinical study, it can be concluded that the combination of an alloplastic bone substitute with EMD provides increased new bone formation in the histologic healing of postextraction sockets when compared to the other treatments tested.

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References


