Three-dimensional Constructs Induce High Cellular Activities for Protein and Cytokine Productions

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Statement of Purpose: Tissues and organs are highly organized hierarchical architectures composed of cells, extracellular matrix (ECM), and signaling molecules. One of the goals of tissue engineering is the creation of three-dimensional (3D) artificial tissues consisting of various cell types and ECM, which resemble the structure and functions of natural tissues closely. Recently, new technologies such as cell sheet engineering[1)] have been reported in the fabrication of layered cellular architectures. We also reported a novel hierarchical cell manipulation technique for developing 3D-cellular multilayers by the fabrication of nanometer-sized layer-by-layer (LbL) films composed of FN and gelatin (G) onto cell membranes.[2)-4)] Studies on the functions of layered cellular architectures as compared with cell monolayer are valuable not only for understanding how a 3D environment composed of cells, ECM, and signaling molecules regulates functions similar to natural tissues, but also for creating 3D-artificial tissues resembling natural tissues. Recently, some researchers have reported the functions of layered cellular architectures in vitro. However, the basic properties induced by 3D-cellular structures, such as the layer number or the cell types, have not been clarified yet. In this study, we evaluated the structural stability of layered constructs consisting of human fibroblast cells (FCs) and human umbilical vascular endothelial cells (ECs) in relation to their layer number. Moreover, the production of heat shock protein70 (Hsp70) and interleukin-6 (IL-6) from the cellular structures were investigated to elucidate any 3D-structural effect on cellular function.[5)]

Methods: Fabrication of cellular multilayer: Briefly, the substrate was immersed into a 50 mM Tris-HCl buffer solution (pH = 7.4) of FN (0.2 mg/ml) for 15 min, and then FCs were seeded onto the substrate at confluent condition and incubated in FC culture medium for 24 h at 37 ^{o}C. The monolayered cells on the substrate were alternately immersed into 0.2 mg/ml FN or G solutions (50 mM Tris-HCl buffer, pH = 7.4) for nine steps, plus a rinse with 50 mM Tris-HCl buffer for 1 min at 37 ^{o}C. The thickness of the FN-G films was approximately 6 nm.[2)] Thereafter, cells at confluent density were seeded as the second layer, and incubated for 24 h at 37 ^{o}C. The FN-G assembly and the cell seeding were repeated for a predetermined time.

Results: To elucidate the structural stability of the layered constructs, four-layered (4L) FCs plus an uppermost layer of ECs (4L-FC/1L-EC) and the 1L-FC/1L-EC were fabricated, and the histological and morphological evaluations were performed. Interestingly, the ECs adhered homogeneously onto 4L-FCs, and tight-junction formation was widely observed at the centimeter scale, while heterogeneous EC domain structures were observed on the 1L-FCs. The production of Hsp70 and IL-6 from the cellular structures was investigated to elucidate the effect of the 3D structures on cellular functions. The Hsp70 expression of the ECs decreased after adhesion onto the 4L-FC structure as compared with the EC monolayer (Figure 1 a, b). Surprisingly, the Hsp70 production response to heat shock increased drastically by approximately 10-fold as compared with a non-heat shock by 3D structure formation, whereas the monolayer structures showed no change (Figure 1 b). Moreover, the production of the inflammatory cytokine IL-6 decreased significantly depending on the layer number of FCs. These results demonstrated that 3D-layered constructs consisting of abundant cells and proteins would provide biocompatible conditions similar to natural tissues.

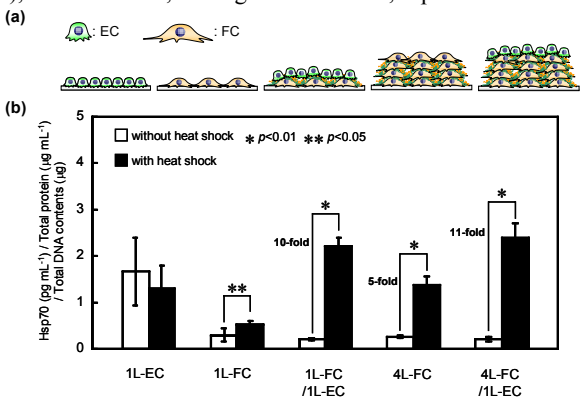

Figure. 1 (a) Schematic illustration of various layered structures composed of FCs and ECs. (b) Hsp70 production versus the total protein from non-heat shocked and heat shocked layered structures composed of FCs and ECs (n=4). The heat shock condition is defined as 20 min of incubation at 45 ^{o}C, followed by a 2 hours recovery period. The asterisks (*, **) denote a statistically significant difference between the samples calculated by a two sample *t*-test.

Conclusions: In conclusion, we investigated for the first time the effects of 3D-cellular structures on cell stability and function in relation to their layer number. The results from this study provide basic and valuable information in the biological and biomedical fields. We are now performing further experiments using 3D-cellular constructs, and layered architectures consisting of various types of cells may be useful as a tissue model for cell biology and pharmaceutical assays.

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References:

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