Biomaterials 33 (2012) 128–136

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Modulation of osteogenic differentiation in hMSCs cells by submicron topographically-patterned ridges and grooves

Shinya Watari [a], Kei Hayashi [a], Joshua A. Wood [a], Paul Russell [a], Paul F. Nealey [b], Christopher J. Murphy [a,c], Damian C. Genetos [d,*]

[a] Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California Davis, Davis, CA 95616, USA
[b] Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madision, WI 53706, USA
[c] Department of Ophthalmology and Vision Science, School of Medicine, University of California Davis, Davis, CA 95616, USA
[d] Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, 2112 Tupper Hall, One Shields Avenue, University of California Davis, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 29 July 2011
Accepted 22 September 2011
Available online 7 October 2011

Keywords:
Mesenchymal stem cells
Nanotopographic ridges and grooves
Osteogenic differentiation
Osteoinductive agents
Bone Morphogenetic Protein (BMP)

ABSTRACT

Recent studies have shown that nanoscale and submicron topographic cues modulate a menu of fundamental cell behaviors, and the use of topographic cues is an expanding area of study in tissue engineering. We used topographically-patterned substrates containing anisotropically ordered ridges and grooves to investigate the effects of topographic cues on mesenchymal stem cell morphology, proliferation, and osteogenic differentiation. We found that human mesenchymal stem cells cultured on 1400 or 4000 nm pitches showed greater elongation and alignment relative to 400 nm pitch or planar control. Cells cultured on 400 nm pitch demonstrated significant increases in *RUNX2* and *BGLAP* expression relative to cells cultured on 1400 or 4000 nm pitch or planar control. Four-hundred nanometer pitch enhanced extracellular calcium deposition. Cells cultured in osteoinductive medium revealed combinatory effects of topography and chemical cues on 400 nm pitch as well as up-regulation of expression of *ID1*, a target of the BMP pathway. Our data demonstrate that a specific size scale of topographic cue promotes osteogenic differentiation with or without osteogenic agents. These data demonstrate that the integration of topographic cues may be useful for the fabrication of orthopedic implants.

1. Introduction

In the United States alone, the annual costs of orthopedic-related injuries are estimated to be between $17–20 billion annually [1]. Many of these costs are associated with complications following adverse events such as skeletal fracture due to traumatic injury or idiopathic or pharmacologically induced osteoporosis. The frequency of such events is predicted to increase as the population ages [2]. High morbidity and mortality in conjunction with increasing healthcare costs are associated with such fractures [3], resulting in a critical need to enhance skeletal repair.

Bone marrow-derived MSCs [4], as well as adipose-derived MSCs, demonstrate the capacity for adipogenic, osteogenic, and chondrocytic differentiation [5] in response to a variety of stimuli such as exogenous growth factor (e.g., BMP [6]) and biophysical conditions (i.e., micromass pellet versus monolayer expansion [7,8]). Cell-based therapies involving autologous or allogeneic MSCs have been investigated as options in cases involving orthopedic complications [9]. The success of tissue engineering in skeletal repair is a function of the scaffold, cells, and exogenous signals that are used [10]. Traditionally, osteoinductive agents such as BMP-2 or BMP-7 have been used for promoting stem cells to differentiate along the osteogenic lineage [6], but potential adverse effects and high costs of such agents create a need for novel methods to promote osteogenic differentiation.

Recent findings underscore that submicron and nanoscale topography can control fundamental cell behaviors including proliferation [11,12], migration [11,12], and differentiation [13,14]. It is important to choose an appropriate topographically patterned substrate to achieve desired cell responses because cell responses vary depending on cell type, topographic feature size and geometry, and substrate compliance [11,15–17]. Two types of geometries, namely nanopits and nanotubes, have been previously investigated in terms of osteogenic differentiation. Nanopits of randomly placed 50 nm holes separated by 250 nm (i.e. 300 nm pitch) as well as nanotubes (100 nm diameter) enhance osteogenic differentiation

* Corresponding author.
E-mail address: dgenetos@ucdavis.edu (D.C. Genetos).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.09.058

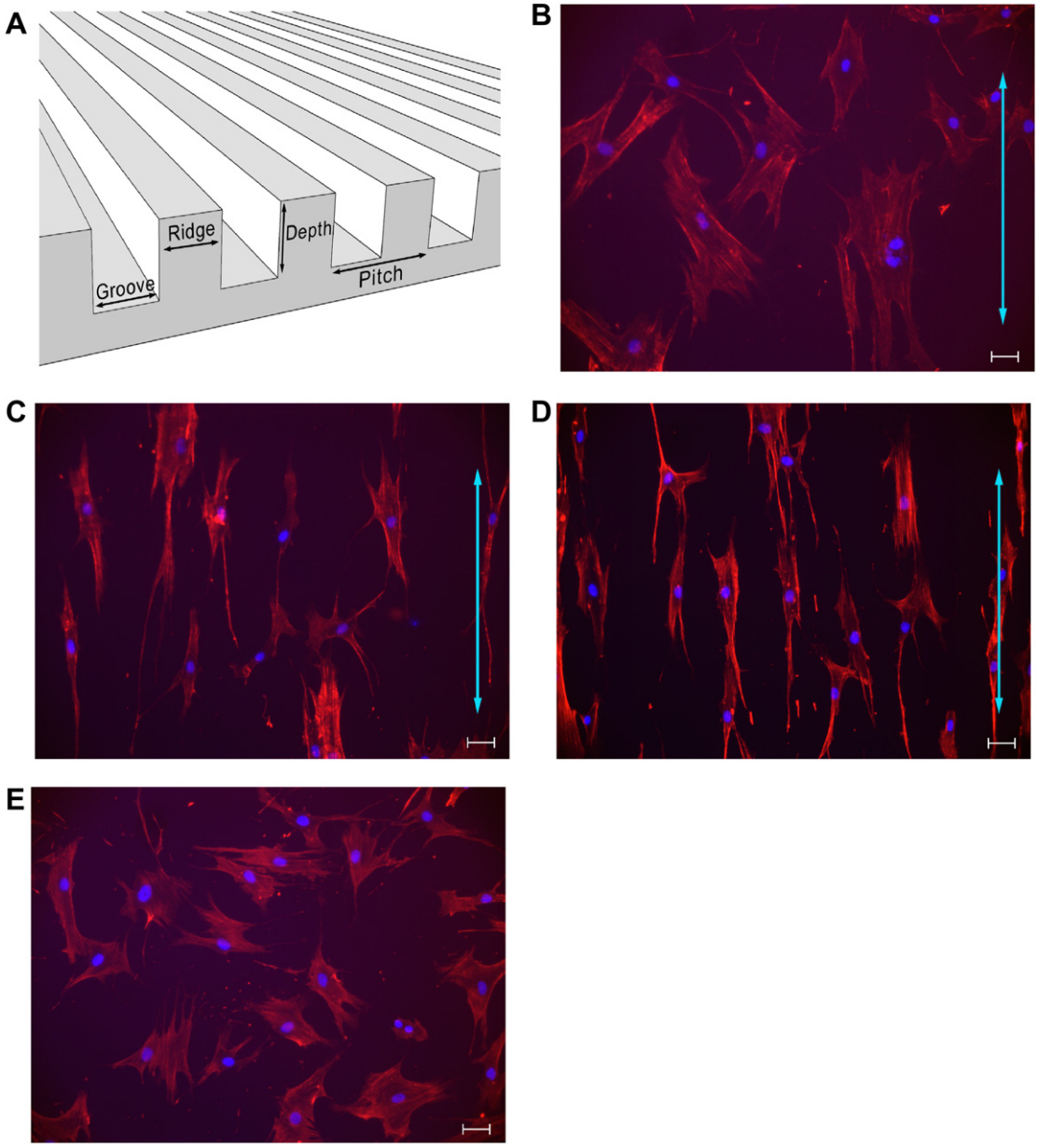

Fig. 1. Influence of topographic surfaces on cell morphology. Pitch is defined as the sum of the width of a ridge and a groove as shown on the schematic diagram of a topographic surface (A). The depth for all the topographic surfaces was 300 nm. Representative images of hMSCs were taken with a Zeiss Axiovert 200 M inverted microscope on 400 nm pitch (B), 1400 nm pitch (C), 4000 nm pitch (D) and planar control (E). hMSCs on 400 nm and planar surfaces showed a more rounded and flattened morphology while hMSCs on 1400 and 4000 nm pitch showed an elongated morphology. Each picture was taken at 10× magnification. Double-headed arrows represent direction of the underlying topography of ridges and grooves. Scale bar: 50 μm.

of hMSCs [18,19]. However, responses to other commonly used geometries [20], including submicron and nanoscale ridges and grooves [16], remain unknown. In addition, the previous investigations of effective feature size were performed on surfaces with feature sizes smaller than 300 nm [18,19,21], and further analysis of additional biologically relevant feature sizes is needed to determine the optimal length scale of topography to promote osteogenic differentiation. Therefore, we focused on surfaces with feature sizes in the range of nanomicron to submicron, as they are biologically relevant but not extensively investigated.

Our hypothesis is that topographic cues in the form of anisotropically ordered ridges and grooves, mimicking biophysical cues provided by collagen fibers, will enhance osteogenic differentiation of MSCs. To optimize the osteogenic differentiation, the effects of feature scale employing dimension values that span the biologically relevant size range of the collagen fibrils (10–300 nm) and fibers (up to several microns) [22] were investigated. Topographic features of ridges and grooves ranging from 200 to 2000 nm (400–4000 nm in pitch) were used to identify the ideal length scale for promoting osteogenic differentiation [11,23]. Osteoinductive effectors were also employed to determine if there was a synergistic or additive effect between soluble signaling molecules and topographic cues.

2. Materials and methods

2.1. Fabrication of micro- and nanoscale surfaces

Patterned silicon surfaces utilized as master stamps were prepared at the Center for Nanotechnology (University of Wisconsin) as previously described [24,25]. The grooves on each substrate were 300 nm in depth and 400, 1400, or 4000 nm pitch.