Fabrication of Anisotropic Cell Sheets for Designing Well-organized Myotube Assembly

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Statement of Purpose: A tissue-like cellular monolayer "cell sheet" can be harvested intact with associated extracellular matrix (ECM) using thermoresponsive poly(*N*-isopropylacrylamide) (PIPAAm) grafted culture substrates simply by reducing culture temperature below the PIPAAm's lower critical solution temperature (LCST) of 32°C. Since the harvested cell sheet can be transplanted onto damaged tissues without additional treatments such suturing, the cell sheet-based tissue engineering are uniquely applicable to regenerative medicine.

An appropriate anisotropy needs to be designed for reconstructing biomimetically complex tissue such as a skeletal muscle tissue. Well-aligned muscle orientation is known as a key factor for producing mechanical functions like in native muscle tissue which has a highly organized structure consisting of long parallel bundles of myotubes. In this study, anisotropic cell sheets composed of well-aligned myoblasts have been fabricated using a stripe-like micropatterned thermoresponsive surface, and the unique features of the cell sheets have been applied for organizing orientation of myoblasts and myotubes three-dimensionally.

Methods: The original procedures for the preparation of micropatterned thermoresponsive surfaces have been reported previously [1]. Briefly, PIPAAm polymer brushes were grafted on glass substrates by a surface-initiated living radical polymerization process, and then hydrophilic polymer poly(*N*-acryloylmorpholine) (PAcMo) was further polymerized spatio-selectively via photolithographic process on the PIPAAm brush surface, resulting in the stripe patterns composted of PAcMo-*b*-PIPAAm block polymer brush and PIPAAm brush regions (50 μm / 50 μm stripes).

Human skeletal muscle myoblasts were seeded onto the patterned or non-patterned polymer brush surfaces. After reached confluency, the cells were harvested as a cell sheet by lowering culture temperature to 20 °C. Myoblast sheets were also manipulated using a gelatin gel-coated plunger [2]. First, a gelatin gel was placed on a myoblast sheet before detachment from the surface, and they were incubated at 28 °C to allow the gelatin gel to attach to the cell sheet. After lowering temperature to 20 °C, the myoblast sheet was transferred onto a normal cell culture dish (e.g., tissue culture polystyrene). For cell sheet layering, on the other hand, a harvested anisotropic cell sheet was transferred onto another cell sheet on a patterned or non-patterned surface. In addition, transferred myoblast sheets were cultured in differentiation media (2% horse serum containing media) for initiating myotube formation.

Results: The stretching direction of myoblasts was guided by the stripe patterns, due to the difference in cell-surface interactions between the two kinds of polymer patterns. Moreover, the cells proliferated with retaining their orientation until reached confluency. Although cell sheets generally shrink after the thermally induced detachment, a gelatin gel-coated plunger allowed the cell sheet to retain the cell alignment even after cell sheet detachment. An anisotropic cell sheet was therefore transferred using the plunger onto a normal cell culture dish. The transferred myoblasts were well aligned even on the non-patterned normal culture substrate for at least 3 weeks. On the other hand, their morphologies were clearly changed by culturing in differentiation media for 5 days. As shown in Figure 1, myotubes were also well aligned along the direction of cell alignment in the transferred myoblast sheet. In addition, the length of aligned myotubes was longer than that of randomly oriented myotubes, due to regulating myoblast alignment.

To design three-dimensional (3D) myotube orientation, the two anisotropic myoblast sheets were layered orthogonally after inducing myotube differentiation. Confocal microscopic observation revealed that the layered myotubes retained their individual myotube orientation in each layer. Therefore, even vertically different orientation can be designed via cell sheet layering process. Taken together, this cell sheet manipulation technique allowing myotube assembly to be manipulated has a potential to construct well-organized 3D myotube assembly.

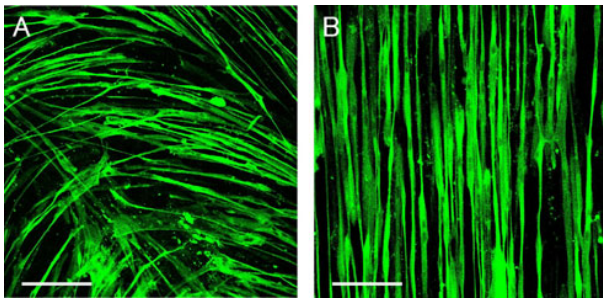

Figure 1. Confocal microscopic images of myotubes on normal cell culture dishes at Day 5 after initiating myoblast differentiation. Myoblast sheets were transferred from (A) non-patterned and (B) patterned thermoresponsive surfaces, and then cultured in differentiation media for 5 days. Myosin heavy chains were stained with fluorescein (green). Scale bar: 200 um.

Conclusions: Anisotropic myoblast sheets can be manipulated and transferred onto desired sites using gelatin gel-coated plunger. Furthermore, the cell sheet layering technique is useful for designing 3D orientation in myotube assembly. This new cell sheet-based technology provides new potential for constructing complex tissues that composed of natively oriented cell assembly, particularly for skeletal muscle tissue.

References: [1] Takahashi H. et al. Biomacromolecules. 2010; 11: 1991-1999. [2] Takahashi H. Biomaterials. 2011; 32: 8830-8838.