Bone marrow derived mesenchymal stem cells (BMSCs) are suitable for bone tissue regeneration because they are easily isolated from a small aspirate of bone marrow and can be differentiated into osteoblasts in vitro. For clinical applications of BMSCs, we have previously demonstrated that transplantation of culture expanded BMSCs into the distracted callus accelerated new bone formation and shortened the treatment period during distraction osteogenesis. The proportion of BMSCs, however, is rather low and the cells generated in primary culture seem to be limited for bone regeneration. Expanding the cell population by several passages would be necessary to obtain sufficient number of cells, but osteogenic potential of BMSCs decreased with pasaging. Establishment of the culture conditions that permit the rapid expansion of the BMSCs while retaining their potential for differentiation will be required.

Materials and methods

BMSCs were obtained from the femora of 8-week old Sprague–Dawley (SD) rats. Adherent cells were expanded as monolayer cultures in a 5% CO₂/95% air atmosphere at 37 °C with medium changes every 3 days. These primary cells were referred to as P0. The confluent cells were subcultured in new 6 well culture dishes at a plating density of 6x10⁵ cells/dish. This was repeated 4 times and these cultures are referred to as P1, P2, P3, P4, and P5. Two groups were prepared – BMP (+): BMSCs continuously cultured with the medium supplemented with 300ng/ml rhBMP-2, and BMP (-): BMSCs cultured without rhBMP-2. To evaluate in vitro osteogenic differentiation of BMSCs seeded in the 6-well dishes during all passages, the concentration of alkaline phosphatase (ALP) and osteocalcin (OC) in the culture media were measured, and the mRNA expression of the ALP and OC were quantified by real time RT-PCR analysis. For in vivo experiments, the individual cultured BMSCs were suspended in 100 ul of alfa-MEM and cultured in vivo. Sufficiently expanded BMSCs cultured with the addition of rhBMP-2, which were capable of inducing and participating in bone formation, can be used in clinical setting to improve bone healing.

Results

In the P0 through P5 cells, the ALP activity and the OC concentration in the supernatant of the BMP(+) group was significantly higher than those of the BMP(-) group (Fig. 1). The expression of the ALP and OC mRNA was demonstrated in the P0 through P4 cells of the BMP (+) group and in the P0 through P3 cells of the BMP (-) group, and different expression levels of the two genes within the passaged cells were ascertained by quantitative real time RT-PCR analysis (Fig. 2).

Calcified tissue formations within the diffusion chambers were radiologically more remarkable in the BMP(+) group than in the BMP (-) group, although the number of calcified nodules were decreased with passages in both groups (Fig 3). Histological examinations of the diffusion chambers demonstrated that massive bone and cartilage formation within the membrane were notable in the P1 through P3 cells of the BMP (+) group (Fig. 4).

Discussion

Loss of osteogenic potential of BMSCs by passages has been a dilemma for autologous cell therapy to promote bone formation. In the BMP (+) group compared to the BMP (-) group, elevated levels of osteogenic markers and more calcified tissue formation observed during all passages. As a result, osteogenic potential of the BMP (+) group was maintained even in the several passaged BMSCs. From our results, we suggested that the stimulation of rhBMP-2 can enhance the osteogenic differentiation of BMSCs in vitro to consequently form bone-like tissue in vivo. Sufficiently expanded BMSCs cultured with the addition of rhBMP-2, which were capable of inducing and participating in bone formation, can be used in clinical setting to improve bone healing.