Collagen Fiber Matrix Coating on Cell Surfaces for Development of Cell-Density Controllable 3D-Thick Tissues

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Statement of Purpose: The creation 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. Accordingly, development of 3D-artificial tissues consisting of not only cells but also ECMs is required. Currently, various technologies have been reported in constructing a multilayered cell construct. We have reported simple and unique technologies, "hierarchical cell manipulation", 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 cell membranes [1,2]. Moreover, the improved and rapid method, termed "cell accumulation technique", can provide thicker tissues containing blood capillary networks with over 100 μm thickness by a couple of days incubation [3,4]. 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 discovered novel tissue engineering technology to control 3D-cell density and ECM thickness in thick 3D-human tissue constructs. Collagen fiber matrices were constructed on single cell surfaces and their thicknesses were easily controlled from 3 ~ 50 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.

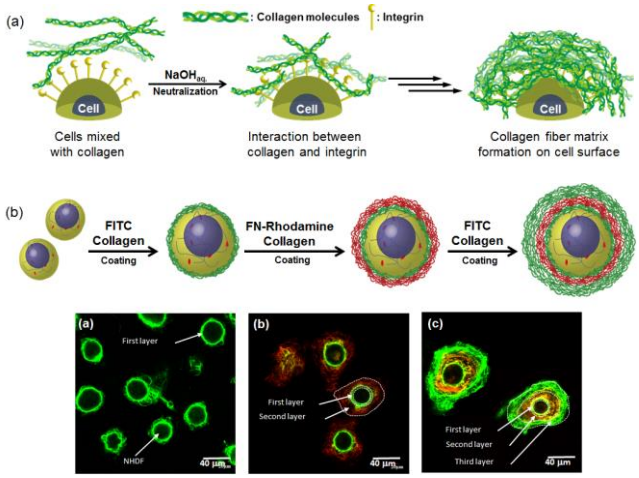

Figure 1. Schematic illustration of the collagen fiber matrix coating on single cell surfaces (a). Illustration and confocal laser scanning microscope (CLSM) images of multi-coating, including addition of the other ECM components e.g. fibronectin (FN) (b).

Methods: The detached cells were suspended in 0.03 wt% type I collagen DMEM solution which was 10 times diluted solution of commercialized collagen solution. After neutralization, the solution was rotated at 50 rpm at 37°C for 90 min. The obtained cells with collagen fiber matrices were washed with PBS twice. For multi-coating, same procedures were employed for two or three times. The coated cells were added in 24-micro well cell-culture insert to construct 3D-tissue structures.

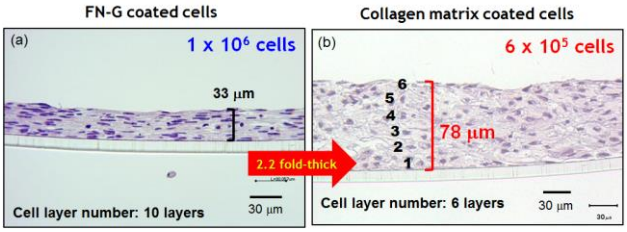

Figure 2. Histological images with hematoxylin and eosin (HE) staining of 3D-human fibroblast tissues. The thin tissues with higher cell density were constructed by our previous cell accumulation technique [3] (a). The thick tissues with about half cell number were constructed by this method (b).

Results: CLSM images clearly showed micrometer-sized collagen fiber matrices on single cell surfaces and the thickness increased with increasing step number drastically (**Figure 1**). We successfully added FN at middle layer in 3-times coated matrices. 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]. These results revealed high potential of the collagen fiber matrix coating method as a next generation tissue engineering technology.

Conclusions: We demonstrate control of cell density and ECM thickness in 3D-tissue constructs using the collagen matrix coating technique. It is expected to fabricate complicated and functional 3D-tissues for biomedical application.

References:

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[4] Matsusaki M et al., Adv. Mater. 2012;24:454-474.