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A PILOT STUDY OF CERASORB AND BIO-OSS ENHANCED NEW BONE FORMATION IN ANIMAL MODEL

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The aim of this pilot investigation was to develop a new animal model for studying the effects on osteogenesis of agents used in the guided bone regeneration technique. As test material, a mixture of two osseoconductive materials with different physico-chemical characteristics was used. One component of the mixture was Bio-Oss, a bovine hydroxyapatite; the other was Cerasorb, a synthetic tricalcium phosphate. The mixture consisited of 50 volume percent of Bio-Oss and 50 volume percent of Cerasorb. In *in vivo* pilot experiment, bone wounds were prepared in the proximal third of both femurs of rabbits. A Cerasorb + Bio-Oss mixture was inserted on the test side and the same amount of sterile buffered physiological solution on the control side. After healing for 4 weeks, the bone segments were embedded and cut without decalcification, using the Exact cutting and grinding system. The density of the newly-formed bone was evaluated histomorphometrically. On the Cerasorb + Bio-Oss test side the bone density was almost 1.5 times higher than that on the control side. These results demonstrated that the applied animal model is appropriate for investigation of the effects on osteogenesis of biocompatible graft materials such as Bio-Oss and Cerasorb.

Keywords: Bio-Oss – Cerasorb – osteogenesis – rabbit model – histomorphometry

INTRODUCTION

Guided bone regeneration has become a routinely applied method in dental implantology. Most of the dentoalveolar regenerative techniques require osseoconductive material in order to establish new bone formation in the necessary anatomical form.

Bio-Oss is a safe, effective xenograft: a deproteinized, sterilized bovine bone with 75–80% porosity. It is reported to be highly osteoconductive and biocompatible

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[3, 5]. It is known that Bio-Oss serves as a scaffold in guided bone regeneration, but, due to its poor resorbability it may exert a negative influence on the structure of the newly-formed bone. It has been found clinically that its resorption is very slow, requiring many years [13].

The chemical characteristics of Cerasorb, another widely used osteoconductive material (pure beta-tricalcium phosphate), allow it to resorb completely and quite rapidly during new bone formation. This may result in too early resorption in some cases, not fulfilling the clinical requirements, the space-maintaining function [1, 15].

The main aim of the present study was to establish a gold standard for the artificial bone growth-accelerating effect of a Cerasorb and Bio-Oss mixture on osteogenesis in order to determine and utilize the most advantageous characteristics of these bone substitutes. Bio-Oss serves as a scaffold, but its resorbability is poor, while Cerasorb is a good bone-developing material, but resorbs too early, not providing a scaffold for the new bone bridges [12, 13].

Bone-substitute materials such as Cerasorb allow targeted bone regeneration as they facilitate construction of a base on which implants can be positioned and further stabilized [12]. Full resorption over a defined period of time, with simultaneous transformation into autologous bone, is of particular significance in this respect. Because of their rounded surface and chemical composition, the Cerasorb particles are remarkably bioinert and is therefore particularly suitable for innovative procedures [11, 15].

The present pilot study reports on the potential of a mixture of Cerasorb and Bio-Oss in implantation technology. A further goal was to develop an *in vivo* animal model suitable for the investigation and comparison of the effects of different materials. Both Cerasorb and Bio-Oss are currently in clinical use, but as far as we are aware their effects in a mixture have never been investigated.

MATERIAL AND METHODS

The applied Bio-Oss had a granule size of 1–2 mm (Geistlich Pharma AG, Switzerland); the Cerasorb was Cerasorb M (1–2 mm) produced by Curasan (Kleinostheim, Germany).

Animals

The pilot study involved adult New Zealand White rabbits. The animal management and the surgical and routine procedures followed “The Guiding Principles for the Care and Use of Animals” approved by the Animal Investigation Review Board of the University of Szeged, in accordance with the principles of the Helsinki Declaration.

Surgical procedures

Narcosis was induced with a cocktail of 5.0 mg/body mass (bm) kg (0.25 ml/bmkg) xylazine (Xylazine 2% inj.), 40.0 mg/bmkg (0.4 ml/bmkg) ketamine (Ketavet inj.) and 0.8 mg/bmkg (0.08 ml/bmkg) acepromazine (Vetranquil inj.) intramuscularly. The rabbits received Ringer lactate infusion therapy at a rate of 0.3 ml/min during narcosis via, a cannula inserted into an ear vein.

After disinfection, isolation and skin incision, the fascia lata was prepared. The femurs were visualized by folding the *m. tensor fasciae latae* and the *m. abductor cruris cranialis*. The site of the bone wound was marked by a round burr in the proximal third of the femur, approximately 2 cm distally from the *trochanter major* of the femur. The diameter of the monocortical bone wound was 3.3 mm. To establish a bone wound of the same size, implantation drills with irrigation were used (pilot-, pre- and form drills, CAMLOG Biotechnologies AG, Germany). In the test (right) femur a 1 : 1 mixture of the bone graft materials Cerasorb and Bio-Oss was inserted. This combination mixed with rabbit blood, was applied to fill the 3.3-mm-diameter monocortical bone wound. On the control side (left femur), sterile buffered physiological salt solution was injected. The bone wound was covered by Surgical (Johnson & Johnson), which was fixed around the hole with Histoacryl (Braun). Suturing was performed with absorbable Vicril 5.0, in three layers (fascia lata, subcutaneous layers and skin).

During the postoperative care, the rabbits received an analgetic [4.0 mg/bmkg (0.8 ml/bmkg) carprofen (Rimadyl) inj. sc.] and antibiotic support [15 mg/bmkg (0.15 ml/bmkg) enrofloxacin (Enroxil) inj. sc.] for 5 days following the operation. All the rabbits remained healthy and the postoperative period was uneventful.

Sample harvesting

After 4 weeks of osteogenesis, the rabbits were sacrificed under general anaesthesia induced by an overdose of an intravenous injection of ketamine.

Specimen preparation

For histological evaluation, perfusional tissue fixation was carried out with 4% neutral buffered formalin solution, after which the cut specimens were subjected to immersional fixation in 4% of neutral buffered formalin solution. X-rays were taken to identify the exact locations of the bone wounds. The specimens were dehydrated and embedded in Technovit 7200VLC resin (Heraeus Kulzer, Germany). Cutting was performed with the Exact cutting and grinding system without decalcification [2]. The thickness of the sections was 20 μm. The slides were stained with toluidine blue.

Histology and histomorphometric analysis

Optical microscopic images (Nikon Eclipse 80i, Japan) were recorded on an Evolution MP 5.1 Mega-pixel FireWire Digital CCD Color Camera Kit (Media Cybernetics, Inc., USA). Measurements were performed with Image-Pro Plus 5.1.1 image-analysing software (Media Cybernetics, Inc., USA).

The histomorphometrical evaluation involved use of the areal bone density, i.e. the ratio of the area of newly-formed bone to the total area of the image [8, 9]. This permitted a quantitative comparison of the new bone formation in the control and the test bone wounds.

RESULTS

Histological observations

In the control samples (Fig. 1a), the reconstruction of the surgically prepared bone wound was not complete. The bone at the border of the wound exhibited signs of resorption and new bone formation. This was young immature lamellar bone with centrifugal orientation of the newly-formed bone in the wound. The outer layer of newly-formed bone was a younger woven bone with cross-oriented collagen fibres.

The closure of the monocortical bone wound bone formation induced by the Cerasorb + Bio-Oss mixture was not complete (Fig. 1b). Around the bone substitutes, new bone formation had started, containing bone bridges between and around the granules. Mostly young immature woven bone was situated around the granules with osteoblastic activity.

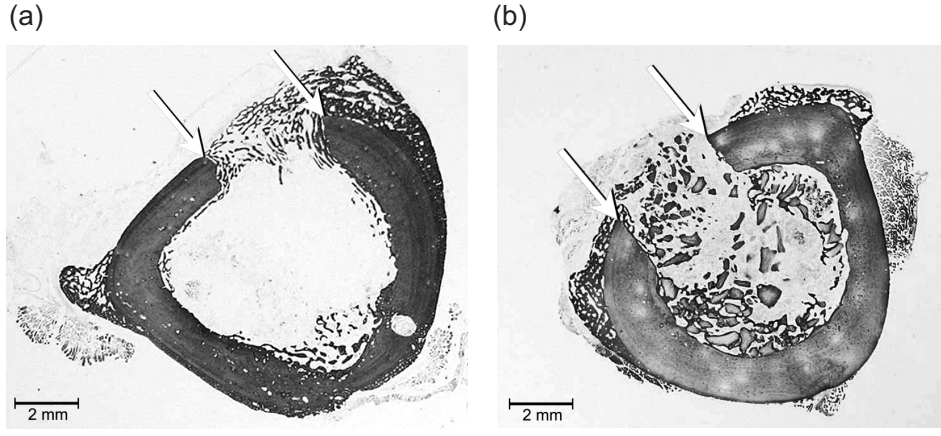

Fig. 1. Macroscopic slides of histological control (a) illustrates the bone formation induced by the Cerasorb + Bio-Oss mixture (b) stained with toluidine blue. Arrows indicate the site of the prepared monocortical bone wound

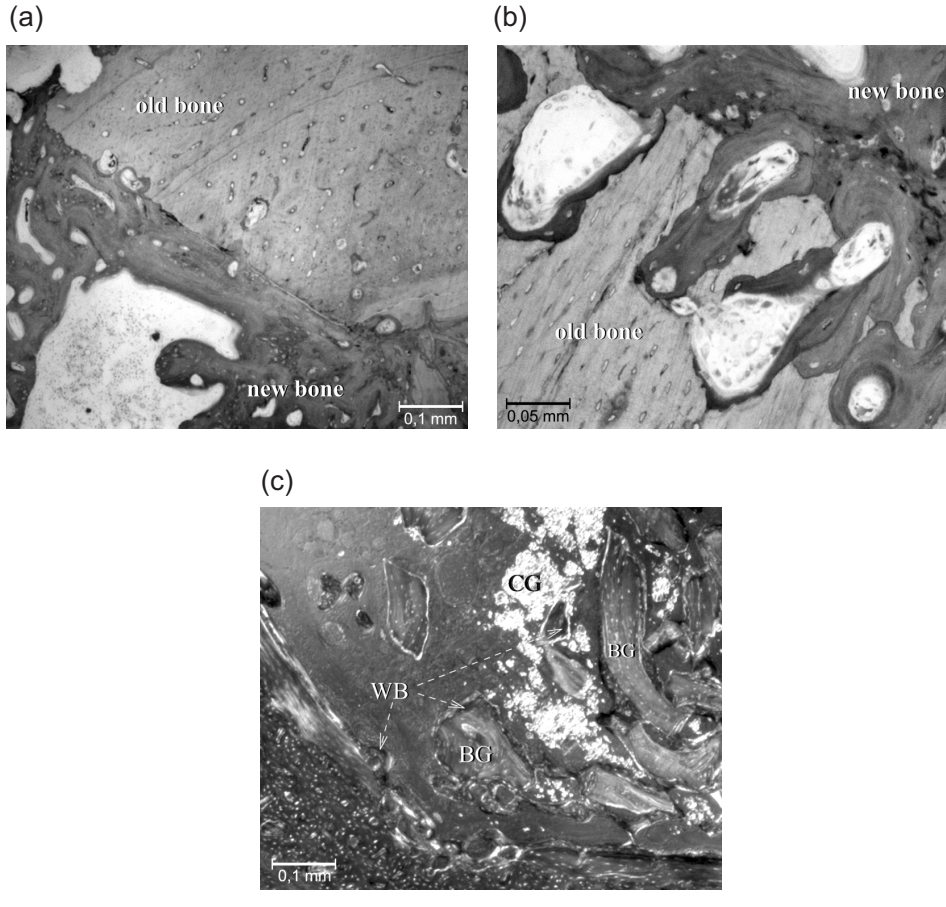

Fig. 2. Picture of new bone formation in the control sample. (a) Bright field (×10 magnification); Picture of the bone formation induced by the Cerasorb + Bio-Oss mixture. (b) Bright field (×40 magnification); (c) polarized light microscopic image (×20 magnification); CG – Cerasorb granules, BG – Bio-Oss particles, WB – woven bone

In Figure 2a, the border of the old and new bone can be clearly differentiated.

In the woven bone, an osteoblast line indicated the formation of bone and primary osteons in the lamellar bone (Fig. 2a, b). Both Bio-Oss (BG) and Cerasorb (CG) granules were visible (Fig. 2c), as a sign that the resorption of the material had not yet finished. At the same time, an osteoid bone (woven bone – WB) network could be discerned around the granules (Fig. 2c).

Histomorphometric measurements

In the Cerasorb + Bio-Oss mixture, the extent of induced osteogenesis was increased almost 1.5-fold, as compared with the control side, where the areal density of the newly-formed bone was 33.9%. For the Cerasorb + Bio-Oss mixture the areal density of the newly-formed bone was 48.7%.

DISCUSSION

In modern dental implantology, the need for bone augmentation techniques demands adequate osteoinductive and osteoconductive effects from the bone substitutes. It is very important that the original anatomical form of the given bone (e.g. the alveolar process as dental implantology concerns) should be reconstructed and also that an appropriate bone structure should be achieved as soon as possible [6].

A variety of biological and synthetic materials are available for reconstruction with augmentative surgical methods of alveolar bone defects. The available bone substitutes exhibit different abilities in guided bone regeneration as regards their biological function in the new bone formation.

Bio-Oss is a highly osteoconductive xenograft material certified for the regeneration of bone defects. It displays very low resorbability and acts as an inert scaffold onto which bone-forming cells and blood vessels creep, forming the new bone [7, 10].

Cerasorb has good bioresorptive properties and as a bone substitute it maintains biological support during healing and is gradually replaced by the newly-formed bone [12]. Its clinical indication differs from that of Bio-Oss due to its excellent biodegradation capacity [5]. For instance, Cerasorb is advantageous for sinus elevation procedures as the floor of the maxillary sinus undergoes rapid regeneration by virtue of its multiple blood vessel supply [12]. Its application has the clinical advantage that it resorbs quite quickly (in some weeks) and totally. However, vertical augmentation of the atrophied alveolar crest, or the regeneration of other load-bearing bone sites, requires a bone substitute with lower resorbability and high stability, such as Bio-Oss.

The goal of this pilot investigation was to study the artificial bone growth-supporting effect of a Cerasorb and Bio-Oss mixture on osteogenesis in order to determine the most advantageous characteristics of these bone substitutes. The two materials were mixed on the supposition that their mixture would provide a qualitatively new effect, i.e. a long-term form-maintaining function with relatively small foreign material imbibition in the bone. In spite of its poor resorbability, Bio-Oss provides an appropriate scaffold. Cerasorb is a good bone-developing material, but in some cases it does not serve long enough as a scaffold for the new bone bridges, as it resorbs too early. With the combination of Bio-Oss and Cerasorb, the most advantageous properties of the bone substitutes could be utilized.

Furthermore, the combination of such osteoconductive bone substitutes (in this case the mixture of Cerasorb and Bio-Oss) with an osteoinductive agent offers promising perspectives for reconstructive bone surgery [14].

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