OSTEOGENIC POTENTIAL OF RAT BONE MARROW DERIVED MESENCHYMAL STEM CELLS AFTER SEVERAL PASSAGES
++Sugiura F; Kitoh H; Ishiguro N
++University of Nagoya, Nagoya, Japan

Introduction
Bone marrow could be a useful donor for bone tissue engineering because it contains mesenchymal stem cells (MSCs) that have the ability to differentiate a variety of mesenchymal lineages including osteoblasts. In clinical applications for bone regeneration, the total number of MSCs generated in primary culture often seems to be limited for bone regeneration. Expanding the cell population by several passages would be necessary to obtain a sufficient number of cells. This presents a problem, since serially passaged MSCs have been shown to lose their capacity to differentiate into osteoblasts. In the present study, osteogenic potential of rat MSCs was analyzed during several passages.

METHODS
MSCs were derived from bone marrow of 8-week-old SD rat. Cells were cultured in two different kinds of culture medium; control medium (CM) and osteogenic medium (OS). OS is consisted of CM supplemented with 0.2mM ascorbic acid, 10nM Na-β-glycerophosphate and 10⁻⁷M dexamethasone to increase MSC osteogenic differentiation. These primary cells are referred to P0. The confluent cells were subcultured in new 6-well culture dishes at a plating density of 6×10⁶ cells/dish. This was repeated 4 times and these cultures are referred to P1, P2, P3, P4. To evaluate in vitro osteogenic differentiation of MSCs seeded in the 6-well dishes during all passages, the concentration of ALP in the supernatants, and the expression of ALP mRNA were studied. For in vivo experiments, the individual cultured MSCs were suspended in 200µl of a-MEM and 0.5% type I collagen gel, and these cell suspensions were then loaded into the diffusion chambers (DC). These chambers were subcutaneously implanted into the dorsal side of 8-week-old male athymic mice in pockets formed by blunt dissection. The mice were sacrificed at 4 weeks postimplantation and the newly formed tissues in the chambers were analyzed for osteogenesis radiographically and histologically.

RESULT
The ALP activity in the supernatant of OS groups was significantly higher than that of CM groups. Within the OS groups, the ALP activity was prominent in P0, P1 cells, but it decreased with passaging (Figure 1). Similarly, the expression of ALP mRNA increased between P0 and P2, but it was extremely low in P3 and later passages (Figure 2). Radiological examination of the chambers showed marked calcified tissue formation in OS groups of P0 and P1. On the other hands, few calcifications were seen in OS P3 and CM groups (Figure 3). These findings were confirmed by histological analysis. Massive bone and cartilage formation within the membrane was demonstrated in OS P0 through P2, while most of the newly formed tissue was filled with fibroblastic cells in P3 (Figure 4).

DISCUSSION
Dexamethasone is thought to increase the number of osteoprogenitor cells of MSCs. In agreement with previous studies, differentiation into the osteoblastic lineage of rat MSCs was induced by osteogenic medium. MSCs continuously cultured in OS, however, lost their osteogenic potential with passaging. Since the differentiated cells may have a limited capacity for proliferation, loss of differentiation may be due to decreased percentage of osteogenic cells in culture by passaging. The failure of in vivo osteogenesis in the implanted P5 cells was a dilemma for autologous cell therapy for bone regeneration. Further investigations are needed to establish the culture conditions that permit the rapid expansion of MSCs while retaining their potential for differentiation.

Figure 1. ALP activity in the OS medium by passaging

Figure 2. The expression of ALP and GAPDH mRNA by RT-PCR analysis

Figure 3. Radiological examinations of diffusion chambers by passaging

Figure 4. Photomicrographs of the histologic section of diffusion chamber at 4 weeks after implantation. Hematoxylin and eosin staining.