New Method for Evaluation of Nano-Drug Carriers by 3D-Arterial Wall Models for Atherosclerosis Treatment

Paninee CHETPRAYOON[1], M. MATSUSAKI[1], U. YOKOYAMA[2], Y. ISHIKAWA[2], M. AKASHI[1].
[1]Graduate School of Engineering, Osaka University, Japan
[2]Cardiovascular Research Institute, Yokohama City University Graduate School of Medicine, Japan
Tel: +81-6-6879-7356, Fax: +81-6-6879-7359, E-mail: akashi@chem.eng.osaka-u.ac.jp.

Statement of Purpose: In the development of drug carriers for treatment of vascular diseases such as atherosclerosis, permeability and accumulation of the drug carrier across the vascular wall are important factors for successful treatment *in vivo*. However, they cannot be evaluated in general arterial wall models (AWMs) which are limited to only monolayer of endothelial cells (1L-ECs), or either co-culture models of monolayered ECs and smooth muscle cells (SMCs) which are cultured on each side of culture membrane. Therefore, they cannot reproduce structure of the native blood vessels which is three-dimensional (3D).

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-AWMs by hierarchical cell manipulation [2], and diffusion of nitric oxide could be analyzed in the 3D-AWMs [3]. The aim of this study is to develop a 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-AWMs will allow us to understand appropriate physical properties such as size, component, and surface property of DDS nanoparticles (NPs) for the accumulation in arterial walls and controlled release of encapsulated drugs for treatment of atherosclerosis.

Methods: The 3D-AWMs consisted of 1L-EC and 4L-SMC were constructed by the hierarchical cell manipulation [2]. Permeability across the 3D-AWM was evaluated using 1 mg/mL of fluorescein isothiocyanate (FITC)-labeled dextran or 0.1 mg/mL phenylanaline-modified poly(γ- glutamic acid), biodegradable NPs (γ-PGA-Phe NPs) [4]. ApoE-deficient mice infused with angiotensinII were used for *in vivo* atherosclerotic model and observed with an In Vivo Imaging System (IVIS).

Results: The histological evaluation indicated the 5L-structures similar to the vascular walls. However, the thickness of 3D-AWMs increased during culture by proliferation of cells. Thus, the 3D-AWMs might represent a model atherosclerosis during early stage.

We evaluated diffusion of various nanomaterials using the 3D-AWMs to understand important factors for diffusion in the vascular walls of atherosclerosis. While the effect of size 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 properties of nanomaterials have significant effect on the diffusion in the vascular wall. Furthermore, by using different sizes of γ-PGA-Phe NPs, we observed that smallest NPs (50 nm) remained highest amount in the 3D-AWMs (**Figure 2**). Following this finding, we then moved to the animal experiment. Interestingly, similar phenomena was found in atherosclerotic mice that the smallest NPs showed highest accumulation in the atherosclerotic plaque (**Figure 3**). The treatment effect of drug-loaded γ-PGA-Phe NPs is under investigation.

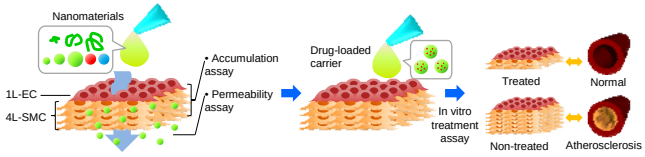

Figure 1. Outline of this study. Permeability and accumulation of nano-drug carriers evaluated by 3D-AWMs, and evaluation of *in vitro* treatment effects of drug-loaded NPs prior to study in animal model.

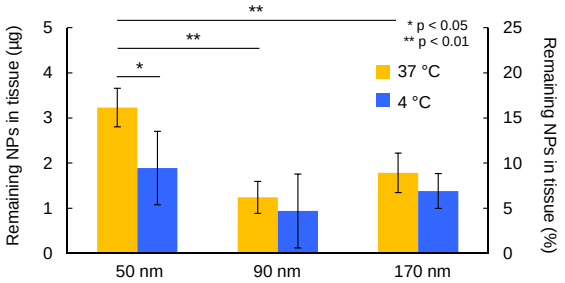

Figure 2. Effect of size and cellular uptake to accumulation of γ-PGA-Phe NPs in 3D-AWMs after 24 h.

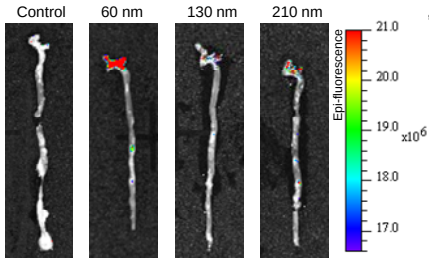

Figure 3. Accumulation of Alexa 633-labeled γ-PGA-Phe NPs with different sizes in the abdominal aorta of atherosclerotic mice after 24 h of injection (10 mg/mL).

Conclusions: The 3D-AWM enable *in vitro* evaluation of nanomaterial diffusion across the vascular wall. The size of material was found to have significant effect to accumulation in the atherosclerotic plaques, which was agreed with the results from animal model. The 3D-AWMs are expected to be useful for development of drug carriers to treat atherosclerosis and other vascular diseases.

References: [1] Matsusaki M, et al., Angew Chem Int Ed. 2007;46:4689- 4692. [2] Matsusaki M, et al., J Biomater Sci Polymer Ed. 2012;23:63-79. [3] Matsusaki M et al., Angew Chem Int Ed. 2011;50:7557- 7561. [4] Kim H. et al., Macromol Biosci. 2009;9:842-848.