Regulation of mesenchymal stem cell differentiation by novel stepwise osteogenesis-mimicking matrices

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Introduction: Stem cells are promising sources of cells in tissue engineering and regenerative medicine because of their ability to differentiate into multiple cell lineages. To apply stem cells in tissue engineering and regenerative medicine, it is necessary to regulate their differentiation into specific cell types. There are many efforts to regulate stem cell differentiation *in vitro* and that focusing on ECM is one of the most important approaches. ECM can regulate stem cell differentiation during *in vivo* tissue development. During the process of tissue development, ECM is dynamically remodeled and its composition is changed based on the stage of stem cell differentiation. Although the mechanics is unknown, ECM remodeling seems to be important for stem cell differentiation and ECM formed at different stages should have different effects. However, the detailed effects of ECM at the different stages of stem cell differentiation are still unclear because there have been no *in vitro* models that mimic *in vivo* stepwise remodeled ECM. Here, we developed matrices that mimicked the stepwise remodeled ECM during osteogenesis. The matrices were referred to as "stepwise osteogenesis-mimicking matrices" and their effects on stem cell differentiation were investigated.

Materials and Methods: MSCs were differentiated into osteoblast with serum, dexamethasone, and β-glycerol phosphate. To check the osteogenic differentiation, alkaline phosphatase (ALP) staining and alizarin red S staining were performed. The production of ECM proteins was evaluated by immunocytochemical analysis. To obtain osteogenesis-mimicking matrices, cultured MSCs were treated with Triton X-100 and NH_4OH following with DNase I and RNase A treatment. MSCs were re-seeded on the stepwise osteogenesis-mimicking matrices. The osteogenic differentiation of MSCs on the stepwise osteogenesis-mimicking matrices was investigated.

Results and discussion: After 1 week of MSCs culture under osteogenic condition, ALP staining was positive whereas alizarin red S staining was negative. After 3 weeks of culture, alizarin red S staining became positive. Therefore, we classified the osteogenesis of MSC into 2 stages *i.e.* early stage and late stage. 1 week-osteogenesis-induced matrices and 3 week-osteogenesis-induced matrices were prepared from MSCs cultured in osteogenic medium for 1 and 3 weeks, and referred as early stage matrices and late stage matrices, respectively. MSCs cultured under non-osteogenic condition for 1 week were used to prepare stem cell matrices.

The stepwise osteogenesis-mimicking matrices showed different effects on the osteogenic differentiation of MSCs. On the early stage matrices, osteogenesis occurred more rapidly than did that on the stem cell matrices and late stage matrices (Figure 1).

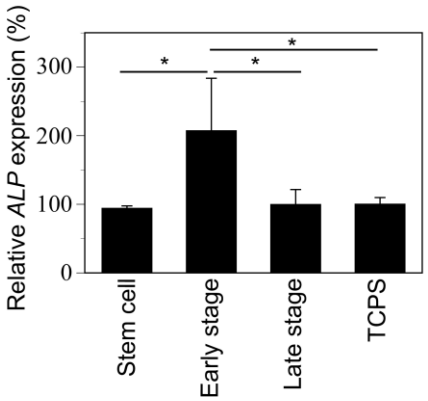

Figure 1: *alkaline phosphatase (ALP)* expression level on stepwise osteogenesis-mimicking matrices. The results were expressed as a percentage normalized to the expression level on TCPS. Data represent means ± SD (n=3). *, $P < 0.05$. TCPS indicates tissue culture polystyrene plates.

To investigate how osteogenesis of MSCs on early stage matrices was enhanced, the expression levels of transcription factors that relate to osteogenesis and adipogenesis were measured. *RUNX2* was similarly expressed when MSCs were cultured on both the early stage and late stage matrices, but decreased on the stem cell matrices, suggesting the osteogenesis of MSCs was directly suppressed on stem cell matrices. *PPARG* expression in the MSCs cultured on the late stage matrices was higher than were those cultured on the stem cell and early stage matrices. Therefore, the expression of osteogenic phenotype might be suppressed by the potential of unexpected adipogenic differentiation when MSCs were cultured on the late stage matrices.

During the osteogenesis, it has been reported that β-catenin regulates *PPARG* expression. To investigate the reason why *PPARG* expression increased on the late stage matrices, the amount of β-catenin in the cells were measured by Western blot analysis. On the stem cell matrices and early stage matrices, the β-catenin amount increased, suggesting that β-catenin suppressed *PPARG* expression on the stem cell matrices and early stage matrices.

Conclusions: These results demonstrate that the stepwise osteogenesis-mimicking matrices had different effects on the osteogenic differentiation of MSCs, and the early stage matrices provided a favorable microenvironment for osteogenesis. The stepwise osteogenesis-mimicking matrices will be a good *in vitro* model that reflects the ECM dynamics during the osteogenesis.

References:

Hoshiba T. *J. Biol. Chem.* 2009; 284: 31164-31173.