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PRELIMINARY STUDY ON THE BIOCOMPATIBILITY AND OSTEOCONDUCTIVE PROPERTIES OF A NEW BOVINE BONE GRAFT

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Abstract

To prevent post-extraction resorption and preserve the integrity of the alveolar ridges, the placement of bone grafts at the time of extraction is recommended. Bovine bone grafts are biocompatibile and osteoconductive, allowing new bone apposition by osteoprogenitor cells. Although there are trademarks recognized internationally regarding bovine bone grafts, they are expensive and even difficult to acquire. Therefore, domestic industry development of high quality biomaterials will reduce the public health high costs in the dental field. Here, we evaluated and compared the effects of an Argentinean manufactured bovine bone graft (Synergy Bone Matrix) with a bovine bone graft recognized for its osteoconductive effects (Bio-Oss), on bone healing in an experimental model in rats. We created critical sized bone defects in rat tibiae and filled them with either one of the bovine bone grafts or control. Clinical responses, X-ray findings, bone mineral density, and histological parameters were evaluated. No abscess, encapsulation, suppuration or inflammation of lymphatic nodes were observed. Radiographically, all implants were amalgamated to the surrounding bony margins, suggesting proper healing. On the other hand, control tibiae exhibited no signs of recovery and remained either unfilled or showed fibrous tissue formation. No statistical differences were observed in BMC and BMD between tibiae filled with Synergy Bone Matrix or Bio-Oss. Histological analysis revealed particles of both bone grafts surrounded by laminar bone tissue indicating osteoconductivity, without any inflammatory sign. This preliminary study suggests that Synergy Bone Matrix, as well as Bio-Oss, present similar properties of biocompatibility and osteoconductivity.

Key words: bovine bone graft, bone formation, critical bone defect, osteoconduction.

Resumen

ESTUDIO PRELIMINAR SOBRE LAS PROPIEDADES DE BIOCOMPATIBILIDAD Y OSTEOCONDUCTIVIDAD DE UN NUEVO INJERTO DE HUESO BOVINO

Para prevenir la resorción post-exodoncia y preservar la integridad de los rebordes alveolares, se recomienda la colocación de injertos

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óseos en el momento de la extracción. Los injertos de hueso bovino son biocompatibles y osteoconductivos, permitiendo nueva aposición ósea por células osteoprogenitoras. Existen marcas internacionales de injertos de hueso bovino, pero resultan caros e incluso difíciles de adquirir. Por ello, la elaboración de biomateriales de alta calidad, nacionales, reduciría los altos costos de salud pública en odontología. En este estudio, se evaluaron y compararon los efectos de un injerto de hueso bovino fabricado en Argentina (Synergy Bone Matrix) versus un injerto de hueso bovino reconocido por sus efectos osteoconductivos (Bio-Oss), en el proceso de cicatrización ósea en un modelo experimental en ratas. Para ello, creamos un defecto óseo crítico en tibia de rata el cual se rellenó con uno de los injertos de hueso bovino o control. Se evaluó: respuesta clínica y radiográfica, densidad mineral ósea e histología. No se observaron abscesos, encapsulación, supuración o inflamación de los ganglios linfáticos. Radiográficamente, todos los implantes se integraron a los márgenes óseos circundantes, sugiriendo una cicatrización adecuada. Por el contrario, las tibias control no mostraron signos de recuperación con formación de tejido fibroso. No se observaron diferencias estadísticas en las BMC y BMD entre las tibias Synergy Bone Matrix o Bio-Oss. La histología reveló partículas de ambos injertos óseos rodeadas por tejido óseo laminar indicando osteoconductividad sin signos inflamatorios. Este estudio preliminar sugiere que Synergy Bone Matrix presenta propiedades similares de biocompatibilidad y osteoconductividad que Bio-Oss.

Palabras clave: *relleno óseo bovino, formación ósea, defecto crítico óseo, osteoconducción.*

Introduction

Bone healing in post-extraction alveolus begins with the formation of a clot, culminating with the alveolus filled with bone and connective tissue covered by epithelium.[1] Full restoration of the original volume of the bone crest after tissue remodeling would be the ideal scenario following the placement of a dental implant, but unfortunately this does not usually happen. Moreover, without a further treatment, resorption of the bone crest is frequently an inevitable process, leading to significant dimensional changes.[2] These changes represent an average vertical bone loss of 1.5 to 2 mm, while the average horizontal bone loss is between 40 and 50% during the first 6 to 12 months of healing.[3] Most of these dimensional changes mainly occur in the first 3 months.[3] However these changes may continue over the time, leading to an additional volumetric bone loss of 11% during the subsequent 5 years.[4] In order to minimize or prevent post-extraction bone resorption and preserve the integrity of the alveolar ridges, the placement of bone filling materials in the socket at the time of extraction is recommended.[1]

In the last few decades, there has been a growing interest in the development and production of new biomedical materials capable to induce the regeneration of bone, on different bone lesions.[5] While autologous fillers have excellent biological properties, such as osteogenic and osteoinductive potential, they also have a number of drawbacks. In this regard, added operative time for graft harvest, donor site morbidity, graft resorption, molding challenges, and limited availability, especially in the pediatric population,[6] have led researchers to think what would be the ideal bone substitute.

Several studies have shown that organic or inorganic matrix derived from bovine bone is biocompatible.[7,8] This source material is desirable because it is readily available and inex-

pensive. On the other hand, this biomaterial requires proper preparation to avoid risks such as transmission of zoonoses.[8] The effectiveness of different bone processing techniques has made possible the use of these materials, of various species, for medical applications[9,10]. Bovine materials are biocompatibile and osteoconductive. These important biological properties allow apposition of newly formed bone by osteoprogenitor cells and the partial remodeling by osteoclast and osteoblast of the host.[5] In addition, the large interconnecting pore volume and its composition encourage the formation and ingrowth of new bone at the implantation sites.

Although on the global the global market, there are recognized bovine bone grafts trademarks, they are very expensive and even difficult to acquire. Therefore, the development of different biomaterials for bone grafting, produced by domestic manufactures, with comparable characteristics and biological effects than those renowned internationally is necessary. The development by the domestic industry of high quality bone grafting materials will reduce the high costs in public health arising from the application of these biomaterials in the dental field.

The aim of the present preliminary study was to evaluate and to compare the effects of an Argentinean manufactured bovine bone graft with a commercial bovine bone graft recognized by its osteoconductive effects, on bone healing process in an experimental model in rats.

Materials and Methods

Animals and experimental design

A total of 12 young male adult Wistar rats (175±10 gr) were housed at room temperature (21±1°C), 55±10% humidity, under 12-hours light/dark cycles. They were fed a standard rodent diet (Ganave SA, Argentina) and deionized water "ad libitum". Body weight was recorded 3 times per week. The rats were maintained in keeping with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.[11]

Drugs

Patented national and commercial bovine bone grafts (Synergy Bone Matrix, Odontit Implant Systems and Bio-Oss, GeistlichSwitzerland) were kindly provided by Odontit Implant Systems, Argentina.

Anesthesia: Ketamine Hydrochloride [0.1 mg/100 g body weight (BW)] and Acepromazine Maleate (0.1 mg/100 g BW) (Holliday-Scott S A, Buenos Aires, Argentina)

Surgical procedure

Rats were anesthetized by intraperitoneal injection. Hind legs were shaved and medial aspect of both tibiae was exposed. A bone defect (1.6 mm × 2 mm) was made in both tibiae with a fissure bur (Figure 1 A, B, C and D).[12]

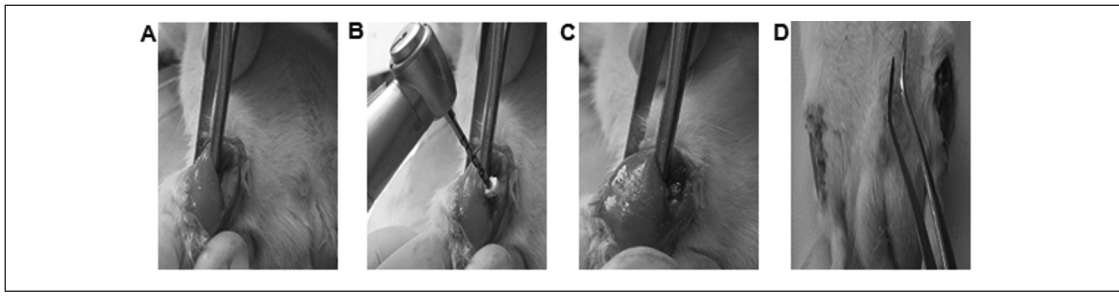

Figure 1. Surgical procedure. **A.** Tissue divulsion and exposure of proximal tibia **B.** Bone defect (1,6 mm × 2 mm) made in rat tibia with a fissure bur. **C.** Bone defect filled with the corresponding bone substitute. **D.** Final suture.

Bone defects were then filled with

Group 1: The right tibia of each rat (n=6) was filled with the bovine bone substitute Synergy Bone Matrix (Lot Nº: E11121216) while the left tibia was unfilled and used as control.

Group 2: The right tibia of each rat (n=6) was filled with the bovine bone substitute Synergy Bone Matrix (Lot Nº: E11121216) while the left tibia was filled with the bovine bone substitute Bio-Oss (Bio-Oss, Lot Nº 100238).

The remodeling phase in the rat takes about 21 days; therefore, a healing period of 4 weeks was used to assess the late healing response. Animals were sacrificed after 24 days.

Clinical evaluation

Animals were clinically evaluated daily throughout the experience. Biological response was evaluated by documenting the macroscopic findings as a function of time, based on ISO 10993-6:2007. Each implant site and the whole animal were examined daily, in order to find clinical alterations of the normal structure. The evaluation included: inspection and palpation of the surgical site in order to assess for signs of inflammation, infection and sensitivity; assessment of the regional draining lymph nodes; the recorded of the nature and extent of any tissue reaction observed such as haematoma, oedema, encapsulation and/or additional gross findings.

X-ray analysis

Radiographs of both tibiae were taken with standard dental X-ray equipment at the beginning, at 2 weeks and at the end of the experiment (4 weeks).

DXA measurements

Total skeleton bone mineral density and bone mineral content (BMD and BMC, respectively) was measured “in vivo” under light anesthesia at the end of the experiment (day 24) using a total body scanner with software designed specifically for small animals (DPX Alpha 8034, Small Animal Softer, Lunar Radiation Corp. Madison WI) following a previously described technique.[13]

All rats were scanned under light anesthesia using an identical scan procedure. The precision of the software in determining total body BMD was assessed by measuring one rat five times after repositioning between scans, both on the same and on different days, as previously described.[13] The coefficient of variation (CV) was 0.9% for total skeleton BMD. A specific region of interest (ROI), was manually traced at the site of the critical size bone defect for the first animal evaluated. Once established the ROI for the first animal, we used the same ROI to evaluate the BMD at the site of the bone defect in all the animals. The BMD CV of the studied area was: 3.5% for the proximal tibia. All the analyses were carried out by the same technician in order to minimize inter-observer variation.

Histological analysis

At the end of the experimental period, tibiae were removed and decalcified in EDTA, pH 7.2, and embedded in paraffin. Sections were obtained at the level of the bone defect and stained with hematoxylin-eosin to qualitatively evaluate the presence of new bone formation.

Statistical methods

Results were expressed as mean ± standard error (SE). Data were analyzed using parametric or non-parametric tests according to data distribution and “a posteriori” tests. Statistical analyses were performed using SPSS for Windows 11.0 (SPSS, Inc., Chicago, IL). A value of p below 0.05 ($p< 0.05$) was considered significant.

Results

Clinical evaluation

Normal and immediate recovery was observed in all animals. Sensory status remained normal and changes in body weight

or other abnormalities were not observed. In addition, no abscess, encapsulation, suppuration or inflammation of lymphatic nodes was observed.

Both groups of animals presented normal behavior habits, locomotion and feeding from surgery until the end of the study. They did not present any sign of inflammation, infection and sensitivity at the implant site.

Radiographic evaluation

By X-ray, a critical-size bone defect was observed in the left tibia of group 1 rats (control) (Figure 2). A radiopaque area compatible with the bone grafts was observed in the right tibiae of group 1 and 2 (Synergy Bone Matrix) and the left tibiae of group 2 (Bio-Oss) (Figure 2). A subsequent resorption of both bone substitutes and its replacement with bone tissue in final radiographs (4 weeks) was observed.

Radiological analysis of tibia showed that the two groups exhibited gradual replacement of bone graft with radiopaque tissue both, at 2 and 4 weeks. After 2 weeks, tissues within the defects were not completely mineralized. Later on, at 4 weeks, the defects treated with the bone grafting materials, were nearly completely filled with mineralized tissue. All implants seemed well integrated to the surrounding bony margins and no differences were observed in X-ray images of Synergy Bone Matrix and Bio-Oss. These findings suggested proper healing. Conversely, the untreated tibiae (control) exhibited no signs of recovery and remained unfilled, at the same time points.

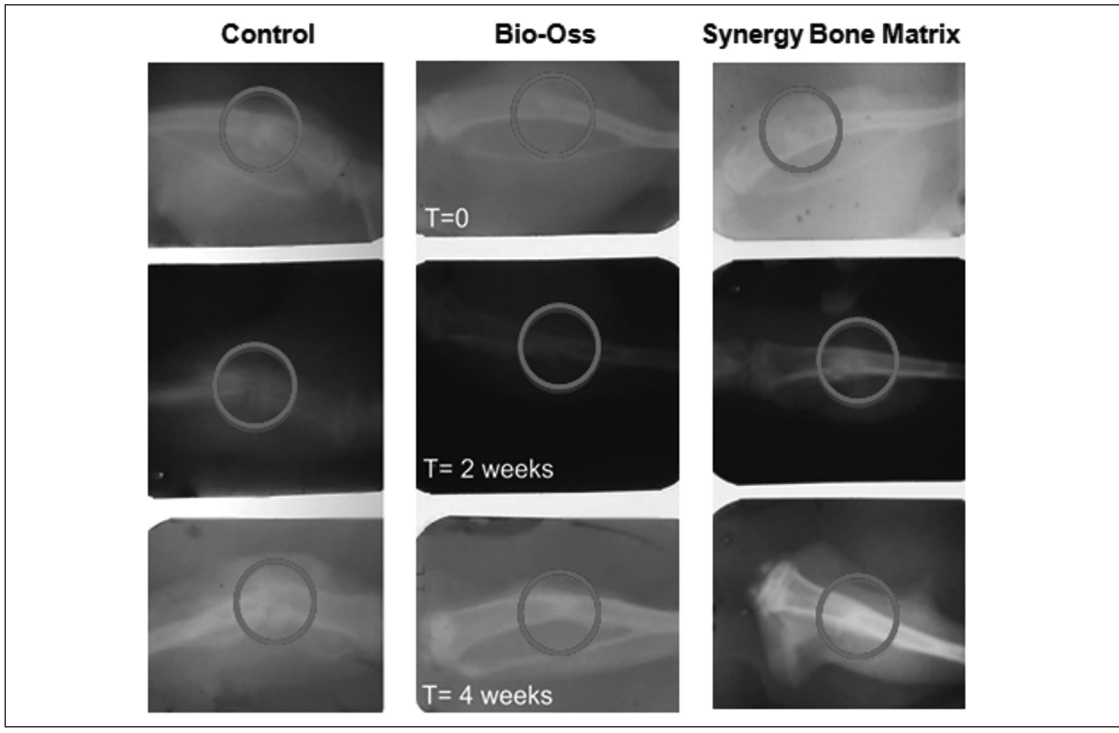

Figure 2. X-Ray images. **Control.** T= 0: a radiolucent circumscribed homogeneous round area was observed T= 2 weeks: a radiolucent area compatible with a bone defect. T= 4 weeks: Left tibia exhibited a fracture in the area where the bone defect was created. A radiopaque zone surrounding that area, compatible with fibrous tissue was observed. **Bio-Oss and Synergy Bone Matrix.** T= 0: a radiolucent area surrounded by a minimum thickness radiopaque cortical was observed. T= 2 weeks: a slightly diffuse radiopaque structure found compatible with bone graft structure was observed. T= 4 weeks: a less heterogeneous diffuse zone with osseointegration of bone graft material was observed, giving a radiological image compatible with alleged bone mineralization.

Bone mineral density evaluation

No significant statistical differences in BMC and BMD between tibiae filled with Synergy Bone Matrix and Bio-Oss were observed (Table 1). The BMC and BMD of the control group were significantly lower than the experimental groups (Table 1).

Histological observation

Cross-sections of tibiae showed remaining particles of each bovine bone graft, where the critical-sized bone defect was made. Multiple particles of Synergy Bone Matrix and Bio-Oss, of different shapes and sizes, surrounded by laminar bone tissue were observed in the medullary space. This finding indicates that both bone substitutes were osteoconductive. Proper bone healing was observed in the tibiae of both groups. No signs of inflammation were observed; this result suggests biological acceptability (Figure 3 A, B and C).

Table 1

Group	T= 4 weeks	
	BMC	BMD
Control	0.0354 ± 0.0188 g	0.1821 ± 0.0182 g/cm^2
Bio-Oss	0.0638 ± 0.0194 g	0.2007 ± 0.0120 g/cm^{2*}
Synergy Bone Matrix	0.0589 ± 0.0160 g	0.2002 ± 0.0167 g/cm^{2*}

* p<0.05 vs control

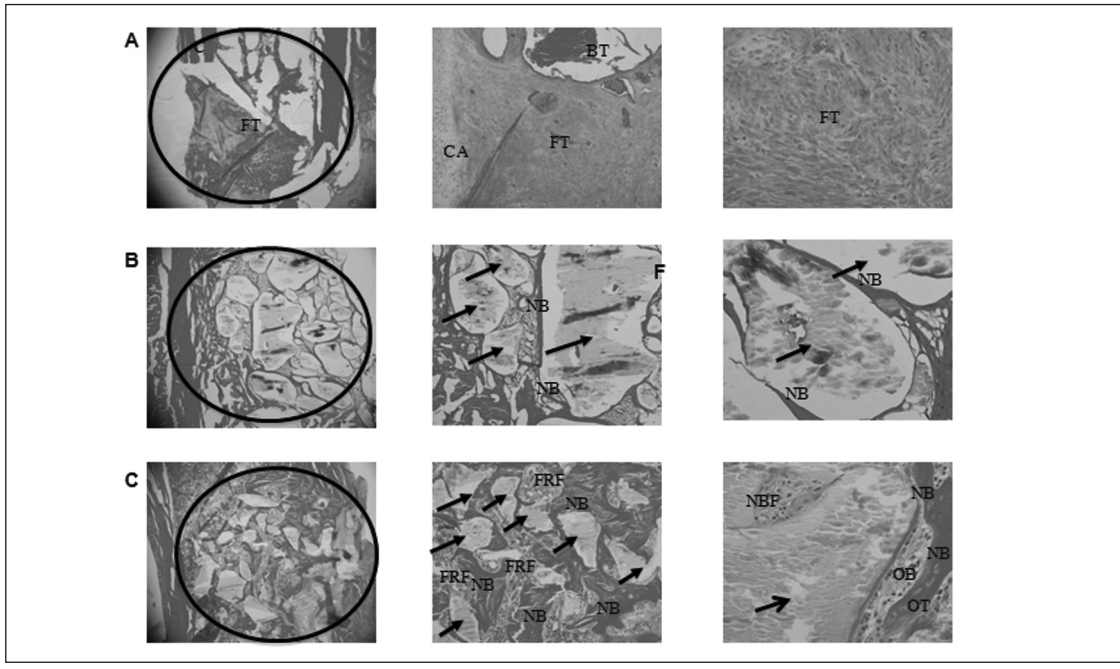

Figure 3. Histological evaluation. Photomicrography of the critical-sized bone defects at 4x, 10x and 40x magnification with Hematoxylin-Eosin staining. **A:** Control group. Circle indicates bone defect area. FT: fibrous tissue. BT: remaining bone trabecula. CA: cartilage. **B:** Critical-sized bone defect filled with Bio-Oss. Circle indicates bone defect area. Black arrows indicate Bio-Oss particles. NB: Newly formed bone trabeculae surrounding the particles. **C:** Critical-sized bone defect filled with Synergy Bone Matrix. Circle indicates bone defect area. Black arrows indicate Synergy Bone Matrix particles.
NB: new bone trabeculae surrounding the particles. FRF: reparative fibrous NBF: new bone formation foci. OB: osteoblasts. OT: osteocytes.

At 4 weeks post-implantation the histological analysis showed that blood vessels with small angiogenesis and re-vascularization foci had formed in the area implanted with either, Synergy Bone Matrix or Bio-Oss. Hematoxilin-eosin staining showed that mature Haversian systems forming a thin interface with the bone forming tissue, new bone formation represented by bone growth and surfaces covered by osteoblasts and fibroblast-like cells were observed surrounding the implanted bone grafts, which implied active osteogenesis. Although further studies are needed, this preliminary studied suggests that the bovine bone graft Synergy Bone Matrix presented similar properties of biocompatibility without inflammatory signs to that of the commercial material Bio-Oss. Moreover, Synergy Bone Matrix also exhibited similar osteoconductive properties to Bio-Oss, allowing a normal bone formation surrounding the particles.

Discussion

The present preliminary report provides evidence for the biocompatibility and osteoconductive properties of Synergy Bone Matrix, a bovine bone graft developed by the domestic industry.

Bone graft implantation is the major treatment modality for bone defect repair and reconstruction.[14] In experimental models, the bone defect above a critical size requires a scaffold to guide bone repair. According to literature, deproteinized bovine bone mineral is osteoconductive and provides excellent biocompatibility because it has similar physicochemical characteristics to that of the mineral component of the original bone.[15] These two important biological properties allow apposition of new bone formed by osteoprogenitor cells located in the host tissue. It is noteworthy that bovine bone inorganic-phase not only promotes the deposition of calcium and phosphate ions, but also it is partially remodeled by osteoclasts and osteoblasts of the host[5]. In addition, the large interconnecting pore volume and its composition encourage the formation and ingrowth of new bone at the implantation sites.

Bio-Oss is a recognized commercial bone defect filling material with osteoconductive properties. Under our experimental conditions, Synergy Bone Matrix, showed similar osteoconductive properties. Radiological finding showed that while untreated bone defects (control) remained unfilled, the bone defects filled with Bio-Oss or Synergy Bone Matrix exhibit gradual replacement of the bone graft with radiopaque tissue both, at the two studied times. Moreover all implants seemed well integrated to the surrounding bony margins.

At the end of the study, the defects treated with the bone grafting materials, were nearly completely filled with mineralized tissue. In addition, histological findings showed neovascularization in the area implanted with either, Synergy Bone Matrix or Bio-Oss. This finding suggests that both bone grafts provided an optimal microenvironment for bone ingrowth. Typically, bone formation starts by bone-forming cells secreting bone matrix (i.e. collagen) into the defect area, followed by mineralization to envelope the implanted graft material.[16] In the present report, collagen fibers had been replaced by mature bone filling the bone defect region confirming active osteogenesis 24 days post-implantation of the graft.

These findings indicate that the bone regeneration process induced by Synergy Bone Matrix and Bio-Oss presented similar characteristics and suggests the possible use of this material to increase bone volume in bones of high turnover as alveolar bone of the jaws. The use of bone grafts is important to preserve the alveolar bone ridge height and volume indispensable for dental implant placement. Despite the highly successful outcomes for the implant-supported overdentures, it seems that a majority of edentulous individuals have

not pursued implant-based rehabilitation. Among the reasons cited for this discrepancy between highly successful therapy and its acceptance is the cost of the treatment.[17] The presence of a biomaterial with similar characteristics than internationally recognized commercial brands, but developed by the domestic industry, will be an important tool to reduce the high cost of these interventions.

Conclusions

Although further studies are needed, this preliminary study suggests that the bovine bone graft Synergy Bone Matrix presented properties of biocompatibility without inflammatory signs similar to those of the commercial available material Bio-Oss. Moreover, Synergy Bone Matrix also exhibited similar osteoconductive properties to Bio-Oss, allowing bone formation surrounding its particles.

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Conflicts of interest: The authors declare no conflicts of interest.

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