Protection and Functionalization of Cell Surfaces Using Nano-Barrier Films

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Statement of Purpose: In recent approaches to tissue engineering, cells face various stresses from physical, chemical, and environmental stimuli. For example, layer-by-layer (LbL) assembly requires many cycles of centrifugation and microfluidic system induces shear stress. These are typical physical stresses. Damage to cell membrane can cause the leakage of cytosol molecules or cell death. Accordingly, we have to consider cell viability and function carefully during tissue engineering approaches. If nanometer-sized barrier films can be prepared to cell membrane, the cells will be protected from the physical stresses. Moreover, further functionalization of cell surfaces could be achieved through the nano-barrier films.

We focused on coating of nanometer-sized extracellular matrix (ECM) films as a nano-barrier film onto cell membrane. Because fibronectin (FN), gelatin (G), type IV collagen (Col IV), and laminin (LN) are important ECM proteins for cell adhesion and protection, FN-G and Col IV-LN nanofilms were prepared on cell surfaces as a nano-barrier (**Figure 1**). Interestingly, both high protection effects from physical stress (centrifugation) and cationic-anionic functionalization were successfully achieved by employing these nano-barrier films (Matsuzawa A. Langmuir 2012, in press, DOI: 10.1021/la303459v). This method will be novel technology for protection and functionalization of cell surfaces in tissue engineering.

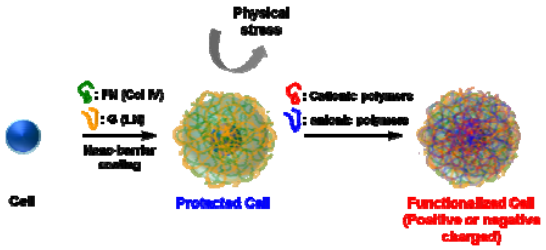

Figure 1. Schematic illustration of protection and functionalization of cell surfaces by nano-barrier films.

Methods: The FN-G or Col IV-LN films were prepared by following our previous paper (Nishiguchi A. Adv. Mater. 2011;23:3506-3510.). Briefly, hepatocyte carcinoma (HepG2) or normal human dermal fibroblast (NHDF) were alternately incubated with 0.2 mg/ml FN and G in 50 mM Tris-HCl (pH 7.4) for 1 min at 37 ^{o}C. After each procedure, the cells were washed with 50 mM Tris-HCl using centrifugation at 2,500 rpm (419 *g*) for 1 min to remove unabsorbed polymers. After nine steps of immersion, FN-G multilayer nanofilms were coated onto single cell surfaces. The Col IV-LN films and poly(ε-lysine)-poly(styrene sulfonate) (ε-Lys-PSS) were also prepared in the same way.

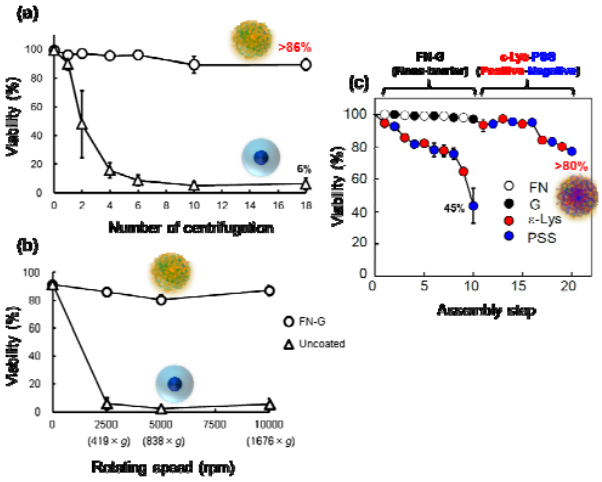

Figure 2. (a) Cell viability of HepG2 with (circle) or without (triangle) FN-G films (nano-barrier) (n=3). (b) The viability of HepG2 with (circle) or without (triangle) nano-barrier at different rotational speeds (n=3). (c) Cell viability of NHDF during the LbL assembly of ε-Lys-PSS with or without nano-barrier (n=3).

Results: Figure 2a shows the viability changes in HepG2 cells with or without coating FN or G in buffer during nine-step LbL assembly including the washing step. Nine steps of LbL assembly include a total of 18 cycles of centrifugation. For LbL assembly, an odd number means centrifugation after incubation in FN or G solution, and an even number means the washing step. The viability of HepG2 without the nano-barrier films drastically decreased to less than half even after the second centrifugation and final value was 6% after 18 cycles. However, when FN and G were employed to fabricate the nano-barrier, the cells maintained a viability of >86% after the final centrifugation. The HepG2 cells should be influenced by the cumulative gravity (physical stress) during centrifugation. In particular, cell membrane structures were probably deformed.

When the rotational speed was increased from 2,500 to 10,000 rpm, uncoated cells showed obvious cell death (<10%). In contrast, surprisingly, a high viability of >85% was achieved even under extreme physical stress at 10,000 rpm which is 10-fold higher than the typical speed for collecting cells (1,000 rpm) (**Figure 2b**). The nano-barrier was useful to functionalize cell surface by additional LbL assembly using cationic and anionic polymers through electrostatic interaction to avoid strong unspecific adsorption of cationic polymers (**Figure 3**).

Conclusions: We observed almost the same barrier effect of Col IV-LN films. This protection method using nano-barrier films will be new strategy to provide strong protected cells for various tissue engineering approaches.