A Trail to Enhance Osteogenic Differentiation of Mesenchymal Stem Cells by Combinational Technology of Gene Therapy and Microfluidic System

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Statement of Purpose: We hypothesized that microscale devices can be potentially used as an *in vitro* model system to examine the targeting efficacy of polymeric gene delivery vehicles to enhance the expression of a plasmid DNA for mesenchymal stem cells (MSC).

Methods: As a carrier of plasmid DNA, dextran-spermine cationic polysaccharide was prepared by reductive-amination between oxidized dextran and the natural oligoamine, spermine. The formed schiff-base imine-based conjugate was reduced with borohydride to obtain the stable amine-based conjugate. Cationized dextran was mixed with plasmid DNA encoding bone morphogenetic protein-2 (BMP-2) to form stable complex. Poly(dimethylsiloxane) (PDMS) molds were fabricated by curing prepolymer (Sylgard 184, Essex Chemical) on silicon masters etched with SU-8 photoresist [1]. PDMS were patterned using microcontact printing and molding to demonstrate the versatility of the approach to pattern microchannels with various soft lithographic techniques [1]. Plasmid DNA-cationized dextran complexes were coated on the surface of each slide. Rat bone marrow mesenchymal stem cells (MSCs) were then seeded on the slide at the density of 1×10^6 cells/ml. The PDMS mold connected to the syringe pump and operated at 37 °C in a 95 % air-5 % CO_2 atmosphere. At different time interval, cells were lysed by 100 µl of a lysis buffer (0.1 M Tris-HCl, 2 mM EDTA, 0.1 % Triton X-100). To measure the expression level of BMP-2 gene, 50 µl of the cell lysate was collected and the BMP-2 protein was determined by a human BMP-2 ELISA Kit (AN'ALYZA, Human BMP-2 Immunoassay System, TECHNE Co., MN, USA).

Results/Discussion: We indicated that the gene expression level of plasmid DNA encoded BMP-2 was enhanced by a combination of using cationized dextran-plasmid DNA complex and microfludic system. The efficiency of transfection with expression vector has been shown to be related to cell cycle activity [2]. Attachment of cells to substrata is a major factor controlling their structure, function and cell cycle. Microfluidic system can provide greater available surface area for cell attachment and spreading than 2-D surfaces (i.e., tissue culture plate). Moreover, microfluidic surface affects cell adhesion, spreading and proliferation, and controls the spatial arrangement of cells and their transmission of biochemical and mechanical signals. Cell adhesion, spreading, and cytoskeletal reorganization initiate signaling cascades that govern gene expression [3].The level of BMP-2 expression was significantly enhanced by plasmid DNA-cationized- dextran complex coated into the PDMS microchannels than by the naked plasmid DNA. Control microfluidic system lacking complex or that coated with naked plasmid DNA did not contribute to create genetically engineered MSC. Osteocalcin (OCN) content of MSCs cultured in microfluidic system signifi ılture metho

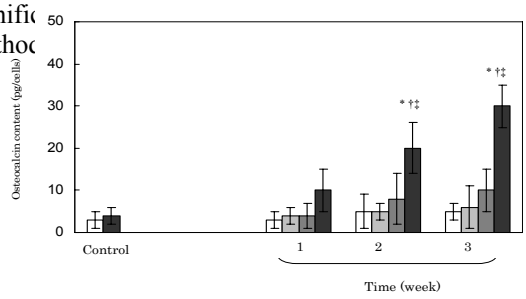

Figure 1. Time-course of OCN of MSCs cultured by 2D culture method (□) and microfluidic system (■) after transfection with naked plasmid DNA-BMP-2 (■) or cationized dextran-plasmid DNA-BMP-2 complex (■) coated into. MSC were cultured by 2D culture method and microfluidic system without any treatment as a control group. The dose of plasmid DNA was 0.01 µg/µl. *, $p< 0.05$; significant against the ALP activity or the osteocalcin contents of control group. †, $p< 0.05$; significant against the OCN of naked plasmid DNA group at the corresponding week. ‡, $p< 0.05$; significant against the OCN of cationized dextran-plasmid DNA-BMP-2 complex group at the corresponding week.

Conclusions: These results demonstrate that the interaction of polymeric gene carriers with cells can be studied under controlled conditions, which may aid in the engineering of MSC.

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