Evaluation of an anorganic bovine bone mineral in post-extraction alveolar sockets: a case series

ABSTRACT

Aim Autogenous grafts have been considered the gold standard in bone grafting. But this often calls for a second surgical site and insufficient bone quantities. Interest exists in having a surgical technique that does not require autogenous bone harvesting and still results in sufficient bone formation within a relatively short time frame.

Materials and Methods The physical-chemical characteristics of an anorganic bovine bone mineral (ABBM) are described, as well as its use as a graft material in extraction sockets of 10 patients. Histology and histomorphometry was performed after 6 months healing.

Results Histomorphometric analysis showed average vital bone content of 26.4 (range 15 to 32%) and residual graft content of 38.4 (range 32 to 48%).

Conclusions The biological and physical-chemical characteristics of the ABBM permits formation of well-vascularized new vital bone in intimate contact with the ABBM particles.

INTRODUCTION

Extractions occur primarily as a result of caries, trauma or periodontal disease. Caries is endemic and a leading cause of tooth loss in the US population, with periodontal disease being responsible for 30-35% of extractions in people over 40 years of age (1). Clinically it is important to replace missing teeth with the most suitable option for the patient, and ridge and site preservation at the time of extraction is critical to long term success, irrespective of the procedure used for tooth replacement (2). Current techniques used for ridge and site preservation include the use of bone graft materials and/or resorbable membranes (3, 4). Ideally, at the end of the process the area will be filled with vital, mineralized bone, with minimal or no bone grafting material remaining.

A variety of materials have been used for bone grafting in sockets, for the purpose of ridge preservation. These include osseous mixtures of donor-retrieved bone particles (autografts), allografts and xenograft particulates, as well as synthetic materials. The use of titanium membranes over extraction sockets - with or without the use of autogenous bone grafts - has been found to favour ridge preservation (5). The synthetic, medical-grade calcium sulfate hemihydrate has been used and found to completely resorb over 3 months and to enable the growth of new trabecular bone (6).

Anorganic bovine bone can be used and has been shown to be safe and effective. One study filled sockets with anorganic bovine bone and then covered the sockets with free gingival grafts, harvested with a soft tissue punch. The gingival grafts were found to have a mean integrated area of 99.7% at 6 months (7). A visual analysis of the soft tissue healing was performed. Bovine bone derivatives have also been used in bone regeneration both for socket preservation (8) and at intra-bony periodontal defects, when used alone (9), or in combination with platelet-rich plasma - with and without guided tissue regeneration (10-12), and with enamel matrix derivatives (9, 13). Bovine porous bone material, in combination with collagen membranes used for guided tissue regeneration at extraction socket sites, has been found to result in greater socket bone fill at 6 months than using the bovine bone materials with autologous fibrinogen/fibronectin instead of the collagen membrane (14). A recent study compared the use of deproteinized bovine bone (Bio-Oss® Osteohealth Co, Shirley, NY, USA) in combination with a resorbable membrane (Bio-Gide®, Osteohealth Co., Shirley, NY, USA), versus use of the membrane alone during immediate implant placement. While bone levels were maintained for both groups, the soft tissue margins were more coronal and therefore more favorable esthetically in the group treated with both the bone grafting material and the resorbable membrane. The study concluded that in peri-implant bone defects, the...
deproteinized bone was able to prevent collapse of the overlying soft tissue into the defect; a very beneficial approach in esthetic areas (15). The present study looks at the results from the use of an anorganic bovine bone mineral (ABBM) called NuOss™ (ACE Surgical Supply Co., Brockton, USA) as a grafting material in human post-extraction alveolar sockets.

**MATERIALS**

Anorganic bovine bone mineral - NuOss™
Bone generally can be divided into two regions; cortical and cancellous. Cortical bone has a higher density than the cancellous bone, due primarily to the difference in the pores distributed in the bone structure. Cancellous bone is a more porous structure, having large pores distributed throughout the bone. The organic component of bone represents 40% of bone content, primarily type I collagen (99%), with minor components of acidic glycoproteins, phosphoproteins, bone morphogenetic proteins (BMPs) and other non-collagenous moieties (1%). The inorganic component of bone is comprised of calcium-based minerals of apatite structure, mainly of carbonate apatite, containing small amounts of magnesium, sodium, potassium, chloride, etc. It has been demonstrated that the organic part of bone can be removed without significantly altering the native structure of the bone mineral (16). A method has been developed that can create this anorganic bone, while maintaining the structure of the mineral similar to that in native bone. This anorganic bone, NuOss™ (cancellous and cortical), is marketed by ACE Surgical Supply Co. (Brockton, MA, USA), and processed by Collagen Matrix, Inc. (Franklin Lakes, NJ, USA). NuOss™ is derived from cows of United States origin and there has never been a reported case of Bovine Spongiform Encephalopathy (BSE) being transmitted in any medical device derived from bovine tissue. This ABBM is isolated and purified from the femur bone of cows less than 30 months old, using a proprietary methodology. The cancellous portion comes from the femur head and the cortical portion from the femur shaft. Essentially the method consists of a chemical extraction process and heat treatment to remove the organic components of the bone resulting in an anorganic bovine bone mineral, a natural calcium phosphate salt in a carbonate apatite structure. The physical and chemical attributes of this anorganic bone were determined by a series of in-vitro characterization studies (17). Data is also on file at Collagen Matrix Inc. (18) and LifeNet Health (19).

Physical characterization
- Pore size determination: Scanning electron micrographs were taken at various magnifications (Image J software, NIH). Pore size was defined as the longest distance across a single pore. NuOss™ Macroscopic pores were found to be in the 250–600 µm range, and the microscopic pores in the range of 0.1-1.0 µm. This determination was not applicable for human cancellous bone, since in intact bone, macro and micropores are filled with organic materials, including bone marrow (20).

- Void Space (Volume fill capacity): The volume per unit weight of bone mineral relates to the empty space available for conducting cellular ingrowth and new bone deposition. The void space is calculated by measuring the volume (cm³) occupied by 1 gram of bone graft material and subtracting the volume occupied by the mineral itself, which indicates the space available for osteoblast ingrowth. Human cancellous/cortical bone, in the 250-1000 µ particle size range, will normally occupy 1.3-2.5 cm³/g. NuOss™ cancellous bone mineral occupies 1.83 cm³/g, whereas the NuOss™ cortical bone occupies 1.77 cm³/g. This indicates that both human bone and NuOss™ are very similar in their ability to provide a matrix with sufficient void space available for osteoblast ingrowth and new bone formation.

- Crystal Size (nanometers): The similarity in crystal size between NuOss™ (30, with a 20-40 range) and human cancellous bone (10-50), indicates that both materials are expected to have similar resorption and remodeling characteristics.

Chemical characterization
- Calcium phosphate index (Ca/P ratio): Calcium was determined by atomic absorption spectrophotometry using a PerkinElmer Analyst 100 (PerkinElmer, Waltham, Mass, USA), and phosphate by using acidic molybdate/acetone as reagent. The phosphate absorbance was read using a Spectronic 21D spectrophotometer (Milton Roy, Ivyland, Penn, USA). The Ca/P Index confirms that NuOss™ has a highly pure mineral content (1.58 ± 0.16), with a close similarity to the 1.71 ratio of human cancellous bone undergoing similar processing (20) and evidenced by the minimal presence of residual organic materials. To determine residual protein content, if any, an analysis was done of residual nitrogen content in the anorganic mineral product. The average nitrogen content for 9 random lots of

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products produced over a span of about 12 months was 0.007±0.001%. Assuming the average nitrogen content in protein is 13.6% w/w (average nitrogen weight in all amino acids ÷ average molecular weight of all amino acids), the residual protein content in NuOss is 0.06 ± 0.01%.

In addition, the chemical composition of this anorganic mineral is natural calcium phosphate in a 5% carbonate apatite structure, approximating the 7.4% evidenced in human cancellous bone (20).

CLINICAL METHODS

The study took place during the period of August, 2006 to August, 2007. There were 10 patients in this case series, 6 women and 4 men, with an average age of 53 years (42-61). Three patients had tooth fracture, two had failed endodontic treatment and the remaining five had extensive caries. All patients underwent tooth removal, after having given written and oral consent. In 8 patients the first or second molar were used; five in the lower and three in the upper arch. In all the extracted molars the roots were separated. In the 2 remaining patients it was an upper incisor tooth.

The inclusion criteria were the following.
> Bone: Buccolingual bone width at the cemento-enamel junction (CEJ) of at least 4 mm, facial bone dehiscence below the soft tissue margin/CEJ of no greater than 5 mm.
> Soft Tissue: Buccolingual width at the CEJ of at least 2mm, facial-apical height below the CEJ of no greater than 5 mm.

The exclusion criteria were smoking, diabetes or autoimmune disease, abscess with soft tissue swelling, oral bisphosphonates, surgical extractions requiring the removal of more than 2 mm of buccal crestal bone and buccal bone dehiscence of more than 5 mm from the soft tissue. Atraumatic extraction was performed under local anaesthetic. The socket was examined for residual debris and to determine whether the amount of bleeding was appropriate. The ABBM grafting material was then thoroughly packed into the socket up to the level of the crestal bone to ensure maximum bone regeneration. A short acting collagen tape or plug (ACE Surgical Supply Co., Brockton, MA, USA) was placed, so as to cover the bone mineral, and a long acting Vicryl® suture (Johnson and Johnson, Langhorne, PA, USA) was placed in both horizontal-mattress and figure-eight fashion. The patient was given a chlorhexidine rinse (0.25%) for a 1 week postoperative period. No antibiotics were given. For the patients who underwent molar extraction, no prosthesis was used during the healing phase. In the case of the incisor tooth removal, a fixed temporary bridge was placed.

Core biopsies
At the implant placement appointment, averaging 6 months after graft placement, a flap was elevated, and a biopsy bone core was taken with a 2.7 mm internal diameter (3.5 mm external diameter) trephine. These cylindrical bone cores were left within the trephine and placed in 10% neutral buffered formalin for fixation.

Histological preparation
All histological preparations were performed at Baylor College of Dentistry, Dallas, Texas, USA and McGill University, Montreal, Quebec, Canada. Upon receipt, specimens were dehydrated with a graded series of alcohols for nine (9) days. The specimens were then infiltrated with a light-curing embedding resin. After a further twenty (20) days, the specimens were embedded and polymerized by 450 nm light. The specimens were then prepared by the cutting/grinding method of Donath (21), and Rohrer (22). The cores were then polished to a thickness of 45-65 µm, followed by a final polish with 0.3 µ alumina polishing paste. The slides were stained with Hematoxylin-Eosin and coverslipped for histologic analysis by means of bright field and polarized microscopic evaluation.

Histomorphometry
Following non-decalcified histologic preparation, the cores were evaluated morphometrically. The cores were digitized at the same magnification using a Leica DMR HC and a Jenoptic ProgRes C14 Digital camera. Histomorphometric measurements were completed using Bioquant Nova Prime Bone Morphometry, version 6.50.10 (Bioquant Image Analysis Corp. - Nashville, Tenn, USA). Parameters evaluated were the total area of the core, the percentage of new bone formation, and the percentage of residual graft material. The remaining area was considered as being soft-tissue, void or osteoid. The primary slide evaluated for each specimen was from the most central region of the obtained core.

RESULTS

Histology
Histomorphometric evaluation of the 10 case are summarized in table 1. They indicate an average vital bone content of 26.4 (range 15-32%) and a residual graft content of 38.4 (range 32-48%) following an average healing time of 6 months. Inspection of the apical and coronal sections of the cores showed no significant differences in bone content between these two areas. A representative example of the
histological evidence may be seen in the series of images in figure 1. A low magnification image of an alveolar core taken 5 months post ABBM placement is shown in figure 1A. The apical direction is toward the right. There is ample evidence of bone infiltration (red) into the region of grafted bone (tan). The remainder of the area consists mostly of connective tissue. A high magnification view of a portion of the previous image shows vital bone apposition directly onto the particles of ABBM (Fig. 1B). It is important to note that the host bone is in intimate contact with the ABBM particles, with no fibrous encapsulation of this ABBM. In addition, there is “bridging” of the particles by the newly formed bone- a cardinal sign of dynamic integration of the graft material into the host bone environment. In figure 1C there is an area of newly formed woven bone with osteoblasts lining the bone.

DISCUSSION

When a patient presents with a need for bone grafting, the material of choice has traditionally been the patient’s own bone. There is a large variation of opinion as to which materials should be used for various clinical procedures, the rationale for their use, and how grafting materials should be combined (23), given that one strives to have materials that possess the triad of osteogenic potential (living cells), osteoinduction (bone-inducing factors) and osteoconduction (scaffolding). Autogenous bone, especially the cancellous portion, has long been considered the gold standard of grafting material, as it contains the complete triad. However, the problems associated with acquiring autogenous material, the need for a second surgical site and the potential associated morbidity, as well as the frequent inability to harvest sufficient supply, have led to the search for alternative materials (24).

There are numerous materials in use; a host of synthetic forms, as well as allografts and xenografts. All of these materials act as matrices for the ingrowth of osteoprogenitor cells, vascular beds and peri-vascular tissues from the surrounding recipient bed (25). Froum (26) did a histological comparison of Bioactive

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Legend

VB = Vital Bone; NVB = Non-Vital Bone; MFT = Marrow & Fibrous Tissue; TT = Total Tissue Area

Table 1 Histomorphometric analysis of bone regeneration with NuOss™ after 6 months.
Anorganic bovine bone mineral in post-extraction alveolar sockets

Glass and Demineralized Freeze-Dried Bone Allograft (DFDBA) in healing extraction sockets. After 6-8 months the differences in percentage of vital bone was not statistically significant, but the residual implant material was significantly higher in DFDBA-treated (13.5%) versus Bioglass-treated sockets (5.5%). Another human extraction socket study, by Carmagnola (8), investigated healing with the use of Bio-Oss xenograft, or a resorbable membrane (Bio-Gide) alone. At time of implant placement the Bio-Gide group showed large amounts of lamellar and bone marrow and small proportions of woven bone. The Bio-Oss sites were comprised of connective tissue and only about 40% of the circumference of the particles in contact with newly formed woven bone.

In a very recent clinical and histologic study of postextraction socket by Cardaropoli (27), the 4 month histologic analysis revealed new bone formation in all sites with a 25% average of residual graft particles. The material studied in this report is an anorganic natural bone mineral of bovine origin. It is produced using a relatively low heat chemical extraction process, resulting in an effective osteoconductive matrix that maintains its trabecular architecture and porosity. The comparisons shown in table 1 make it clear that from the standpoint of the various physical characteristics, this ABBM is very similar to human cancellous bone. In particular, the open trabecular architecture of the ABBM, and human cancellous bone, enhances clot stabilization and later revascularization of the graft site. The open network of trabeculae gives rise to a very large inner surface area and high porosity. This permits progenitor cells to easily migrate through the site and eventually allow osteoblasts to lay down new bone (28).

Human bone is characterized by relatively small apatite crystals, as is this ABBM. This type of compact crystalline structure is important, since osteoclasts in the bone matrix will ultimately be required to resorb these crystals, to begin the remodeling process. If the crystal structure of the graft material is significantly different from that of human bone, the graft material may ultimately not succeed in being properly remodeled and replaced with new host bone (29). It has been known for some time that human bone mineral is deposited as carbonate apatite crystals (30), containing a little more than 5% carbonate, 5-10% hydrogen phosphate, as well as other assorted minerals. Biological apatite deposits more quickly than synthetic hydroxylapatite crystals onto grafting material containing calcium. This attachment serves as a substrate for later bone cell and protein attachment. This evidence would suggest that grafting material containing natural bone mineral is more likely to be incorporated sooner into host bone (31).

The biological nature of ABBM has been studied in a number of animal and human studies. Schlickewei (32), using Bio-Oss® observed the remodelling of bone in the rabbit femoral bone over a 1 year period. Analysis from 1 month through to 12 months showed new bone contact to the ABBM increasing over the observation period, with a 90.8% bone cover of the graft material after a year. The histomorphometric analysis over that period also showed that the quantity of Bio-Oss® decreased during that time period, as new bone increased, together amounting to 60% of the total bone tissue. The bone marrow represented the remaining 40%.

Boyne (33), using a porous anorganic bovine bone mineral in rhesus monkeys, was able to show that during slow remodelling the surgical area increased in bone density. This highlighted the possible use of these grafting materials in areas of decreased bone density, such as the posterior maxilla, to enhance support of loaded implants.

Histologic evidence of bone specimens in animal and clinical studies have confirmed the osteoconductive properties of ABBM (34, 35). In the present study the histological evidence shows active osteoblastic activity, with these cells lining up and creating the mineralization process. The new osteoid covers the ABBM, interconnecting the particles. This leads to bridging by the new bone and a stabilization of the host bone. In the histomorphometrics for this present study, the percentage newly generated bone volume falls within the values seen in studies of bone volumes in other studies. Trisi and Rao, in a 1999 study (36), correlated a hand clinical assessment of bone quality to the histologic structure quantified by histomorphometric evaluation of bone density. The results were expressed as percentage of bony trabeculae over the total biopsy area, and ranged from 76.5% ± 16.19 in D1 density to 28.28% ±12.02 for D4 (37). However, there can be great variability in this trabecular bone volume, with values as high as 51.93% and as low as 6.73%, with averages of 17.1% for females and 23.4% for males (38).

The results of this study show that the biological and physical-chemical characteristics of the ABBM, NuOss™, allow for formation of new vital bone that appeared to be in intimate contact with the ABBM particles.

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REFERENCES