Vertical Bone Augmentation with Simultaneous Dental Implantation Using Crestal Biomaterial Rings: A Rabbit Animal Study

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ABSTRACT

Background: Ceramic biomaterial blocks like hydroxyl apatite are too brittle for simple simultaneous vertical augmentation and dental implant placement. Biological scaffolds of xenogenic or allogenic origin are known to be advantageous.

Purpose: The aim of this study was the proof of principle for combined vertical bone augmentation and dental implantation with marginal cuffs made of biological scaffolds with interconnecting porous system and titanium dental implants.

Materials and Methods: Cylindrical porcine biomaterial rings (processed, mineralized bone matrix) were placed in combination with titanium dental implants in the tibia model using six chinchilla bastard rabbits (n = 12 samples). Histological examination included undecalcified histological examination with toluidine blue staining and fluorescence microscopy. Animals were sacrificed after 30 days.

Results: The results showed bony healing in the scaffolds with immature bone tissue ingrowth following the trabecular structure, showing lamellar cancellous bone healing. Fluorescence microscope showed analogous results.

Conclusion: The biological scaffold proved a biocompatibility in a xenogenic setting. The vertical bone augmentation with simultaneous implantation was successful and proved the feasibility of the concept.

KEY WORDS: biomaterials, rabbit, vertical bone augmentation

INTRODUCTION

Successful functional and esthetic placement of dental implants requires a sufficient bony bed in the jaws. The loss of teeth can lead to various defects in the bone resulting from different pathological mechanisms.[1] Periodontal inflammation, pressure of removable prosthetics, and physiological resorption in the edentulous jaws lead to various, mostly combined, defects, including horizontal and vertical bony deficiency. Whereas horizontal defects allow standard guided bone regeneration (GBR) techniques and similar approaches, it is necessary to provide mechanical stability in vertical defects.[2–6]

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Vertical bone augmentation is still a challenging problem in dental implantology.[1,3–5,7] The most common techniques for these surgeries are bone block grafts, distraction osteogenesis or particulate biomaterials, and bone in combination with either sandwich osteoplastic or membrane techniques (GBR).[2,6,8–17] Other options are cylindrical autologous bone grafts as presented previously.[18,19] The use of bone cylinders and rings derived from the well-known technique of trephine drill harvesting for bone grafts.[20,21] Using these graft designs allows to prepare appropriate implant sites for better fit compared with the hand crafted block grafts.[18–23]

The use of these graft types in dental implantology can be diverted in the trias of bone ring cuffs around implant necks, assisted press-fit cylinders with osteosynthesis screw fixation, and those with press-fit not requiring a screw as discussed earlier.[18] The later ones demand a second procedure for the placement of a dental implant. The first approach bears several advantages including the on-step procedure. However, the harvesting of the graft results in donor site morbidity. Using alloplastic, allogenic or xenogenic materials could avoid this second injury for the patient. Additionally, it was histologically shown that autologous cortical bone grafts show only limited osseous healing in rabbits.[24] This implies that a cancellous structure with interconnecting pores could be a better option. We referred to previous clinical results with vertical bone augmentation, used a processed cancellous bone material described earlier as cuff ring for simultaneous vertical bone augmentation in the rabbit tibia model, and evaluated bony healing in this type of bone augmentation.[18,25]

MATERIALS AND METHODS

Experimental Setup

The animal study of a biphasic implant consisting of a cancellous bone-derived material ring and a commercial dental implant was done in six chinchilla bastard rabbits using the tibia model on both sides ($n = 12$ samples). Samples were taken after 30 days representing the final acute bone healing in rabbits.

Figure 1 Biphasic implant consisting of a porcine steam-resistant mineralized bone matrix ring and an Alfa Gate 3.75/8 mm SCI implant (VHX-600 digital three-dimensional microscope, Keyence Inc., Osaka, Japan).

Preparation of the Biphasic Implant Device

Steam-resistant mineralized bone matrix (SMBM) was obtained by the preparation process described earlier.[25] This technique involves mechanical cleaning, defatting, and steam sterilization without enzymatic fermentation. We used cancellous bone from the porcine pelvic bone (Mainz slaughter house, Germany). The bone rings were harvested using diamond hollow drills (diamond trephine control system, Hager & Meisinger Inc., Neuss, Germany). Bone cylinders measuring 7.5 mm (diameter) x 2.0 mm (height) were obtained by drilling the bone blocks at an angle of 90° with a diamond drill size of 8.0 mm (outer diameter) and then cutting to the correct length with a scalpel. Alfa Gate Bioactive titanium implants with calcium phosphate coating were obtained from the company as free samples (Alfa Gate Dental Implants, Kfar Qari, Israel). The biphasic implant consisting of both parts was assembled under sterile conditions under the laminar flow bench (see Figure 1). Samples were stored at −30°C before surgery.

In Vivo Experiments

The animal experiments were approved by the government of Moldova and performed in compliance with the guidelines for animal experimentation in the animal experimentation institute of the University of Moldova (Chisinau, Moldova). Female ex-breeder chinchilla bastard rabbits were chosen for all experiments as their growth plates were closed, which ensured normal bone healing. The animals were held in a combined indoor and outdoor paddock containing up to four rabbits each; the rabbits had unrestricted mobility. The number of rabbits was chosen according to the standards in medical device research for representative descriptive histology. Anesthesia included ketamine 100 mg/xylazin 20 mg/5 kg adapted to the individual weight of the animal. The biphasic implants were placed in both medial tibia bones of the six animals via an anterior transdermal approach ($n = 12$ samples) following the drill protocol of the manufacturer after planar drilling of the bone surface with a planar drill (Ring Control Professional System, Hager & Meisinger Inc., Neuss, Germany). Wound closure included running sutures using Vicryl 3-0 (Ethicon, Inc., Somerville, NJ, USA). Animals received up to 30 mg phenylbutazone after the operation. The rabbits were sacrificed by an overdose of ketamine 200 mg decapitation. Samples were harvested and fixated with 4% paraformaldehyde.

Histological Examination

Samples were cut in appropriate bony pieces after immersion fixation for 4 weeks as described earlier. Histological preparation was described elsewhere.[26,27] Dehydration

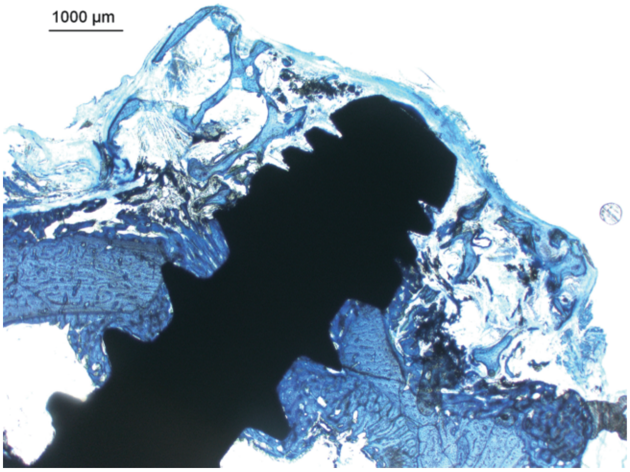

Figure 2 Histological example with toluidine blue staining (50×) showing early lamellar bone healing and marginal bone mineralization.

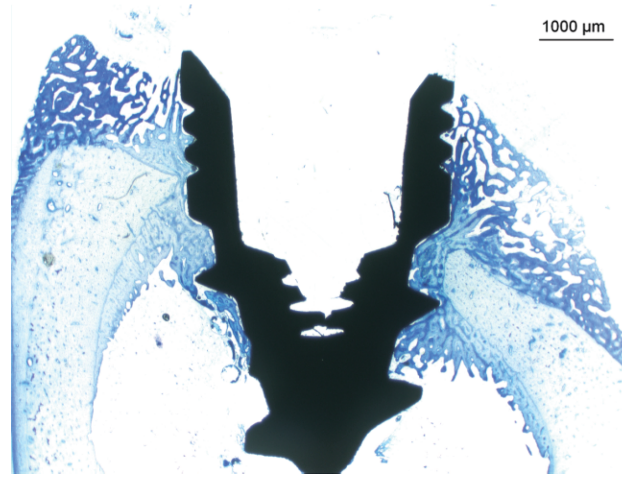

Figure 3 Histological example with toluidine blue staining (50×) showing advanced immature bony healing and mineralization of marginal triangle area.

with increasing percentages of alcohol (70–100%) was followed by Technovit® embedding (Heraeus Kulzer GmbH & Co., Wehrheim, Germany), toluidine blue staining, and diamond grinding of microscopic samples down to a thickness of 20 µm. Light and fluorescence microscopic examination was performed under a Leica DM8000 M microscope (Leica Microsystems GmbH, Wetzlar, Germany). Pigs are regularly treated with tetracycline resulting in fluorescence of the porcine mineralized bone matrix (SMBM) used in this study. This effect was applied to show newly formed bone (no fluorescence) within the fluorescence-marked biomaterial more clearly. Data were analyzed as means and standard deviations. 'Prism' version 5.0d (GraphPad Software Inc., La Jolla, CA, USA) was used for the analysis.

RESULTS

Light Microscopic Examination

Samples showed a good osseointegration of the titanium implant in the local bone in all samples (see Figures 2 and 3). Examination of the biomaterial rings showed bone ingrowth with most mature mineralization in the marginal bone area forming a mineralization triangle from the local bone surface toward the supracrestal implant surface. The structure of the scaffold material was either completely replaced by newly formed cancellous bone or in the process of ingrowth. Most mature bone with advanced mineralization was observed in the marginal bone triangle between supracrestal rough implant body and the horizontal, marginal, crestal bone.

Fluorescence Microscopic Examination

Corresponding fluorescence examination showed newly mineralized bone tissue in the described triangular area as well as in the implant interface in all samples (see Figure 4). The newly mineralized, marginal, vertical bone growth was measured (see Figure 5). The height of

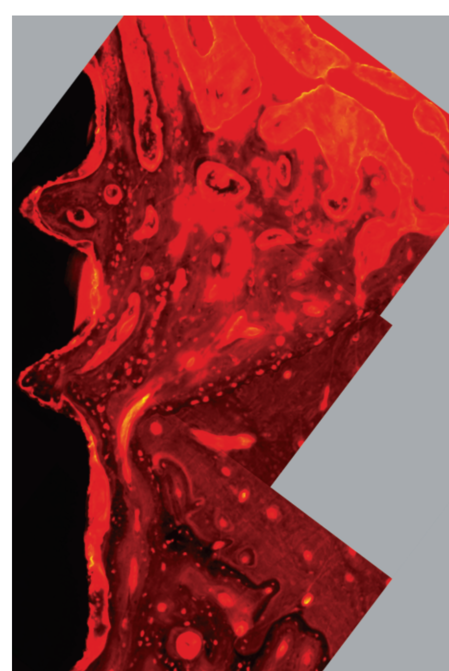

Figure 4 Fluorescence microscopy emphasizes the newly formed marginal bone due to remains of the matrix fluorescence (200×, assembled from multiple images following digital mosaic technique).

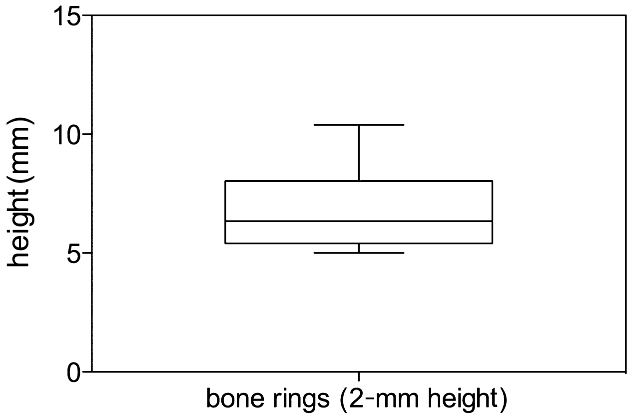

Figure 5 Vertical, marginal mineralized bone growth height with mean and standard deviation.

the newly formed mineralized bone was between 5.1 and 10.4 mm (mean 6.8 mm), covering the rough surface of the implant completely.

DISCUSSION

Samples were taken after 30 days representing the end of acute bony healing in rabbits.[25,28,29] The rabbit tibia is an accepted animal model for biomaterial research and technically favorable.[30–32] Therefore, it was the preferred choice. The Alfa Gate dental implants are advantageous for bony healing due to their calcium phosphate coating.[33] The biocompatibility of the SMBM material was proved in an earlier study and was reproducible in this experiment.[25] Bony ingrowth and mineralization was fast following the cancellous SMBM scaffold structure. The use of porcine material also proved biocompatibility of the SMBM processing in xenogenic bone biomaterials. This has some impact on actual product developments such as the equine bone block from Geistlich Inc. (Wolhusen, CH, Switzerland) and supports the idea of xenogenic biomaterials.[34] This pilot study provides valid descriptive data. We also analyzed the vertical mineralized marginal bone growth with mean and standard deviation. However, due to the limited size and structure volatility, these data must be considered as preliminary. Metric data with high statistic power demand more animals and a cancellous bone model like the patellar groove to avoid influence of surrounding muscle movements as given in the tibia model.[35]

A previous study in rabbits showed a limited healing of cylindrical autologous bone grafts fixed with osteosynthesis screws after 3 months, indicating an uncertain fate for the cortical bone rings described for vertical augmentation in several publications clinically as mentioned earlier.[24] This study reported fibrous healing in the interface in 50% of the cases and complete fibrous resorption of the bone transplant in another 50% of the cases.[24] The aim of our current animal study was to evaluate the option of cancellous bone material processed from xenogenic bone. The histological results indicate a successful osseous healing in all cases with a complete immature bone tissue ingrowth. Beginning calcification can be observed in the marginal bone triangle as typical location for this process. These results support the current clinical success of vertical bone augmentation with this kind of material in our group (unpublished data) and emphasize the importance of a cancellous structure with interconnecting pores for vertical bone augmentation materials.

Biomaterial blocks offer advantages compared with particulate vertical bone augmentation.[11,16,36] No stabilizing medical devices such as titanium mesh or metal-enhanced membranes are required. Reduced resorption of block grafts was compared with particulate augmentation with GBR techniques.[37] Osteosynthesis screws are a disadvantage of most block augmentations. However, this can be avoided if the dental implant is placed simultaneously or a press-fit is achieved.[18,19] The need for bone grafts or bone-derived materials containing protein matrix derives from the strong stress within the material block when performing simultaneous implantation. Alloplastic materials such as hydroxylapatite, tricalciumphosphate, and others are too brittle concerning internal stress forces, resulting in fracture of the material block. Using bone-derived biomaterial matrices with mechanical stiffness avoids donor site morbidity resulting from the harvesting of autologous bone blocks.

CONCLUSION

The results of this pilot study show that vertical bone augmentation using a biological scaffold with interconnecting porous system of a cancellous structure combined with simultaneous dental implant placement is successful in the rabbit tibia model. It results in good bony ingrowth of scaffold and osseointegration of the dental implant.

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