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The use of anisotropic cell sheets to control orientation during the self-organization of 3D muscle tissue☆

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ABSTRACT

In some parts of native tissues, the orientation of cells and/or extracellular matrixes is well organized. We know that because anisotropy produces important tissue functions, an appropriate anisotropy needs to be designed to biomimetically construct complex tissue. Here, we show the unique features of anisotropic myoblast sheets for organizing the three-dimensional (3D) orientation of myoblasts and myotubes. Utilizing a micropatterned thermoresponsive surface, human skeletal muscle myoblasts were aligned on the surface, and manipulated as a single anisotropic cell sheet by reducing the culture temperature. Consequently, layering of anisotropic myoblast sheets using gelatin gel allowed 3D myotube constructs to be built up with the desired anisotropy. We also discovered a surprising myoblast behavior. An anisotropic cell sheet placed on top of other cell sheets in fabricating thick tissue was able to change the cell orientation in several layered cell sheets underneath. This self-organization is believed to provide the uniqueness required in designing 3D cell orientation architecture for reconstructed muscle tissue.

1. Introduction

Some parts of native tissues have the well-organized orientations of cells and/or extracellular matrixes (ECM) [1–3]. The well-aligned orientation of muscle tissue is a known key factor for producing mechanical functions in native skeletal muscle which has a highly organized structure consisting of parallel bundles of muscle fibers. Early in the development of mature skeletal muscle, myoblasts, precursor muscle cells fuse to form myotubes, and the newly formed myotubes orient themselves parallel to each other before finally maturing to muscle fibers. As a result, regulating myoblast alignment has been an important step to construct biomimetic muscle tissue. To date, numerous microfabricated materials have been developed for regulating myoblast alignment [4–8]. In the tissue engineering field, however, it has not been successful because the aligned cells are basically impossible to separate from these microfabricated surfaces. This indissociable relationship has hampered the design of three-dimensional (3D) oriented myoblast and myotube structures.

Tissue-like cellular monolayers, called "cell sheets", have been developed to establish a new class of tissue reconstruction technology. A thermoresponsive polymer, poly(*N*-isopropylacrylamide) (PIPAAm), grafted on a cell culture substrate allows confluent cells to be harvested intact as a single cell sheet by reducing the culture temperature below the PIPAAm's lower critical solution temperature (LCST) of 32 °C [9,10]. Since a cell sheet can be harvested with its associated ECM intact, it can be transplanted onto damaged tissues without any additional treatments such suturing [11,12]. Based on this technology, human epithelial cell sheets have already been applied in human clinical studies (e.g., cornea reconstruction), and myoblast sheets have been implanted to treat severe heart failure [13,14]. Moreover, this cell sheet-based tissue engineering allows us to create scaffold-free 3D tissues by layering multiple cell sheets [15,16]. Layered cell sheets stratify tightly because of the preserved ECM on individual cell sheets, and can communicate with each other both physically and biologically [17,18]. In this study, myoblast sheets with well-aligned orientation were fabricated to create 3D oriented myoblast and myotube constructs.

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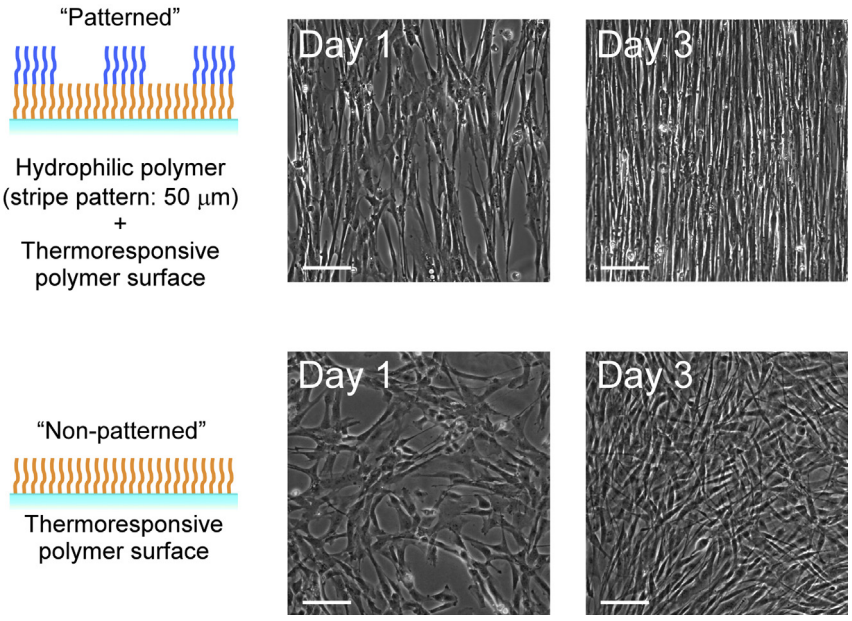

Fig. 1. Alignment of human skeletal muscle myoblasts on patterned and non-patterned thermoresponsive cell culture substrates. In schematic illustrations, hydrophilic PAcMo segments and thermoresponsive PIPAAm brushes are shown as the blue and orange brushes, respectively. Microscopic images show adhesion of myoblasts at Day 1 and Day 3 after cell seeding onto thermoresponsive surfaces. Scale bar: 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Preparation of micropatterned thermoresponsive surfaces

The original procedures for the preparation of micropatterned thermoresponsive surfaces have been reported previously [19]. Briefly, PIPAAm brushes were fabricated on glass substrates by a surface-initiated reversible addition-fragmentation chain transfer radical (RAFT) polymerization process [20], and then positive photoresist (OFPR-800 LB, 34 cp) (Tokyo Ohka Kogyo, Kanagawa, Japan) was spin-coated at 8000 rpm for 30 s by a spin coater ACT-300D (ACTIVE, Saitama, Japan) onto the PIPAAm brush surfaces. After being irradiated by UV light with a photomask (stripe width: 50 μm/50 μm), the photoresist at the irradiated areas was removed

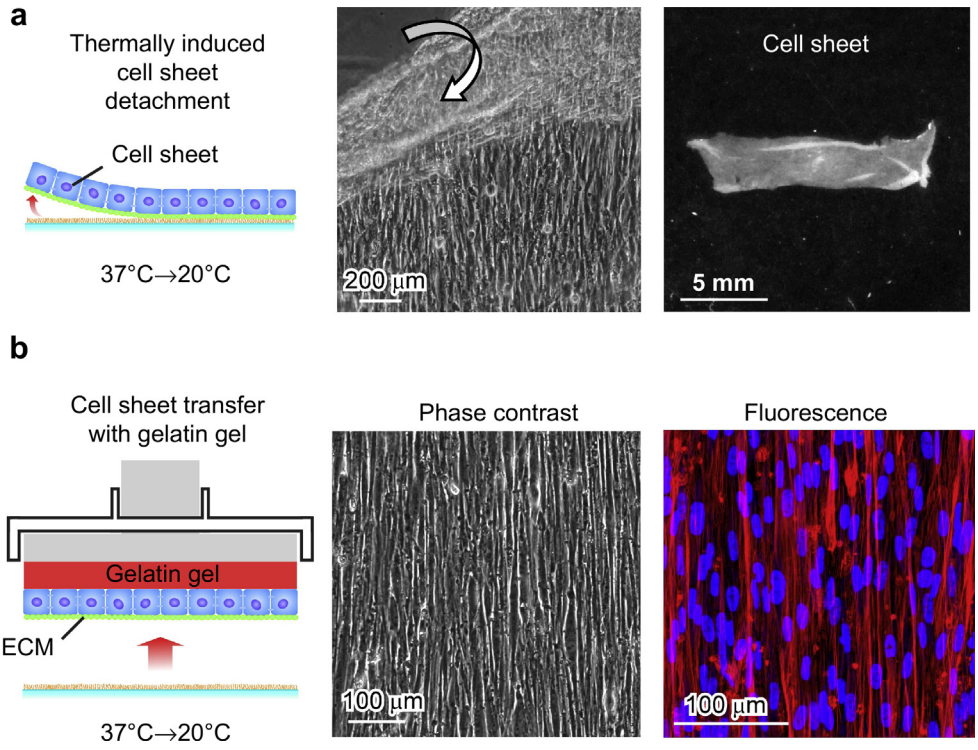

Fig. 2. Thermally-induced detachment and transfer of an anisotropic myoblast sheet. (a) The aligned myoblasts were harvested as an anisotropic cell sheet simply by reducing culture temperature (20 °C). In a photograph, the cell sheet shows a unique shrinking that originated from the cell alignment (the original size of adherent cell sheet: 20 × 20 mm). (b) A myoblast sheet attached to gelatin gel was harvested from the surface and transferred onto a normal TCPS dish. Microscopic images show that the transferred cell sheet maintains its anisotropic orientation even after 3 weeks post transfer. A fluorescence image shows that actin fibers (red) and nuclei (blue) are also well-aligned. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)