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Research paper

Effects of shear stress on the cellular distribution of polystyrene nanoparticles in a biomimetic microfluidic system

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ABSTRACT

Effects of shear stress on the intracellular uptake of nanoparticles were originally investigated using a calibrated biomimetic microfluidic system (BMS) that mimics the dynamic environment of cells. Positively or negatively charged polystyrene nanoparticles (PSNs) were chosen as a model. PSNs were delivered to HEK 293T and MS1 cell lines using a BMS. To evaluate intracellular uptake of PSNs under static and dynamic conditions (0.5, 1.0, 3.0 dyne/cm^2), the fluorescence intensity of intracellular PSNs was measured by flow cytometric analysis and confocal laser scanning microscopy. When delivering cationic PSNs to cells, the intracellular uptake increased as the exposure time and PSN concentration increased under both static and dynamic conditions. Under dynamic conditions, the intracellular uptake of cationic PSN was highly increased in both HEK 293T and MS1 cell lines compared to static conditions. However, intracellular uptake of cationic PSNs was maximized when shear stress was at 0.5 dyne/cm^2 and then gradually decreased as the magnitude of fluidic shear stress increased to 3.0 dyne/cm^2. Contrarily, the anionic PSNs showed no significant difference of cellular uptake in presence of shear stress. Thus, shear stress should be considered to investigate the cellular distribution of various nanoparticles and drug delivery systems.

1. Introduction

For the distribution and cellular uptake of drug and drug delivery systems, it requires an understanding of the interactions between nanoparticles and cells within a physiological environment. Fluidic stimuli at different shear stress levels contribute interactions between nanoparticles and cells and may affect intracellular uptake of nanoparticles during drug delivery. Various physicochemical and biochemical properties, including pH, temperature, oxygen tension, soluble factors, gradients and shear stress influence interactions between nanoparticles and cells [21]. Among them, shear stress is one of critical factors to show the difference of in vitro and in vivo cellular distribution of drug and nanoparticles because of the presence of a dynamic physical environment exerting forces on cells in vivo. However, most of in vitro cellular uptake of drug and nanoparticles has been performed under the static environments.

Fluidic shear stress is generated by blood flow in the vascular microenvironment and interstitial flow in the extracellular matrix, with the average shear stress ranging from 0.5 to 30.0 dyne/cm^2 and ~0.1 dyne/cm^2, respectively [15,17]. Fluidic shear stress in the extracellular environment affects the distribution and interaction of nanoparticles with cells and regulates specific cellular processes. It may affect cellular uptake and ultimately efficacy of drug as depicted in Fig. 1, but the role of shear stress in drug delivery remains largely unexplored. There are relatively a few studies evaluating interactions between nanoparticles and cells within a biomimetic dynamic environment. Among these reports, results show decreased targeting of particles [3,4,11]; and increased delivery efficiency [8,9,18] to endothelial cells or epithelial cells with increasing shear stress. Other studies considered the charge of nanoparticles and determined that the interaction of positively charged carriers with myoblast cells was enhanced in the presence of shear stress [10]. In addition, concerning magnitude of shear stress, low shear stress led to an increase nanoparticle uptake in endothelial cells [14,16]. From these findings, it suggested that externally modulated stimuli to form a biomimetic dynamic microenvironment must be carefully considered when modeling cells and tissues in vitro.

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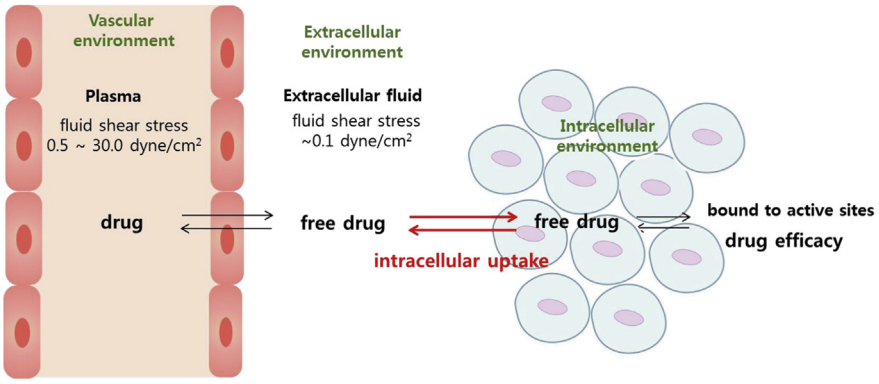

Fig. 1. Schematic process of intracellular drug uptake for drug efficacy under extracellular dynamic environment.

Moreover, complex physical and biochemical factors within the cellular microenvironment may be affected by nanoparticles properties such as their size, charge and concentration [2]. Thus, a more detailed investigation of nanoparticulate behaviors with cells under biomimetic microfluidic conditions, where shear stress and physicochemical factors may change the intracellular uptake of nanoparticles, is required. However, most of in vitro cellular behaviors and experiments have only been studied under static conditions.

The purpose of this study was to investigate how the shear stress in dynamic environment affects intracellular uptake of nanoparticles using calibrated biomimetic microfluidic system (BMS). In this study, cationic and anionic polystyrene nanoparticles (PSNs) were chosen as model nanoparticles for their fluorescence (imaging) and wide availability [12,13]. The PSN provides size homogeneity, colloidal stability in biological media and the ease of surface modifications for various applications as drug carrier systems [7]. Effects of physicochemical properties of PSNs such as concentration and surface charge on cellular uptake and fluorescent imaging under BMS were compared with static condition. Additionally, cell type and magnitude of fluidic shear stress was also varied under BMS.

2. Materials and methods

2.1. Materials

Fluorescently labeled polystyrene latex beads were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Amino-modified (NH_2^+) and sulfate-modified (SO_4^{2-}) PSNs were used. Accurate particle size, surface charge, and morphology of cationic and anionic PSN are shown in Fig. S2. Cell media and supplements for cell culture were purchased from Gibco (Grand Island, NY). Tube (Silicone Tubing, Ibidi), connector (Elbow Luer Connector, Ibidi) and fluidic cell chamber (μ-Slide VI 0.4) were purchased from Ibidi GmbH (Munich, Germany). Hoechst 33342 and trypan blue solution were purchased from Invitrogen (USA), and 4% paraformaldehyde were purchased from Electron Microscopy Science, EMS (USA). The other reagents were from Sigma–Aldrich.

2.2. Characterization of polystyrene nanoparticle (PSN)

The average particle size and surface charge of cationic and anionic PSN were evaluate by PAR-III Laser Particle Analyzer System (Otsuka Electronics, Japan) which measures using a laser light from a suspension in distilled water with pH value of 7.0. All measurements were performed 3 times, and the average size and size distribution were determined. The morphology of the PSN was confirmed by transmission electron microscopy, TEM (TECNAI G2 F30 S-TWIN, FEI Company, USA). For observed TEM images, the nanoparticle suspension in distilled water was mounted on a copper grid covered with formvar film and dried in a vacuum dryer before loading on to the microscope.

2.3. Cell culture

A fluidic cell chamber was used for HEK 293T (human embryonic kidney epithelial cell line) and MS1 (mouse pancreatic endothelial cell line) cell culture. Thirty microliters of cell suspension (8×10^5 cells/mL) was seeded into each channel and allowed to attach and stabilize for 24 h. Cells were maintained in Dulbecco's modified Eagle's media (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

2.4. Establishment of a biomimetic microfluidic system (BMS)

2.4.1. BMS design

To investigate in vitro intracellular drug delivery of PSNs within dynamic environment, a BMS was used fluid cell media. Fig. 2 shows a schematic diagram of the BMS (top), and a cross section of a fluidic cell chamber with flow direction of cell media (bottom). It consisted of peristaltic pump, media, a fluidic cell chamber, a bubble trap and tube. With the exception of the pump, the BMS was placed in the incubator (37 °C and 5% CO_2) during the fluidic experiment. When the peristaltic pump was running, the fluidic media reached the cell chamber after passing through a bubble trap to remove air bubbles.

2.4.2. Calibration of BMS as a function of shear stress

To calibrate the BMS for different levels of fluidic stimuli (shear stress levels) on cells within the cell chamber (μ-slide, 2 channel), fluidic media volume changes were measured for 3 min at different micropump speeds (gage settings: 1, 2, 4, 6, and 8). From the standard calibration curve of the BMS between fluidic speed and volumetric flow rate (Q), the Q value increased linearly with increasing speed (Fig. S1). The Q can be extrapolated from a specific speed using the equation ($Q = 0.4653\times - 0.3721$) from a standard calibration. Shear stress (τ) was then calculated using the Hagen–Poiseuille equation:

$$\tau\ [\mathrm{dyne/cm^2}] = (6\mu Q) \div (bh^2)$$