Enhanced bone healing with BCP granules implanted with BMP-2 and MSCs in a rabbit calvarial defect

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Abstract

The aim of this study was to evaluate the *in vivo* osteogenic potential of biphasic calcium phosphate (BCP), bone morphogenetic protein 2 (BMP-2), and/or mesenchymal stem cell (MSC) composites by using a rabbit calvarial defect model. Bone formation was assessed by using three different kinds of implants in rabbit calvarial defects, BCP alone, BCP/recombinant human (rh) BMP-2, and BCP/rhBMP-2/MSCs composite. The implants were harvested after 2 or 8 weeks, and the area of new bone formation was quantified by micro-computed tomography (micro-CT) and histologic studies. The highest bone formation was achieved with the BCP/rhBMP-2/MSCs treatment, and it was significantly higher than that achieved with the empty or BCP-alone treatment. The quantity of new bone at 8 weeks was greater than at 2 weeks in each group. The relative density of osteocalcin immunoreactivity also increased during this interval. These results indicate that the combination of BCP, rhBMP-2, and MSCs synergistically enhances osteogenic potential during the early healing period and could be used as a bone graft substitute.

Background and Aim

Calcium phosphate ceramics have been widely applied as bone substitutes because of their resemblance to bone in orthopedic, maxillofacial, and dental surgery. Biphasic calcium phosphate (BCP) is composed of various concentrations of hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP). BCP shows efficient incorporation into bone tissue; its biocompatibility, degradability, and porous structure facilitate intergrowth through newly formed bone. However, the osteoinductive properties of synthetic ceramics, including BCP, are insufficient for the healing of extensive bone defects [1].

To overcome the problem, several growth factors, such as bone morphogenetic proteins (BMPs), fibroblast growth factors, and vascular endothelial growth factors have been used for bone tissue engineering [2]. BMP-2 is a promising therapeutic agent promoting bone regeneration when delivered locally in combination with bone substitute.

The bone-forming ability of the bone substitute/BMP-2 composite is insufficient because of the time required for osteoblast differentiation and for recruiting mesenchymal stem cells (MSCs) to the injury site. To overcome this problem, recent studies have reported cell-based therapy using MSCs. The cell source is an important factor for successful bone tissue regeneration [3].

Therefore, the aim of this study was to evaluate the osteogenic therapeutic efficacy of BCP, BMP-2, and MSCs in combination by using a rabbit calvarial defect model. The osteogenic capacity was evaluated by a micro-computed tomography (micro-CT) analysis and histologic studies.

Methods and Materials

Preparation of mesenchymal stem cell

Rabbit bone marrow was collected from the iliac after anesthesia using 18-gauge needles. The extracted bone marrow was cultured in α -Minimum Essential Medium (Gibco, Gaithersburg, MD, USA) containing 20% fetal bovine serum (Gibco) and 1% antibiotics (Gibco), in a humidified CO₂ incubator at 37 °C for 1 week. Before the *in vivo* transplantation experiment, cells were dissociated with TrypLE Express (Invitrogen), washed with PBS, and collected. The collected cells were resuspended at a concentration of 1×10^7 cells/mL and used for *in vivo* study.

Implantation of BCP ceramic

BCP was purchased from Dio Implant (Busan, Korea; HA: β -TCP = 70:30). The rhBMP-2 supplied by the Korea Bone Bank (Seoul, Korea) was dissolved in a buffer at a concentration of 1.5 μ g/ μ L. Then, 10 μ L of the rhBMP-2 solution was soaked with 30 mg of BCP and freeze dried for 48 hours. To transplant the MSC, 30 mg BCP or BCP/rhBMP-2 was soaked with 10 μ L of prepared MSC suspension solution (at 1×10^7 cells/mL).

Loading of BCP ceramics with BMP or/and MSCs

The animal experiments were performed according to the protocols approved by the Institutional Animal Care Committee of Wonkwang University. To determine the osteogenic potential in a calvarial defect model, we prepared four experimental groups of implants as follows: empty (non-implanted), BCP, BCP/rhBMP-2, and BCP/rhBMP-2/MSCs.

Micro-CT analysis

The specimens were scanned using micro-CT (Sky-Scan 1172TM; Skyscan, Kontich, Belgium). The bone volumes percentiles were calculated as the ratio of radio-opaque regions of interest to the total defect volume, and residual BCP volumes were also measured from the micro-CT data.

Histological examination

The harvested specimens were dehydrated in a graded series of alcohol solutions (80–100%), decalcified in a 10% EDTA solution, and embedded in paraffin. 5- μ m sections of the specimens were cut and stained with hematoxylin and eosin (H&E) stain and Goldner's Masson trichrome stain. To quantify the osteogenic responses, immunohistochemistry stain performed with anti-osteocalcin.

Results

Figure 1. The 2-dimensional reconstructed image of calvarial defects 8 weeks after implantation, using a micro-CT analysis.

Figure 2. Quantification of the newly formed bone volume (A), Image of the residual BCP granules (B) and quantification of the volume (C) at 8 weeks, calculated from micro-CT data. Each column represents the mean $\pm$ standard deviation (SD) (*, # $P < 0.05$)

Figure 3. Hematoxylin and eosin staining at a higher magnification for the bone generated after implantation for 2 weeks (A) and 8 weeks (B) in the marginal defect. Arrow, new bone near a BCP granule; Asterisk, BCP granule; HB, host bone; NB, new bone. Scale bar = 250 μ m.

Figure 4. Histologic view of the bone generated 2 and 8 weeks after implantation, in the central area of bone defects. Arrow, new bone near BCP granule; CT, connective tissue; asterisk, BCP granule. Scale bar = 250 μ m.

Figure 5. The immunohistochemical localization of osteocalcin in the margin of bone defects 8 weeks after implantation. Arrow, osteoblast-like cells surrounding lamellar bone matrix; Asterisk, BCP granule; HB, host bone; NB, new bone; Scale bar = 250 μ m.

Conclusion

This study provided histologic evidence that the combination of BCP, rhBMP-2, and MSCs could synergistically enhance BCP resorption rate and new bone formation in rabbit calvarial defects.

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References

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