Short Communication: Collagenated Cortico-Cancellous Porcine Bone Grafts. A Study in Rabbit Maxillary Defects

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ABSTRACT

Background: Bone substitutes of collagenated porcine bone (CPB) have previously been shown to have osteoconductive properties and to be resorbed with time. The influence of different ratios between bone particles and collagen on bone response is not yet known.

Purpose: The objective of the study was to evaluate the effect of different collagen ratios on the bone tissue responses to CPB grafts.

Materials and Methods: Eight rabbits were used in the study. Bilateral bone defects, $5 \times 8 \times 3$ mm, were created in the maxilla and were filled with 60% CPB/40% collagen gel or with 80% CPB/20% collagen gel. Animals were killed after 8 weeks for histological and morphometrical evaluations.

Results: There were no differences between the two biomaterials tested. Both materials showed a high degree of bone formation, 42% and 46%, respectively. Both materials were showing signs of resorption at time of sacrifice.

Conclusions: Different collagen/CPB ratios do not influence the bone tissue responses to CPB. Both materials exhibited osteoconductive properties and were starting to be resorbed at 8 weeks.

KEY WORDS: rabbit maxilla, bone substitute, collagen, osteoconduction

INTRODUCTION

In a recent study we evaluated the bone tissue responses to collagenated porcine bone (CPB), with and without prehydration.[1] This study showed that CPB exhibits good biocompatibility, osteoconductive properties, and that the material was resorbed by surface osteoclasts as well as part of the remodeling with the formation of osteons. Also, it was recorded that the dehydration process made the graft material sticky, facilitating clinical handling.

The model used has earlier been employed to study regeneration of defects with and without the use of barrier membranes and to study the influence of mechanical trauma on bone density.[2–4] The defects will heal spontaneously within 4 weeks but with a remaining buccal concavity.[2] In the present investigation, the model was used to study the bone tissue response to the material but not to evaluate the effects on the overall morphology of the maxillary bone.

Most bone substitutes are regarded as osteoconductive and serve as substrates/scaffolds for bone formation, that is, they support vessel ingrowth, cell migration/differentiation, and subsequent formation of new bone in osteogenic environments. With time, the interparticular spaces will be filled with newly formed bone and the bone substitute will be incorporated in the bone tissue. Some biomaterials will be completely resorbed with time, while others will remain more or less intact with time.

Xenogeneic biomaterials have long been used as bone substitutes because they share features with human bone, that is, a similar morphology and a potential of being resorbed. Deproteinized bovine bone is one of the most well documented bone substitutes with osteoconductive properties and satisfactory incorporation in bone

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tissue[5] and clinical histology has demonstrated integration of titanium implants in areas previously treated with bovine bone.[6,7] From a biological point of view, it seems that xenogeneic biomaterials work well and, clinically, the addition of prehydrated collagen to the bone granules will create a sticky and moldable material, which simplifies application. However, the tissue response to various ratios of collagen is not known.

The purpose of the present experimental study was to evaluate the bone tissue response to CPB, with different collagen content, when placed in defects in the rabbit maxilla.

MATERIALS AND METHODS

Animals and Anesthesia

Eight adult (>7 months) female New Zealand white rabbits were used in the study. The animals were kept free in a purpose-designed room and were fed *ad libitum* with water and standard laboratory animal diet and carrots. Prior to surgery, the animals were given general anesthesia by an intramuscular injection of fluanison and fentanyl (Hypnorm, Janssen Pharmaceutica, Brussels, Belgium) 0.2 mg/kg and intraperitoneal injection of diazepam (Stesolid, Dumex, Copenhagen, Denmark) 1.5 mg/kg body weight. Additional Hypnorm was added when needed. Local anesthesia was given using 1 mL of 2.0% lidocain/epinephrine solution (Astra AB, Södertälje, Sweden). Postoperatively, the animals were given antibiotics (Intenpencillin 2,250,000 IE/5 ml, 0.1 lm/kg body weight [LEO, Helsingborg, Sweden]) and analgesics (Temgesic 0.05 mg/kg (Reckitt and Colman, NJ, USA) as single intramuscular injections for 3 days. The study was approved by the local committee for animal research.

Surgery

The bilateral edentulous areas between incisors and molars of the maxilla were used as experimental sites. The bone surface was exposed via a 10-mm long incision between buccal and palatal mucosa. A muco-periostal flap was raised. A 5 × 8-mm wide and 3-mm deep defect was drilled with the use of a 5-mm trephine drill followed by a large round burr (3 mm in diameter) under irrigation with saline. The defects were filled with prehydrated (20% collagenI/III) collagenated cortico-cancellous porcine bone mix (granulometry 300 μm [Putty, Tecnos, Turin, Italy]) (A) or prehydrated (40% collagenI/III) collagenated cortico-cancellous porcine bone mix (granulometry 300 μm [Gel40, Tecnos, Turin, Italy]) (B). The wounds were closed with resorbable sutures. All animals were killed after 8 weeks. The experimental areas were retrieved and immersed in 2.5% paraformaldehyde, 0.1% glutaraldehyde, and in 0.1 M cacodylate buffer (pH 7.4) for 24 hours.

Tissue Processing and Analyses

Specimens were decalcified in ethylenediaminetetraacetic acid (15%) for a period of 2 weeks. Specimens were again x-rayed in order to verify the decalfication procedure. After dehydration in graded series of ethanol the specimens were embedded in paraffine, sectioned (3 to 5 μm sections) and stained with hematoxyline-eosine and mMAB (modified Mallory Aniline Blue).

Examinations were performed in a Nikon Eclipse 80i microscope (Teknooptik AB, Huddinge, Sweden) equipped with an Easy Image 2000 system (Teknooptik AB, Huddinge, Sweden) using ×1.0 to ×40 objectives for descriptive evaluation and morphometrical measurements. The histomorphometrical evaluations comprised measurements of the area of bone and porcine particles in relation to the total measurement area.

RESULTS AND DISCUSSION

The postoperative healing was uneventful; clinically healthy mucosa, without signs of infection, had already covered the defects in all animals after 5 to 6 days. No loss of appetite was recorded. At 8 weeks, an active resorption was taking place in both materials tested and the bone found in the defects was mature with an active reorganization (Figure 1, A and B). All types of vessels were found both in the mineralized part and in the soft tissue. The morphometrical measurements revealed a high amount of mineralized bone with no significant differences between the two materials, A – 42.3% (standard deviation [SD] 12.3) and B – 46.2 % (SD 15.1%), respectively.

Bone substitutes of xenogeneic origin are commonly used as grafting materials, for example, in bone defects, maxillary sinus floor augmentation procedures, and extraction sockets. Studies have, in general, demonstrated good biocompatibility of the grafts and high success rates.[7,8] Also, it has been shown by Aghaloo and Moy[9] that implants inserted in xenogeneic grafts might even have a better survival than implants placed in different combinations, for example, autogenous/composite grafts or alloplast/xenograft materials.

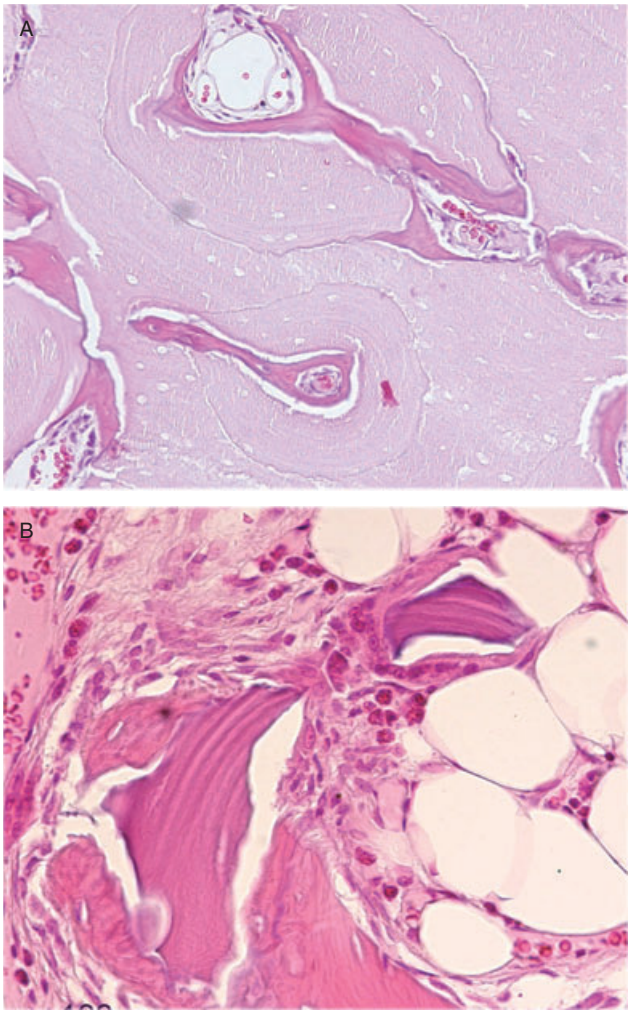

Figure 1 *A,* Light micrograph showing new bone formation inside a granule of Gel40. Note the extensive vessel formation (orig magn ×20). *B,* Light micrographs showing resorption of granules within Putty graft. No signs of inflammation can be seen (orig magn ×40).

The present study clearly demonstrates that CPB with different collagen content induce bone formation in defects in rabbit bone, and that resorption of the porcine bone particles takes place. The high presence of collagen might induce adhesion of both mesenchymal cells and osteoclasts to the surface of the material because these cells are shown to have integrins linking to different proteins, for example, ostepontin, which may be important for both migration, adhesion, and subsequent resorption.[10] Also, collagen has been shown to have a chemotactic and differentiation effect on mesenchymal stem cells.[11]

Controlled clinical investigations with histology are however needed to establish the resorption properties of the porcine bone graft with added collagen.

It is concluded that CPB with different ratios of collagen exhibits good biocompatibility and osteoconductive properties. In this model, the two materials were equal with respect to both bone formation and resorption which had started at the endpoint at 8 weeks.

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