

Control of Cellular Functions by Layer-by-Layer Nanofilms Prepared on Cell Surface

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Statement of Purpose: Surfaces play an important role in biology and biotechnology with most biological reactions, such as cell surface/biomaterials, extracellular matrix (ECM)/biomolecules, and ECM/cells, etc. The design of novel biocompatible surfaces at the molecular level is of great interest for the scientific and engineering communities because of their potential for applications in the fields of biology and biomaterials. Recently, we reported a novel hierarchical cell manipulation technique for developing three-dimensional cellular multilayers similar to natural tissues by the fabrication of nanometer-sized Layer-by-Layer (LbL) films composed of fibronectin (FN) and gelatin (G) onto the cell membrane.¹ Although some researchers have already reported the fabrication of LbL films on the surface of cells, to the best of our knowledge, there are no reports on the effects of the various LbL films prepared on the cell membrane directly on the cellular functions. In this study, various multilayers were fabricated onto the surface of mouse L929 fibroblast cells using LbL assembly (Figure 1 A), and the cell viability, morphology, and proliferation were investigated in relation to the LbL film components.²

Methods: LbL assembly on cell surface: Briefly, a substrate was immersed in 50 mM Tris-HCl buffer solution (pH = 7.4) containing 0.2 mg/ml FN for 15 min, and L929 fibroblast cells were seeded onto the substrate and incubated in Dulbecco's modified eagle medium with 10% fetal bovine serum for 12 h at 37 °C. The cells on the substrate were alternately immersed into a 50 mM Tris-HCl buffer solution (pH = 7.4) containing proteins or polymers for 1 min at 37 °C. The substrate with the cells was then rinsed with 50 mM Tris-HCl buffer solution.

Results: The species of LbL nanofilm strongly affected the cell viability, morphology, and growth. All polyelectrolyte (PE) multilayers on L929 fibroblast cells showed cytotoxicity depending on the film thickness (Figure 1 B), although each component in solution showed high cytocompatibility except poly(allylamine hydrochloride) (PAH). On the other hand, FN-G or FN-dextran sulfate (DS) multilayers with FN-binding domain interactions (FN films) did not show any cytotoxicity, even at over 100 nm. Furthermore, PE films induced a round-shaped morphology of the adhered cells and inhibited the cell proliferation, although the cells survived during the culture period, whereas FN films did not show any effect on the cell morphology and proliferation as compared with the control cells (without films). Fluorescence microscopic and scanning electron microscopic observations clearly suggested a nanometer-sized meshwork morphology of the FN films on the cell surface after 24 hours of incubation, whereas the PE films showed homogeneous film morphologies on the cell surface. These nano-meshwork morphologies seemed to be similar to the fibrous structure of the natural ECM.

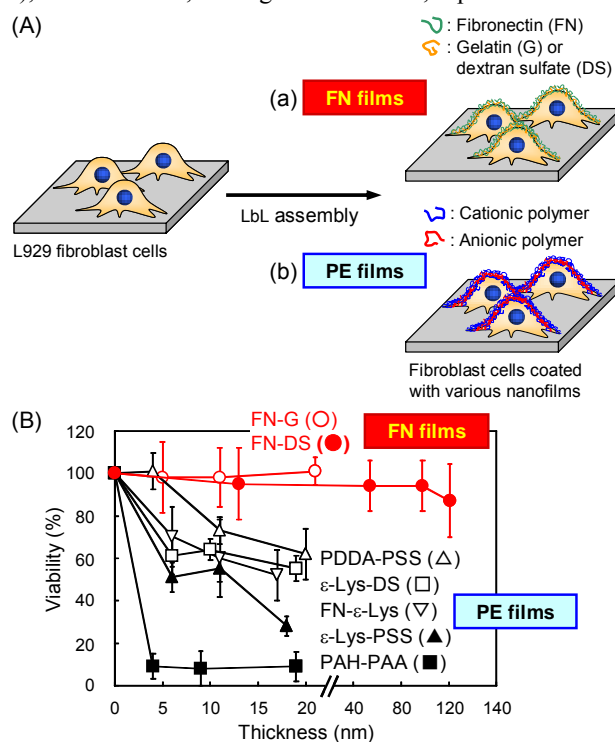


Figure 1 (A) Schematic illustration of preparation of various nanofilms on cell surface by layer-by-layer assembly. (a) : FN-G or FN-DS, (b) : Polyelectrolyte (PE) nanofilms. (B) Effect of various nanofilms prepared on cell surface to cell viability. FN : Fibronectin, G : Gelatin, DS : Sodium dextran sulfate, PDDA : Poly(diallyldimethylammonium chloride), PSS : Poly(styrenesulfate sodium salt), ε-Lys : ε-Poly(lysine hydrochloride), PAH : Poly(allylamine hydrochloride), PAA : Poly(acrylic acid).

Accordingly, the FN films FN-G and FN-DS prepared by collagen and heparin binding domain interactions did not show any effect on the cell functions. Furthermore, the meshwork structure of the FN films would have great potential for controlling the cellular interfaces and functions as an artificial ECM meshwork.

Conclusions: In conclusion, we found that the components, charge, and morphology of LbL nanofilms prepared directly on the cell surface strongly affected cell functions. The preparation of LbL nanofilms onto a cell surface might be a novel and interesting technique to control cell surface and functions.

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References:

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2. Kadowaki, K. et al., *submitted*.