Construction of 3-Dimensional Artificial Tissues by Layer-by-Layer Assembly Technique

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Statement of Purpose: Recently, the in vitro construction of cell-polymeric material composites for the treatment of skin or cartilage has been reported by adhesion and proliferation of cells on/in biodegradable matrices.[1] However, 3-dimensional artificial tissues have not been achieved yet, because organs and tissues in the body require the appropriate components, cells, materials and signal molecules, with sizes typically in the micro- or nano-scale.[2] Our challenge for the development of tissue engineered constructs is the development of cell and matrix structures that better mimic the complex tissues found in vivo. In this study, we report a novel type of 3-dimensional artificial tissue constructed by the layer-by-layer assembly of nano-meter sized polymeric films (nano-ECM) and L929 fibroblast cells. In addition, the effects of the nano-ECM formation on the structure of 3-dimensional cell-polymeric organisms were investigated.

Methods: Gelatin was obtained from Wako (Japan). Bovine plasma fibronectin (FN, lyophilized from 0.05 M Tris buffered saline at pH 7.5) was obtained from the Sigma-Aldrich (Japan).

Results / Discussion: Here, we chose gelatin and FN as the component materials of the nano-polymeric films. Gelatin is a denatured collagen, and is often utilized in tissue engineering. FN belongs to the family of glycoproteins, and is responsible for the key function of cell attachment. FN connects with collagen (gelatin) through its binding site in the extracellular matrix, which motivated us to construct the layer-by-layer assembly of these polymers. Figure 1 shows the frequency shift plotted against the assembly step of the layer-by-layer assembly in gelatin and FN solutions. The frequency shifted with an increasing number of assembly steps. This result supports stepwise, alternative polymer deposition. The frequency shift after an 8-step assembly was 759 Hz, which translates to a mean film thickness of about 20 nm. In this study, both assembly polymers are found in the extracellular matrix, and therefore we termed this type of nano-polymeric film "nano-ECM".

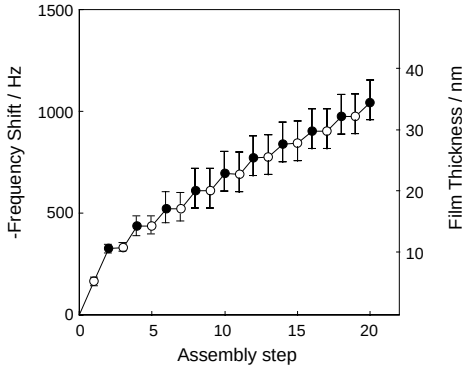

Figure 1. Frequency shift of the QCM plotted against the assembly step for a gelatin-FN assembly in 50 mM Tris buffer solutions (pH = 7.4). The open and closed symbols represent the gelatin and fibronectin steps, respectively.

The 3-dimensional artificial tissue was constructed by the following experimental procedures. First, a precursor film (2-step) composed of gelatin and FN was prepared on a slide glass. Next, the substrate was immersed into a suspension of L929 fibroblast cells, and nano-ECM film (8-step) was prepared on the substrate by the layer-by-layer assembly. The outermost surface was FN layer to facilitate cell attachment. These manipulations were repeated to build-up the ambient layers.

Figure 2 shows phase-contrast and fluorescence images of the L929 fibroblast cells (1 x 10^5) adhesion without and with the nano-ECM after 12 hours of incubation in Eagle's MEM medium containing 10% FBS (blood serum) at 37 ^{O}C. In the absence of the nano-ECM, we couldn't observe any 3-dimensional cell organisms at all, and all of the cells were adhered onto the slide glass. On the other hand, in the presence of the nano-ECM, 3-dimensional assembly structures of L929 fibroblast cells could be observed. This result clearly shows that the nano-ECM contributes to the construction of a 3-dimensional cell organism.

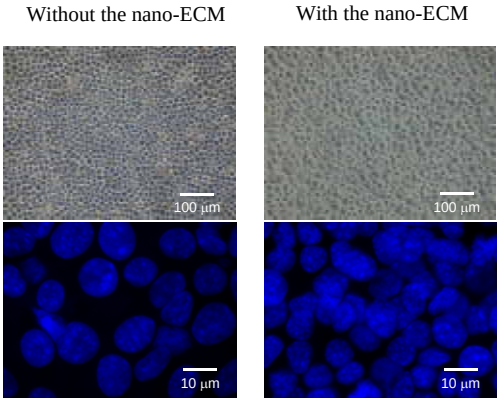

Figure 2. Phase-contrast and fluorescence microscope images of the L929 fibroblast cells (1 x 10^5) adhesion without and with the nano-ECM after 12 hours of incubation in Eagle's MEM medium (10% FBS) at 37 ^{O}C. The nuclei were stained with DAPI (blue).

Conclusions: Here, we present a novel design for a 3-dimensional artificial tissue. Nanostructured-biodegradable nano-ECM composed of gelatin and FN was prepared by layer-by-layer assembly technique, and 3-dimensional cell-polymeric assembly structures were constructed by the alternative assembly of nano-ECM films (film thickness: 20 nm) and L929 fibroblast cells. We believe that this method will be one of the basic techniques in tissue engineering.

References: (1) Lee, K. M.; Mooney, D. M. *Chem. Rev.* **2001**, *101*, 1869. (2) Whitesides, G. M.; Ostuni, E.; Takayama, S.; Jiang, X.; Ingber, D. E. *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335.