Grooved PLGA Films Incorporated with RGD/YIGSR Peptides for Potential Application on Skeletal Muscle Tissue Engineering

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Statement of Purpose: Skeletal muscle is composed of highly-aligned bundles of parallel multinucleated myotubes, formed by fusion of differentiated mononuclear myoblasts during early development. Grooved surface topography has been studied widely for cell alignment, but rarely regarding in skeletal muscle tissue engineering. In this study, PLGA films with submicron grooves on the surface and blending with either poly-L-lysine grafted RGD- or YIGSR-containing peptide was fabricated. The myogenesis of C2C12 skeletal myoblasts was further investigated.

Methods: A silicon wafer containing grooved structure (800 nm in width of ridges and grooves and 700 nm in depth) was fabricated by electron beam lithography and dry etching. The patterned silicon wafer was employed as a master to fabricate PDMS molds, which were later used to imprint grooved PLGA films (Fig.1A). Flat PLGA films, used as a control substrate, were fabricated by the same procedure from flat silicon. 10% (w/v) PLGA/HFP or PLGA/PLL-g-peptide/HFP solution (PLGA:PLL-peptide = 10:1 w/w) was dropped onto a glass slide and then imprinted by a PDMS mold. After HFP evaporation, PDMS molds were removed to obtain PLGA films on the glass slide. The films were treated with 0.1 N NaOH for 10 min in order to expose the peptides. PLGA films blended with PLL-RGD and PLL-YIGSR were abbrivated to PLGA/RGD and PLGA/YIGSR, respectively.

Results: We found that base treatment of the membrane using NaOH increased surface concentration of peptides (approximately 8-fold) compared with untreated membrane. Cell proliferation on PLGA/peptide membrane was enhanced (1.5-fold for RGD and 1.2-fold for YIGSR) compared with PLGA membrane. Cell morphology was guided the underlying grooves, e.g. cells aligned with the direction of grooves, while cell proliferation and differentiation were improved by the peptide presentation (myogenic index was further enhanced approximately 10%-16% on peptide-presented membrane compared with PLGA membrane) (Fig.1B-C).. Both RGD- and YIGSR-containing peptides had positive effect on myogenesis of C2C12 skeletal myoblasts, suggesting that improvement of integrin-ligand signalling may benefit myoblast fusion (Fig.1D).

Conclusions: This study elucidates the effects of both surface biochemical cue and topographic feature on the proliferation and differentiation of C2C12 myoblasts on biodegradable polymer films. The presentation of RGD or YIGSR peptides enhances the growth and fusion of C2C12 myoblasts, while the grooved structure guides cell arrangement to form parallel myotubes. The combination of surface topography and peptide presentation shows a great potential in designing scaffolds for skeletal muscle tissue engineering.

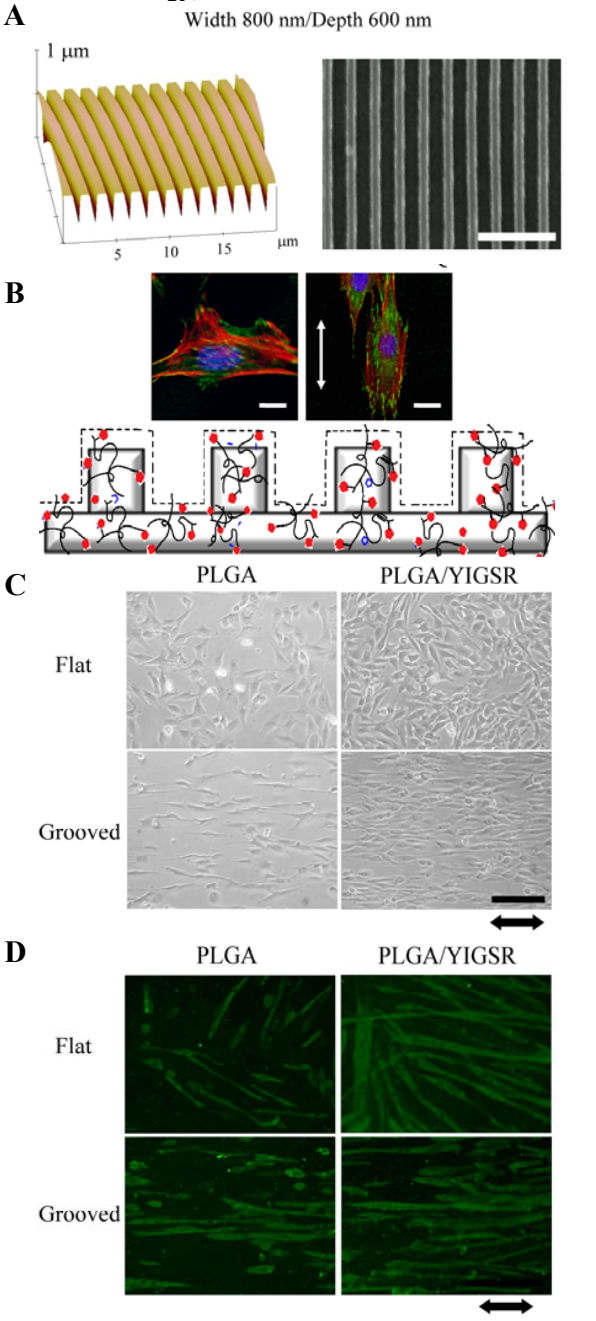

Figure 1. (A) AFM and SEM images of PLGA membrane surface. (B) Vinculin staining of cells on surfaces with peptide presentation. Illustration shows the peptide present and expose on the membrane surface. (C) Cell morphology on the surfaces after 1 day. (D) Myosin Heavy Chain staining of myotubes after cell fusion for 3 days. Bar = 5 μm in A, 25 μm in B, 200 μm in C, and 100 μm in D.