Direct laser machining-induced topographic pattern promotes up-regulation of myogenic markers in human mesenchymal stem cells

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Abstract

The engineering of tissue is preferably done with stem cells, which can be differentiated into the tissue of interest using biochemical or physical cues. While much effort has been focused on using biological factors to regulate stem cell differentiation, recently interest in the contribution of physical factors has increased. In this work, three-dimensional (3-D) microchannels with topographic micropatterns were fabricated by femtosecond laser machining on a biodegradable polymer (poly(ε-lactide-co-e-caprolactone)) substrate. Two substrates with narrow and wide channels respectively were created. Human mesenchymal stem cells (hMSCs) were cultured on the scaffolds for cell proliferation and cellular organization. Gene expression and the immunostaining of myogenic and neurogenic markers were studied. Both scaffolds improved the cell alignment along the channels as compared to the control group. Microfilaments within hMSCs revealed significant up-regulation of several hallmark markers associated with myogenesis for hMSCs cultured on the scaffold with narrow microchannels, while osteogenic and neurogenic markers were down-regulated or remained similar to the control at day 14. Immunostaining of myogenin-specific and neurogenin-specific differentiation markers were used to further confirm the specific differentiation towards a myogenic lineage. This study demonstrates that femtosecond laser machining is a versatile tool for generating controllable 3-D microchannels with topographic features that can be used to induce specific myogenic differentiation of hMSCs in vitro, even in the absence of biological factors.

1. Introduction

Tissue engineering aims to develop biocompatible cell–scaffold constructs that integrate with native tissue in situ to repair or replace damaged tissue [1]. To achieve this aim, it is imperative to use cells which can augment the cellular functions in these cell–scaffold constructs. Stem cells, which are unspecialized precursor cells with the ability to self-renew and differentiate into different cell types, are such cells [2]. Adult human mesenchymal stem cells (hMSCs) isolated from bone marrow have been proven capable of self-renewal and differentiation into a wide variety of lineages in response to proper cues [3–5]. The cues include soluble/immobilized factors, chemical and physical signals from the extracellular matrix [6]. Molecular mediators (e.g. growth factors, transcription factors) capable of regulating stem cell fates are well characterized. However, increasing evidence has shown that non-molecular stimuli like physical environmental factors (e.g. matrix compliance [7] and surface topography [8]) can also control stem cell activities [9], whilst avoiding side effects such as uncontrollable cell growth, tumorigenesis and even cell death.

Topography, particularly controllable nanoscale topography, is one of the most important physical inductions, as increasing evidence has demonstrated that topography has great potential in regulating cell behavior, especially in the presence of biological or chemical induction media [10,11]. Interestingly, some recent studies have revealed that, in the absence of any inductive biological agents, topographical cues alone may direct stem cell differentiation [8,12,13]. Electrospinning and lithography are two commonly used techniques to construct scaffold with controllable topographic features. Dang and Leong [12] have demonstrated that aligned electrospun hydroxybutyl chitosan fibers can up-regulate myogenic genes of Pax-43, Pax-7 and myogenin of hMSCs to indicate myogenic lineage commitment. Similarly, Yim et al. [13] found that nanogratings of PDMS induced neuronal proteins expression of Tuj-1 and MAP2, as confirmed by both microarray