Cell Micropatterning on Cell Surfaces Using Multi-Armed-Poly(Ethylene Glycol)-Eicosapentaenoic Acid

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Statement of Purpose: The fabrication of artificial three-dimensional (3D) tissues with similar properties to natural tissues is a key challenge for implantable tissues in tissue engineering, and for 3D-human tissue models in pharmaceutical assays. Tissues and organs are composed of multiple types of cells and their locations are complex in three dimension. For example, hepatic lobule has radiates blood capillary and hepatocytes are sandwiched in between the capillaries. Because specific 3D-alignment or localization of each cell type is important for tissue function, reconstruction of 3D-cell location inside tissue is crucial in tissue engineering. We reported simple technology, "cell accumulation technique", to construct the controlled cell multilayers by fabrication of nanometer-sized (~ 6 nm) layer-by-layer (LbL) films composed of fibronectin and gelatin onto the single cell surfaces [1]. This method successfully provides tissues containing blood capillary networks with over 100 μm thicknesses [2]. However, it was difficult to control cell location inside the 3D-tissues. Although cell micropatterning technologies on "substrate" have been reported [3], cell micropatterning on "cell surfaces" is still a big challenge. Only a few reports of cell patterning on adhered cell surface were reported [4]. However, cell patterning resolution was limited.

In this study, we develop a cell micropatterning technology using a cell-cell anchor molecule. We focused on fatty acids (FAs) for anchoring to cell membrane because FAs are main component of cell membrane. To screen the best FA, fluorescently labeled-saturated and unsaturated FAs were synthesized and then evaluated their adsorption properties on adhered cell surfaces (Figure 1a). Furthermore, the effect of the number of unsaturated bond was also investigated. Finally, the optimized FA was conjugated to the end group of 4-arm poly(ethylene glycol) (4-arm-PEG) and their anchoring property was evaluated.

Methods: 9-(bromomethyl)acridine (9Br) was mixed with saturated (stearic acid: Ste, arachidic acid: Ach, and melissic acid: Mel) and unsaturated (oleic acid: Ole, eicosenoic acid: Δ_1Arc, eicosadienoic acid: Δ_2Arc, eicosatrienoic acid: Δ_3Arc, arachidonic acid: Δ_4Arc, eicosapentaenoic acid: EPA, and docosahexaenoic acid: DHA) FAs solution for fluorescently labelling. The 0.5 μL of the obtained 9Br-FAs was added to the surfaces of pre-cultured normal human dermal fibroblasts (NHDFs) and then incubated for 15 min. Fluorescent images were observed by confocal laser scanning microscope (CLSM) after 1 times washing with phosphate buffered saline (PBS). The fluorescent area was quantitatively estimated by ImageJ software.

Results: The saturated FAs indicated carbon number independent lower anchoring effect, whereas unsaturated FAs showed increased manner with the number of carbon and double bond (**Figure 1a**). EPA, carbon number is 20 with 5 double bond, revealed highest adsorption amount on cell surface. However, DHA did not show high adsorption amount, might be due to high melting point. From the results of screening, we discovered that EPA has highest anchoring property. EPA conjugated 4-arm-PEG (4-arm-PEG-EPA) was synthesized, and evaluated their anchoring property (**Figure 1b-e**). NHDF treated with 4-arm-PEG-EPA in DMEM with 10% FBS was printed on the surface of adhered NHDF using a dispenser, and the printed cells were stable and growth well on NHDF surfaces.

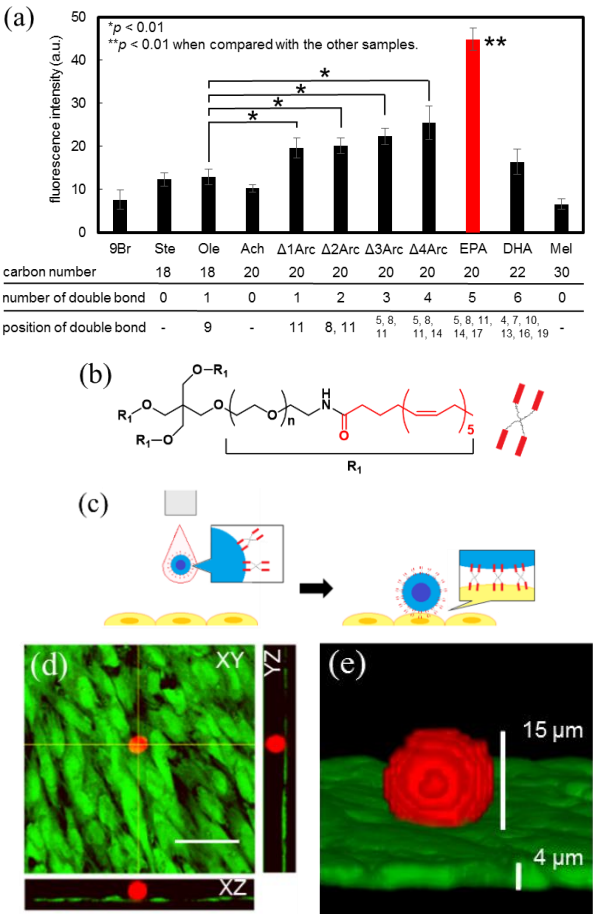

Figure 1. (a) Quantitative evaluation of adsorption properties of FAs on adhered cell surfaces (n=3). (b) The structure of 4-arm-PEG-EPA. (c) Schematic illustration of cell micropatterning on cell surfaces using 4-arm-PEG-EPA. (d,e) CLSM images of the printed NHDF on the surfaces of NHDF after 2 hours of incubation. Scale bar in (d) is 50 μm.

Conclusions: We optimized highest anchoring propery of EPA and synthesized 4-arm-PEG-EPA. The designed "molecular-anchor" would be useful to achieve cell micropatterning on cell surfaces.

Reference:

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