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3D microenvironment of collagen hydrogel enhances the release of neurotrophic factors from human umbilical cord blood cells and stimulates the neurite outgrowth of human neural precursor cells

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ABSTRACT

The umbilical cord blood (UCB) cells have been reported to secrete therapeutic signals, including a series of neurotrophic factors. This suggests the cell source provides suitable therapeutic environments for nerve regeneration that ultimately finds a possible cell therapy for nerve tissue. In this study, we observe a collagen hydrogel provides human UCB cells a proper 3D environment that stimulates the release of various neurotrophic factors. When compared to 2D culture, the 3D hydrogel culture significantly enhanced the expression of a series of neurotrophic factors, including neurotrophins, nerve growth factor, brain-derived neurotrophic factor, and ciliary neurotrophic factor as verified by the gene and protein analysis. To confirm the effects of neurotrophic factors secretion, we allowed an indirect interaction of the UCB-environment with human neural precursor cells (hNPCs). Results showed significantly enhanced neurite outgrowth of hNPCs. Collectively, our findings demonstrate that the collagen-based 3D hydrogel provides excellent environment for UCB-derived cells to release neurotrophic factors that will be ultimately useful for the neural repair and regeneration purposes.

1. Introduction

Stem cells play key roles in the regenerative process of damaged and diseased tissues [1–4]. Among the stem cell sources that have been indentified, the umbilical cord blood (UCB) derived stem cells have also gained great attention. UCB cells are easy to harvest and expand for practical applications and have shown relatively low immunogenicity and side effects after transplantation [5,6]. While the major population of UCB stem cells is hematopoietic stem cells, a great fraction of them is mesenchymal stem cells, and many therapeutic efficacies resulted from both cell types [7–11].

One of the major goals of the stem cell therapy is in fact to restore or replace injured tissues through a controlled stem cell transdifferentiation into a cell type of interest. However, recently, a modulatory role of stem cells i.e., immune-modulation of anti-scar formation and nerve protection by their secreted cytokines and neurotrophic factors, has been highlighted [12–14]. UCB cells have also shown to produce several neurotrophic factors, including nerve growth factors (NGFs), brain-derived neurotrophic factors (BDNFs), ciliary neurotrophic factors (CNTFs), neurotrophin-3 (NT3), and -4 (NT4) [15]. Therefore, UCB cells are considered as a possible therapeutic cell source for the treatment of neurotrauma and neurodegenerative diseases [14].

Here we thus hope to make use of the UCB cells for this neural repair and regenerative purposes. In particular, we consider the culture environments of UCB cells would significantly affect their biological functions, particularly their releasing behaviors of neurotrophic factors. We introduce 3-dimensional (3D) hydrogel made of collagen to culture UCB cells. 3D hydrogel environment is considered to mimic the native soft tissue structure, providing stem cells appropriate physical and chemical cues for adherence, spreading, growth and even switch to a lineage differentiation [16]. Collagen, the most abundant extracellular matrix component in human, constitutes a primary structural unit that preserves a gel-like tissue structure, and has been the most-widely studied as the cell culture matrix [17,18]. In fact, stem cell-based therapy has been mainly conducted by transplanting the cells directly to the sites or intravenously. However, this method has been resulted

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in a loss of stem cells at large fractions and a dilution of secreted factors that might come from stem cells, which eventually limit their therapeutic efficacy and clinical applicability [14,19]. In this manner, the collagen hydrogel matrix is considered as a potential UCB cell carrier for neural regeneration.

Here we culture UCB cells within collagen hydrogel matrix, analyze the release of neurotrophic factors and compare the results with those cultured in 2D culture dish. We further demonstrate the biological effects of neurotrophic factors released from UCB cells by co-culturing with neural precursor cells (NPCs) and examining their neurite outgrowth behaviors.

2. Materials and methods

2.1. Isolation and preparation of umbilical cord blood cells

The umbilical cord was obtained with mother's informed consent and approval by Institutional Review Board of Dankook University Hospital at Cheonan, S. Korea. The cord blood was collected after a delivery of the placenta by puncturing the umbilical cord vein with a syringe (>40 ml/sample). Cell numbers, viability, and blood sterility were evaluated before blood storage, which did not exceed 12 h. The mononuclear cell fraction was isolated from the blood on ficoll/hypaque gradient techniques (Pharmacia LKB, Gaithersburg, MD) followed by a washing and resuspension in DMEM/F12 medium (Gibco BRL, Grand Island, NY) at a final concentration of 1×10^6 cells/ml. The cell fractions that proliferated in tight adherence to the plastic culture dish were considered in this study.

2.2. Flow cytometry

For flow cytometric analysis, UCB-derived cells were washed twice with PBS, resuspended and fixed with 4% paraformaldehyde for 15 min, and treated with 0.2% Triton X-100. Blocking was conducted by incubating the cells in 1% BSA at 4 °C overnight, and the cells were probed with either mouse anti-Oct3/4 antibody, mouse anti-Sox2 antibody, anti-SSEA-4 antibody, anti-NGF antibody or rabbit anti-BDNF antibody (Santa Cruz Biotech, Santa Cruz, CA) at 4 °C overnight. The cells were then incubated for 1 h with either Alexa 555-conjugated goat anti-mouse antibodies (Santa Cruz Biotech) for NGF or FITC-conjugated donkey anti-rabbit antibody (Santa Cruz Biotech) for BDNF. After washing in PBS, the flow cytometry was conducted with FACS Caliber flow cytometer (BD Biosciences, San Jose, CA) and analyzed using the Cell Quest-Pro software (BD Biosciences).

2.3. RT-PCR analysis for gene profiling of neurotrophic factors

Reverse transcriptase polymerase chain reaction (RT-PCR) was performed to detect the mRNA levels of neurotrophic factors, including nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT3), neurotrophin-4 (NT4), and basic fibroblast growth factor (bFGF). Total RNA was isolated from each cell pellet by using the RNA isolation kit (RNeasy Mini Kit 74104, Qiagen, Germantown, MD). About 1 μg total RNAs were reverse-transcribed by using the Quantitect RT-PCR Kit (#205311, Qiagen) according to the manufacturer's protocol. Then, the cDNA was subjected to PCR amplification with neurotropic factor-specific primers as listed in Table 1 in the pre-mixed PCR solution (Bioneer, DaeJon, S. Korea) by 35 cycles of an incubation consisting of 95 °C for 30 s, 58 °C for 30 s, followed by 75 °C for 60 s.

2.4. Western blotting for protein profiling of neurotrophic factors

NGF and BDNF protein levels were analyzed by the Western blotting with anti-NGF and anti-BDNF antibody, respectively. About 80 μl of cell lysates was boiled for 5 min with 20 μl of 5× sample buffer. Twenty microliters of boiled protein samples was loaded into each well of a gradient polyacrylamide gel (10%, Bio-Rad, Hercules, CA), and then transblotted to a nitrocellulose membrane. Transblotted membrane was blocked in 5% fat-free milk Tris buffer with 0.5% Tween-20 (TBST) for 1 h at room temperature, and then incubated with primary antibody (mouse anti-NGF antibody or anti-BDNF antibody (Santa Cruz Biotech) that was previously diluted at 1:1000 in the 2% fat-free milk TBST solution overnight at 4 °C. The blotted membrane was washed in 0.5% TBT and incubated with horseradish peroxidase-conjugated anti mouse IgG 1 h, and the immunoreactive bands were detected by using

Table 1
Primer sequences, annealing temperatures, and expected product sizes in PCR reactions.

Target gene	Primer (forward and backward)	Annealing temperature	Product size (bp)
NGF (NM_002506.2)	5-TCACCCCGTGTGCTGTTTAG-3 5-ATTCGCCCCTGTGGAAGATG-3	60	148
BDNF (M61176.1)	5-TTTGGTTGCATGAAGGCTGC-3 5-GCCGAACTTTCTGGTCCTCA-3	60	199
CNTF (AK314118.1)	5-AGGGATGGCTTTCACAGAGC-3 5-CGCAGAGTCCAGGTTGATGT-3	60	163
NT3 (NM_001102654.1)	5-TCTCTTCATGTCGACGTCCC-3 5-TTACCTTGGATGCCACGGAG-3	60	137
NT4 (NM_006179.4)	5-TGACAGGTGCTCCGAGAGAT-3 5-GGGAGAGAAGGTCCCACTCA-3	60	148
RGMa (BC015886.1)	5-GGGAGAGGAGCAGGGTCTTA-3 5-GCACTTTTGGGAGTGAAGCC-3	60	220
RGMb (NM_001012761.2)	5-TGCCCTTGTAATCGGTGTCC-3 5-ACCAGTGTTTCCCCCGTTTT-3	60	242
Netrin-1 (NM_004822.2)	5-CTGAGTGCCTGCTTTACGGA-3 5-TGTCCCTCCCTCCCACATAG-3	60	170
bFGF (NM_004465.1)	5-GTGCGGAGCTACAATCACCT-3 5-ATGCTGTACGGGCAGTTCTC-3	60	131
GAPDH (M33197.1)	5-GAGAAGGCTGGGGCTCATTT-3 5-AGTGATGGCATGGACTGTGG-3	60	231