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Research paper

Investigation of effective shear stress on endothelial differentiation of human adipose-derived stem cells with microfluidic screening device

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ABSTRACT

Mesenchymal stem cells (MSCs) can differentiate into not only mesenchymal lineage cells, such as osteoblasts, adipocytes and chondrocytes, but also into endothelial cells *in vitro* when they are exposed to specific biochemical factors. A shear stress stimulus combined with biochemical factors has been reported to promote endothelial cell differentiation of MSCs more effectively than the case with biochemicals only. Although many previous studies suggested that the specific shear stress levels can increase the differentiation potentials of MSCs into endothelial cells, no studies have investigated the effects of shear stress levels within a physiological range on the endothelial differentiation at once. In this paper, we utilized a microfluidic screening device with an equilateral triangular channel, which generates a shear stress gradient in the physiological range of 0–19.8 dyne/cm^2 across the channel, to examine shear stress effects on endothelial differentiation of MSCs. Human adipose-derived stem cells (hASCs) were differentiated into endothelial cells by applying the shear stress gradient with an endothelial induction medium to find out an effective shear stress range on endothelial differentiation. As a result, a shear stress range of 7.8–13.7 dyne/cm^2 was found to be the most effective level of shear stress stimulus for the endothelial differentiation of hASCs.

1. Introduction

Mesenchymal stem cells (MSCs) can differentiate not only into mesenchymal lineage cells including osteoblasts, adipocytes, chondrocytes and myocytes but also into other lineages, such as neurons and endothelial cells, with target-specific biochemical factors [1–3]. It has been recently known that additional mechanical forces combined with the biochemical factors can promote differentiation of MSCs more effectively than the use of biochemical factors only. For example, mechanical strain was found to be involved in enhancing osteogenic [4] and chondrogenic [5] differentiation, and the fluid shear stress was reported to promote endothelial differentiation of MSCs [6–13].

Endothelial cells that line the inner wall of blood vessels are continuously subjected to hemodynamic shear stress *in vivo*, and the shear stress ranges applied to the vessel are 1–5 dyne/cm^2 in veins and 10–40 dyne/cm^2 in arteries [14]. Many previous reports showed that the shear stress levels within the physiological range, about 0–20 dyne/cm^2, were positive for the endothelial differentiation of MSCs [6–12]. For example, Kim et al. reported that the shear stress level of 2.5 dyne/cm^2 enhanced the endothelial surface marker, PECAM-1 [6]. Bai et al. reported that the discrete shear stress levels of 10 and 15 dyne/cm^2 upregulated the expression of the endothelial marker, while the higher discrete shear stress levels of 20 and 25 dyne/cm^2 decreased the expression of endothelial marker [7]. Although many previous reports have been investigated on endothelial cell differentiation regarding effective discrete shear stress, it has not been established which level of shear stress effectively enhances the endothelial differentiation of MSCs with a wide range of shear stress gradient. Therefore, it would be valuable to screen the effective shear stress levels for promoting endothelial differentiation of MSCs by applying an appropriate shear stress gradient.

Various types of devices are used for endothelial differentiation of MSCs, including parallel plate flow chambers [7–9], tubular shear stress systems [6,10] and orbital shakers [11,12]. The parallel plate flow chambers are a simple system that contains two plates with a controllable gap to generate a uniform fluid flow between them. Also, the tubular shear stress systems are composed of tubular scaffolds where the cells are cultured on the inner layer. A uniform parabolic fluid flow profile can be easily predicted through the circular channel of tubular scaffold. Though the parallel plate flow chambers and tubular shear stress systems can generate a controlled, uniform fluid flow, i.e., a uniform flow-induced shear stress on the substrate, multiple cell experiments should be conducted to investigate the effects of different shear stress levels due to the single shear stress generation per flow experiment. In the case of orbital shakers, which are the commercially available system, different levels of shear stress along the radial direction can be

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generated at a single experimental condition; however, the shear stress distribution is unexpected due to the disturbed flow from the shakers.

On the other hand, a microfluidic device has been regarded as a useful tool for generating a stable laminar flow, enabling the manipulation of the flow-induced shear stress on the substrate. Recently, we developed a microfluidic screening device, which consists of an equilateral triangular channel (called, ETRIC), generating a shear stress gradient across the channel [15]. Due to the unique equilateral triangular cross-sectional design of the ETRIC (1) a shear stress gradient can be easily predicted and modulated across the channel and (2) the prescribed shear stress gradient can be readily established in the channel at a single flow condition. Using the ETRIC, a shear stress gradient of 0–20 dyne/cm^2, which is the well-accepted physiological shear stress range, was applied to human adipose-derived stem cells (hASCs) at once to figure out the effective shear stress range promoting endothelial differentiation.

2. Design and experiments

2.1. Design of experimental conditions with ETRIC

Fig. 1 illustrates the experimental scheme for screening the effective shear stress levels to facilitate endothelial differentiation of hASCs with the ETRIC. To achieve the desired shear stress range of 0–20 dyne/cm^2, we have newly designed the geometrical parameter of an ETRIC along with the flow condition. The shear stress (τ) distribution in the ETRIC can be determined with a fluid viscosity, η, a channel size, a, and a flow rate, Q, according to the following equation [15]:

$$\tau = 40\frac{\eta Q}{a^5}\left(a^2 - y^2\right), \quad (1)$$

where y corresponds to the channel position, perpendicular to the flow direction.

The working fluid was determined to be a cell culture medium whose η is about 0.001 Pa·s. According to Eq. (1), it can be found that the shear stress is linearly proportion to Q, while it is inversely proportion to the third power of a. Due to the sensitive dependency of shear stress on the channel size, we first determined a as 1.5 mm where the average size of hASCs that is 80–100 μm [16] was considered. The present channel size ($a = 1.5$ mm) provides a space on which more than 10 hASCs can be adhered across the channel where a shear stress gradient is developed. Finally, Q was determined as 10 mL/min through Eq. (1), which results in the shear stress gradient of 0–19.8 dyne/cm^2.

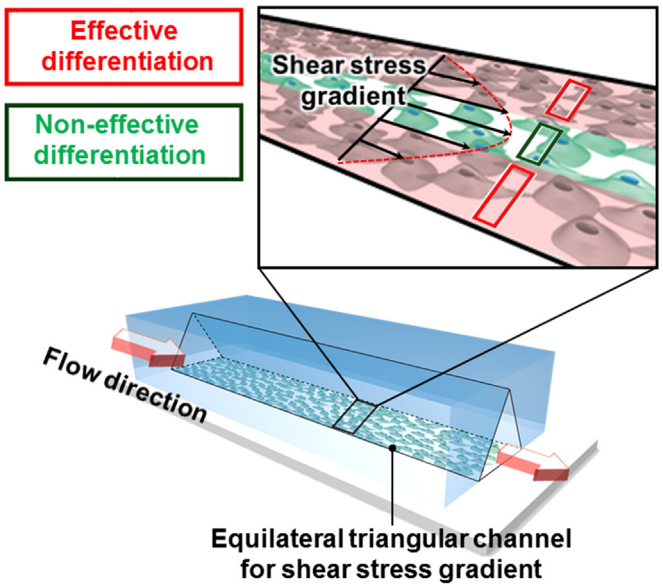

Fig. 1. Schematic diagram for screening effective shear stress levels to facilitate endothelial differentiation of hASCs by applying shear stress gradient with an ETRIC.

2.2. Fabrication of microfluidic screening device with ETRIC

A microfluidic screening device with the designed ETRIC was fabricated through a two-step micromilling process, polydimethylsiloxane (PDMS) replica molding and bonding of the PDMS microchannel to a polystyrene (PS) substrate. The fabrication process was similar except for the different ETRIC size and bonding process from our previous work [15]. Briefly, the two-step micromilling process was conducted with two different types of end mills, a standard end mill and a 60° tapered end mill, to fabricate a master template having a relief of protruded equilateral triangular channel with a of 1.5 mm and a length of 100 mm. A PDMS ETRIC was fabricated through PDMS replica molding again the master template with a mixture of base and curing agent in a 10:1 weight ratio (Sylgard 184, Dow Corning). The microfluidic screening device was finally fabricated by bonding the PDMS ETRIC with a PS substrate that is a material commonly used for cell cultureware. For bonding, the PDMS ETRIC was treated with oxygen plasma (100 W for 1 min) followed by coating with a 5% w/v 3-aminopropyltriethoxysilane (APTES) solution at 80 °C for 20 min [17]. After treating the PS substrate with oxygen plasma (50 W for 15 s), the PDMS ETRIC was bonded firmly to the PS substrate.

2.3. Culture of human adipose-derived stem cells (hASCs)

hASCs were isolated from the fat tissue obtained from donors with informed consent in accordance with the requirements of the Seoul St Mary's Hospital of Korea IRB (IRB permit No. KC11TNMS0095), as previously described [18]. hASCs were cultured in low glucose-Dulbecco's modified Eagle's medium (LG-DMEM, Hyclone) with 10% fetal bovine serum (FBS, Hyclone), 1% penicillin/streptomycin (Gibco) and incubated at 37 °C, 5% CO_2. The medium was changed every three days and all experiments were conducted with less than five cellpassages.

2.4. Applying shear stress and induction of endothelial differentiation of hASCs

The endothelial differentiation of hASCs was evaluated with and without fluid shear stress. For both conditions, hASCs were seeded on the PS substrate in the microfluidic screening device with a density of 1×10^4 cells/cm^2. After 24 h of culture, an endothelial induction medium, which was composed of LG-DMEM supplemented with 2% FBS, 50 ng/mL vascular endothelial growth factor (VEGF, R&D) and 10 ng/mL basic fibroblast growth factor (bFGF, R&D), was applied. In the group applying shear stress, the microfluidic screening device was connected to a series of a medium reservoir, a peristaltic pump and a damper. The medium reservoir contains the endothelial induction medium, and the peristaltic pump was set to transmit the medium from the medium reservoir to the microfluidic device through the damper, resulting in a steady flow rate in the ETRIC. The fluid flow was applied discretely at days 6, 13 and 20 with a duration of 24 h at a flow rate of 10 mL/min resulting in a shear stress gradient of 0–19.8 dyne/cm^2 as explained above. Otherwise, for the group without shear stress, the endothelial induction medium was replaced every 24 h for static culture condition.

2.5. Acetylated-low density lipoprotein (Ac-LDL) uptake assay

Ac-LDL assay was conducted to investigate the level of endothelial differentiation of hASCs. After 21 days of cell culture, cells were washed with PBS three times and incubated with LG-DMEM including 10 μg/mL Dil-Ac-LDL (Life Technology) at 37 °C for 4 h. Then, the cells were washed with PBS for three times and incubated another 10 min with