3-Dimensional Artificial Blood Vessel Architectures by Layer-by-Layer Technique

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Statement of Purpose: In tissue engineering, in vitro construction of cell-polymeric material composites for implantation attracts much attention by controlling cell adhesion and proliferation on/in biodegradable matrices[1]. However, it is difficult to construct 3-dimensional (3D) artificial tissues because organs and tissues in the body are complicated organization of the appropriate components such as cells, extracellular matrices (ECM), and signal molecules. In particular, some organs such as blood vessel and skin have a well-organized and laminated 3D-structure composed of cells and ECM layers. We have developed a novel 3D-cell assembling technique via layer-by-layer (LbL) assembly (Figure 1). We prepared the nano-meter sized ECM layers (fibronectin-gelatin layers: nano-ECM) onto monolayered cell surfaces by LbL assemblies. The nano-ECM on the surface of first-cell layer can provide the nano-level scaffold for the adhesion of the second cells. Recently, Rajagopalan and co-workers reported bilayer constructs of hepatocyte-hepatocyte, hepatocyte-endothelial cell and hepatocyte-fibroblast by preparing chitosan-DNA polyelectrolyte multilayer on hepatocyte surface[2]. They reported chitosan-DNA multilayer provides a cell-adhesive surface on which a second layer of cells can be cultured, resulting in layered architectures.

In this study, we have successfully prepared 5 layers architectures of mouse L929 fibroblast cells or human umbilical artery smooth muscle cell (UASMC) by continuing the nano-ECM preparation and the cell adhesion process. Furthermore, 3-dimensional artificial blood vessel structure composed of UASMC and human umbilical vascular endothelial cell (HUVEC) also fabricated. The 3D-cell assembling technique would have a possibility to provide well-organized 3D-artificial tissues for tissue engineering.

Methods: Briefly, cover glass was immersed in 0.2 mg/mL of FN/50 mM Tris buffer (pH=7.4) for 15 min at 37 ^{o}C, and then immersed into 50 mM Tris buffer (pH=7.4) for 1 min to remove excess adsorption of FN. 8 x 10^4 cell/cm^2 of L929 or 4 x 10^4 cell/cm^2 of UASMC stained with cell tracker green was seeded onto the cover glass and incubated for 6 hours. The cover glass was rinsed in 50 mM Tris buffer for 1 min, and then immersed in 0.2 mg/mL of FN/50 mM Tris buffer for 15 min at 37 ^{o}C. After immersion in 50 mM Tris buffer, the cover glass was immersed again into 0.2 mg/mL of G/50 mM Tris buffer (pH=7.4) for 15 min at 37 ^{o}C, and then immersed in 50 mM Tris buffer for rinse. The alternative deposition was repeated 7 times to prepare about 6.2 nm of nano-ECM (FN-G multilayer). After the LbL step, the same amount of L929 or UASMC was seeded onto the first cell layer for assembling of the second cell layer. 3D-cell assembly was fabricated by repeating of these assembling steps.

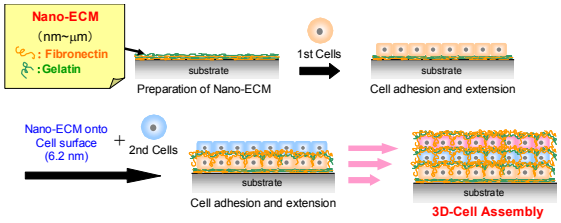

Figure 1. Schematic illustration of 3D-cell assembly.

Results/Discussion: When the cells seeded onto the first cell layer without nano-ECM, bilayer structure could not observe and the cell density of the first cell layer clearly increased. However, 4 or 5 layers structure of the cells was successfully fabricated via preparing 6.2 nm of nano-ECM on cell surface. The thickness of 3 and 4 layers of L929 was ca. 15.0 and 24.6 μm, and these well agree with theoretical thicknesses. We successfully fabricated 3D-blood vessel like artificial bilayer of UASMC-HUVEC by preparing 6.2 nm of nano-ECM layer on surface of UASMC (Figure 2B), although heterogeneous monolayer was obtained without nano-ECM (Figure 2A).

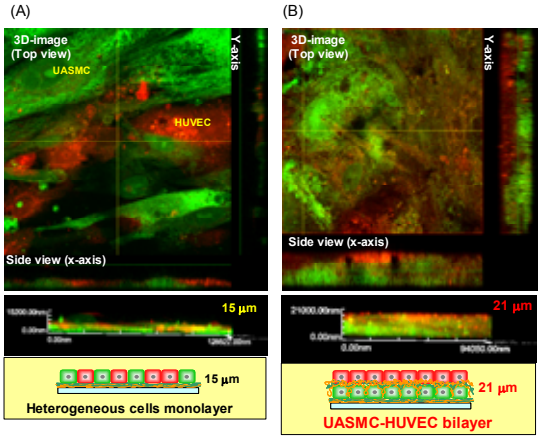

Figure 2. Conforcal fluorescent microscopic images of UASMC-HUVEC monolayer (A: without nano-ECM) and bilayer (B: with nano-ECM).

Conclusions: We have successfully fabricated 3D-artificial blood vessel architecture by preparing nano-ECM on cell surfaces. The 3D-assembly method via LbL technique will be useful for in vitro fabrication of 3D-artificial tissues.

References:

1) Lee KM, Mooney DM, Chem Rev. 2001;101:1869.
2) Rajagopalan P et al, Tissue Eng. 2006;12:1553.