The Use of Anisotropic Cell Sheets for Controlling Orientation during the Self-organization of 3D Tissue Construct

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Statement of Purpose: An appropriate anisotropy needs to be designed for constructing 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.

A tissue-like cellular monolayer "cell sheet" can be harvested intact with associated extracellular matrix (ECM) using thermoresponsive polymer grafted cell culture substrates by reducing culture temperature. Since the intact ECM layer functions as a natural glue to bond a cell sheet to another one, multiple cell sheets can be layered simply using gelatin gel-coated plunger. Therefore, the cell sheet-based tissue engineering are uniquely applicable to produce 3D cell-dense tissue construct.

In this study, to mimic cell orientation in muscle tissue, anisotropic cell sheets composed of well-aligned myoblasts have been fabricated using a stripe-like micropatterned thermoresponsive surface. Utilizing cell sheet layering technique, the use of anisotropic myoblast sheets has potential for organizing 3D cell orientation in tissue constructs.

Methods: The original procedures for the preparation of micropatterned thermoresponsive surfaces have been reported previously [1]. Briefly, thermoresponsive polymer poly(*N*-isopropylacrylamide) (PIPAAm) was 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 through photolithographic process on the PIPAAm grafted 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 thermoresponsive surface. After reached confluence, the cells were harvested as a single cell sheet by lowering culture temperature to 20 °C. This myoblast sheet was also manipulated using a gelatin gel-coated plunger. After allowing the gelatin gel to attach to the cell sheet, the myoblast sheet was transferred onto another cell sheet. Moreover, for initiating myotube formation, transferred myoblast sheets were cultured in differentiation media (2% horse serum).

Results: Myoblasts were aligned on the patterned surfaces by one-pot cell seeding. The cells recognized the difference in cell affinity between the two different regions, and consequently spread in the same direction as the stripe patterns. After cultured in differentiation media, aligned myoblasts differentiated efficiently into myotubes, and they also remained well-aligned [2].

Unlike myoblasts aligned on conventionally micro-fabricated substrates, the anisotropic myoblast sheet can be manipulated using a gelatin gel-coated plunger after the thermally-induced detachment. Interestingly, when layered with an anisotropic cell sheet (Aligned sheet), randomly oriented myoblasts (Random sheet) changed their orientation, and were finally aligned with the layered Aligned sheet. This self-organization behavior was also observed in multilayered myoblast sheets. When an Aligned sheet was layered on top of two Random sheets, randomly oriented cells self-organized their orientation to align with the top sheet (Fig. 1a). The mechanism has not yet been elucidated; however, it is possible that several biological factors influence with each other complicatedly for orientated structure formation, as in the development of muscle tissue.

The self-organization behavior was useful to produce a well-aligned myotube construct composed of multiple cell sheets. As described above, the use of one Aligned sheet induced to form aligned myoblast orientation in triple-layered cell sheets. After differentiation into myotube, the myotube construct has a single myotube orientation (Fig. 1b). That is, only one Aligned sheet was essential to produce an aligned myotube construct from multiple cell sheets. Therefore, adjusting the alignment of multiple cell layers is unnecessary to produce thick tissue constructs with a single cell orientation.

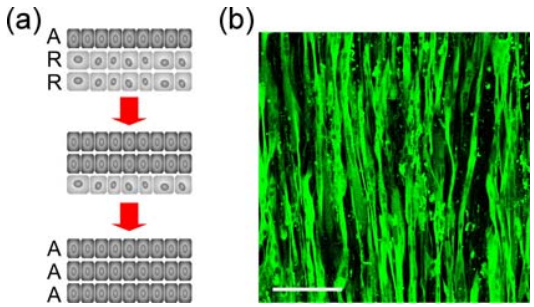

Figure 1. Formation of aligned myotube construct through the self-organization process in multilayered cell sheets. (a) Myoblasts of two Random sheets (R) change their orientation and consequently all myoblasts were aligned with the layered Aligned sheet (A). After the self-organization, this construct was cultured in differentiation media, and then myosin heavy chain-positive myotubes were stained (green). Scale bar: 200 um.

Conclusions: Since all myoblasts were aligned with the top Aligned sheet, 3D anisotropy in tissue constructs can be flexibly designed by simply layering multiple cell sheets. This new cell sheet-based technology provides potential for constructing complex tissues composed of natively-oriented cell assemblies, particularly for skeletal muscle tissue.

References: [1] Takahashi H. et al. *Biomacromolecules*. 2010; 11: 1991-1999. [2] Takahashi H. *Biomaterials*. 2013; 34: 7372-7380.