Biomaterials 31 (2010) 2470–2476

Contents lists available at ScienceDirect

Biomaterials

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

The regulation of stem cell differentiation by cell-cell contact on micropatterned material surfaces

Jian Tang, Rong Peng, Jiandong Ding*

Key Laboratory of Molecular Engineering of Polymers of Ministry of Education, Department of Macromolecular Science, Advanced Materials Laboratory, Fudan University, Shanghai 200433, China

ARTICLE INFO

Article history:
Received 24 November 2009
Accepted 1 December 2009
Available online 22 December 2009

Keywords:
Micropattern
Mesenchymal stem cell
Cell-cell contact
Differentiation
Hydrogel

ABSTRACT

Using the material technique recently developed by us, we prepared a micropattern on poly(ethylene glycol) (PEG) hydrogel to keep background resistant to cell adhesion for a long time, which made examination of differentiation of localized stem cells available. Our micropattern designed in this paper prevented or ensured contact between cells adhering in arginine-glycine-aspartic acid (RGD) microdomains, and thus afforded a unique way to study the effects of cell-cell contact on the lineage differentiation of stem cells while ruling out the interference of soluble factors or cell seeding concentration etc. As demonstration, mesenchymal stem cells derived from rats were examined in this study, and both osteogenic and adipogenic differentiations were found to be regulated by cell-cell contact. Isolated cells exhibited less significant differentiation than paired or aggregated cells. For those stem cells in contact, the extent of differentiation was fairly linearly related to the extent of contact characterized by coordination number. Additionally, we revealed the existence of some unknown cues besides gap junction responsible for such effects of cell-cell contact.

1. Introduction

A very important fundamental research of biomaterial is studies of cell-material interaction and cell-cell interaction. This paper strengthens that cell-cell interaction could be well controlled by cell-material interaction by a careful design of substrate surface. As cell studies are concerned, while differentiation of stem cells has been paid much attention upon during the latest decade [1–11], relatively little is focused upon the relationship between cell differentiation and communication. Straightforward cell-cell contact is the most popular way of cell-cell interaction. It is, however, very hard to rule out the effects of soluble factors due to paracrine of cells and the effects of different concentrations of nutrients during cell culture, if one just examines differentiation of stem cells under a series of seeding concentration. An unambiguous research approach is thus desired.

Based upon a material design, this paper put forward a unique methodology to examine the effects of cell-cell contact between stem cells per se on their lineage differentiation, as schematically presented in Fig. 1a. We employ a micro-patterning technique to localize cells before and during cell differentiation. The prototype of micropatterns is generated via photolithography, which is a necessary procedure in microelectronic industry. Surface patterning has been used to control "cell geometry" [12], by which a single cell could be constrained in a microisland of an appropriate size. A key technique in such cell-patterning is the generation of a cell-adhesion-resistant background, most popularly by introduction of a self-assembly monolayer (SAM) of poly(ethylene glycol) (PEG) molecules [12,13]. However, the PEG monolayer gradually falls off from the substrate and thus narrows the period of cell culture. Considering that differentiation of stem cells must proceed for at least several days, the availability of the methodology as shown in Fig. 1a is also dependent upon a better adhesion-resistant background. To this end, we select a PEG hydrogel as substrate. A transfer strategy schematically shown in Fig. 1b is employed to generate Au microislands on PEG hydrogel substrate. Before culture cells, a thiol-ended linker containing oligopeptide of arginine, glycine and aspartic acid (RGD), a sequence existing in some extracellular matrix proteins, is further bound to gold via S-Au bonds, and the Au microisland is then converted into a RGD microisland. Because all of microdomains undergo the same conditional culture medium and those microdomains with different numbers of microislands are spatially very close to each other, the influences of soluble factors are ruled out. Despite of a small distance, cells on one microdomain cannot migrate to another one due to very strong and persistent cell-adhesion

* Corresponding author. Tel.: +86 21 65643506; fax: +86 21 65640293.
E-mail address: jdding1@fudan.edu.cn (J. Ding).

0142-9612/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2009.12.006

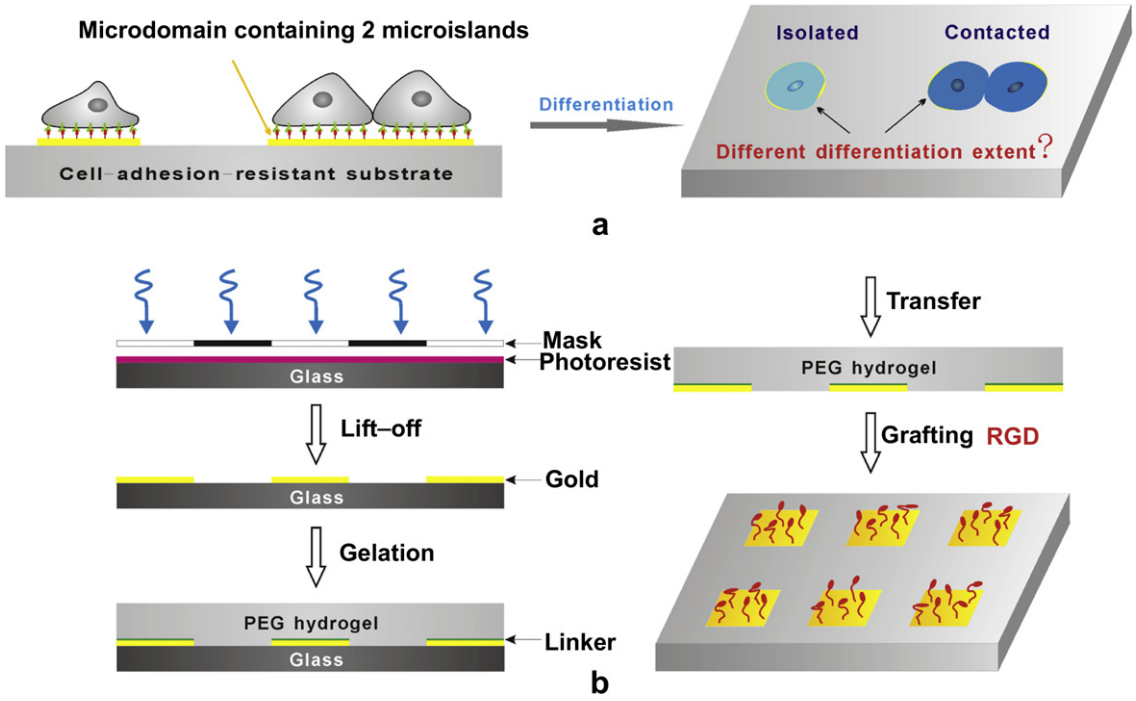

Fig. 1. (a) Schematic presentation of the methodology to explore the effects of cell-cell contact on differentiation of stem cells based upon designing of an appropriate micropattern. Each adhesive microdomain is composed of a pre-defined number of adhesive microislands on a cell-adhesion-resistant PEG background. A microisland here is a very thin gold sheet coated with RGD. Then the difference of the lineage differentiation extent of isolated and contacted cells can be examined simultaneously. (b) Schematic presentation of the transfer strategy to prepare adhesive microdomains on a PEG hydrogel. Briefly, a gold micropattern on glass is fabricated by lift-off photolithography, then a PEG hydrogel is formed (a hetero-bifunctional linker with a thiol end group and an acryloyl end group reacted with gold first). Last, glass is removed to complete the transfer, resulting in a micropatterned PEG hydrogel. Before cell culture, a thiol-ended RGD agent is added, and a RGD micropattern on a PEG hydrogel is eventually obtained.

resistance of PEG hydrogel. The cells are hence well localized on the microdomains, which prevent or ensure cell-cell contact persistently and makes the later spontaneous examination of differentiation of isolated or contacted stem cells available.

While cell studies based upon patterned surface have been reported in the literatures [14–26], the present study provides a semi-quantitative investigation into the relationship between the extent of stem cell differentiation and the extent of cell-cell contact. Bone marrow stromal cells (BMSCs) from neonatal Sprague Dawley (SD) rats are employed as demonstration. Their osteogenic and adipogenic differentiations are examined in this paper.

2. Materials and methods

2.1. Micropattern preparation

The fabrication process includes mainly two steps: preparation of gold microislands on glass slips via a lift-off photolithography, and transfer of these patterns onto a PEG hydrogel [27]. In lift-off photolithography, we first cleaned glass slides in piranha solution [H_2SO_4/H_2O_2, 3:1(v/v)] for half an hour, sonicated in deionized (DI) water, and baked at 100 °C for half an hour. (Caution: The piranha solution reacts violently with organic materials and must be extremely careful.) Second, the cleaned glass slides were spin-coated with a layer of positive photoresist (Ruihong, Suzhou, China), baked, then exposed to ultraviolet light through a chrome mask, and developed. Last, a layer of gold was sputtered onto these slides with the resulting thickness estimated as about 13 nm, and the photoresist was then removed.

In the transfer procedure, we first grafted allyl mercaptan (Fluka) to the gold microislands in vacuum. Poly(ethylene glycol) diacrylate (PEGDA) (M_n 700, Sigma) mixed with photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone (D2959, Sigma) was casted onto the micropatterned glass and photocrosslinked under UV irradiation. Micropatterned PEG hydrogel was finally obtained after mechanically separating the hydrogel covalently bound with gold micropatterns from the glass slides.

2.2. Cell isolation and culture

Bone marrow stromal cells or bone mesenchymal stem cells (BMSCs) were isolated from neonatal SD rats. Briefly, the rats were sacrificed by cervical dislocation, and femurs and tibias were removed. The marrow was flushed out, centrifuged, and resuspended in low-glucose Dubecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco). Cells were cultured in humidified incubator at 37 °C with a 5% CO_2 atmosphere. After 3 days, nonadherent cells were removed and fresh media were added. The cells were passaged upon almost confluence. Only early-passage BMSCs were used for later experimental studies.

2.3. Differentiation on micropatterns

The substrate was soaked in DI water, and then a layer of cyclo(RGDfK)-thiol (R: arginine, G: glycine, D: aspartic acid, f: D-phenylalanine, and K: lysine) (25 μM, Peptides International, USA) were grafted onto the pre-prepared gold microislands at 4 °C for 4 h. The substrates were sterilized and then placed into 12-well plates. BMSCs in growth medium (low-glucose DMEM, 10% FBS) were seeded at a density of 5×10^4 cells/well. 1.5 h later, nonadherent cells were removed. After about 6 h, the stem cells were cultured in differentiation media. For osteogenic differentiation, BMSCs were cultured in a cycle of 3 days of osteogenic induction medium composed

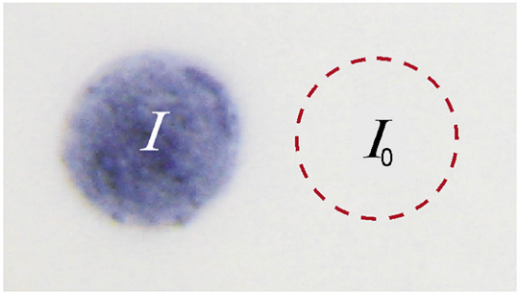

Fig. 2. A local brightfield micrograph of a micropatterned surface after osteogenic differentiation of BMSCs was used to indicate the method to quantify ALP activity. First, the image was converted into RGB slides by the software ImageJ. Then, cells were outlined, and the grey level for each pixel within cells was recorded as I; near the cells, a background region with size similar to a cell was framed, and the grey level for each pixel within the frame was recorded as I_0.