Design of Drug Delivery Nanoparticles Using 3D-Human Vascular Wall Models for Atherosclerosis Treatment

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Statement of Purpose: For the treatment 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 (BVMs) 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 [1]. 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-BVMs by hierarchical cell manipulation [2], and diffusion of nitric oxide could be analyzed in the BVM [3]. The aim of this study is to develop the new method for nanoparticle design for drug delivery systems (DDS) by using the 3D-human vascular wall models consisted of layer of ECs and multilayer of SMCs for atherosclerosis treatment (**Figure 1**). The constructed 3D-BVMs will allow us to understand appropriate physical properties such as size, component, and surface property of DDS nanoparticles (NPs) for the accumulation in blood vessel walls and controlled release of encapsulated drugs for treatment of atherosclerosis.

Methods: The BVMs 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 BVM was evaluated using 1 mg/mL of fluorescein isothiocyanate (FITC)-labeled dextran or phenylanaline-modified poly(γ-glutamic acid), biodegradable NPs (γ-PGA-Phe NPs) [4].

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, suggesting that the 5L-BVMs might represent a 3D model of atherosclerosis. We evaluated diffusion of various nanomaterials using the 3D-BVMs to understand important factors for diffusion in the vascular walls of atherosclerosis. Biodegradable γ-PGA-Phe NPs were used in this study. While size effect to permeability was observed in dextran, γ-PGA-Phe NPs showed distinctly higher permeability regardless of having comparable size to the dextran. The results suggest that physical properties of NPs have significant effect on the diffusion in the vascular wall. Furthermore, we observed the effect of size to an accumulation amount of NPs in BVMs. The NPs with 200 nm showed significantly higher amount of remaining in the BVMs as compared to smaller-sized NPs with 15 and 50 nm (**Figure 2**). It is expected that the 200 nm sized NPs will induce sustained-drug release at the atherosclerosis area.

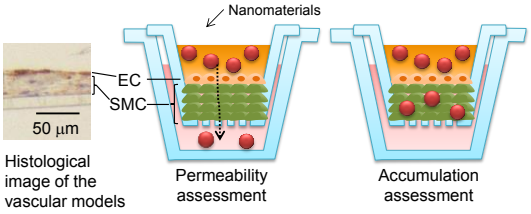

Figure 1. Histological of the BVMs consisted of 1L-EC and 4L-SMC and schematic representation of this study.

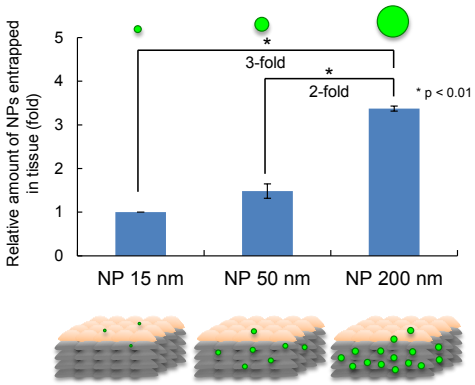

Figure 2. Effect of NP size to the entrapment amount of γ-PGA-Phe NPs which remained in BVMs after 24h of incubation with medium containing NPs.

Conclusions: The *in vitro* constructed 3D-BVM provides us a system for evaluation of NP diffusion, accumulation, and drug release at the wall of atherosclerotic blood vessel. This system will be powerful *in vitro* 3D-BVM for the design of NP carriers that have suitable transport properties to the vascular wall for the treatments of atherosclerosis or other vascular diseases.

References:

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