Acta Biomaterialia 8 (2012) 2193–2202

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Alignment of multi-layered muscle cells within three-dimensional hydrogel macrochannels

Stephanie L. Hume [a,1], Sarah M. Hoyt [a], John S. Walker [b], Balaji V. Sridhar [a], John F. Ashley [a], Christopher N. Bowman [a], Stephanie J. Bryant [a,*]

[a] Department of Chemical and Biological Engineering, University of Colorado Boulder, Boulder, CO 80309, USA
[b] Division of Cardiology, Department of Medicine, University of Colorado Denver, Aurora, CO 80045, USA

ARTICLE INFO

Article history:
Received 26 October 2011
Received in revised form 26 January 2012
Accepted 1 February 2012
Available online 8 February 2012

Keywords:
Macrochannels
Muscle
Photolithography
Alignment
Hydrogel

ABSTRACT

This work describes the development and testing of poly(ethylene glycol) (PEG) hydrogels with independently controlled dimensions of wide and deep macrochannels for their ability to promote alignment of skeletal myoblasts and myoblast differentiation. A UV-photopatterned thiol-ene mold was employed to produce long channels, which ranged from ~40 to 200 μm in width and from ~100 to 200 μm in depth, within a PEG–RGD hydrogel. Skeletal myoblasts (C2C12) were successfully cultured multiple cell layers deep within the channels. Decreasing channel width, increasing channel depth and, interestingly, increasing cell layer away from the channel base all contributed to a decreased interquartile range of cell angle relative to the long axis of the channel wall, indicating improved cell alignment. Differentiation of skeletal myoblasts into myotubes was confirmed by gene expression for myoD, myogenin and MCH IIb, and myotube formation for all channel geometries, but was not dependent on channel size. Qualitatively, myotubes were characteristically different, as myotubes were larger and had more nuclei in larger channels. Overall, our findings demonstrate that relatively large features, which do not readily facilitate cell alignment in two dimensions, promote cell alignment when presented in three dimensions, suggesting an important role for three-dimensional spatial cues.

1. Introduction

Skeletal myopathies, such as muscular dystrophy, and traumatic injuries, can lead to muscle loss [1]. Unfortunately, no therapies exist that can regenerate or restore damaged muscle to a fully functioning muscle. One potential solution offering hope is tissue engineering and the use of cell-seeded biomaterial scaffolds to regenerate muscle in vitro. An important design requirement for engineering functional muscle is myoblast alignment. Cell alignment prior to fusion of myoblasts into multinucleated myotubes is a prerequisite to achieving efficient contraction in an engineered skeletal muscle [2]. While significant research has focused on strategies to guide cell alignment in two dimensions, a three-dimensional (3-D) approach is clearly needed to engineer and re-create the dense and highly organized muscle tissue where cells are aligned in a parallel orientation.

For cells to orient in a particular direction in culture, some form of external cue must be presented to the cells. Towards this end, spatial cues like material-induced contact guidance have been used to facilitate cell alignment along a common axis [3]. Several approaches have proven effective and include patterned structures made by soft lithography [4,5], hot embossing [6], photolithography and solvent casting [7] or alternatively, electrospun networks [4,8,9]. These approaches often use small-scale topography on the order of tens of nanometers to a few microns [4–12] and have achieved excellent alignment and elongation of cells in two dimensions. This range of feature sizes represents the dimensions of native grooves in which myoblasts align in vivo during development, just prior to their fusion into myotubes [13]. Aligning of myoblasts in two dimensions in vitro has been shown to lead to improved differentiation evidenced by the formation of long multinucleated cells [5,7,9], orientation of nuclei along a common axis [5], presence of sarcomeric myosin, a key molecule of the contractile unit [6,7,9], and the development of aligned sarcomeres [9].

Contact guidance cues, however, are typically limited to features that are less than ~50 μm, where sizes greater than this often fail to promote cell alignment [6]. Alternatively, cyclic strain [15–19], static strain [20] and/or electrical stimulation [14,21,22] have all been used to achieve cell alignment, most notably in 3-D cultures. While these stimuli have resulted in improved cell alignment, the contractile forces generated by the engineered muscle

* Corresponding author. Tel.: +1 303 735 6714; fax: +1 303 492 4341.
E-mail address: stephanie.bryant@colorado.edu (S.J. Bryant).
[1] Present address: National Institute of Standards and Technology, Boulder, CO, USA.

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2012.02.001