Bone regeneration in critical size defect by hBMSCs mediated BMP-2 non-viral gene transfer

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INTRODUCTION
Bone morphogenetic protein-2 (BMP-2), have demonstrated potential for enhancing therapeutic approaches for treatment of an array of musculoskeletal problems, and for bone and cartilage tissue engineering. Mesenchymal stem cells are suitable cell sources for tissue regeneration and gene therapy, since they have the ability to differentiate into mesodermal cell lineages such as muscle, bone, cartilage, and fat under appropriate culture conditions, while circumventing ethical issues facing embryonic stem cells. Recently, stem cells were successfully transduced with therapeutic genes via viral vehicles. However, the probabilities of inducing toxicity, immune and inflammatory responses by virus remain to be the main concern. Our previous study demonstrated that microporation is an excellent non-viral method for gene transduction in hBMSCs. In this study, we further tested the effects of the non-viral delivery of a small amount of plasmid (<2.5 μg) encoding BMP-2, to human bone marrow-derived mesenchymal stem cells (hBMSCs) on osteogenesis in vitro and test its effect on bone healing in a null mice critical size calvarial defect model.

METHODS
Bone marrow sample was obtained by tapping from the iliac crest of patients undergoing orthopaedic surgery. Human bone marrow stromal cells (hBMSCs) were isolated using Percoll (70% in PBS) and cultured in K-NAC medium [Keratinocyte-SFM (Gibco-Invitrogen Corporation) supplemented with 2 mM N-acetyl-L-cysteine (NAC) and 0.2 mM L-ascorbic acid 2-phosphate (Asc 2P)]. The medium was changed every two to three days. hBMSCs were transfected with either pEGFP-N1 (Clontech, BD Biosciences) or pcDNA-BMP2 by Microporator (Digitalbiotechnoloty, South Korea). One day after the transfection, the osteogenetic medium was completely exchanged. BMP2 concentration was determined by BMP2 ELISA kit (R&D Systems). Mineralization was determined at 21st day after osteo-induction using Alizarin Red S staining. Cultures of murine neonatal calvaria incubated for 10 days in the presence of pcDNA-BMP-2 transfected hBMSCs or hBMSCs only. In vivo study, a round-shape defect (diameter: 3.0 mm) were created in the calvarial bones in 6 null mice. The mice were 8 weeks old and weighed 20-23g. The PLGA scaffolds were prepared as disks with pore size within 300-400 μm. The hBMSCs were seeded in PLGA scaffold for over night and implanted to the site of defect. Calvarial were harvested and analysed using a micro-CT machine (Skyscan 1076; Skyscan, Aartselaar, Belgium) at the 4th weeks after surgery.

RESULTS:
Our results showed that pcDNA-BMP-2 increased the protein expression of BMP-2 on hBMSCs in a dose-dependent manner after transfection (Fig. 1). For subsequent time points up to day 9, the concentration of BMP-2 produced by the hBMSCs transfected with pcDNA-BMP-2 and grown in osteogenetic medium appeared to be stable at approximately 1 ng/mL. We also found that a 21-day pellet culture of pcDNA-BMP-2 transfected hBMSCs significantly increased mineralization (Fig. 2). We further confirmed the effect of new bone formation by pcDNA-BMP-2 transfected hBMSCs on neonatal calvaria (Fig. 3). In vivo study, the micro-CT analysis showed that the new bone formation was enhanced in pcDNA-BMP-2 transfected hBMSCs group comparing with vehicle control group (Fig. 4).

DISCUSSION
Our results demonstrate that hBMSCs transfected with a low load (<2.5 μg) of a BMP-2 plasmid using microporation can sustain production of BMP-2, with the accumulated amounts reaching therapeutic levels. In this study, we found that the higher BMP-2 production in the pcDNA-BMP-2 group was due to a higher concentration of plasmid (2.5 μg > 1.5 μg > 1.0 μg). According to this study, we suggest that the future applications of microporation may be useful for select bone tissue engineering applications.

REFERENCE