Sandwich culture using bio-functional hydrogels as a three-dimensional culture model for mesenchymal stem cells

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Introduction: Lineage specification of stem cells *in vitro* is a major challenge for successful tissue regeneration and cell transplantation. Recently, the immobilization of cell signaling proteins [1] and the elasticity of culture substrates [2] have been extensively investigated as biological and physical signals to regulate the lineage specification of stem cells. On the contrary, it has been demonstrated that three-dimensional (3-D) culture could enhance biological activities of cells compared with two-dimensional (2-D) culture [3]. The objective of this study is to obtain the fundamental knowledge of mesenchymal stem cells (MSC) cultured in a sandwiched condition with bio-functional hydrogels as a 3-D culture model. The bio-functional hydrogels were prepared through the immobilization of a cell signaling protein on poly(acrylamide) hydrogels with different crosslinking densities. As the cell signaling protein, ephrin B2 of an Eph signal ligand was employed to stimulate the osteoblastic differentiation of MSC [4]. A direct binding between ephrinB2 and EphB4 via the cell-cell contact is required to activate the ephrinB2-EphB4 signaling [4]. In this study, based on the site-specific interaction between IgG Fc domain and protein A, the immobilization of ephrinB2 in an orientation-regulated manner was designed to achieve the efficient ligand-receptor binding.

Methods: Acrylamide and *N,N'*-methylenebisacrylamide (BIS) were co-polymerized to prepare polyacrylamide hydrogels with varied elasticity. The elasticity was assessed in terms of hydrogels storage modulus measured by a rheometer (Rheostress I, Thermo Haake, Inc.). To allow MSC to attach on the surface of the hydrogel, rat tail collagen type I was immobilized by the sulfosuccinimidyl ester activation method. A recombinant chimeric protein of ephrinB2 and Fc domain (ephrinB2-Fc) was immobilized via protein A in an orientation-regulated manner on the surface of hydrogels with varied elasticity [1]. Briefly, protein A was immobilized on *N*-hydroxysuccinimide ester-conjugated hydrogels. The protein A-immobilized hydrogel was exposed to the solution of ephrinB2-Fc to fabricate ephrinB2-immobilized substrates. Human MSC were sandwiched between the resulting bio-functional hydrogels and cultured to evaluate their proliferation and osteoblastic differentiation comparing with those normally cultured. RUNX2 gene expression of MSC was measured by quantitative polymerase chain reaction (qPCR) to evaluate their osteoblastic differentiation.

Results: The elasticity of hydrogels increased with a decrease in the BIS concentration. The orientation of ephrinB2-Fc was evaluated according to the method previously described [1] and ephrinB2-Fc was immobilized on hydrogels in an orientation regulated-manner that allows ephrinB2 to achieve the efficient ligand-receptor binding.

When sandwich-cultured between the ephrinB2-immobilized bio-functional hydrogels, MSC showed enhanced osteoblastic differentiation in terms of the RUNX-2 gene expression, which is similar to those on the corresponding bio-functional hydrogel in the 2-D culture condition (Figure 1). The RUNX-2 gene expression could be modulated by not only the bottom bio-functional hydrogels in the 2-D culture but also the upper bio-functional hydrogels in the sandwich culture. This result suggests that cell signaling pathways for osteoblastic differentiation in MSC could be activated by immobilized ephrinB2 even in the sandwiched condition.

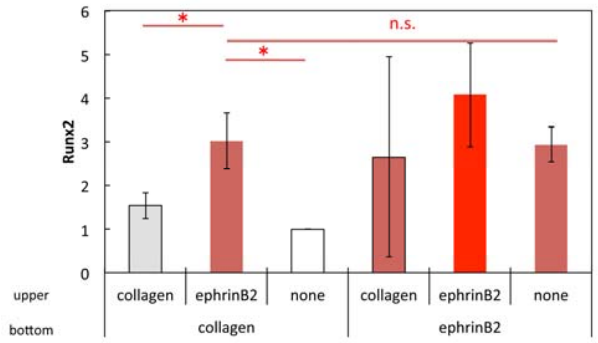

Figure 1. Runx2 expression of MSC cultured for 5 days in different culture conditions. *p<0.05; significance against culture conditions without ephrinB2.

Conclusions: The ephrinB2-Fc was immobilized onto the protein A-conjugated hydrogels in an orientation-regulated manner. The bioactivity of ephrinB2 in the osteoblastic differentiation of MSC could be modulated by the immobilization manner. In the sandwich culture condition, MSC showed enhanced osteoblastic differentiation by activating cell signaling pathways, as seen in the 2-D culture condition.

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References:

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