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Bone repair of critical size defects treated with mussel powder associated or not with bovine bone graft: Histologic and histomorphometric study in rat calvaria

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ABSTRACT

The objective of this study was to evaluate the bone repair of critical size defects treated with mussel powder with or without additional bovine bone. Critical size defects of 5 mm were realized in the calvaria of 70 rats, which were randomly divided in 5 groups – Control (C), Autogenous Bone (AB), Mussel Powder (MP), Mussel Powder and Bovine Bone (MP-BB) and Bovine Bone (BB). Histological and histomorphometric analysis were performed 30 and 90 days after the surgical procedures (ANOVA e Tukey $p < 0.05$). After 30 days, the measures of remaining particles were: 28.36% (MP-BB), 26.63% (BB) and 8.64% (MP) with a statistically significant difference between BB and MP. The percentage of osseous matrix after 30 days was, AB (55.17%), 23.31% (BB), 11.66% (MP) and 10.71% (MP-BB) with statistically significant differences among all groups. After 90 days the figures were 25.05% (BB), 21.53% (MP-BB) and 1.97% (MP) with statistically significant differences between MP-BB and MP. Percentages of new bone formation after 90 days were 89.47% (AB), 35.70% (BB), 26.48% (MP-BB) and 7.37% (MP) with statistically significant differences between AB and the other groups.

Within the limits of this study, we conclude that mussel powder, with or without additional bovine bone, did not induce new bone formation and did not repair critical size defects in rat calvaria.

1. Introduction

The repairing of major bone losses is still a great challenge for modern Regenerative Medicine, especially in craniomaxillofacial and orthopaedic surgery. Despite the great potential of the bone tissue for repair, in some situations, according to the size of the defect, regeneration cannot be completely achieved because the defect may be invaded by surrounding connective tissue, which has a faster speed of cellular proliferation and migration than those of bone tissue (Melcher, 1976). Thus, the use of bone grafts should be considered, in conjunction with bone repair.

Bone grafts are classified into autogenous (the same individual is the donor and receptor of the graft), allogenous (donor and receptor individuals of the same species), xenogenous (donor and receptor individuals of different species) or alloplastic (synthetic) (Castro-Silva et al., 2009).

Although autogenous bone has been considered the first choice or "gold standard" for bone grafts because of its osteogenic, osteoinductive, and osteoconductive potentials, its availability is limited and their use results in greater morbidity (increased sensibility) to the patients. Therefore, the predictability of the clinical results with the use of allogenous, xenogenous and alloplastic bone grafts leads to consideration of these as valid options for tissue repair, mainly because of the lack of volume resorption, one surgical site, and decreased post-operative morbidity (Daelemans et al., 1997).

Xenografts have been produced for more than twenty years by biomaterial companies around the world, who have invested in the development of biocompatible materials capable of increasing and/or accelerating the bone repairing (Castro-Silva et al., 2009). The most common application of xenografts in Dentistry has been in the treatment of periodontal defects (Richardson et al., 1999), post-extraction sites (Gonçalves et al., 2009), maxillary sinus augmentation (Gonçalves et al., 2009) and alveolar ridge augmentation (Gonçalves et al., 2005). These grafts guide the new bone formation, that is, they have been considered as osteoconductive grafts.

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Xenografts of bovine origin, properly processed, biocompatible and osteoconductive have a separate role in helping bone repair. In a retrospective study conducted in humans, Block et al. (2012) performed surgery for horizontal augmentation of the anterior region of the maxilla with a particulate bovine xenograft and membrane. Through tomographic analyses, Block et al. (2012) concluded that the use of particulate bovine bone graft associated with membrane was efficient in the horizontal augmentation of the anterior maxilla, which was stable during the study period of 500 days.

Among the bovine commercially available xenografts, Bio-Oss® (Geistlich Biomaterials, Wolhusen, Switzerland) is the most commonly used. In Brazil, Orthogen® (Genius, Baumer, São Paulo, Brazil) is one of the viable options for particulate xenografts of bovine origin. In addition to the bovine xenografts, other sources can be used to obtain the grafts, such as porcine (Suckow et al., 1999), equine (Di Stefano et al., 2009), marine algae (Buser, 2009) or bio coral grafts (Yukna and Yukna, 1998; Shafiei-Sarvestani et al., 2012).

Roux et al. (1988) used coral fragments as bone substitutes in cranial surgery. Of the 167 grafts implanted, 150 were used to fill defects of 10 mm of diameter performed with burs; five were larger implants (length of 20–40 mm) to repair cranial defects and 12 were coral blocks to reconstruct the floor of the anterior nasal fossa. According to the authors, the coral implants are biocompatible and their resorption occurs while new bone tissue is formed. Roux et al. (1988) concluded that corals are promising biomaterials for the use in cranial reconstructive surgeries. Currently few studies are available on the use of corals as bone substitutes.

Similar to the corals, mussels have been considered as calcium sources, because 96% of its chemical composition is composed of calcium oxide (CaO). As far as we could find out there are no studies on the use of mussels as an alternative biomaterial for bone repairing.

This present study aimed to assess the bone repairing of defects of critical size created in the rat calvaria, treated with mussel powder graft with or without bovine bone graft histologically.

2. Material and methods

This present study was submitted and approved by the Ethical Committee in Research of the Positivo University, under protocols number 015/2011 and 34/2011. Seventy male rats (*Rattus norvegicus*, *albinus*, Wistar) were used. They had a mean age of 7 months with weight ranging from 365 to 480 g. The animals were randomly divided into five groups according to Table 1.

2.1. Anaesthetic protocol

For the experimental surgical procedures, the animals were positioned inside a campanula individually and anaesthesia was induced with oxygen and isoflurane (Cristália, Itapira, SP, Brazil) followed by an intramuscular injection on the posterior part of the thigh with 2.3 g xylazine (0.52 mg/kg) (Vetbrands, Paulínia, SP, Brazil) and 1.16 g ketamine (1.04 mg/kg) (Vetbrands, Paulínia, SP, Brazil). Anaesthesia was maintained with isoflurane vaporization (Cristália, Itapira, SP, Brazil) by facial mask if necessary.

Table 1
Distribution of groups: C (Control); AB (Autogenous bone); MP (Mussel powder); MP-BB (Mussel powder + Bovine bone graft) and BB (Bovine bone graft).

Groups	30 days	90 days	Treatment
GROUP C	07 animals	07 animals	Without treatment
GROUP AB	07 animals	07 animals	Autogenous bone
GROUP MP	07 animals	07 animals	Mussel powder
GROUP MP-BB	07 animals	07 animals	Mussel powder + bovine bone graft
GROUP BB	07 animals	07 animals	Bovine bone graft

2.2. Surgical procedures

After induction of anaesthesia, shaving and antisepsis of the areas to be operated (calvaria) was carried out. After antisepsis, a "U" shaped incision was performed with the aid of a size 15c scalpel blade for surgical access to the calvaria area and a flap was raised posteriorly.

A critical size defect (CSD) of 5 mm of diameter (Fig. 1) (Schmitz and Hollinger, 1986) was created with a trephine bur (Neodent, Curitiba, PR, Brazil) mounted in a contra-angle handpiece for implant (20:1, Kavo, Joinville, SC, Brazil), under copious irrigation with sterile saline solution.

With the aid of a millimetric probe (PCPUNC 15, Hu-Friedy, Chicago, IL, USA) and a size 701 tapered bur mounted in straight handpiece, two "L-shape" marks were performed at 2 mm towards anterior direction and 2 mm towards posterior direction to the surgical defect margins. The long axis of each "L" was localized on a longitudinal imaginary line which divided the surgical defect by half and the marks were then filled with dental amalgam (Permite – SDI, Victoria, Australia). These marks were created to identify the middle of the original surgical defect during the laboratorial processing and to locate the original bone margins of the surgical defects during the histological and histomorphometric analyses.

In group C, the surgical defect was filled with only the blood clot as a negative control. In group AB, the defect was filled with triturated autogenous bone graft as a positive control. The autogenous bone was gathered from the calvaria portion that had been removed to create the surgical bone defect. The bone was ground up with the aid of a pestle-type bone grinder (Kopp, Curitiba, PR, Brazil).

In group MP, the defects were filled with mussel powder. This was obtained from *Perna* mussels or brown mussel, which is a genus of freshwater mussel (family MYTILIDAE, class BIVALVIA). The molluscum was removed from its shells, which were washed with neutral detergent and cleaned with a brush. The shells were then washed in running water and immersed into water for 30 min and again washed individually. Next, the shells were stored in a refrigerator at 3 °C up to trituration.

The shells were initially ground up in a hand grinder (Kopp, Curitiba/PR, Brazil), resulting in a powder, so-called dirty powder. Next, the dirty powder was ground up in an electrical motor (Polidora, Knebel, Porto Alegre/RS, Brazil) resulting in a clean powder. Then, the clean powder was manually sieved through 60 meshes resulting in the mussel powder used to fill the bone defects. The mussel powder was placed onto a Petri plate, wrapped into surgical paper and sterilized in autoclave (Cristófoli, Campo Mourão/PR, Brazil). This powder was then inserted into the bone defects with the aid of a Molt surgical curette.

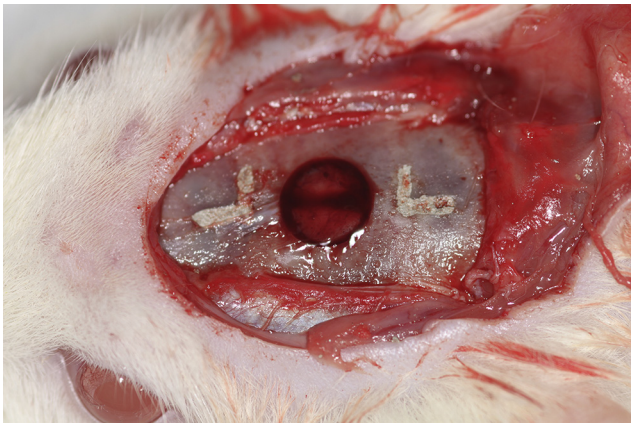

Fig. 1. A critical size defect (CSD) of 5 mm of diameter in rat calvaria.