The influences of nano-groove/ridge surfaces on the alignment and differentiation of skeletal myoblasts

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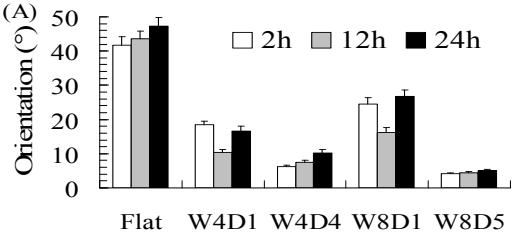
Introduction: Skeletal muscle is formed of highly ordered fibers of differentiated muscle cells. Alignment of myoblasts is an important step in musculoskeletal tissue engineering. The ability of myoblasts to form parallel and straight myotubes *in vitro* would correlate with the vertical muscle contraction. Cells are known to respond to topographic cues. It is previously shown that myoblasts aligned along topographically micro-patterned substrates [1]. In this study, we investigated the influence of nano-groove/ridge topography on alignment and differentiation of adult mouse myoblast cell-line, C2C12.

Materials and Methods: Nano-patterned polystyrene (PS) surfaces were fabrication by solvent casting method on a PDMS master, which was replicated from silicon wafer. Nano-patterns with different groove/ridge widths (400 and 800 nm) and with different depths (100, 400 and 500 nm) were used and labeled as W4D1, W4D4, W8D1, and W8D5. After 2 days of incubation, C2C12 cells were cultured in the differentiated medium. Cell alignment was determined by orientation angle. Elongation = the major axis/the minor axis of cells. Actin filaments and myosin heavy chain (MHC) was stained by phalloidine-TRITC and antibodies, and observed under a confocal microscopy.

Results: After 2 hr of culture, C2C12 cells alignment and elongation was enhanced by the nano-grooved surfaces (Fig. 1). Cell alignment was enhanced by increasing groove depth apparently (Fig. 1A). Cells elongated more on the patterned surfaces than on the flat surfaces. Cell area was larger on the flat control than nano-grooved surface (Fig. 1C). Cell proliferation rate was higher on flat control than nano-grooved surface (Fig. 1D). After myogenesis for 5 days, myogenic index of the patterned surface was higher than flat surface in average. After 8 days, the width of myotubes was higher on the flat control than grooved surface (Fig. 2A). The myogenic index was increased on W8 and flat surface significantly. Noticeable, myogenic index of W8D5 was highest (Fig. 2B). Fluorescent images revealed that F-actin as well as MHC aligned with the pattern direction (Fig. 3). Cell nuclei were also aligned with the directions of patterns.

Conclusions: C2C12 cells were aligned on the nano-groove/ridge patterns. The alignment and elongation of C2C12 cells depend on groove depth and width. The nano-groove/ridge patterns provide guidance to design substrates for the development of tissue-engineered skeletal muscle.



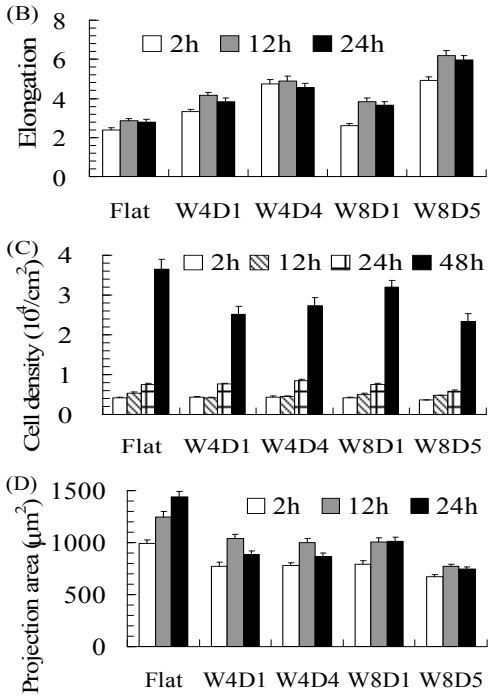


Figure 1. Orientation angles (A), elongation (B), cell projection area (C), and cell proliferation (D) of C2C12 at different substrates. (n = 200).

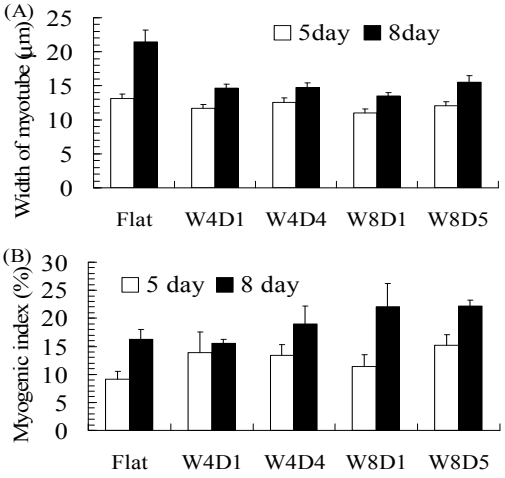


Figure 2. Width of myotubes (A) and myogenic index of myotubes (B). n = 100

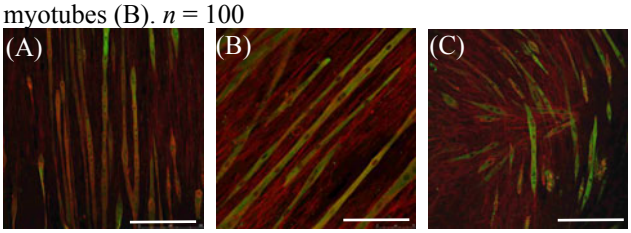


Figure 3. Fluorescence images of myotubes on W4D4 (A), W8D5 (B), and flat surface (C) after 8 days culture. Scale bar = 250 μm.

References: [1] Lam et al. (2006) *Biomaterials*, 27:4340.