Collagen NanoFiber Coating on Cell Surfaces for Construction of Cell-Density Controlled 3D-Thick Tissues

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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. In the body, nearly all tissue cells in the body reside in the micrometer-sized fibrous meshwork of the extracellular matrix (ECM), and ECM plays an important role in controlling cellular functions. We have reported simple and unique technologies, "cell accumulation technique", to construct controlled cell multilayers by fabrication of nanometer-sized (~ 6 nm) layer-by-layer (LbL) films composed of fibronectin (FN) and gelatin (G) onto the single cell surfaces [1]. This method successfully provides thicker tissues containing blood capillary networks with over 100 µm thicknesses by a couple of days of incubation [2,3]. However, current technologies cannot easily control 3D-cell density and ECM thickness and component inside the 3D-tissues. To fabricate complicated and functional 3D-artificial tissues constructs, solution for the above requirements will be crucial.

We recently reported novel tissue engineering technology to control 3D-cell density and ECM thickness in thick 3D-human tissue constructs [4]. Collagen nanofiber matrices were constructed on single cell surfaces and their thicknesses were easily controlled from 3 ~ 30 µm by repeating the same steps for three times (**Figure 1**). Moreover, ECM components were easily added to the collagen matrices and their locations were also controllable. Finally, cell density was successfully altered by changing the thickness of the coated collagen matrices (**Figure 2**). This method has great potential to fabricate 3D-thick and complicated tissue constructs.

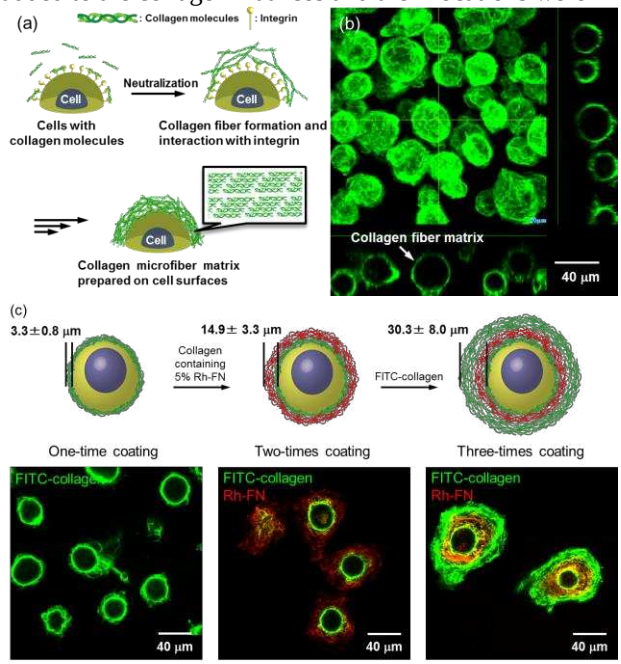

Figure 1. Schematic illustration for coating a collagen nanofiber on single cell surface (a). 3D-reconstructed confocal laser scanning microscope (CLSM) image of coated cells (b). illustration of CLSM images of the multiple coated cells (c).

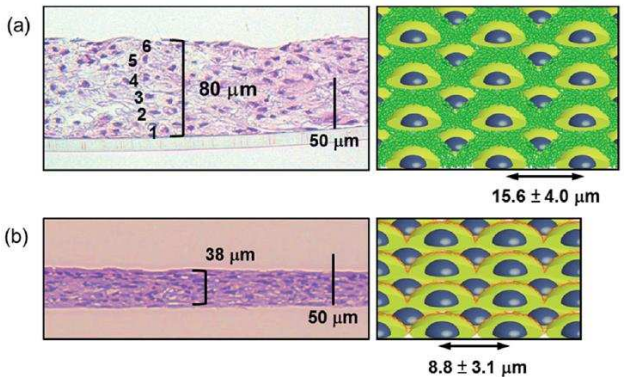

Figure 2. Histological HE staining images (left) and schematic illustration (right) of 3D tissues constructed by one-time collagen nanofiber coating (a) and FN-G nanofilm coating [1] (b). The mean distances between nuclei-nuclei were estimated from 50 nuclei in HE images.

Results: The trypsinized cells were suspended in 0.03 wt% type I collagen DMEM solution and then rotated at 50 rpm at 37°C for 20 min. CLSM images clearly showed micrometer-sized matrices of collagen nanofibers on single cell surfaces and the thickness increased with increasing step number drastically (**Figure 1b**). Furthermore, when the collagen-coated cells were added in cell-culture inserts, thick 3D-tissues with 78 µm thickness and lower cell density were obtained (**Figure 2**). On the other hand, thin 3D-tissues with higher cell density were obtained by coating with 6 nm sized FN-G nanofilms when our previous cell accumulation technique was employed [3]. The cell density of the present method is a 3.5-fold lower cell density in 3D-tissues by a collagen nanofiber coating method against that of our previous cell accumulation technique [4].

Conclusions: We demonstrate control of cell density and ECM thickness in 3D-tissue constructs using the collagen matrix coating technique. Moreover, millimeter-sized thick but lower cell density tissues were successfully constructed by this method. It is expected to fabricate complex and functional 3D-tissues for biomedical application.

References:

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[4] Liu C.Y. et al., RSC Adv. 2014;4:46141-46144.