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Early healing after ridge preservation with a new collagen matrix in dog extraction sockets: preliminary observations

C. Cioban[1], Raluca Zăgănescu[2], Alexandra Roman[1], A. Muste[3], F. Beteg[3], R. S. Câmpian[4], Bianca Boşca[5]

[1]Department of Periodontology
[2]Student
"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca
[3]Department of Surgery,
University of Veterinary Medicine and Agriculture Science, Cluj-Napoca
[4]Department of Oral Rehabilitation
[5]Department of Histology
"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca

Abstract

The aim of the present animal study was to investigate the early healing processes developing in the post-extraction sockets preserved with a new-marketed collagen matrix as, to our knowledge, such investigations have not been reported so far. In both quadrants of the mandible of a mongrel dog, the distal sockets of the second premolars served as experimental sites for ridge preservation. The experimental site 1 was protected with a resorbable membrane and then with the collagen matrix. The experimental site 2 was filled with a xenograft and then covered with the collagen matrix. The samples were harvested after one month of healing. In both experimental sites, the bundle bone lining the inner surface of the alveolus was replaced with trabecular bone containing areas of woven bone. A continuous layer of osteoblasts could be observed on the surface of woven bone areas. Osteoclasts encased within resorptive lacunae lined the outer portions of bone walls for the experimental site 1. The trabecular bone occupied only the apical third of the socket in experimental site 1, but it was obviously more abundant in the experimental site 2, occupying also the central compartment of the socket. Moreover, the trabeculae of the bone occupying the inner area of the alveolus were thicker for the experiment site 2 than for experiment site 1, suggesting an increased osseous deposition in the latter situation. Our preliminary results suggest that the association collagen matrix plus xenograft may be a valuable method for ridge preservation.

Keywords: tooth extraction, barrier membrane, wound healing, remodeling.

Introduction

In the majority of the circumstances, post-extraction healing proceeds uneventfully, but the removal of the tooth generally results in some alveolar bone loss [1]. A narrower and shorter ridge can be an expected sequela of the resorptive process resulting in the relocation of the ridge to a more lingual position [2].

Natural healing of post-extraction socket induces a vertical reduction of the ridge of about 1.24±0.11 mm on buccal plate [3]. Percentage changes in vertical dimension are expected to be less than 11–22%, which means that there might be 78–89% bone fill of the original socket height [3]. The amount of horizontal dimensional change is greater than that of the vertical dimension. Horizontal reduction is about 3.79±0.23 mm or 29–63% from the original dimension at six month post-operatory [3].

There is scientific evidence demonstrating the potential benefit of socket preservation therapies, which result in significantly less vertical, and horizontal ridge loss in comparison with natural healing making the prosthetic and implant treatments more predictable.

However, no clear guidelines in regards to the type of biomaterial or technique to be used are provided [4]. A thorough understanding of the resorptive pattern and alterations in bony and mucosal contours following tooth extraction would greatly increase the ability of the practitioners to optimally rehabilitate the patients using prosthetic or implant devices.

A new bilayer pure collagen matrix (Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland) was designed as an alternative to autologous soft tissue grafts. This matrix was considered to be effective in increasing the width of the keratinized gingiva [5, 6] and a viable alternative to connective tissue graft when used associated with coronally advanced flap to cover gingival recessions [4, 7]. Having in view the favorable results reported on the use of this collagen matrix, in realizing this study it was assumed that Mucograft® might provide supplementary protection of the post-extraction remodeling processes and possibly a supplementary space for tissue gain. Consequently, the aim of the present animal study was to investigate the early healing processes developing in the post-

extraction sockets preserved with Mucograft® associated with a resorbable membrane (BioGide®, Geistlich Pharma AG, Wolhusen, Switzerland) or with a bovine xenograft (Bio-Oss Collagen®, Geistlich Pharma AG, Wolhusen, Switzerland) as, to our knowledge, such investigations have not been reported so far.

⊡ Materials and Methods

Design of the study

A mongrel dog in good health, 12-month-old and weighing 11 kg was used in the experiment. Ethical approval was obtained from the Ethical Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy of Cluj-Napoca, Romania (No. 442/2011).

In both quadrants of the mandible, the distal sockets (resulted after the removal of the distal roots) of the second premolars served as experimental sites for ridge preservation.

Surgical procedure

The dog was anesthetized with intravenously administered Ketamine 10% (Propofol®, Fresenius Kabi Austria GMBH, Austria) (3 mg/kg) and maintained with the inhaled anesthetic Isoflurane (Isoflurane®, Lunan Pharmaceutical Group Corporation, Shandong, China) 2% combined with oxygen. Before the onset of the surgery, the crowns of the mandibular second premolars were ground to their cervical third to expose the pulp chamber. The pulp of each mesial roots was removed, the canals prepared with manual instruments No. 15–40 (K-File®, Dentsply Maillefer), rinsed with sodium hypochlorite 1% and filled with gutta-percha (Meta®, MetaDental Co. Ltd., Korea) and a temporary root filling material (Hydrocal®, CERKAMED Dental-Medical Company, Poland).

The entrance of the pulp chamber on the remaining mesial root was restored with glass-ionomer cement (Ketac Molar®, 3M ESPE AG, Germany).

Pocket incisions were made in the first three premolar (P1, P2, P3) regions in both quadrants of the mandible. Buccal and lingual full thickness flaps were elevated to disclose the bone and a periosteal incision was performed in order to mobilize the flap.

The two second premolars were hemisected using a high-speed fissure bur (Edenta AG, Switzerland) (Figure 1A). Thin luxators gentle moved the distal roots in horizontal direction in relation to the long axis of the tooth and then the forceps were used with circular movements without jingling [8] in order to carefully remove the distal roots (Figure 1B).

One experimental site (named experimental site 1) was protected first with BioGide® (30×40 mm), which intimately covered the post-extraction alveolus and then with Mucograft® (20×30mm) (Figure 1C). The other experimental site (named experimental site 2) was filled with Bio-Oss Collagen® and covered with Mucograft® (20×30 mm). The preserved post-extraction sites were completely covered with the mobilized flaps. The flaps were retained with horizontal mattress and interrupted 4-0 resorbable suture (Vicryl®, Ethicon Inc., Johnson & Johnson, USA).
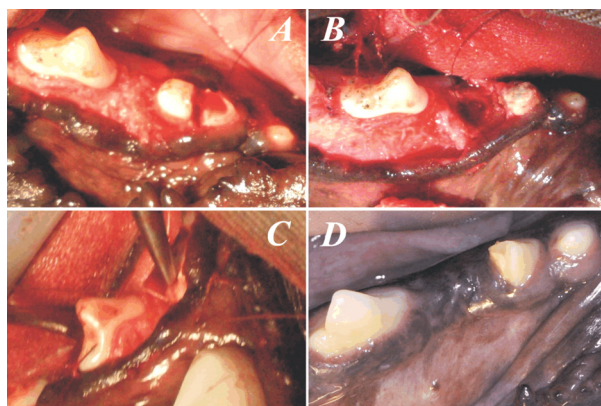
Figure 1 – *Surgical steps of ridge preservation: (A) The hemisected mandibular second premolar; (B) The distal socket of the second premolar; (C) Collagen membrane placement; (D) One month healing of the alveolus.*

After the surgery, the following regimen was administrated: (1) the animal was observed once a day for any clinical abnormality; (2) an antimicrobial prophylaxis used an association of Benzathine Benzyl-penicillin 112.5 mg/mL and Procaine Benzylpenicillin 150 mg/mL (Duphapen®, Wyeth-Lederle Pharma GmbH, Wien, Austria), 1 mL/day for seven days; (3) for post-operative pain control the dog received 1 mL of Sodium Dipyrone (Algocalmin®, Antibiotice, Iassy, Romania) two times/day after the surgery and on the following day; (4) the dog was placed on a soft diet throughout the entire observation period; (5) the dog was placed on a plaque control regimen that included tooth cleaning twice a week.

Termination procedure and sampling

After one month of healing, the animal was euthanatized, with an overdose of Ketamine and perfused, through the carotid arteries, with 300 mL of 10% formaldehyde in phosphate buffer pH 7 [9].

The jaw segments corresponding to the premolar sites that included the mesial root and the distal socket area were dissected using diamond burs and fixed in 10% buffered formalin solution.

For each experimental site, the width of the remaining mesial root was calculated and initiating from the distal border of the root, half of the measurement was calculated to the distal. In this spot, assuming to be centre of the former distal root, a tattoo mark was performed to facilitate the location of the histological section.

Histological analysis

The samples underwent routine histological procedures. Immediately after extraction, the samples were placed in 10% neutral buffered formalin and fixed for 48 hours. The samples were decalcified in 10% nitric acid for 12 days and than dehydrated in progressive concentrations of ethanol (alcohol 80% two times for 30 minutes; alcohol 95% two times for 30 minutes; and alcohol 100% two times for 30 minutes). The samples were embedded in paraffin. Serial sections, 5 μm thick, in bucco-lingual direction were sampled from the central area of the sockets using a Microtom Gm BIT HN 310 (Germany) and were stained with Hematoxylin and

Eosin. The sections were fixed with DPX medium. The sections were examined by one author (BB) by light microscopy (Leica DM 750, Germany) and were photographed with Leica ICC 50 HD (Germany) camera connected to the microscope.

⊟ Results

Clinically, the extraction sockets healed uneventfully (Figure 1D).

In the experimental site 1, the bundle bone lining the inner surface of the alveolus was replaced with trabecular bone containing areas of woven bone. A continuous layer of osteoblasts could be observed on the surface of woven bone areas (Figure 2).

The outer portions of both buccal and lingual bone walls were lined with osteoclasts encased within resorptive lacunae and being more abundant onto the surface of the buccal wall; several osteoblasts were also found on both outer surfaces of the alveolus (Figures 3 and 4).

The apical internal compartment of the socket was occupied by trabeculae of lamellar bone alternating with islands of woven bone spicules (Figures 5 and 6). Large amounts of provisional matrix resided in the central and outer compartment of the socket and they were mainly formed by fibroblasts, irregular distributed collagen fibers and newly formed vessels. Thin trabeculae of lamellar bone surrounded bone marrow areas containing adipocytes. Scattered osteoclasts could be observed on the surface of the trabeculae and were indicative for the remodeling activity of the bone (Figures 5 and 7).

For experimental site 2, the same characteristics of the bundle bone lining the inner surface of the alveolus as for the experimental site 1 were observed. No osteoclasts could be observed on the outer portions of both buccal and lingual bone walls (Figure 8).

The trabecular bone occupying the socket was mainly comprised of the well-developed trabeculae of lamellar bone alternating with small islands of woven bone; large amounts of trabecular bone occupied the apical and central compartments of the socket (Figure 9).

Residual xenograft particles contained in the provisional matrix were observed in the outer and central portion of the socket and they were not connected with the osseous trabeculae. A continuous layer of osteoblasts could be observed on the surface of unresorbed xenograft particles (Figures 10 and 11).
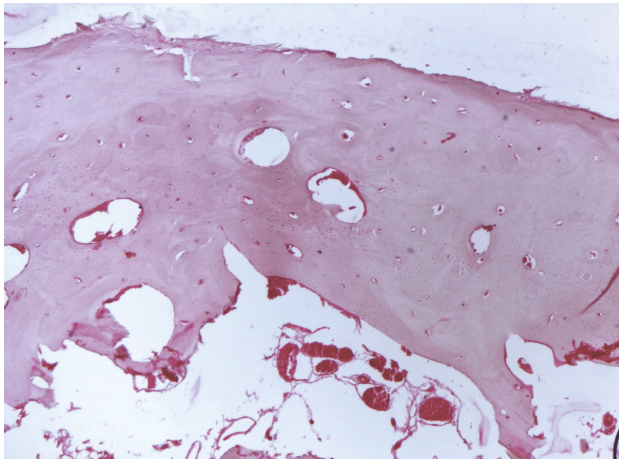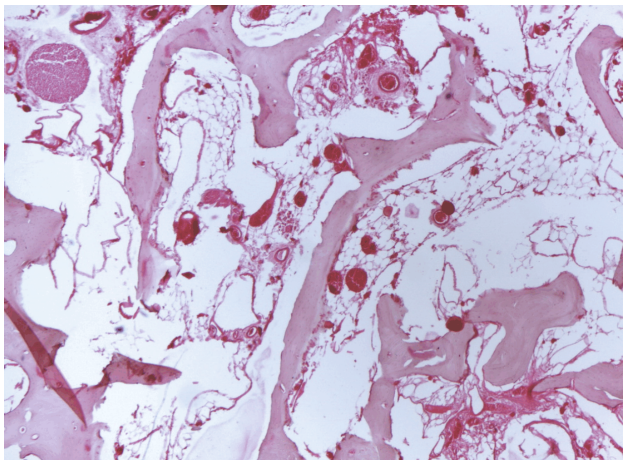

Figure 2 – *Histological feature of the biopsy harvested from the experimental site 1: trabecular bone replaced internal alveolar layer of bundle bone (HE stain, ob. 4×).*
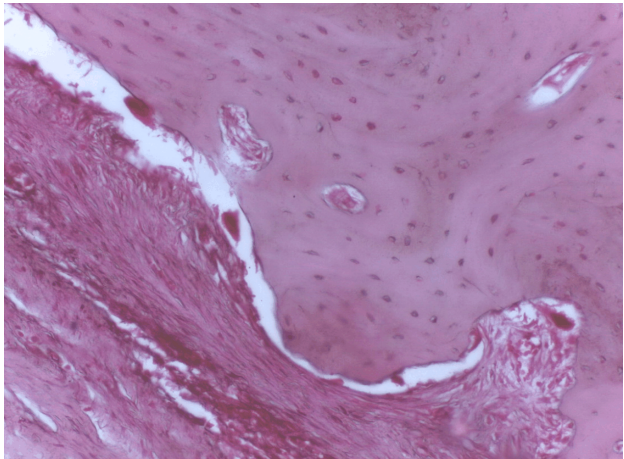
Figure 3 – *Histological feature of the biopsy harvested from the experimental site 1: osteoclasts on the outer surface of the lingual wall (HE stain, ob. 20×).*
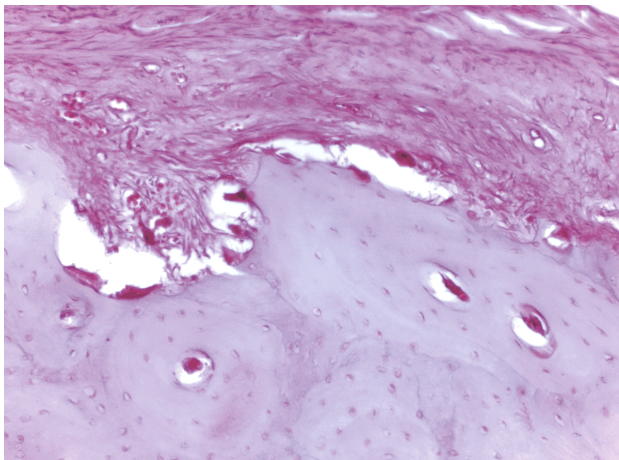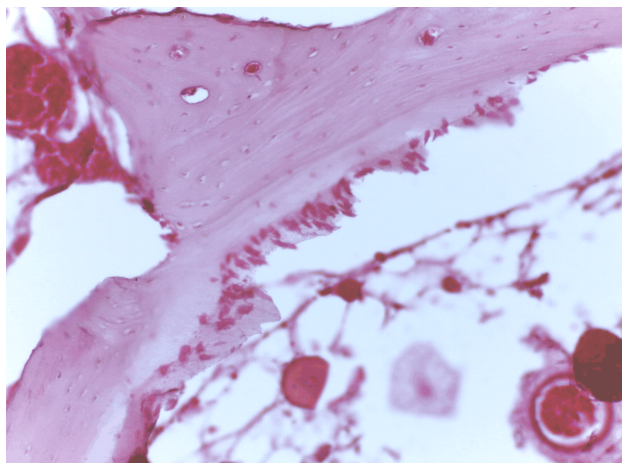

Figure 4 – *Histological feature of the biopsy harvested from the experimental site 1: osteoclasts on the outer surface of the buccal wall (HE stain, ob. 20×).*
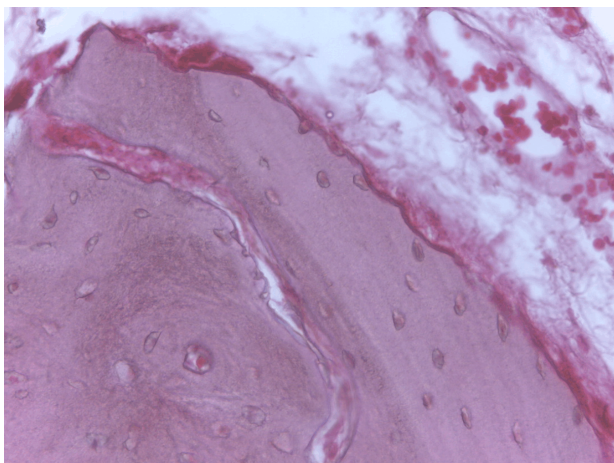

Figure 5 – *Histological feature of the biopsy harvested from the experimental site 1: trabeculae of lamellar and woven bone in the apical part of the socket (HE stain, ob. 4×).*
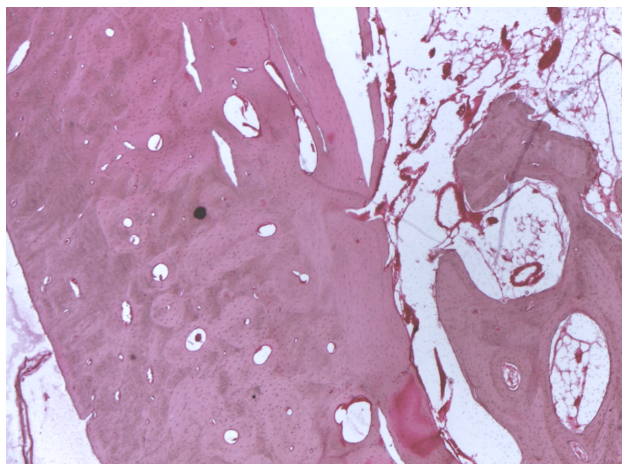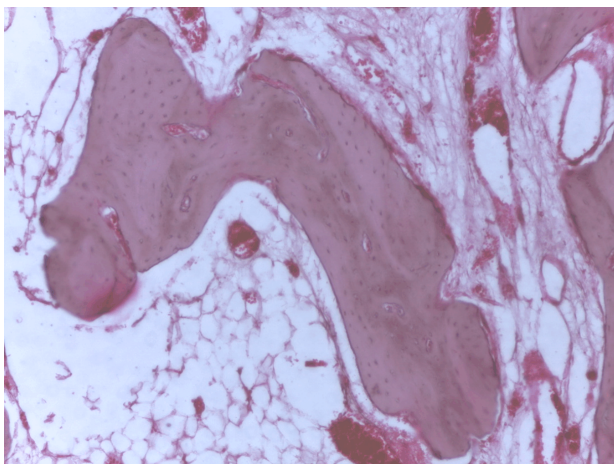
Figure 6 – *Histological feature of the biopsy harvested from the experimental site 1: trabeculae of lamellar and woven bone in the socket and many osteoblasts lining the surface of the woven bone areas (HE stain, ob. 20×).*
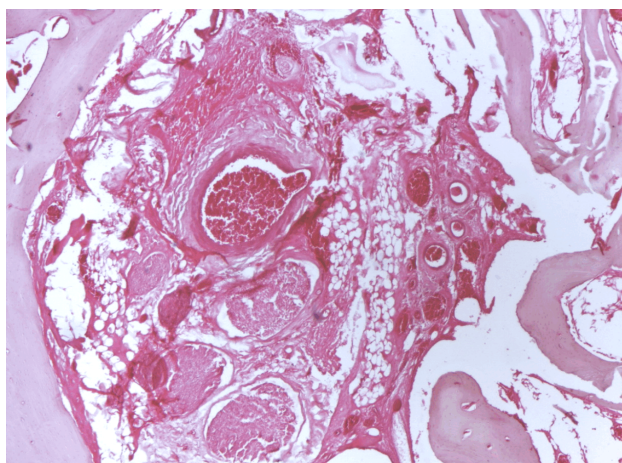

Figure 7 – *Histological feature of the biopsy harvested from the experimental site 1: scattered osteoclasts on the surface of the trabeculae of lamellar bone (HE stain, ob. 40×).*

Figure 8 – *Histological feature of the biopsy harvested from the experimental site 2: overview of the buccal bone wall (HE stain, ob. 4×).*
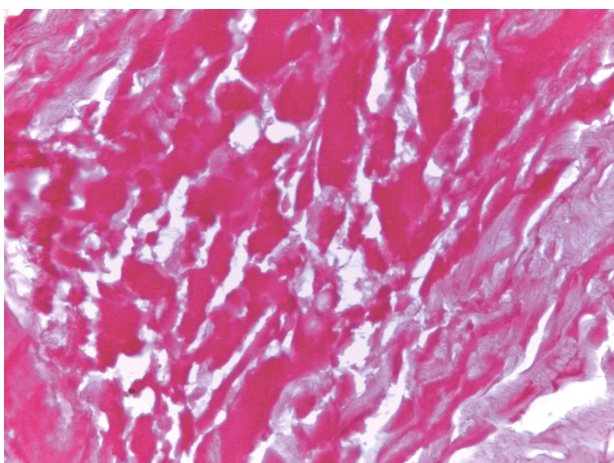

Figure 9 – *Histological feature of the biopsy harvested from the experimental site 2: well-developed osseous trabeculae in the socket surrounded by bone marrow (HE stain, ob. 10×).*

Figure 10 – *Histological feature of the biopsy harvested from the experimental site 2: unresorbed residual graft particles lined by osteoblasts within connective tissue stroma (HE stain, ob. 4×).*

Figure 11 – *Histological feature of the biopsy harvested from the experimental site 2: active osteoblasts on the surface of residual graft particles (HE stain, ob. 40×).*

⊟ Discussion

The present animal study investigated the early remodeling processes taking place in preserved post-extraction sockets with a new-marketed 3D collagen-matrix (Mucograft®) associated with a resorbable membrane (BioGide®) or with a xenograft (Bio-Oss

Collagen[®]). This study is the first part of a complex research investigating the dimensional changes and remodeling processes of post-extraction sockets when the new 3D collagen matrix was used as a ridge preservation technique (Roman *et al.*, unpublished data).

In realizing the surgical protocol for the present study, the actual recommendations of the literature were followed: raising a flap, primary wound closure, and the use of materials with a low resorption and replacement rate [10].

The surgical procedure using a flap elevation approach was chosen because flapped surgical techniques demonstrated a significantly lesser horizontal resorption of the socket, when compared to flapless surgeries [4], probably because of the importance of achieving full closure and first intention healing [4]. However, the insertion of the membranes and the coverage of the alveoli would have been difficult without a flap elevation. In the same time, the extractions were performed with caution in order to preserve both buccal and lingual walls of the alveoli, as it is well known that, their integrity influences the post-extraction remodeling processes [11, 12].

The use of socket preservation therapies could not prevent the post-extraction resorption of the ridge but significantly limited the horizontal and vertical dimensional changes [4, 13]. In our study, the presence of a large number of osteoclasts on both the buccal and lingual bone walls of the socket in the experimental site 1 is thus not surprising. Moreover, the fact that the surface of the lamellar bone was replaced with woven bone is a consequence of the remodeling process in which osteoclasts played an important role. In adult canine model, a daily osteoclastic resorption rate of 50–60 μm was reported [14]. The present study recorded much more abundant osteoclasts on the surface of the buccal wall than on the surface of the oral wall of the socket in experimental site 1, which is in agreement with the current knowledge that the resorptive processes are more pronounced at the level of the alveolar buccal compartment of the ridge [1, 15]. An interesting aspect recorded by the present study was the lack of the osteoclasts on the surface of the socket walls in the experimental site 2.

The present study recorded that after one month of healing the bundle bone lining the inner surface of the alveoli was replaced with trabecular bone alternating with areas of woven bone in both experimental sites. This is in agreement with other studies, which reported that bundle bone inside the socket as well as in the crestal regions was not found in specimens representing 4–8 weeks of healing [16]. After only two weeks following the extraction, most of the bundle bone at the mesial and distal aspects of the extraction sockets has been replaced with woven bone [17]. In humans, a similar remodeling activity was noticed not earlier than six months after tooth removal [1], which might be equivalent to approximately 1–2 months of healing in dog models [18].

The presence of lamellar bone and marrow inside the sockets was in agreement with the results of the other studies [19].

Even if there was a resemblance between the remodeling processes developed in both preserved alveoli, some differences in the composition of the post-extraction sockets were recorded by the present study. Since the trabecular bone occupied only the apical third of the socket in experimental site 1, it was obviously more abundant in the experimental site 2, occupying also the central compartment of the socket. Moreover, the trabeculae of the bone occupying the inner area of the alveolus were thicker for the experiment site 2 than for experiment site 1, suggesting an increased osseous deposition in the latter situation. This may be explained by the particular properties of Bio-Oss Collagen[®], which contains calcium carbonate apatite particles identical to natural human bone minerals that allow the prompt attachment of the osteoblasts [20]. Its porous structure allows Bio-Oss Collagen[®] to be a highly effective osteoconductive grafting material, facilitating vascular ingrowths and osteoblastic cell migration throughout. This is in agreement with the presence of the osteoblasts covering the grafting material noticed by the present study. Our data showed that the presence of the xenograft particles stimulated bone synthesis inside the socket and was far from inhibiting the osteogenesis as other studies had reported [21]. The presence of Bio-Oss Collagen[®] may promote bone healing and compensate, at least partially, for marginal ridge contraction [22].

To conclude with, our study recorded an enhanced osseous synthesis when the post-extraction alveolus was preserved with Mucograft[®] plus Bio-Oss Collagen[®] in comparison with the use of a double membrane layer, which is in agreement with other results demonstrating a more uniform bone structure of the sockets grafted with mineral grafts particles plus membranes [23].

A recent report clarified the mechanisms of incorporation of Bio-Oss Collagen[®] in the host tissue. The xenograft is first trapped in the fibrin network of the coagulum and becomes covered by neutrophilic leukocytes, which are later replaced by osteoclasts. Osteoclasts apparently remove the material from the surface of the graft particles and disappear 1–2 weeks later. Osteoblasts migrate and colonize the surface of the Bio-Oss Collagen[®], and lay down bone mineral in the collagen bundles of the provisional matrix. In a subsequent phase Bio-Oss Collagen[®], particles become osteointegrated [24].

One of the aims of ridge preservation therapies is to generate a good soft and hard tissue volume for the time of implant placement [10]. Our preliminary results suggest that the association Mucograft[®] plus Bio-Oss Collagen[®] may be a valuable method to be used for ridge preservation.

⊟ Conclusions

The combination Mucograft[®] plus Bio-Oss Collagen[®] was associated with an increased osseous deposition in the alveolus in comparison with the use of a double membrane layer. Moreover, less osteoclastic activity was observed in the post-extraction socket preserved with Mucograft[®] plus Bio-Oss Collagen[®].

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Corresponding author
Alexandra Roman, Professor, PhD, Department of Periodontology, "Iuliu Haţieganu" University of Medicine and Pharmacy, 8 Victor Babeş Street, 400012 Cluj-Napoca, Romania; Phone +40722–627 488, Fax +40264–590 287, e-mail: veve_alexandra@yahoo.com