**Biomimetic substrate-dependent myogenic commitment of iPSC-derived cells**

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**Statement of Purpose:** Recent development in stem cell technology has demonstrated iPSCs as a possible cell for cell-based therapy and studies of particular differentiation program. However, spontaneous and heterogeneous differentiation of these cells may limit the potential use. Here, we describe the efficient generation of non-tumorigenic myogenic progenitor cell population from iPSCs and their commitment into myoblast. Furthermore, we demonstrate that iPSCs-derived myogenic progenitor cells can undergo efficient myogenic differentiation by topographical cues and unique surface chemistry present in their environment. We have created substrates from biomimetic materials that can replicate the mico- and nanoscale topography of fully differentiated skeletal myoblast. Using UV-assisted lithography, an optically transparent cellular pattern of fully differentiated myoblasts of a UV curable poly(urethane acrylate) (PUA) resin is fabricated and employed as a cell-culture substrate for myogenic pattern of iPSCs-derived cells. When iPSCs-derived cells were cultured on these biomimetic patterns, the cells followed the underlying myoblast pattern. Furthermore, myogenin staining indicates efficient myogenic commitment on these substrates. These results demonstrate that myogenic potential of iPSCs-derived cells are highly dependent on the micro- and nanoscale topographical cues.

**Methods:** Cell Culture and derivation of myogenic precuorsors from iPSCs**.** iPSCs were culture as reported. For myogenic progenitor cells derivation, iPSCs were plated onto gelatin-coated (0.1% wt/vol) plates and cultured for 10 days with TGF-b inhibitors. The outgrowth cells were selectively isolated and subcultured in myogenic cell growth media (Lonza). In vitro chondrogenic differentiation was induced in RGD-modified PEG-based hydrogel. For adipogenic differentiation, cells were exposed to adipogenic induction medium (1 μM dexamethasone, 200 μM indomethacin, 10 μg/ml insulin, and 0.5 mM methylisobutylxanthine; Cambrex Biosciences) for 3 weeks. For osteogenic differentiation, cells were cultured in osteogenic differentiation medium (50 μM ascorbic acid-2-phosphate, 10 mM β-glycerophosphate, and 100 nM dexamethasone) for 2 weeks. Fabrication of the cell-patterned PUA substrate.

Human skeletal myoblast were plated on tissue culture plate for 3-7 days to reach near-confluence and induced for myotube formation. Cells were fixed with 4% paraformaldehyde and post-fixed with Karnovsky's fixative overnight, incubated in 1% $OsO_4$ in 0.1M cacodylate buffer for 1h. Cells were gradually dehydrated with ethanol, subsequently replaced with HMDS, and dried in desiccator. The fixed cells were used as template to generate poly (urethane acrylate) (PUA) substrate with indented cell impression, and these substrate with myoblast cell-shaped feature was used to culture iPSCs-derived myogenic precursor cells.

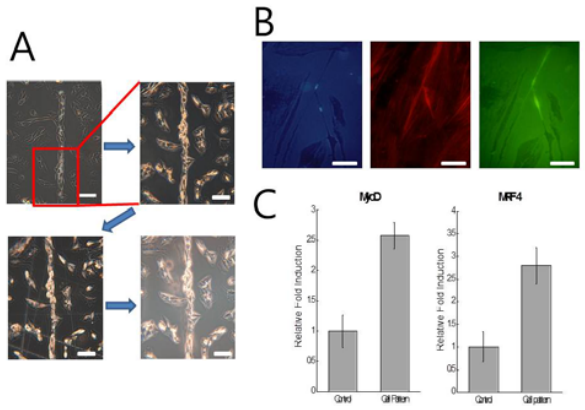


Figure : (A) Well-defined replication of myogenic cells via PUA-based photopolymerizing resin. (B) Myogenic differentiation marked by expression of MHCIIa staining of stem cells on substrate topography replicating myoblast cells. (C) Myogeni gene markers MyoD and MRF4 pression on cell patterned substrate vs. control substrates after 15 days of culture

**Results and Conclusions:** Recent progress in tissue engineering has focused on the possibility of using iPSCs to produce unlimited numbers of specialized cell populations [1]. One requirement for such applications is that differentiated or tissue restricted cells must be isolated from the iPSCs before they can be used safely and effectively in clinical application. The main objective of this work was to demonstrate that iPSCs-derived myogenic precursor cells can undergo efficient myogenic commitment in vitro. This precursor cell population was generated without genetic manipulation or growth factors. In this study, we created new biomimetic substrate that replicated differentiated myoblast morphology at the nanoscale. The major finding of this study was that materials containing the topography with nanoscale features can enhance alignment and modulate cell morphology as compared to control flat substrates. Furthermore, a change in cell morphology due to biomimetic substrate was observed.

**References:** [1] Takahashi K, Yamanaka S, *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors*, Cell 126(4): 663-76, August 2006.