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Osteogenic efficacy of bone marrow concentrate in rabbit maxillary sinus grafting

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ABSTRACT

Maxillary sinus grafting is required to increase bone volume in the atrophic posterior maxilla to facilitate dental implant placement. Grafting with autogenous bone (AB) is ideal, but additional bone harvesting surgery is unpleasant. Alternatively, bone substitutes have been used but they limit new bone formation. The strategy of single-visit clinical stem cell therapy using bone marrow aspirate concentrate (BMAC) to facilitate new bone formation has been proposed. This study aimed to assess bone regeneration capacity of autologous BMAC mixed with bovine bone mineral (BBM) in maxillary sinus grafting. Twenty-four white New Zealand rabbits were used and their maxillary sinuses were randomly assigned for grafting with 4 different materials. Rates of new bone apposition in augmented sinuses were measured and bone histomorphometry were examined. Significant increase in the quantity of nucleated cells and colony forming unit-fibroblasts were confirmed in BMAC. Mesenchymal stem cells in BMAC retained their in vitro multi-differentiation capability. Higher rates of mineral appositions in the early period were detected in BBM + BMAC and AB than BBM alone, though they are not significantly different. Graft volume/tissue volumes in BBM and BBM + BMAC were found to be higher than those in AB and sham.

1. Introduction

Alveolar bone deficiency in the posterior maxilla from severe bone loss after tooth extraction with or without pneumatization of the maxillary sinus is problematic for dental implant placement (Devlin et al., 1998; Balshi and Wolfinger, 2003; Sharan and Madjar, 2008). Bone quality in this area is generally poor because it has low bone density, loose trabecular bone and thin cortical bone (Lekholm et al., 1985; Bryant, 1998; Drage et al., 2007). In addition, the posterior maxilla is considered as a high loading area because it is near the temporomandibular joint (Tolstunov, 2007). Consequently, the highest rate of dental implant failure was reported in this area (Moy et al., 2005).

In order to reduce dental implant failure, bone augmentation is required to accommodate dental implant placement as well as provide mechanical support. Vertical bone augmentation in an atrophic maxilla knows as "maxillary sinus grafting". The technique using a lateral window approach was first described by Tatum (Boyne and James, 1980). The standard grafting material using bone augmentation is autogenous bone (AB). It possesses osteogeneic, osteoconductive and osteoinductive properties which correspond with the key triad factors of tissue regeneration including cells, extracellular matrix and signaling molecules (Piattelli et al., 1999; Griffth and Naughton, 2002; Bruder and Scaduto, 2005). Sources of AB include cavarium, mandibular ramus, maxillary tuberosity, iliac bone and tibia (Hung, 2012). The bone harvesting procedure at the donor site is unpleasant, traumatic to patients, and susceptible to risks of morbidities (Block and Kent, 1997).

Currently, various bone substitute materials have been used for bone grafting (Damien and Parsons, 1991). Bovine bone mineral (BBM), a xenograft, is well-accepted as one of the alternatives to AB. It has been proven success in supporting dental implant treatment (Ramírez-Fernández et al., 2011). BBM contains more than 95% hydroxyapatite particle, size of 1–3 μm, and pore size of 100–1,500 μm with interconnecting pores (Gierse and Donath, 1999; Briem et al., 2002). It only has osteoconductive property and the amount of newly formed bone has been found to be

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histologically limited (Gazdag et al., 1995; Stavropoulos, 2008; Testori et al., 2009). Therefore, supplements to bone substitution have been introduced to improve bone regeneration such as cell-based and/or osteogenic factor-based therapy (Nandi et al., 2010).

Bone substitutes incorporated with stem cells have been introduced as a promising strategy to enhance bone regeneration. Stem cell-based tissue engineering aims to increase viable bone cells by cell transplantation. Undifferentiated mesenchymal stem cells (MSCs) or osteogenic cells have been cultured and expanded on scaffolds before being grafted into patients (Chen et al., 2008). Recently, the strategy of immediate stem cell application by transplantation of bone marrow aspirate concentrate (BMAC) has attracted considerable attention in regenerative medicine (Connolly et al., 1991; Hernigou et al., 2005; Jäger et al., 2009; Farré-Guasch et al., 2013).

The prevalence of MSC in bone marrow has been reported to be as low as 0.00001–0.01% of nucleated cells (Pittenger et al., 1999; Muschler et al., 2003; Wexler et al., 2003; Hernigou et al., 2005; Pountos et al., 2007; Murawski and Kennedy, 2011). Bone marrow enriched by a concentration technique has been utilized in which the quantity of nucleated cell was reported to increase by a factor of 3–5 (Connolly et al., 1989; Gessmann et al., 2012; Betsch et al., 2013). BMAC contains several types of nucleated cells including MSCs, endothelial stem cells and platelets (Haig et al., 1992; Hernigou et al., 2005; Massberg et al., 2006; Marx and Tursun, 2013). In view of bone formation, co-existence of MSCs and endothelial progenitor cells showed a synergistic effect for osteogenesis (Moioli et al., 2008; Atesok et al., 2010). Likewise, signaling molecules released from MSCs, lymphocytes, granulocytes and platelets are also known to play a role in increasing vascularization which subsequently enhances new bone formation (Andrew et al., 1994; Kanczler and Oreffo, 2008; Langer and Gawaz, 2008; Schroeder and Mosheiff, 2011). The use of autologous BMAC application has shown a benefit in tissue regeneration, for example, in treatment of delayed-union, non-union in limbs and avascular necrosis of bone (Connolly et al., 1989; Hernigou et al., 2005; Sen and Miclau, 2007; Hernigou et al., 2008). This procedure has been shown to be a simple, safe, less time consuming and inexpensive technique without malignant transformation (Hendrich et al., 2009; Jäger et al., 2009; Coelho et al. 2012).

The present study aimed to assess bone regenerative efficacy of BMAC in a rabbit maxillary sinus grafting model using BBM as a scaffold. Quantitative evaluation of nucleated cells of BMA and BMAC was carried out and BMAC-derived MSCs were tested for their multilineage differentiation in vitro. The osteogenic efficacy of BMAC was also examined in a rabbit maxillary sinus grafting model using micro-CT analysis and histological investigation.

2. Materials and methods

Twenty-four female adult New Zealand white rabbits aged 6 months (3.0–4.0 kg) were utilized in this study. Animal license was issued by the Department of Health. Ethical approval of this study was granted by the Committee on the Use of Live Animals in Teaching and Research (CULATR), Li Ka Shing Faculty of Medicine, the University of Hong Kong. Care and management of the experimental animals was conducted in accordance with the CULATR guidelines. Before the surgical procedures, the rabbits were (s/c) injected subcutaneously with antibiotics, Baytril® (enrofloxacin: 5–10 mg/kg) and Temgesic (buprenorphine: 0.05 mg/kg) for analgesic. The rabbits were anaesthetized with ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (1 mg/kg) via intramuscular injection. Four different materials including normal saline solution (sham control), AB (standard control), BBM (Endobon®, Biomet3i, USA) and BBM with BMAC were randomly assigned for grafting to 4 maxillary sinuses at each healing periods of 2, 4, and 8 weeks survival groups. Finally, the rabbits were euthanized with intravenous pentobarbital (120 mg/kg) at 2, 4, and 8 weeks post-operatively.

Diagram for the experiment is shown in Fig. 1.

2.1. Surgical procedures

2.1.1. Bone marrow aspiration and concentration

Bone marrow aspiration was performed using needle aspiration technique from both tibias of rabbit. The inner surface of a spinal needle (18 GA, BD) and 10 ml-syringes were coated with heparin (1000 U/ml; DKSH, Leo pharmaceutical product). Thereafter, 2½ ml heparin was pre-loaded in a 10 ml syringe for anticoagulation. After skin preparation and disinfection, bone marrow was aspirated into the 10 ml-syringe and immediately mixed with heparin by gentle rotation. Subsequently, BMA was centrifuged at 400 *g* for 10 min at room temperature (RT). Twenty-microliter of pellet was collected by micro-pipette and used as BMAC for mixing with BBM. Likewise, BMA and BMAC were sent for evaluation in laboratory.

2.1.2. Maxillary sinus grafting procedure

The surgical procedure was performed using a modified method as reported by Xu (Xu et al., 2003). In brief, 2% xylocaine (0.5 ml) with epinephrine 1:80,000 (Xylestesin™-A, 3M) was given subcutaneously (s/c) along the dorsal surface of the nasal bone. A para-sagittal incision was made at the midline of nasal bone. Skin and periosteum were elevated to expose the nasal bone and fronto-nasal suture (sutura frontonasalis). Two bone windows were created at approximately 1.5 cm anteriorly, 0.8–1.0 cm laterally to the internasal suture (sutura internasalis) on both sides using a round diamond bur under copious irrigation with normal saline (Fig. 2a). An osteotome was used to displace the bone window inward. A plastic instrument (Dentsply, USA) was used to gently elevate the antral membrane. Sufficient space in each maxillary sinus was created and the graft was inserted. Four different grafts including normal saline solution (negative control), AB (standard control), BBM (Endobon®, Biomet3i, USA) and BBM + BMAC were randomly assigned for grafting in each maxillary sinus. Wound was closed with 4/0 Vicryl (Johnson & Johnson Medical, USA).

After the operation, Baytril® (enrofloxacin: 5–10 mg/kg) was given s/c for the first 3 days. Thereafter, it was incorporated in the drinking water (100 mg/l) for 14 d or until the sutures were removed. For pain relief, Temgesic was injected for the first 3 days post-operatively. This was followed by Meacham injection (meloxicam: 0.2 mg/kg) s/c for 14 d or till sutures were removed.

2.1.3. Autogenous bone harvesting procedure

The lateral right hind limb of a rabbit was shaved and prepared with antiseptic solution. Local anesthesia was s/c injected with 0.5 ml of 2% xylocaine with epinephrine 1:80,000 (Xylestesin™-A, 3M). A 1 cm longitudinal incision was made over the lateral side of femoral head. Superficial tissue and periosteum were incised and reflected. Cortico-cancellous bone was harvested using 3.5 mm diameter-trephine bur under copious irrigation with normal saline. Afterward, the defect was packed with a hemostatic sponge (Spongostan® Dental, Johnson & Johnson Medical, USA) and the wound was closed with 4/0 Vicryl (Johnson & Johnson Medical, USA). The cortico-cancellous bone was weighted to 100 mg. It was chipped into small particles before grafting. Bone marrow aspiration and AB harvesting procedures were not performed in the same rabbit in view of increased morbidities causing additional suffering to the animals or potential increase in mortality rate.