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An evaluation of a collagen-based material osseointegration

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Abstract

The aim of this study is to evaluate the osseointegration of a collagen-based material Alveoprotect, suitable for socket preservations, while preserving the vestibular bone lamella. This material is intended to be used in the field of implantology for smaller bone defects. Thus, 3-mm diameter experimental cavities were carried out on the calvaria and maxilla of 18 laboratory Wistar rats. The cavities were augmented with the collagen fleece Alveoprotect or left unaugmented as a control group and half of the laboratory animals were sacrificed after two months, and the other half after four months in order to evaluate *in vivo* the way in which the inserted material is osseointegrated. The obtained samples were submitted to a histological and immunohistochemical study. The bone defects healing followed the same pattern in all the three groups, but the bone wound healing evolution in the cases augmented with material Alveoprotect offered spectacular results especially in the cavities prepared in the calvaria. On the histological samples, we generally noticed the experimental defects filling with connective tissue with various bone ingrowths from the surrounding bone tissue. The collagen-based material Alveoprotect appeared as a biocompatible augmentation material that can be used successfully for the maintenance of bone volume in the case of small bone defects.

Keywords: collagen, bone graft, histological osseointegration.

Introduction

Osseointegrated implants have revolutionized clinical dentistry and they have an enormous potential in dental prosthodontics [1]. The dental implants are made usually from titanium [2], but there is increasing interest also for ceramic materials [3]. Beside the implant material and the level of the implant surface preparation (machining, sandblasting, acid etching) [4] for a good osseointegration a sufficient bone volume it is mandatory.

Preservation of alveolar dimensions after tooth extraction is crucial to achieve optimal esthetic and functional prosthodontic results. Atraumatic extraction and socket preservation techniques have been introduced to minimize bone resorption after tooth extraction [5]. To prevent loss of the alveolar process following tooth extraction, filling of the extraction socket with various materials has been recommend for quite some years now [6]. To restore alveolar bone loss and support efficient placement of dental implants, many different bone substitute such as autografts, allografts, xenografts, synthetic biomaterials and osteoactive agents have been proposed. Even autografts remain a golden standard, in order to avoid harvesting and thereby eliminating additional surgical procedures and risks, other bone grafting materials and substitutes are alternative filler materials to be used for ridge augmentation [7]. Collagens, being the most abundant proteins in the body, and having suitable properties such as biodegradability, bioabsorbability with low antigenicity, high affinity to water and the ability to interact with cells through integrin recognition, are a very promising candidate as a bone augmentation material [8].

This study aimed to assess the biological integration of a collagen material used in guided bone regeneration techniques after a tooth extraction: Alveoprotect (Bredent, Senden, Germany).

Materials and Methods

For this study, we made three study groups, each of them consisting of six laboratory Wistar rats. On calvaria and maxilla of these animals, 3-mm diameter experimental cavities were carried out. The surgical procedures were performed under general anesthesia using Ketamidor 100 mg/mL 20 IU (0.2 mL) and Xilazyn Bio 2% 0.3 mL within the Laboratory Animal Facility of the University of Medicine and Pharmacy of Craiova, Romania, and the study protocol was approved by the Ethics Committee of our University (No. 101/12.12.2014).

After incision and periosteum elevation, the experimental cavities were carried out using a physiological saline cooled handpiece with a bone round carbide bur with a diameter of 3 mm. The cavities from the first two study groups were then filled with the Alveoprotect material. The third group was the control group to which the experimental cavities were left unaugmented the healing being achieved without an external intervention.

After augmentation, the wound was closed and sutured with 5.0 non-resorbable thread.

Laboratory animals were kept under observation and fed according to the standard diet. The laboratory rats from the first study group and three animals from the control group were sacrificed after two months, and those from the second study group and the other three from the control group after four months in order to perform a histological evaluation of the bone wound healing. The euthanasia of laboratory animals was performed according to the current standards by administration of an overdose of anesthesia. Samples were obtained from calvaria and maxilla bone, which had been cut to adequate sizes using a handpiece to cover both the areas of bone healing and adjacent normal bone.

Finally, the obtained samples were submitted to the classical phases necessary to the histological study. Bone samples were fixed in 10% buffered formalin for two weeks and then decalcified in an ethylenediaminetetraacetic acid (EDTA) solution, which was refreshed at regular intervals. Decalcification was considered complete when the samples reached a rubber consistent. Samples were then dehydrated in increasing degrees of alcohol (50%, 75%, 100%), cleaned with xylene and then embedded in paraffin. The paraffin-embedded samples were serially sliced in cross-sections with 5-µm thickness and mounted on glass slides. The sections were deparaffinized, hydrated and stained with Hematoxylin–Eosin (HE) and Masson's trichrome stainings. Some sections were subjected to the immunohistochemical study, where we used two primary antibodies: CD68 and lectin. The sections were examined with an Olympus CX 20 microscope attached to a camera and a computer.

Results

As an overview of the results of our study, the bone defects healing followed the same pattern in all the three groups, but the bone wound healing evolution in the cases augmented with material Alveoprotect offered spectacular results especially in the cavities prepared in the calvaria. Thus, after two months from the material insertion, the cavities from the calvaria were already occupied by a bony tissue, even if less dense than neighboring structures, while after four months the bone healing tissue had a more dense, homogeneous aspect making difficult to differentiate it from the native adjacent bone tissue. For the cavities prepared in the maxilla, the integration speed of the Alveoprotect material appears to have been reduced than for those prepared in the calvaria. Thus, after two months from the material insertion, we could see the appearance of a dense fibrous connective tissue at the level of the defect created and filled with this material (Figure 1).

On the histological samples, we generally noticed the experimental defects filling with connective tissue with various bone ingrowths from the surrounding bone tissue (Figure 2). The intensity of the inflammatory reparative processes can be observed in the examined defects by assessing the lymphocyte infiltration (Figure 3), as well as the presence of foreign body reaction, manifested by the existence of multinucleated giant cells in a granulomatous-type inflammatory response (Figure 4).

Typical structures of a bone forming process were seen, with many blood vessels, thin bone containing numerous bone cells, a mineralization front with osteoblasts in a loose and cell-rich connective tissue (Figure 5).

However, even the formation of the new bony tissue seems to begin and develop especially at the periphery of the defect, centers of mineralization may be seen also in the center of the defect (Figure 6).

CD68 is a glycoprotein that is highly expressed by human monocytes and tissue macrophages and could be used as a marker of the proliferation and differentiation of osteoblasts and osteoclasts for bone regeneration and remodeling (Figure 7). Most of the immunohistochemical findings of bone remodeling do not correlate with the age of the defect, which indicates a permanent imbalance of bone formation and resorption. Lectin plays multiple roles in the different stages that occur during bone fracture healing: inflammation, reconstruction and remodeling (Figure 8). Blood vessels invasion brings the cells elements for the collagen structure reorganization and replacement with a newly formed tissue.

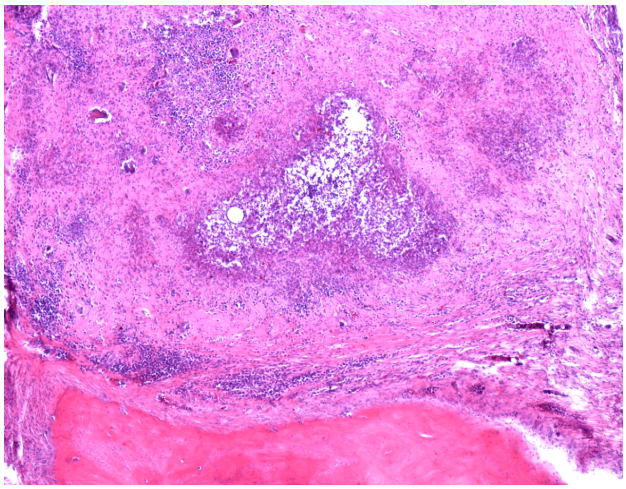

Figure 1 – ***Overview of the bony defect augmented with the Alveoprotect material at two months after the material insertion. HE staining, ×100.***

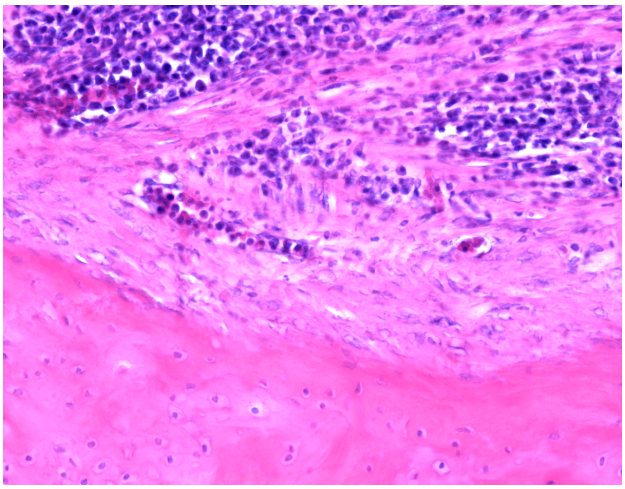

Figure 2 – ***Detail highlighting the young bone tissue rich in cells, the connective tissue with collagen fiber bundles with a specific layout and the rich cellular infiltration. HE staining, ×400.***

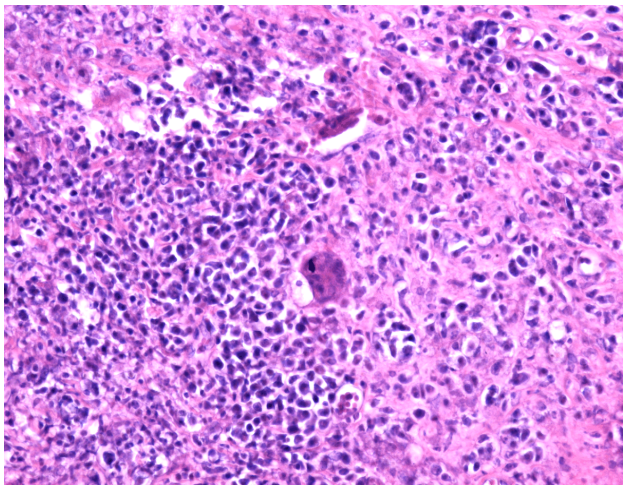

Figure 3 – ***Rich cell infiltrate in the defect filled with Alveoprotect material. HE staining, ×200.***

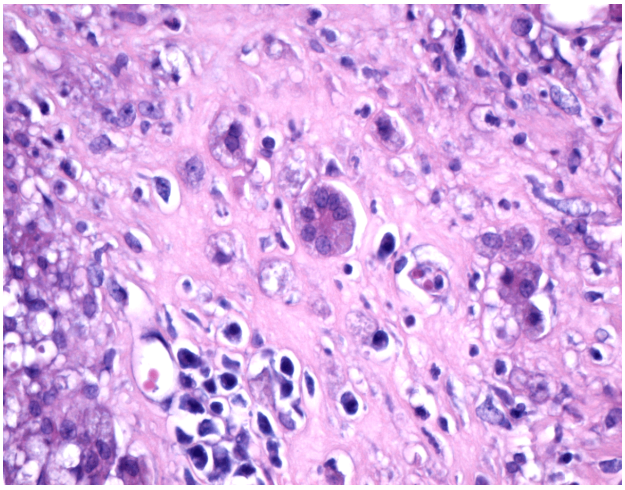

Figure 4 – ***Polynuclear cells showing the intensity of the repairing inflammatory response. HE staining, ×400.***

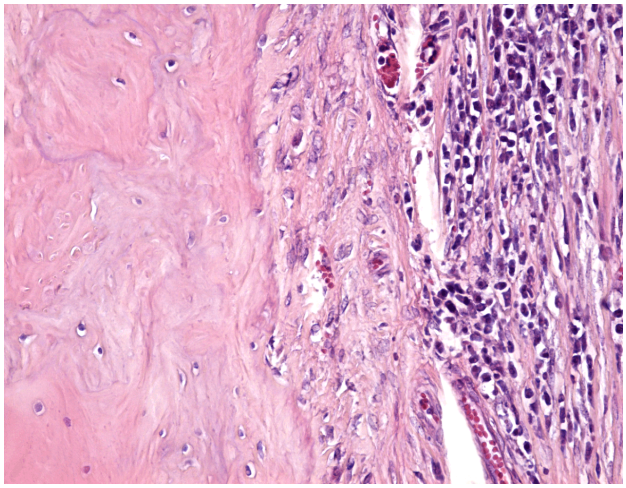

Figure 5 – ***Highlighting the osteoid front departed from the periphery of the bony defect with young bone tissue in course of mineralization and connective tissue rich in cells. HE staining, ×200.***

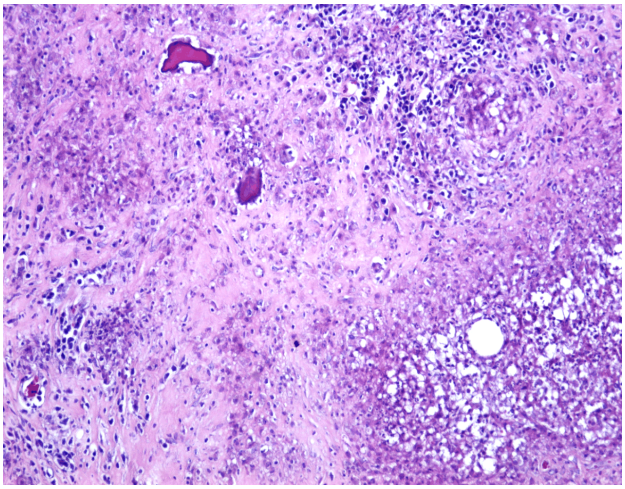

Figure 6 – ***Overview of the defect occupied with repairing connective tissue with cell rich infiltrate areas and ossification centers inside the repairing tissue. HE staining, ×100.***

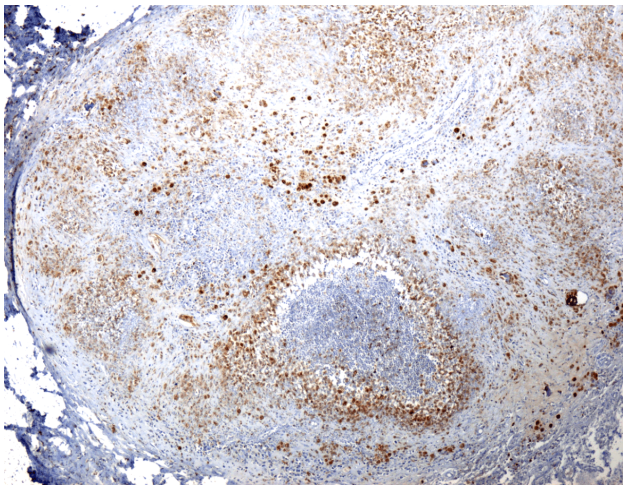

Figure 7 – ***Overview of the CD68 immunohistochemical expression in the bony defect augmented with the Alveoprotect material (×100).***

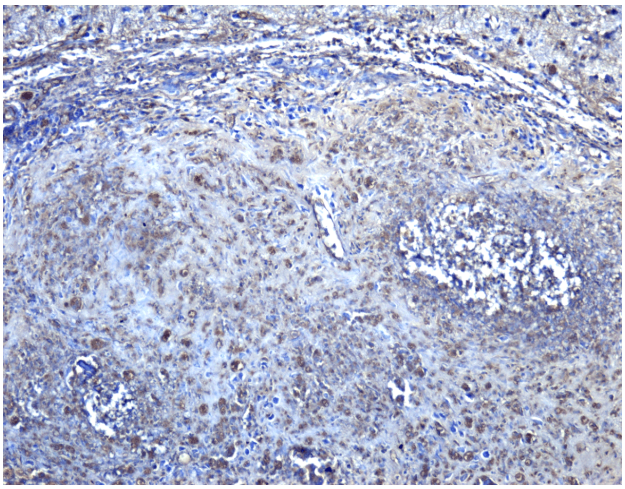

Figure 8 – ***Overview of the lectin immunohistochemical expression in the bony defect augmented with the Alveoprotect material (×200).***

Discussion

Major changes of an extraction site occur even during one year after tooth extraction [9]. Healing of the rat tooth extraction socket occurs rapidly, indicating a mechanism for cancellous bone formation occurring swiftly throughout a matrix. The residual periodontal ligament, with its rich collagen type III fiber content, may form a template for future cancellous bone formation. The pattern of distribution of collagen fibers are similar shows they passed from the bone margin towards the centre of the socket – in the same direction as the forming bone trabeculae. Bone formation occurs by rapid movement of the osteoprogenitor cells along these collagen fibers to allow a rapid healing [10].

The alone implant placement without any augmentation material may fail to preserve the hard tissue dimension of the ridge following tooth extraction. The lingual and especially the buccal bone walls may resorb resulting in some marginal loss of osseointegration. Bone loss at molar sites is more pronounced than at the premolar locations [11]. The autograft materials offer a golden standard, as autologous bone leads to a higher degree of osseointegration of the dental implants placed in this material compared to those placed in alloplastic material, but without a statistically significant difference. However, alloplastic grafts have a lower rate of resorption compared to autologous grafts [12]. More than that, the use of autografts is limited by complications from the receptor site, mainly related to infections and undetectable necrotic areas on initial microscopic examination, which prevent proper incorporation of autografts, but also those of the donor situs [13]. The bone augmentation using material of animal origin proved to be successful in treating massive bone defects in order to insert dental implants [14]. The alloplastic commercial biomaterials have proven their osteoconductive properties, but bone regeneration in this case needed more than four months to be performed. Grafts obtained *in vitro* through tissue engineering using adult stem cell prove also osteogenic properties but this is a laborious and expensive technique [15].

However, in the daily practice the clinicians are often dealing with small post-extraction defects, which may affect the future dental implant osseointegration. Even from 2007, a study used several bone fillers placed, in a randomized fashion, under a membrane: autogenous bone, demineralized freeze-dried bone, inorganic bovine bone, tricalcium phosphate granules, and collagen sponge. Following an eight-month healing period, titanium implants were placed and three months later, all sites demonstrated high percentages (50% to 65%) of bone-to-implant contact, with no significant differences across the various treatment groups [16]. In 2016, an overview of 184 publications noticed that the extent of defect repair and the complication rate were affected by the barrier material used and collagen-based barriers provide a higher defect fill and the lowest percentage of complication rate [17].

In our study, we found good results for the Alveoprotect collagen material with a faster healing speed and a higher degree of bone formation especially for the calvaria defects, compared with the control group. Our results are sustained by a 2011 study, which performed a detailed evaluation of the healing of extraction sockets covered with a resorbable collagen membrane. Using histological evaluation, subtraction radiography, and of micro-computed tomography (μ-CT) analysis, this study demonstrated that adequate bone formation for implant placement occurs as early as 12 weeks following tooth extraction, with insignificant changes in alveolar ridge dimensions [18]. Many investigations reported on the use of products derived from types I and III porcine or bovine collagen. Collagen has been reported to be superior to other materials, due to its active role in coagulum formation. Although these membranes are absorbable, collagen membranes have been demonstrated to prevent epithelial down-growth along the root surfaces in the periodontal defects during the early phase of wound healing [19].

The collagen structure may be used as a support matrix for different biologically active materials, which fasten and improve the new bone tissue formation. Thus, a 2015 study indicated that the alveolar bone of the patients treated with bone matrix gelatin, delivered on an absorbable collagen sponge, compared to a placebo (collagen sponge alone) in human alveolar socket defects had better bone quality and quantity compared to the controls and the bone density and histology revealed no differences between the newly induced and native bone [20]. The combination of recombinant human bone morphogenetic protein-2 (rhBMP-2) delivered on a bioabsorbable collagen sponge had also a striking effect on *de novo* osseous formation for the placement of dental implants [21]. Another 2015 study found a statistically significant bone loss prevented by the collagen alone (22.8%) and even more for the collagen soaked in alendronate group (44.38%), at the end of four months [22]. Moreover, further studies should explore more intimately these mixed structures as a 2016 study underlined that a biomimetic mineralized collagen showed better clinical outcomes in the bone formation for extraction site preservation than an ordinary physically blended hydroxyapatite/collagen [23].

Grafting materials like collagen have a high resorption rates, which allow the formation of a replacing bone tissue with no residual graft particles at the time of implant placement and loading, but their ability to sustain alveolar ridge volume in the long term might be inferior to that of mineralized grafts [24]. To increase the elastic modulus of the collagen sponges incorporation of starch in a 1:4:10 ratio (collagen–HA–starch, by weight) was proposed which also increased the cell proliferation, as well as the blood-clotting capability of the scaffolds [25].

Conclusions

The collagen-based material Alveoprotect appeared as a biocompatible augmentation material that can be used successfully for the maintenance of bone volume in the case of small bone defects. The material did not change the natural histological pattern of the regeneration process, but offered a support for the bone wound healing which enhanced the bone formation speed and it can be used in the guided bone healing process to prevent the bone loss in areas with small bone defects.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

All the parts of this study took place in the University of Medicine and Pharmacy of Craiova. The interventions on the laboratory animals were made within the laboratory animal facility and the histological study was made within the Research Center for Microscopic Morphology and Immunology.

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