NEURAL STEM CELL DIFFERENTIATION IN VASCULAR MICROENVIRONMENT

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ABSTRACT

Here, we present a novel method to create *in vivo*-like vascular microenvironment of neural stem cell (NSC) and to quantify the influences of three-dimensional vascular microenvironment for guiding the differentiation of NSCs embedded in extracellular matrix using quantitative real-time polymerase chain reaction (qRT-PCR). Using our new assay, we found that NSC differentiation in reconstituted vascular microenvironment differs from the one in macro *in vitro* system (Qin Shen et al., *Science*, 2004).

KEYWORDS

neural stem cell (NSC), microenvironment, *in vitro*, differentiation, microfluidics

INTRODUCTION

The mechanisms underlying neural stem cell (NSC) differentiation and self-renewal are keys to understand neurodegenerative disorders and central nervous system (CNS) injury.[1-4] NSC microenvironments, including brain endothelial cells, extracellular matrices (ECMs), neural stem cells and differentiated cells (e.g. neuron and glia), regulate differentiation of NSC through soluble factors, cells-cells interactions and cells-ECMs interactions in micro-scale **(Fig.1)**.[5-7]

Most *in vitro* studies of NSC differentiation, however, have been concentrated on effects of soluble factors on NSC differentiation using macro systems.[8-10] Qin Shen et al. [8] reported that endothelial cell (EC)-derived soluble factors stimulate self-renewal and delay NSC differentiation. In their report, a transwell assay was used to examine the interaction between ECs and NSCs. In macro-scale, however, the molecules secreted by the NSCs and the ECs cannot be accumulated, but can easily diffuse away.[11] Recently, we reported 3D ECM-mediated NSC differentiation in microfluidic device.[12] In our previous work, we have successfully shown the significance of 3D microenvironmental cues on NSC differentiation using a microfluidic device. For more physiological model, however, ECs should be co-cultured with NSCs in 3D micro-scale.

EXPERIMENT

Here, we report a new assay to create *in vivo*-like vascular microenvironment of NSC and to quantify NSC differentiation in 3D micro-scale using qRT-PCR. For 3D vascular microenvironment, an upper part of the microfluidic device was fabricated with poly(dimethylsiloxane) (PDMS) by a soft lithography so as to have one channel for 3D NSC culture in the center channel and two side channels for EC culture **(Fig.2)**. For qRT-PCR analysis, PDMS membrane (~80 μm; Korea Bio-IT Foundry center, South Korea) was used as a bottom part of the device. The upper and bottom part were then boned using oxygen plasma. NSCs were isolated from E13 embryos of imprinting control region (ICR) mice (Samtako, South Korea). These isolated NSCs were then cultured in T75 tissue culture flask at a density of 2×10^5 cells/ml. The NSCs were cultured in Dulbecco's Modified Eagle Medium /Nutrient Mixture F-12 medium (DMEM/F12; Gibco, USA) supplemented with 20 ng/ml of basic fibroblast growth factor (bFGF; Sigma, USA), 20 ng/ml of epidermal growth factor (EGF; Sigma, USA), and N-2 supplement (Gibco, USA). The culture medium was added to the tissue culture flask every 48 hours. Type I collagen (3.44 mg/ml, rat tail collagen type I; BD, USA) solution was prepared for 3D NSC culture in the microfluidic device. Collagen solution was mixed with 10X phosphate buffered saline (PBS, Thermo Scientific, USA), 0.5 N NaOH and distilled deionized water. Final concentration of the collagen solution was 2.0 mg/ml at pH 7.4. NSCs were then suspended with the collagen solution at a density of 5×10^6 cells/ml. Collagen with NSCs was allowed to gel in a humid chamber for 30 minutes. After gelation, ECs were seeded into the side channels and cultured in DMEM/F12 in the absence of mitogenic factors (bFGF, EGF). The growth medium was replaced every 24 hours. Four days after culturing NSCs embedded in collagen gel, the PDMS membrane was detached from the upper part of the device for qRT-PCR. The NSCs were harvested with micropipette, and then these cells were analyzed with qRT-PCR system (StepOne Plus; Applied Biosystems, USA)

Interestingly, our result differs from [8] in the macro-scale NSC differentiation in the presence or absence of EC **(Fig.3)**. This might reflect the ability of micro-scale ECM to accumulate soluble factors released from EC and/or NSCs. Therefore, microenvironments reconstituted in our microfluidic system, which mimics *in vivo* regional conditions of NSC differentiation, might amplify autocrine/paracrine signals originating from EC/NSCs-derived soluble factors.

We expect that understanding NSC differentiation in vascular microenvironment could be of great significance for stem cell research targeting, such as Alzheimer's disease and multiple sclerosis.[13, 14] Details regarding the differentiation in the vascular microenvironments will be presented in the conference.

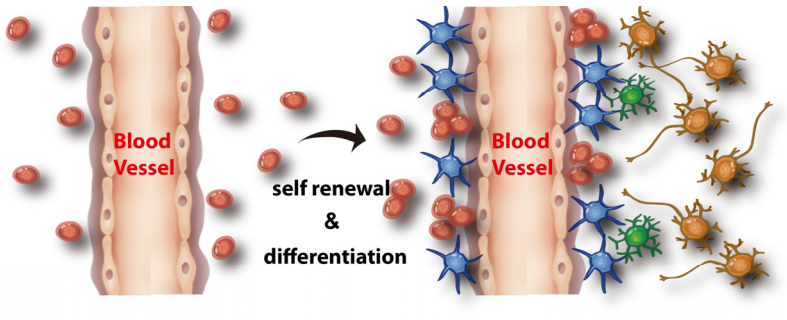

Figure 1. Differentiation of neural stem cells in vascular microenvironment

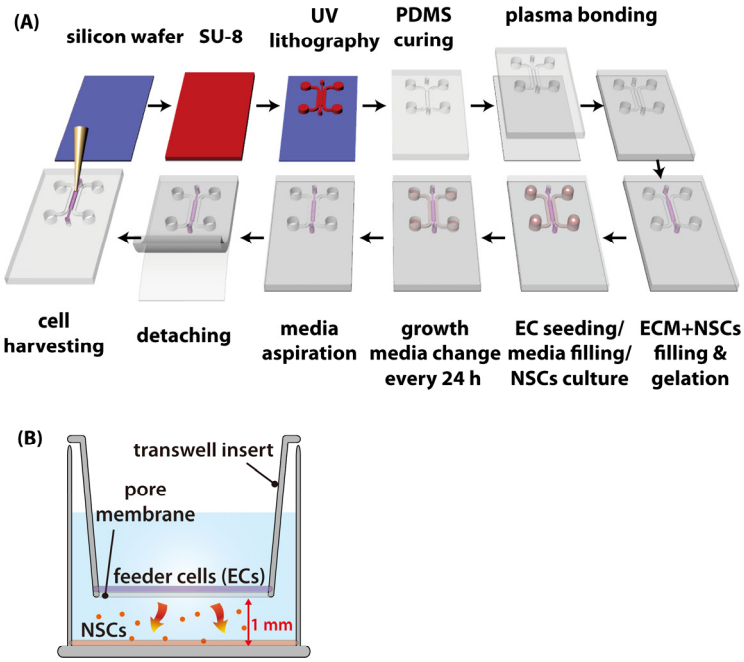

Figure 2. (a) A schematic diagram depicting microfluidic device fabrication and cell collection for quantitative real-time polymerase chain reaction (qRT-PCR). (b) The macro-scale co-culture system in [8]. Height between endothelial cells (ECs) and NSCs: 1mm.

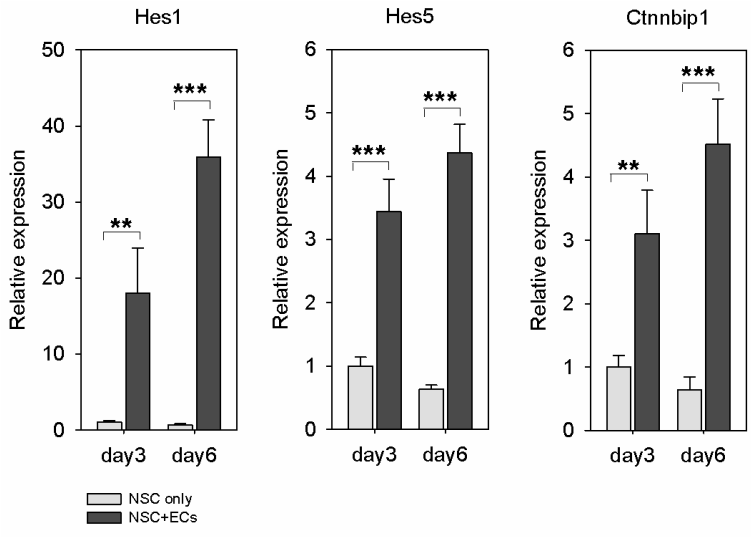

Figure 3. Hes1, Hes5 and Ctnnbip1 are up-regulated in the NSC/EC microfluidic co-culture. n=4; **P < 0.01 and ***P < 0.001; Student's t-test

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