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Vertical Bone Augmentation Using Two Bioactive Glasses in a Rabbit Tibia Model: A Comparative Study with Literature Review

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Abstract

Background: Recently, synthetic materials based on bioactive glasses have been in special demand in dental implantology, which, according to the mechanism of their action, are not only osteo-conductors, but also osteo-inductors. Of even greater interest is the fact that the biological degradation of these materials causes alkalization of the area and inhibits the growth of many pathogens. Considering the unique properties of this group of osteoplastic fillers and the problems associated with healing after guided bone regeneration, this study aimed to compare the effectiveness of using bioactive glasses S53P4 and 45S5 when performing vertical bone augmentation in an experiment applying a rabbit tibia model.

Methods and Results: Six adult outbred rabbits aged from 1.5 to 2 years and weighing from 2.5 kg to 3.2 kg were used in the study. A titanium mesh was used to perform a true vertical guided bone regeneration technique. In each animal, two titanium tents were placed: one on the left tibia and one on the right tibia. On the left limb, empty spaces were filled with bioactive synthetic bone filler NovaBone® Morsels (USA); on the right limb, we used a mixture of bioactive glass Bonalive® granules CMF (Finland) and mineralized bone MedPark Bone-D XB (Bovine Xenograft, South Korea) in a ratio of 1:1. The quantity and quality of regenerated tissues were assessed after 8-10 weeks. The NovaBone application has allowed a gain of 2.6±2.67 mm in extra-skeletal hard tissue growth. No signs of guided bone regeneration were observed in all cases of application of an osteoplastic mixture of Bonalive and Bone-D XB granules.

Conclusion: Within the limits of this study, it was found that the use of a mixture of bioactive glass Bonalive (S53P4) with bovine hydroxyapatite in a 1:1 ratio was not effective in vertically guided bone regeneration, but the application of NovaBone (45S5) could promote a new bone formation.(**International Journal of Biomedicine. 2023;13(3):148-153.**)

Keywords: bioactive glasses • vertical bone augmentation • rabbit tibia model

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Introduction

Dental implantation has become a traditional and affordable method of mouth rehabilitation for a large number of patients. One of the main conditions for achieving satisfactory and stable results at the stage of healing and implant loading is considered to be an adequate volume of the alveolar ridge, which is necessary for the correct placement of the infrastructure. Therefore, to avoid serious clinical problems, the existing horizontal and vertical bone deficiency must be replenished using various tissue regeneration techniques.[1-8]

Guided bone regeneration (GBR) is one of the most widespread and studied methods for restoring lost bone. It has been established that the favorable formation of a new bone may occur in the absence of ingrowth of fibrous tissue and epithelium, while the migration of osteogenic cells into the site of a bone defect is maintained.[9-11] Therefore, the increase in the desired volume of bone tissue is difficult to predict due to the significant difference in the rate of osteogenesis and fibrogenesis.

In accordance with many studies, the success rate of the GBR technique depends on a positive coincidence of the following factors: primary wound closure, biocompatibility and bioactivity of a bone graft, volumetric maintenance of augmented part, and negligible connective tissue ingrowth.[9,10] However, from a practical viewpoint, it comes to the quality of materials used, operator skills, and individual response of the patient.

It is believed that osteogenesis, osteo-induction, and osteo-conduction are the three main mechanisms of bone regeneration. To date, none of the known osteoplastic materials works in three directions, except for autogenous bone, which contains osteoblasts and necessary proteins and minerals for bone formation.[12-14] However, the main disadvantages associated with using autologous bone are additional surgery in the donor site, difficulty obtaining a sufficient amount of graft, morbidity, high resorption rate, and, not infrequently, the poor quality of transplant tissue.[15-18] In this regard, the development of new materials for predictable bone regeneration when applying a simultaneous or staged approach of implant placement in an augmented alveolar ridge remains relevant.

Recently, synthetic bone grafts based on bioactive glasses have been in special demand in implant dentistry. In terms of mechanism of action, they are both osteo-conductors and osteo-inductors. Among the other unique properties of these bone substitutes is a multi-component chemical composition, which can be modified by adding certain oxides to the basic formula to impart distinctive properties to the graft. Also, these substitutes have the ability to form a strong bond with bone and chemical type of resorption without the participation of osteoclasts. A beneficial bacteriostatic feature of bioactive glasses is due to the increased pH at time of interaction with tissue fluid.[19-22]

The basic formula of any bioactive glass is mainly presented by oxides of SiO_2, Na_2O, CaO, and P_2O_5 in various proportions. This composition is considered traditional and is typical for the first generation of grafts, such as 45S5 and S53P4.[23]

Considering the significant advantages of bioactive glasses compared to other synthetic bone substitutes, since their discovery by Larry Hench, many experimental and clinical studies have been carried out to identify the opportunities for their field application.[21-23]

A review of scientific databases revealed a lot of knowledge on bioactive materials with the 45S5 composition at the preclinical and clinical stages of application. According to the results of several observations, they contributed to a significant increase in the volume of the alveolar ridge when using the GBR technique despite minor postoperative complications.[21,22]

Primary wound closure is among the main prerequisites for the successful outcome of bone grafting in the case of alveolar ridge augmentation. However, the technical sensitivity of surgery, along with the low regenerative capacity of injured soft tissues and the patient›s morbidity at the early stages of the healing period, may be the reason for wound dehiscence followed by suppuration and loss of the augmented site. That is why using bioactive bone grafts with pronounced antibacterial properties could help reduce the risk of infectious complications.[24-28]

Previously, it was found that bioactive osteoplastic material S53P4 may inhibit the growth of many aerobic and anaerobic pathogenic microorganisms and can integrate with living tissues after implantation through stimulation of a new bone formation.[29-32]

This study aimed to compare the effectiveness of using bioactive glasses S53P4 and 45S5 when performing vertical bone augmentation in an experiment applying a rabbit tibia model.

Materials and Methods

Six adult outbred rabbits aged from 1.5 to 2 years and weighing from 2.5 kg to 3.2 kg were used in the study. Before and after surgery, the rabbits were kept separately in a room for experimental animals where the required temperature and humidity were maintained. The animals were fed with a standard laboratory diet.

All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals (Laboratory Animal Resources Institute, 1996). The aim and methods of the research did not require the euthanasia of the animals at the end of the experiment. The study protocol was reviewed and approved by the Ethics Committee of the Tashkent State Dental Institute.

A titanium mesh (Oss Builder, Tutanium Membrane OB3 Vertical 20[BW]×11[BL]×11[LL], Osstem Implant, Korea) was used to perform a true vertical guided bone regeneration technique. It was trimmed and bent in a standard manner to get the desired fit in each case. The shape of the experimental titanium tent resembled a rectangular basket (dimensions 10-11[L]×5-6[W]×6-7[H] mm), which was fixed on the inner surface of the tibia upside down using four titanium screws (1-1.2/3-4 mm, CONMET) (Fig. 1).

Therefore, in each animal, two titanium tents were placed: one on the left tibia and one on the right tibia. On the left limb, empty spaces were filled with bioactive synthetic bone filler NovaBone® Morsels (USA) (Fig. 2); on the right limb, we used a mixture of bioactive glass Bonalive® granules CMF (Finland) and mineralized bone MedPark Bone-D XB (Bovine Xenograft, South Korea) in a ratio of 1:1 (Fig. 3a, b).

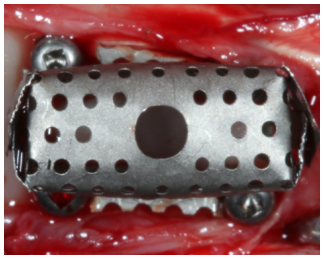

Fig. 1. *Titanium tent mounted on the inner surface of rabbit tibia.*

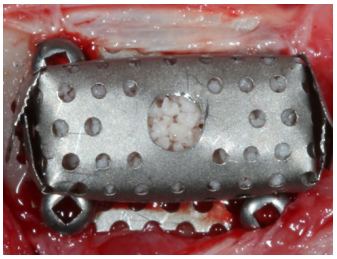

Fig. 2. *Titanium tent filled with NovaBone granules.*

Fig. 3a. *Mixture of Bonalive and Bone-D XB granules.*

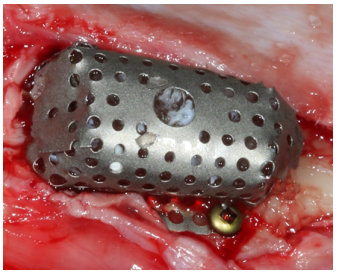

Fig. 3b. *Augmented site filled with a mixture of Bonalive and Bone-D XB granules.*

Surgery was conducted under general intravenous anesthesia with 1% etaminal sodium solution in a dosage of 3ml/kg of body weight through the ear vein. The limbs

around the site of surgery were shaved and disinfected with a 5% alcohol solution of iodine. Soft tissues were additionally infiltrated with 2-4 ml of 2% lidocaine hydrochloride solution before making a full-thickness incision.

The median incision was made longitudinally to the axis of the limb on the inner surface of the tibia down the knee joint by 1-1.5cm. The incision length in each case varied between 4 and 5cm. After the skin-periosteal flaps were elevated and mobilized, a flat cortical surface of the tibia was exposed, and the titanium tent was securely screw retained. Augmented spaces were filled with osteoplastic materials in accordance with the study protocol. Flaps were repositioned and wounds closed in each case with interrupted sutures using absorbent suture material (Vicryl 4/0).

Reentry surgery was performed after 8-10 weeks. A trephine drill with an outer diameter of 5 mm was used to collect hard tissue samples from the augmented site. A biopsy was made under constant irrigation with saline solution at a handpiece rotation speed of 1200 rpm. The height of the obtained bone cylinders was measured in millimeters. The bone gains up from the cortex border were considered. For each sample, four measurements were made (Fig. 4 a, b). After that, tissue samples were fixed in a 10% neutral buffered formalin solution followed by decalcification, washing, and drying. Samples were then embedded in paraffin in accordance with the standard procedure. Sections of 4-6μm thickness were prepared from paraffin blocks and stained with hematoxylin and eosin. Light microscopy of tissues was performed using a Leitz HM-LUX microscope (Germany).

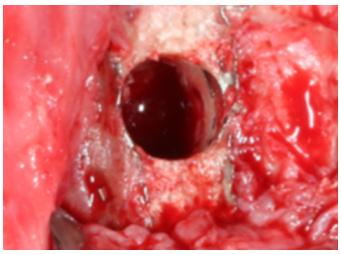

Fig. 4a. *Bone defect after tissue sampling from the augmented site of the tibia (NovaBone).*

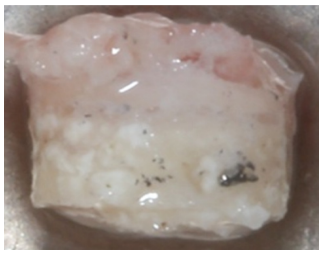

Fig. 4b. *A tissue sample taken from the augmented site of rabbit tibia (NovaBone).*

Statistical analysis was performed using StatSoft Statistica v6.0. For descriptive analysis, results are presented as mean±standard deviation (SD). The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P<0.05$ was considered statistically significant.

Results

At the time of reentry surgery, the general health condition of the animals was assessed as normal. In cases of using NovaBone, skin palpation over the augmented site of the tibia had no signs of pathology. In all animals, protruding portions of the left limbs were dense and immobile. However, examination of augmented sites on opposite limbs revealed fluctuation, and cold abscesses were diagnosed in three out of six cases. After drainage of cysts, the altered and granulomatous tissues were excised along with the titanium membranes, and wounds were thoroughly washed with a 0.05% chlorhexidine solution. No signs of guided bone regeneration were observed. In the remaining three cases of application of an osteoplastic mixture of Bonalive and Bone-D XB granules, there were fibrous and granulomatous tissues noted, nor was there any visual or histological evidence of a new bone formation. At the same time, vertically guided bone regeneration performed on the tibia was positive in all six animals after application of NovaBone bioactive granules (Fig. 5a). Despite no precedents of a complete graft maturation into a dense mineralized tissue, the average new bone gain was 2.6±2.67mm (Fig. 5b).

Microscopic examination of stained sections showed the presence of mature, viable bone tissue with a uniform distribution of osteocytes (Fig. 6a). Some sections showed residual biodegradable fragments of NovaBone granules surrounded by osteoid and woven bone (Fig. 6b).

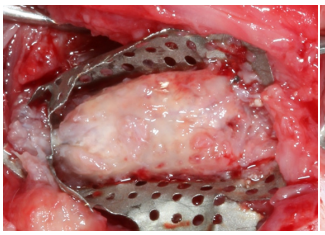

Fig. 5a. *Augmented portion of rabbit tibia (NovaBone).*

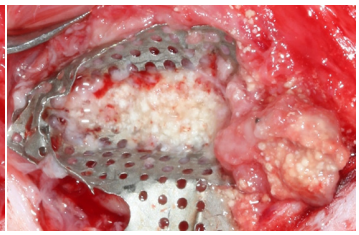

Fig. 5b. *Regenerated bone and graft particles in granulomatous tissue (NovaBone).*

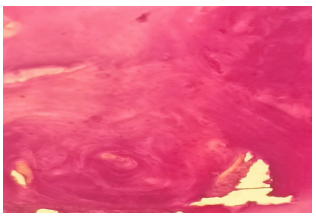

Fig. 6a. *Histological slice taken from the augmented site (H&E, ×40): non-mineralized osteoid and newly formed bone (NovaBone).*

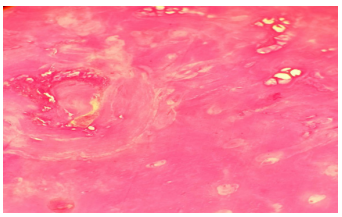

Fig. 6b. *Histological slice taken from the augmented site (H&E, ×20): fragments of graft surrounded by the osteoid matrix and woven bone (NovaBone).*

Discussion

The present study tested an extra-skeletal guided bone regeneration technique using a rabbit tibia model to assess the osteo-promotive capacity of applied bioactive glasses. Despite the difference in the mechanisms of ossification during embryonic development, the geometry of rabbit tibia and atrophied jawbone is very similar, which allows us to simulate a complicated clinical situation corresponding to the third class of alveolar ridge defects according to the Sibert classification.[33]

The main source of blood supply to the augmented site of the alveolar ridge is the residual bone. The surrounding soft tissues also take part in transporting nutrients and growth factors, but to a much lesser extent. The latter are associated

with periosteal trauma during flap elevation and mobilization, the presence of a barrier membrane, and soft tissue edema at the initial stages of healing.[34-36]

Thus, the probability of incomplete bone graft remodeling into viable hard tissue during guided bone regeneration remains significantly high. According to the results of most studies, the amount of new bone gain ranges from 30%-70% of the total volume of the graft.[37-40]

In the present study, the NovaBone application has allowed a gain of 2.6±2.67 mm in extra-skeletal hard tissue growth. Generation of new bone was mainly noted around fixing screws and along the lower border of the titanium membrane. The unreacted portion of the osteoplastic graft was replaced by granulomatous and granulation tissues containing remnants of filler particles. A similar type of regeneration was noted earlier in other studies.[38-40]

On the other hand, the necessary gain in bone volume also depends on the type of membrane. It maintains the space for directed bone tissue growth despite using the particulate graft. For this purpose, commercially available titanium membranes were used in the present study. They were trimmed and bent into a three-dimensional structure resembling an inverted basket. It was retained with the help of four titanium screws on a flat surface of the tibia and filled with bone graft substitute.[40-41] Additional holes in the tibia cortex to induce bleeding from the marrow space were not made.

Titanium meshes are non-resorbable membranes and are widely used in oral and reconstructive surgery. They serve to increase the volume of the alveolar ridge as well. The most common titanium meshes are perforated and have a thickness of more than 100 μm, which gives sufficient rigidity to the structure and makes it possible to protect the internal space from external mechanical impact.[15,16,41,42]

The issue of perforation size in titanium membranes remains disputable. According to some studies, the presence of the 40-60 μm pores promotes the free transport of nutrients to the augmented site but does not prevent the ingrowth of fibrous tissue. Other data indicate the optimal perforation size of 20 μm because it still facilitates a free diffusion of tissue fluid and cell migration while obstructing connective tissue and epithelium invasion. Experimental studies have also been conducted on non-perforated hemispherical titanium caps, which have been used for vertical bone regeneration. Despite the paucity of data, most of these studies concluded in favor of non-perforated membranes due to the more significant growth of hard tissue.[42,43]

In the present study, titanium membranes of 0.3 mm thickness with a pore diameter of 0.5–1 mm were used. During the entire observation period, there was no incidence of titanium tent exposure. However, with respect to the new bone gain, there were significant differences between the bone substitutes. It was revealed that a mixture of Bonalive bioactive glass with bovine hydroxyapatite in a 1:1 ratio did not contribute to the formation of new bone and, in 50% of cases, was accompanied by the formation of cold abscesses, despite the results of previous microbiological studies indicating the bacteriostatic properties of this material.[29-32] At the same time, the use of NovaBone alone in all animals demonstrated the absence of infectious complications and the formation of new mineralized tissue. However, a complete transformation of the synthetic graft into a living bone was not achieved.

Thus, within the limits of this study, it was found that the use of a mixture of bioactive glass Bonalive (S53P4) with bovine hydroxyapatite in a 1:1 ratio was not effective in vertically guided bone regeneration, but the application of NovaBone (45S5) could promote a new bone formation.

Competing Interests

The authors declare that they have no competing interests.

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