Acta Biomaterialia 7 (2011) 683–690

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Hydrophobic nanopillars initiate mesenchymal stem cell aggregation and osteo-differentiation

Karla S. Brammer [a], Chulmin Choi [a], Christine J. Frandsen [a], Seunghan Oh [b], Sungho Jin [a,*]

[a] Materials Science & Engineering, University of California, San Diego, La Jolla, CA, USA
[b] Department of Dental Biomaterials, College of Dentistry, Wonkwang University, Iksan, South Korea

ARTICLE INFO

Article history:
Received 7 June 2010
Received in revised form 9 September 2010
Accepted 16 September 2010
Available online 21 September 2010

Keywords:
Hydrophobic
Gold
Nanopillar
Mesenchymal stem cell
Osteo-differentiation

ABSTRACT

Surface engineering approaches that alter the physical topography of a substrate could be used as an effective tool and as an alternative to biochemical means of directing stem cell interactions and their subsequent differentiation. In this paper we compare hydrophobic micro- vs. nanopillar type fabrication techniques for probing mesenchymal stem cell (MSC) interaction with the surface physical environment. The roles played by the topography of the nanopillar in particular influenced MSC growth and allowed for regulatory control of the stem cell fate. The nanopillar induced large 3-D cell aggregates to form on the surface which had up-regulated osteogenic specific matrix components. The ability to control MSC differentiation, using only the topographical factors, has a profound effect on both MSC biology and tissue engineering. This study aims to highlight the importance of the physical material carrier in stem cell based tissue engineering schemes.

1. Introduction

Influencing cell behavior, from proliferation to differentiation, using the material design of the substrate or implant topography, is a desirable approach for many regenerative medicine applications [1]. The role of a surface provides physical, geometrical, mechanical, and structural signals which act together as an intelligent entity for guiding cellular responses. By designing surface features on the micro- and nanoscale the topography becomes in the size regime comparable to natural extracellular matrix (ECM) structures, and thus has potential to provide functional ECM cues as well. New fabrication technologies and new nanotechnologies have provided biomaterial scientists with enormous possibilities when designing customized tissue culture substrates and scaffolds with controlled micro- and nanoscale topography [2]. Micro- and nanoscale fabrication techniques are particularly suitable for probing stem cell interactions with their microenvironment because they allow for levels of precision compatible with the delicate regulatory control necessary to determine stem cell fates [3].

It is well known that mesenchymal stem cells (MSCs) are multipotent cells that have the capacity to differentiation into stromal lineages such as adipocyte, chondrocyte, and osteoblast cell types by generating the appropriate intermediate progenitors for each. Although much as been studied on the effects of biological factors, such as growth factors, hormones, biochemicals, and vitamins, in the induced differentiation of mesenchymal stem cells (MSCs), the roles of the physical environment in the differentiation process are just starting to emerge. Analyses of biochemical characteristics of MSC differentiation do not necessarily translate to in vivo applicability and interactions with complementary or competing signaling pathways active under the physical physiologic conditions may be overlooked [4]. Therefore, by examining the cellular behavior of MSCs cultured in vitro on various micro- and nanotopographies, it can provide some indication and understanding of the effects that the physical environment may have on the stem cell's response, and possibly how to regulate control of stem cell fates. A key principle in stem cell engineering is to reduce the use of potentially toxic, unpredictable biochemical inducing reagents or uncontainable soluble factors by stimulating differentiation locally by use of the scaffold materials instead [5].

Various surface micro- and nanofabrication techniques have been explored for creating topographic surface structures and chemical patterns that have been used to create different effects in cells, including cell adhesion [6–9], orientation, morphology, cytoskeletal arrangements, proliferation, gene expression, and most recently differentiation [10–22]. In fact, topography has been proven to have significant and favorable effects on stem cell commitment into mature lineages (for a review see Ref. [2]). Most studies involve grooved, pore, or short (<100 nm tall) pillar structures, but in this study we have investigated micro- and nanoscale diameter pillars with a height of ~2.5 μm. In addition, we have chosen to investigate a hydrophobic metallic surface, namely gold (Au) coated silicon substrates, seemingly different from the polymeric

* Corresponding author. Tel.: +1 858 534 4903; fax: +1 858 534 5698.
E-mail address: jin@ucsd.edu (S. Jin).

1742-7061/$ - see front matter © 2010 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2010.09.022

[16,23–27], hydrophilic type surfaces investigated in the realm of MSC differentiation. In order to shed light on the possibility of controlled differentiation on tall (>2.0 μm) high aspect pillars and hydrophobic, biologically inert Au chemically coated Si surfaces, this study was conducted to evaluate this type of topography as a differentiation tool for biotech applications utilizing convenient micro/nanofabricated silicon as a base substrate. We hope to aid in the understanding of surface feature effects on differentiation. Biomedical devices and materials have defined features, thus introducing new research on different types of surface features would be helpful for design rationale.

Adipogenic, chondrogenic, and osteogenic differentiation paths were examined on experimental surfaces with different physical and topological features, keeping the chemistry constant. We have compared flat vs. micro- vs. nanopillar configurations and found that pillars with nanoscale diameters have greater impacts on MSC growth and lineage specific, osteogenic (in our case) differentiation. Our aim was to explore the effects of not only the size of the topographic features, but also the hydrophobic physical nature of the Au surface on MSC culture.

2. Materials and methods

2.1. Culture substrate fabrication

2.1.1. Micropillar array fabrication

A typical photo-lithography process for fabrication of the Si microtemplates is schematically illustrated in Fig. 1. The microtemplates were fabricated on (1 0 0) silicon wafers patterned with photo-lithography and a cryo reactive-ion-etch (RIE) process with a metal mask to achieve the desired fine-scale geometries as described below. A positive photoresist was spin coated on the silicon wafers (see Fig. 1a). Then it was exposed to UV to create a pattern of holes, which was performed by a Karl Suss MA6 Mask Aligner system (Fig. 1b). After exposure, the resist was developed (Fig. 1c). Ni thin metal layer (~50 nm thickness) is sputter-deposited on the whole surface of Si (Fig. 1d). Only the Ni layer in the "holes" has direct contact with Si substrate remaining on the surface after a lift-off process (Fig. 1e). A metal mask of 50 nm Ni was used to transfer this fine lithographic definition by cryo RIE steps (Fig. 1e). After RIE process, the Ni layer was etched with Ni etchant (Fig. 1f). Micropillars with ~2 μm diameter and 2.5 μm height were fabricated by photo lithography and RIE (Fig. 1g). Finally, Ti 2 nm/Au 20 nm were tilted sputter-deposited on the micropillars (Fig. 1h). A thin titanium pre-coating was used as an adhesion layer between the gold layer and silicon substrate. The morphology of the Au coated micropillar arrays were visualized using an FEI field emission scanning electron microscope (SEM).

2.1.2. Nanopillar array fabrication

First, silicon nanopillar arrays were prepared using the electroless chemical etching method [28–30]. Briefly, the samples were immersed in a solution composed of 4.6 M HF/0.02 M $AgNO_3$ for 20 min. The etching procedure was performed at 50 °C. After the etching process, the samples were immersed in H_2SO_4/H_2O (1:1) to remove the silver dendrites formed during the etching process. The samples were washed several times with distilled water and ethanol. During these steps, the samples were kept in the solution. The regular drying process for the wet etched nanopillar samples was performed using nitrogen gas environment. For the purposes of this study, a thin layer of gold (Au) was tilt sputtered on the surface the in the same manner as the micropillars. The morphology of the Au coated nanopillar arrays was visualized using an FEI field emission scanning electron microscope.

A flat Si wafer surface with the same Au coating was used as a control surface without topographic features for the cell assays. All experimental samples (flat, micro-, and nanopillar) were cut into identical size pieces (0.5 × 0.5 in.^{2}) and autoclaved before use.

2.2. Contact angle measurement

The measurement of contact angle for the experimental surfaces was carried out by a video contact angle measurement system Model No. VSA 2500 XE (by AST Products, Inc.).

2.3. Cell culture

For this study, rat marrow stromal cells, or mesenchymal stem cells (MSCs), isolated from rat bone marrow (Cell Applications, Inc.) were used. The growth media consisted of alpha minimum

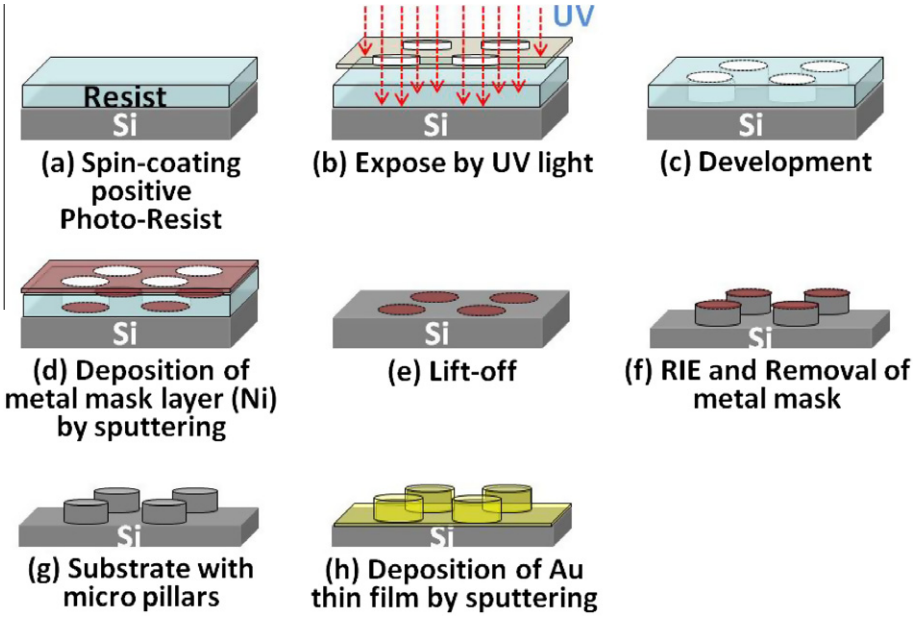

Fig. 1. Schematic illustration of a typical photo-lithography process for fabrication of the micropillar surfaces used as one of the MSC experimental culture substrates.

ID	Title	Pages
976	Hydrophobic nanopillars initiate mesenchymal stem cell aggregation and osteo-differentiation	8