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Control of Cellular Inflammation by Layer-by-layer Nanofilms through Different Driving Forces

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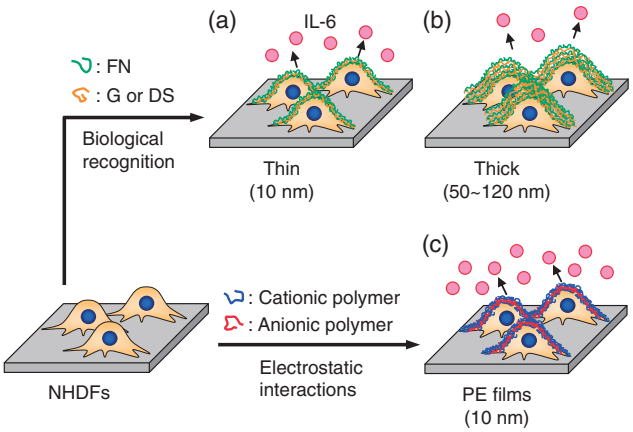
Layer-by-layer-assembled nanofilms prepared by different driving forces were directly fabricated onto a cell surface to control cellular inflammation. Polyelectrolyte multilayers (electrostatic interaction) induced an over 2.5-fold higher production of the inflammatory cytokine interleukin-6 (IL-6) than fibronectin (FN)-based multilayers (biological recognition), which did not show any significant production of IL-6. Moreover, thick FN-based nanofilms showed lower expression of IL-6 than thin ones. This is the first report of controlling cellular inflammation by directly prepared nanofilms on a cell surface.

Nearly all tissue cells in the body are surrounded by extracellular matrix (ECM) fibers, typically composed of collagen and fibronectin (FN), and this fibrous meshwork of the ECM regulates cellular activities through direct interactions with cell surface integrin receptors.[1] The composition of the cellular surface determines its interactions with the environment, and its ability to communicate with other cells.[2] Recently, the surface modification of living cells with functional molecules has attracted much attention because of the potential for applications of cell manipulation in the fields of biology, biomaterials, and tissue engineering. Up until now, few methods such as DNA transfection,[3] metabolic pathways,[4] or the direct reaction of macromolecules with the cell surface[5] have been reported to modify the surface of living cells. Although these methods are intriguing, complicated manipulation is required, and the composition and thickness of the layer are not controllable. Recently, layer-by-layer (LbL) assembly[6] has been explored for cell surface modification,[7] because LbL assembly has many advantages over cell surface modification such as ease of preparation, versatility, and a tunable structure and composition under ambient and physiological conditions.[6b] However, researchers have focused on the modification of relatively simple organisms such as bacteria and yeast by LbL films formed through electrostatic interactions. Much less work has examined the surface modification of living mammalian cells, focusing on the effect of LbL films prepared onto the cell surface on cellular functions.[8]

Recently, we reported that the components, charge, and morphology of LbL nanofilms prepared directly onto the cell surface strongly affected cellular functions.[8c] On the other hand, in order to understand the effects of these materials on cellular function in detail, it is essential to analyze the inflammatory responses of these cells in relation to their properties.[9] Although the inflammatory response of cells on LbL films has been already investigated,[6c,10] in general, the adsorption of serum proteins onto LbL films occurs quickly before cell adhesion. Accordingly, it is difficult to evaluate the direct effects of LbL films on the inflammatory responses of these cells.

In this study, two types of LbL films were prepared directly onto the surface of cells through different driving forces, FN-binding domain interactions (FN films: FN–G and FN–dextran sulfate sodium salt (DS)) and electrostatic interactions (PE films: FN–poly(ε-lysine hydrochloride) (ε-Lys) and ε-Lys–poly(styrene sulfate sodium salt) (PSS)). The production of interleukin-6 (IL-6) was then investigated to elucidate the effects of the nanofilm driving force on the inflammatory responses of these cells (Scheme 1). To the best of our knowledge, this is the first report on protein production from cells modified by nanometer-sized multilayers, focusing on the thickness, composition, and driving force of the LbL films. This cell surface control without inflammation will be important as a cell manipulation technique for tissue engineering.[8d]



Scheme 1. Schematic illustration of IL-6 production from NHDFs with (a) thin FN films, (b) thick FN films, and (c) PE films.

Normal human dermal fibroblasts (NHDFs) were seeded onto a plastic disk with a FN base layer and incubated for 12 h at 37 °C. PE films and FN films were prepared directly onto the cell surface following the procedure described in our previous report.[8c] All of the detailed experimental procedures are shown in the Supporting Information.[11] The thickness of the LbL films was estimated by quartz-crystal microbalance.[8c] To analyze the effects of the LbL films prepared onto the cell surface on cellular adhesion and morphology, the actin skeleton was stained with tetramethylrhodamine isothiocyanate (TRITC)–phalloidin after 24 h of incubation with PE films or FN films. Figure 1 shows phase contrast and fluorescent images of the cells with and without the LbL films. The cells showed a good extended morphology and actin filament under all conditions, suggesting that the LbL films on the cell surface did not have any effect on cell adhesion. Thus, the effects of the driving force of the LbL nanofilms on the cell surface on cellular inflammation can be evaluated.

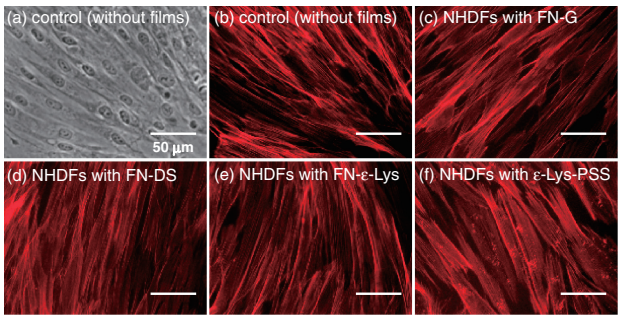


Figure 1. (a) Phase contrast and (b) fluorescent images of NHDFs without films (control). Fluorescent images of (c) NHDFs with 11-nm thick FN–G films, (d) 13-nm thick FN–DS films, (e) 11-nm thick FN–ε-Lys films, and (f) 11-nm thick ε-Lys–PSS films for 24 h of incubation after nanofilms were prepared on the cell surface, respectively. (b–f) The actin skeleton was stained with rhodamine–phalloidin. All scale bars are 50 μm.

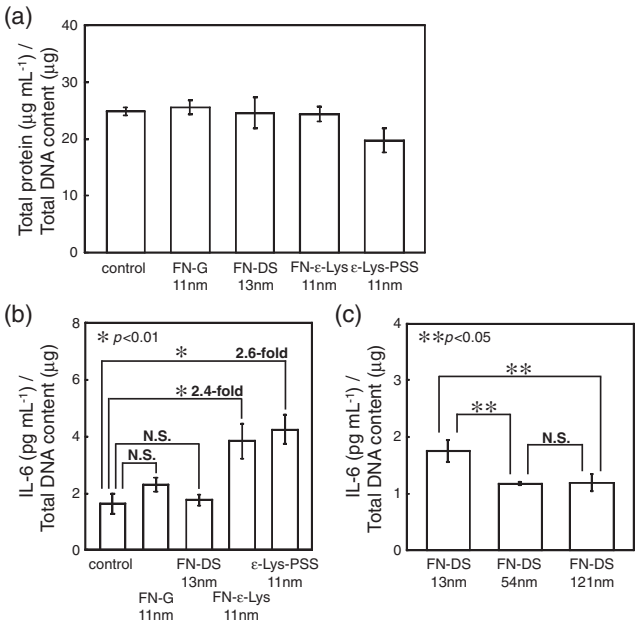


Figure 2. Production of (a) total protein or (b, c) IL-6 versus the amount of DNA from NHDFs without films (control) and with various nanofilms for 24 h of incubation after nanofilms were prepared on the cell surface ($n = 3$). The asterisks (*, **) and N.S. mean a statistically significant difference and no significant difference between the samples calculated by a two sample t-test, respectively.

The production of the inflammatory cytokine IL-6 with or without the LbL films was then analyzed (Supporting Information), because IL-6 plays an important role in intercellular communication and the inflammatory response.[12] In all cases, the production of total protein from the cells was almost the same (Figure 2a), whereas the expression of IL-6 depended strongly on the species of LbL film used (Figure 2b). The FN films did not induce a significant production of IL-6, probably due to the high cytocompatibility of the FN films.[8c] On the other hand, the cells with PE films showed two- to threefold higher IL-6 production than controls. The PE films on the cell surface might have induced inflammatory responses due to the condensation of the cationic components on the cell membrane, because it is well known that cationic polymers strongly interact or aggregate with anionic glycoproteins on the cell membrane.[13] Although the ECM protein FN, which has a weak negative charge, was employed as the counterpart component to the cationic polymer, IL-6 expression was clearly observed at the same level, suggesting that the predominant reason should be the cationic polymer under the electrostatic driving force. Accordingly, the usage of binding domain interactions between FN and negative components enables the direct fabrication of cytocompatible nanofilms on cell surface. This concept might be applicable to other specific interactions of negatively charged antibody–antigens, enzyme–substances, etc.[8d]

Interestingly, the production of IL-6 decreased upon increasing the thickness of the FN films (Figure 2c). Although the reasons for this finding are still unclear, thicker FN films would provide a more favorable environment for the cells than the thinner one to suppress the inflammatory response. In fact, the actual ECM layers in the body under physiologic conditions are much thicker than our nanometer-sized FN films (at least micrometer-size).

In conclusion, the cytocompatibility of LbL films on the cell surface were evaluated directly, focusing on the inflammatory responses of the cells. We found that the production of IL-6 depended strongly on the species and thickness of the LbL films. This LbL nanofilm coating without inflammation on cell surfaces would be useful technology for three-dimensional cell manipulation in biomaterial or tissue engineering fields.

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References and Notes

1 a) R. O. Hynes, A. T. Destree, *Cell* **1978**, *15*, 875. b) Y. Mao, J. E. Schwarzbauer, *Matrix Biol.* **2005**, *24*, 389.
2 M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **2005**, *23*, 47.
3 a) J. K. Scott, G. P. Smith, *Science* **1990**, *249*, 386. b) M. Kato, M. Mrksich, *J. Am. Chem. Soc.* **2004**, *126*, 6504.
4 a) J. A. Prescher, D. H. Dube, C. R. Bertozzi, *Nature* **2004**, *430*, 873. b) J. A. Prescher, C. R. Bertozzi, *Nat. Chem. Biol.* **2005**, *1*, 13.
5 a) M. E. Medof, S. Nagarajan, M. L. Tykocinski, *FASEB J.* **1996**, *10*, 574. b) A. J. Link, D. A. Tirrell, *J. Am. Chem. Soc.* **2003**, *125*, 11164.
6 a) G. Decher, *Science* **1997**, *277*, 1232. b) K. Ariga, J. P. Hill, Q. Ji, *Phys. Chem. Chem. Phys.* **2007**, *9*, 2319. c) T. Boudou, T. Crouzier, K. Ren, G. Blin, C. Picart, *Adv. Mater.* **2010**, *22*, 441.
7 a) B. Neu, A. Voigt, R. Mitlöhner, S. Leporatti, C. Y. Gao, E. Donath, H. Kiesewetter, H. Möhwald, H. J. Meiselman, H. Bäumler, *J. Microencapsulation* **2001**, *18*, 385. b) A. Diaspro, D. Silvano, S. Krol, O. Cavalleri, A. Gliozzi, *Langmuir* **2002**, *18*, 5047. c) A. L. Hillberg, M. Tabrizian, *Biomacromolecules* **2006**, *7*, 2742.
8 a) N. G. Veerabadran, P. L. Goli, S. S. Stewart-Clark, Y. M. Lvov, D. K. Mills, *Macromol. Biosci.* **2007**, *7*, 877. b) A. J. Swiston, C. Cheng, S. H. Um, D. J. Irvine, R. E. Cohen, M. F. Rubner, *Nano Lett.* **2008**, *8*, 4446. c) K. Kadowaki, M. Matsusaki, M. Akashi, *Langmuir* **2010**, *26*, 5670. d) M. Matsusaki, H. Ajiro, T. Kida, T. Serizawa, M. Akashi, *Adv. Mater.* **2012**, *24*, 454.
9 a) A. Kishida, S. Kato, K. Ohmura, K. Sugiyama, M. Akashi, *Biomaterials* **1996**, *17*, 1301. b) Y. Iwasaki, S.-i. Sawada, K. Ishihara, G. Khang, H. B. Lee, *Biomaterials* **2002**, *23*, 3897.
10 P. Tryoen-Tóth, D. Vautier, Y. Haikel, J.-C. Voegel, P. Schaaf, J. Chluba, J. Ogier, *J. Biomed. Mater. Res.* **2002**, *60*, 657.
11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
12 J. Van Snick, *Annu. Rev. Immunol.* **1990**, *8*, 253.
13 a) D. Fischer, Y. Li, B. Ahlemeyer, J. Krieglstein, T. Kissel, *Biomaterials* **2003**, *24*, 1121. b) D. Fischer, H. Dautzenberg, K. Kunath, T. Kissel, *Int. J. Pharm.* **2004**, *280*, 253.

