Preparation of Artificial Fibrous Nano Extracellular Matrices on Cell Surface for Layered Tissues

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Statement of Purpose: In the body, tissues and organs consist of a complex organization of cells, extracellular matrix (ECM), and signaling molecules. In particular, blood vessels and skin are a highly organized hierarchical layer composed of various types of cells and ECM layers. However, an effective methodology to fabricate a 3D multilayer composed of cells and an ECM layer with the appropriate components and thickness has not yet been achieved. We focused on a layer-by-layer (LbL) technique, which is an appropriate method to prepare nanometer-sized films on a substrate through the alternate immersion into interactive polymer solutions. The preparation of fibronectin (FN)-gelatin (G) nanofilms (Nano-ECM films) on the surface of the first layer of cells will provide a suitable cell-adhensive surface that is similar to the natural ECM for the second layer of cells.[1] Herein, we report the preparation of FN-G nanofilms and morphological change of the nanofilms on cell surface for creation of layered tissues (Figure 1 a).

Methods: LbL assembly of FN and G or Dextran sulfate (Dex): A 9 Hz quartz crystal microbalance (QCM) plate (USI System, Japan) with polished gold electrodes and a diameter of 4.5 mm was used as the substrate. The QCM was alternately immersed into a 50 mM Tris-HCl buffer solution (pH=7.4) of FN (0.2 mg/mL) and a 50 mM Tris-HCl buffer solution of G (0.2 mg/mL). Each immersion was for 1 min and at 37 °C. A sample was taken and rinsed with 1 mM Tris-HCl buffer solution (pH=7.4), dried with nitrogen gas, and frequency of the sample was then measured.

Fabrication of cellular multilayer: Briefly, cells were seeded on the substrate at confluent condition. Preparation of the FN-G nanofilms on the cell surface was performed in the same manner as that of the above-mentioned LbL assembly. After 9-step assembly of FN and G, cells as second layer were seeded at confluent condition and incubated for 6 hours at 37 °C. Repeating these LbL steps, the multilayered architectures of cells were obtained.

Results: The LbL assembly of FN and G on the cell surface was analyzed quantitatively by using a QCM as the assembly substrate and with a phospholipid bilayer membrane as a model cell membrane. The alternate immersion of the QCM substrate with the phospholipid bilayer into a Tris-HCl buffer solution (pH=7.4) of FN followed by a Tris-HCl solution of G resulted in a stepwise decrease in the frequency, indicating the formation of FN-G multilayers. The SEM and fluorescence images showed morphological change of the homogeneous nanofilms to fibrous structure like the natural ECM on cell surface during 24 hours of incubation (Figure 1 b and c). We tried to fabricate an artificial blood vessel architecture composed of 4-layered human smooth muscle and monolayered endothelial cells by LbL assembly of cells with FN-G nanofilms. HE staining image clearly indicated the 5-layered architecture (Figure 1 d). Furthermore, SEM and CD31 staining images suggested that the top surface of this layered tissues was covered with endothelial cells (Figure 1 e).

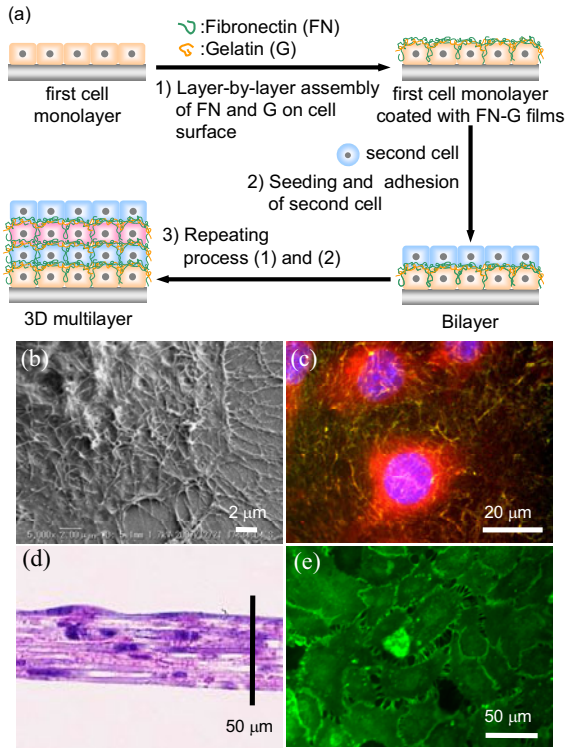

Figure 1. (a) Fabrication process of 3D-cellular multilayers by preparing FN-G nanofilms onto the cell surface. (b) SEM image of L929 fibroblast cells with 121 nm of FN-Dex nanofilms after 24 hours of incubation. (c) Confocal microscopic image of L929 fibroblast cells with Rhodamine-FN (red)-FITC-G (green) nanofilms after 24 hours of incubation. Nuclei was stained with DAPI (blue). (d) HE staining of blood vessel like 5-layered tissues and (e) confocal microscopic image of CD31-stained HUVEC on the surface of 5-layered structure.

Conclusions: In summary, we found that FN-G nano fibril acted as an artificial ECM matrix like the native one on cell surface for cellular multilayer. The present methodology may be applied as one of the biomedical applications of LbL assembly to fabricate various cellular multilayers composed of target cells and ECMs.

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References:

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