Quantitative In vitro 3D Analysis of Nanomaterial Diffusion in a 3D-Atherosclerosis Model

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Statement of Purpose: In the study of vascular disease such as an atherosclerosis, an evaluation of the drug permeability and transport across the thickened vascular wall is extremely important. General blood vessel models for the *in vitro* study is limited to construction of only monolayer of endothelial cells (ECs), or monolayer of ECs and monolayer of smooth muscle cells (SMCs) which are cultured on each side of the culture membrane. Therefore, they cannot reproduce structure of the native blood vessels. We have reported a novel hierarchical cell manipulation technique by fabrication of nanometer-sized layer-by-layer (LbL) films composed of fibronectin (FN) and gelatin (G) onto the cell membrane (Matsusaki M. Angew Chem Int Ed. 2007;46:4689-4692.). This technique enables us to construct 3D-multilayered tissues with controllable cell types and layer number. Recently, we have reported the construction and characterization of 3D-vascular models by hierarchical cell manipulation (Matsusaki M. J Biomater Sci Polymer Ed. 2012;23:63-79.), and diffusion of nitric oxide could be analyzed in the vascular model (Matsusaki M. Angew Chem Int Ed. 2011;50:7557-7561.). The aim of this study is to develop an *in vitro* drug or nanomaterial assessment method by using the 3D-human vascular wall models consisted of layer of ECs and multilayer of SMCs (Figure 1). The 3D-structered tissues will allow us to evaluate the transport of substance across the tissue depth instead of the *in vivo* animal models.

Methods: The vascular models were constructed by the hierarchical cell manipulation. In brief, FN-G nanofilms were prepared onto a layer of umbilical artery SMCs (UASMCs) grown on a culture insert membrane. The cellular layer was alternately incubated with 0.04 mg/mL FN solution (step 1) and 0.04 mg/mL G solution (step 2), for totally 9 steps. The trypsinized SMCs were seeded onto the FN-G coated SMC layer to construct the second SMC layer. Cells were incubated for at least 6 hours to allow a firm cellular adhesion. After construction of 4-layered (4L-) SMCs, EC layer was constructed onto the top layer of SMCs. Permeability of the blood vessel model was evaluated using fluorescein isothiocyanate (FITC)-labeled dextran or phenylanaline-modified poly(γ-glutamic acid), biodegradable nanoparticles (γ-PGA-Phe NPs) (Kim H. Macromol Biosci. 2009;9:842-848.). Culture medium containing the drug models (1 mg/mL) was added to the upper chamber, and concentration below the chamber was measured by fluorescence intensity to evaluate the permeability. 3D-structure and morphology of the vascular models were evaluated by immunological staining of their histological specimens.

Results: The histological evaluation indicated the 5L-structures similar to the vascular walls. However, mRNA expression of the contractile markers of SMCs was slightly lower than the actual artery, thus the obtained 5L-models can be useful as a model of 3D atherosclerosis. We evaluated diffusion of various nanomaterials using the 3D-model to understand important factors for diffusion in the vascular walls of atherosclerosis. We employed two nanomaterials, dextran-2000k and biodegradable nanoparticles (γ-PGA-Phe NPs). Both have almost the same hydrodynamic radius of about 30 nm, but their morphologies are different (free chain VS solid NP). Interestingly, solid NPs showed approximately 10-fold higher permeability than the dextran-2000k (Figure 2). These results suggested that the physical properties of nanomaterials have significant effect on the diffusion in the vascular wall.

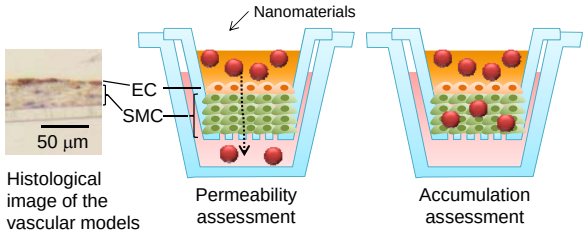

Figure 1. Histological of the vascular models consisted of 1L-EC and 4L-SMC and schematic representation of this study.

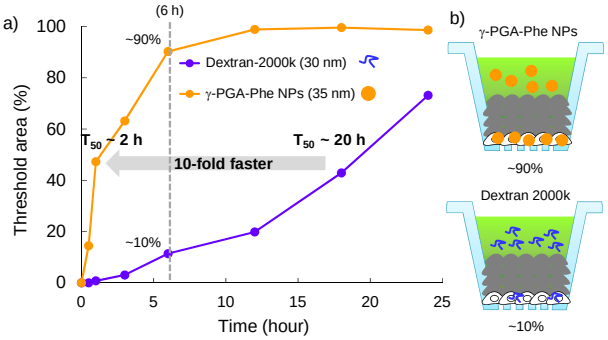

Figure 2. a) Fluorescence coverage at the bottom layer of vascular models representing the transport of dextran and γ-PGA-Phe NPs. b) Illustration of the transport of dextran and γ-PGA-Phe NPs to the bottom layer of the vascular models at 6 h.

Conclusions: The *in vitro* constructed vascular wall model provides us a system for evaluation of nanomaterial diffusion across the vascular wall of atherosclerosis. The results showed that morphology or physical properties of the nanomaterials have significant effect to their diffusion in the vascular wall. This system will provide us useful information for the design of drug carriers that have suitable transport properties across the vascular wall for the treatments of atherosclerosis or other vascular diseases.