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Enhancement of human mesenchymal stem cell infiltration into the electrospun poly(lactic-co-glycolic acid) scaffold by fluid shear stress

Min Sung Kim [a, b], Mi Hee Lee [a], Byeong-Ju Kwon [a, b], Min-Ah Koo [a, b], Gyeung Mi Seon [a, b], Jong-Chul Park [a, b, *]

[a] Cellbiocontrol Laboratory, Department of Medical Engineering, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea
[b] Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, Republic of Korea

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ABSTRACT

The infiltration of the cells into the scaffolds is important phenomenon to give them good biocompatibility and even biodegradability. Fluid shear stress is one of the candidates for the infiltration of cells into scaffolds. Here we investigated the directional migration of human mesenchymal stem cells and infiltration into PLGA scaffold by fluid shear stress. The human mesenchymal stem cells showed directional migrations following the direction of the flow (8, 16 dyne/cm^2). In the scaffold models, the fluid shear stress (8 dyne/cm^2) enhanced the infiltration of cells but did not influence on the infiltration of Poly(lactic-co-glycolic acid) particles.

1. Introduction

A scaffold is one of the key components in the tissue engineering paradigm in which it can function as a template to allow new tissue growth and also provide temporary structural support while serving as a delivery vehicle for cells and/or bioactive molecules [1,2]. An ideal scaffold for tissue regeneration should possess mechanical properties similar to the tissues being replaced, good biocompatibility with surrounding tissue, large porosity and pore size for good infiltration of cells, high pore interconnectivity for tissue ingrowth, and biodegradability such that it is gradually replaced by growing tissues [3]. For a scaffold that requires minimal cellular infiltration (e.g., a vascular graft) and proliferation limited to the surface may be acceptable or even desirable. A large number of studies have been devoted to characterize the in vitro new tissue regeneration capability of hMSCs cultured within biodegradable porous scaffolds. The results of these studies evidenced that parameters such as surface topography and chemistry as well as hMSC seeding density and 3D spatial distribution/organization strongly influence cell–material interaction and extracellular matrix deposition [4–9]. Nevertheless, cell cultivation in 3D porous scaffolds is often impaired by the difficulty of achieving a homogeneous cell seeding and by the diffusion constraints within the cell–scaffold constructs [6,9–11].

Fluid shear stress enhances cell migration in the direction of flow and is called "mechanotaxis" [8]. Studies have shown that shear stress can regulate MSC proliferation and differentiation into osteoblasts, ECs, or cardiomyocytes [9–11], suggesting that MSCs are also sensitive to mechanical stress produced by fluid flow.

Human bone marrow-derived mesenchymal stem cells (hMSCs) are ideal candidates for tissue engineering research because they are multipotent, uncommitted cells with the ability to become specialized cells and which can be relatively easily isolated [12]. They contribute to the development, regeneration and maintenance of various mesenchymal tissues including cartilage, bone, muscle and adipose [4,5,13–16]. In this study, we investigated the effect of fluid shear stress on the migration of hMSCs and figure out the enhancement of hMSCs infiltration into PLGA scaffold by fluid shear stress.

2. Materials and methods

2.1. Cell culture

Human bone marrow-derived mesenchymal stem cell (hMSCs, Lonza, Basel, Switzerland) were cultured in Mesenchymal Stem Cell

Abbreviations: hMSC, human mesenchymal stem cell; PLGA, poly(lactic-co-glycolic acid).

* Corresponding author. Cellbiocontrol Laboratory, Department of Medical Engineering, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, Republic of Korea. Fax: +82 2 363 9923.
E-mail address: Parkjc@yuhs.ac (J.-C. Park).

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Growth Medium (MSCGM, Lonza). Cells were incubated at 37 °C in a 5% CO_2 atmosphere. hMSCs between passages 5 and 9 were used in all experiments.

2.2. Applying the fluid shear stress to hMSCs

We used the parallel plate chamber system to apply shear stress to hMSCs [17]. The parallel plate chamber system was made of incubator system installed with the microscope to observe live cells and the flow chamber to apply shear stress to the cells. The incubator was regulated by temperature and gas composition controlling program (CCP ver. 3.8) under appropriate environment for the cells (CO_2 5%, 37 °C). The flow chamber consisted of the main body, bottom plate and silicon gasket (Fig. 1A). The main body had the inlet and outlet for tubing (inner diameter, 2 mm) to apply the fluid shear stress to the cells. The hMSCs were seeded on the coverslip before mounted on the bottom plate. The main body and the silicon gasket (200 μm in height, 2 mm in width) were combined with the coverslip and bottom plate together. The medium was flowed through the inlet and outlet tube.

2.3. Analysis of cell migration by fluid shear stress

The parallel plate chamber was placed on the microscope stage. The cell images were recorded every 5 min using a charge-coupled device (CCD) camera (Electric Biomedical Co. Ltd., Osaka, Japan) attached to an inverted microscope (Olympus Optical Co. Ktd., Tokyo, Japan). The images were stored to the computer by using the Tomoro image capture program; images were saved as JPEG files. Captured images were imported into ImageJ (ImageJ 1.37v by W. Rusband, National Institutes of Health, Bethesda, MD, USA). Image analysis was carried out by the manual tracking and chemotaxis tool plug-in (v. 1.01, distributed by ibidi GmbH, Munchen, Germany). The XY coordinates of each cell were obtained by using the manual tracking program. The data were imported into the chemotaxis plug-in. The cell migration speed was computed automatically and the cell migration pathway was plotted by the chemotaxis tool. The directedness of migration was assessed as cosine θ, where θ is the angle between the flow shear stress vector and a straight line connecting start and end positions of a cell (Fig. 1B). A cell moving directly to the down (direction of the flow) would have a directedness of 1; a cell moving directly to the up (opposite direction of

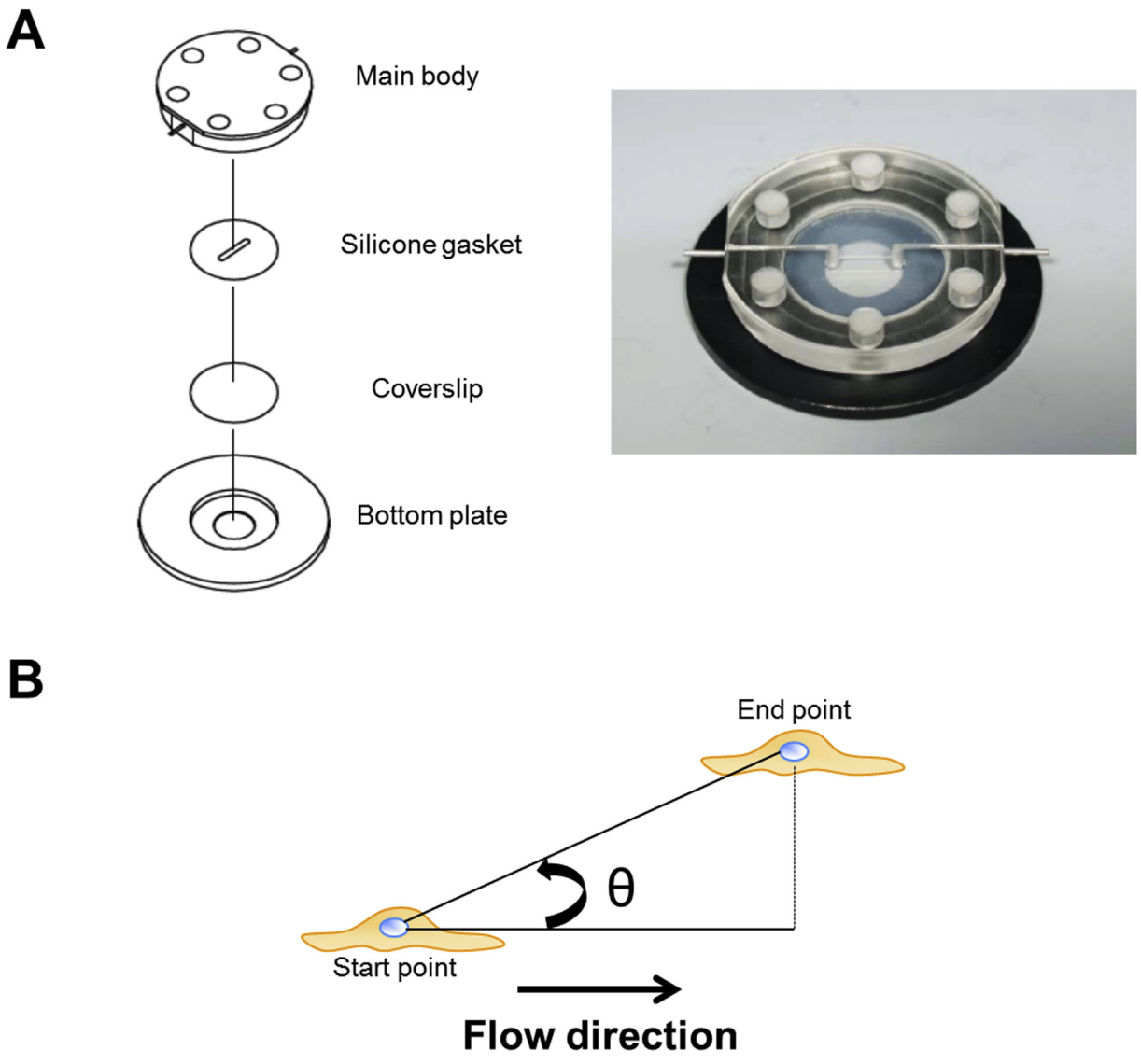

Fig. 1. The schematic of mechanotaxis chamber and directedness. (A) The parallel plate chamber system. (B) Schematic of the directedness.