Immobilization of ephrinB2 in an orientation-regulated manner on the surface of hydrogels with different elasticities.

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Introduction: Recent advances in stem cell biology have demonstrated the importance of chemcial cues, such as growth factors and cell signaling molecules in regulating stem cell behaviors. In addition to the chemical cue, physical cues, such as the substrate elasticity play a crucial role in directing the lineage specification of mesenchymal stem cells (MSC) [1]. The objective of this study is to investigate the synergestic effect of the cell signaling and the substrate elasticity on osteoblastic differentiation of MSC. EphrinB2 was empoyed as a chemical cue to enahnce osteoblastic differentiation of MSC, since EphrinB2 on osteoclasts was reported to promote bone formation by activating the EphB4 siginaling in osteoblasts. To activate the ephrinB2-EphB4 signaling, a direct binding between ephrinB2 and EphB4 via the cell-cell contact is required [2]. In this study, based on the site-specific interaction between protein A and Fc domain, the immobilization of ephrinB2 in an orientation-regulated manner was designed to achieve the efficient ligand-receptor binding. The osteoblastic differentiation of MSC was evaluated on the ephrinB2-immobilized hydrogels with different elasticities.

Methods: Acrylamide and *N,N'*-methylenebisacrylamide (BIS) were co-polymerized to prepare polyacrylamide hydrogels with different elasticities. The elasticity was assessed in terms of the storage modulus of hydrogels 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 use of Sulfosuccinimidyl ester.

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 different elasticities [3]. 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. As a control, ephrinB2-Fc was chemically conjugated on the collagen type I-immobilized hydrogel without the protein A immobilization. To investigate the orientation of ephrinB2, the adsorption of protein A was examined for different hydrogels by using ^{125}I-labeled protein A.

Human MSC were cultured on the surface of ephrinB2-immobilized hydrogels with different elasticities. The osteoblastic differentiation of MSC was quantitatively evaluated in terms of the RUNX2 gene expression.

Results: The elasticity of hydrogels increases with a decrease in the BIS concentration. The protein A adsorption was significantly suppressed for the ephrinB2-Fc-immobilized hydrogels, in contrast to the ephrinB2-Fc-conjugated ones (Figure 1). If the Fc domain is not exposed on the surface of ephrinB2-Fc-immobilized hydrogels due to the orientation-regulated immobilization, it is likely that the adsorption of protein A can be suppressed, in remarked contrast to hydrogels with ephrinB2-Fc conjugated in a random manner where there are some Fc domains exposing to the surface. As a result, significantly lower protein A adsorption was detected for the ephrinB2-Fc-immobilized hydrogels than the ephrinB2-Fc-conjugated ones. The amount of protein A adsorbed onto the ephrinB2-Fc-immobilized hydrogels was as low as that of hydrogels immobilized with rat tail collagen of no Fc domain to bind to protein A.

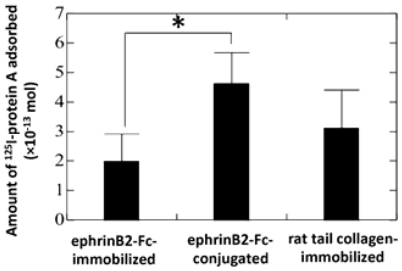

Figure 1. The amount of ^{125}I-labeled protein A adsorbed on ephrinB2-Fc-immobilized, ephrinB2-Fc-conjugated, and rat tail collagen-immobilized hydrogels. *$p < 0.05$; significance between the two substrates.

Runx2 expression was enhanced by hydrogels with ephrinB2-Fc immobilized in an orientation-regulated manner, in contrast to those with ephrinB2-conjugated in a random manner. Similar to the result reported by Engler et al. [1], MSC showed the highest Runx2 expression on rat tail collagen-immobilized hydrogels with an elasticity of 2.9 x 10^4 Pa, while the enhanced Runx2 expression for ephrinB2-immobilized hydrogels was independent on the elasticity.

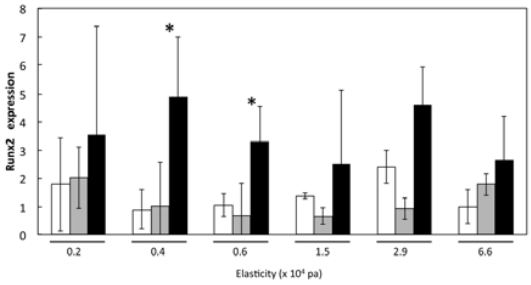

Figure 2. Runx2 expression of MSC cultured for two days on (■) ephrinB2-Fc-immobilized, (▨) ephrinB2-Fc-conjugated, and (□) rat tail collagen immobilized hydrogels. *$p < 0.05$; significance against the other two hydrogels with the same elasticity.

Conclusions: These results suggest that ephrinB2-Fc was immobilized onto the protein A-conjugated hydrogels in an orientation-regulated manner. The bioactivity of ephrinB2 in osteoblastic differentiation of MSC could be modulated by the immobilization manner.

References:

1. Engler A. et al. Cell 2006;126:677-689.
2. Pennisi A. et al. Blood 2009;114:1803-1812.
3. Toda H. et al. Biomaterials 2011;32:6920-6928.