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Subcellular cell geometry on micropillars regulates stem cell differentiation

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ABSTRACT

While various material factors have been shown to influence cell behaviors, recent studies started to pay attention to the effects of some material cues on "subcellular" geometry of cells, such as self-deformation of cell nuclei. It is particularly interesting to examine whether a self deformation happens discontinuously like a first-order transition and whether subcellular geometry influences significantly the extent of stem cell differentiation. Herein we prepared a series of micropillar arrays of poly(lactide-*co*-glycolide) and discovered a first-order transition of nuclear shape as a function of micropillar height under the examined section area and interspacing of the pillars. The deformed state of the nuclei of mesenchymal stem cells (MSCs) was well maintained even after osteogenic or adipogenic induction for several days. The nuclear deformation on the micropillar arrays was accompanied with smaller projected areas of cells, but led to an enhanced osteogenesis and attenuated adipogenesis of the MSCs, which is different from the previously known relationship between morphology and differentiation of stem cells on flat substrates. Hence, the present study reveals that the geometry of cell nuclei may afford a new cue to regulate the lineage commitment of stem cells on the subcellular level.

1. Introduction

One of the fundamental topics in the fields of biomaterials and regenerative medicine is cell-material interactions [1–5], which are dynamic and intricate. Cells are able to sense the external material features and integrate physiochemical signals, which eventually result in altered cell functions and gene expressions [6,7]. In order to reveal cell-material interactions, various patterning techniques have been developed and applied in cell research [8–13]. Although there are increasing understandings on cell responses to material surfaces via cytoskeleton remodeling, relatively little attention has been paid to the responses of cell nuclei, such as nuclear deformation.

Nucleus is the largest as well as the stiffest organelle in a mammalian cell that makes a great contribution to the mechanical properties of the cell [14]. In a typical mechanotransduction pathway, a nucleus is connected to extracellular matrix (ECM) through the cytoskeleton to facilitate adaptive cell responses [15–17]. Most of genetic material is located in the nucleus for a eukaryote. Study of nuclear geometry and its influence on cell behaviors is thus crucial for the understanding of cell-material interactions on a subcellular level.

A few studies on deformation of cell nuclei such as nuclear elongation under transiently applied forces have been reported by techniques including micropipette aspiration [15,18,19], atomic force microscopy [19], microplate compression [14], microfluidic system [20] and so forth. A persistent nuclear elongation was achieved by adhesive microislands, which restricted aspect ratios (ARs) of cells to some extents [21–24]. Nanostructures were found to generate nuclear deformation as well [25,26]. Recently, groups of BX Cui and Y Cui discovered in their collaborative research that vertical nanopillar-induced nuclear deformation could be applied in probing *in situ* subcellular perturbation of nuclear mechanics [26]. Meanwhile, micropillar arrays were reported to deform nuclei severely [27–30]. The nuclear distortion of osteosarcoma-derived cell lines was observed on micropillars of poly(dimethyl siloxane) and poly(L-lactic acid) by Anselme and co-workers [27,30]. Our group has also achieved the control of nuclear deformation of mesenchymal stem cells (MSCs) by applying different micropillar patterns [29]. Nevertheless, a very basic question has never been addressed: a nucleus changes continuously or discontinuously with material dimension, or in light of statistical physics, whether or not

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such a biological process obeys a first-order (discontinuous) transition. The present report will answer this question by a semi-quantitative observation of self-deformation of MSCs on micropillar arrays.

Another critical question is the effect of nuclear deformation on stem cell differentiation. Although it is known that the cells could survive and differentiate in spite of serious nuclear deformation [29], it remains to be explored whether or not the nuclear deformation affects the extents of stem cell differentiation. Various cues have been known to dictate fate decisions of stem cells in the past decade. Regardless of soluble factors, lineage-specific differentiation of stem cells is influenced by cell cues including cell shape [31–33], cell size [34,35], cell-cell contact [35–37] and matrix cues containing stiffness [38–41], topography [42], functional groups [43], nanoscale distribution of ligands [44], and even molecular chirality on material surfaces [45]. This paper reports that the nuclear deformation remains after cell differentiation. Upon that, we hypothesized that a persistent nuclear deformation might be a potential new cue to determine cell fate. This hypothesis was rationalized from the concept of chromosome territories to depict the non-random distribution of chromosomes and compartmentalization of nuclear architecture [46,47]. Positioning of the chromosome territories is related to gene contents and exerts profound influence on gene expression [46]. In order to verify our hypothesis, we utilized micropillar arrays to study the nuclear deformation and the resulting stem cell differentiation, as well as the corresponding gene expression.

In this study, we examined osteogenic and adipogenic differentiations of MSCs on micropillar arrays of poly(lactide-*co*-glycolide) (PLGA). PLGA is a very useful biomedical polymer [48], and here its micropillar arrays were fabricated by molding from silicon micropit templates. Appropriate pillar width and pillar spacing were chosen to induce a severe self-deformation of cell nuclei. Under these size dimensions, MSCs derived from bone marrow of rats were cultured on micropillars with varied pillar heights to validate the existence of a critical pillar height for nuclear deformation. Stem cell differentiation was then induced in osteogenic or adipogenic medium. The degrees of differentiation were measured on high micropillars, with those on low micropillars and smooth PLGA films as controls. Based on these experiments, the effects of nuclear deformation on stem cell differentiation were explored and summarized.

2. Materials and methods

2.1. Preparation and characterization of micropillar patterns

PLGA (Purac Inc., Netherlands) composed of 85 wt% lactide (LA) and 15 wt% glycolide (GA) units (PLGA85/15) with number-average molecular weight of 3.59×10^5 and molar mass dispersity of 1.72 was used to fabricate micropillars. The PLGA micropillar arrays were made by molding from a series of silicon wafer templates with micropits [29], and the micropits were ordered from Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences. A PLGA solution in dichloromethane (5 wt%) was casted onto these templates. The dried PLGA micropillars were carefully peeled off from the silicon wafers. As a control, a smooth PLGA film was also prepared by depositing the above PLGA solution on a smooth silicon wafer. The resulting samples were gold sputtered and observed under field-emission scanning electron microscope (FE-SEM, Ultra 55, Zeiss, Germany). The micropillar heights were then calculated using the software ImageJ 1.48v (National Institutes of Health, USA).

2.2. Isolation and culture of MSCs

MSCs were isolated from marrow of the tibiae and femora of neonatal Sprague Dawley (SD) rats [49]. The marrow was flushed out thoroughly using a sterile injection syringe filled with low-glucose Dulbecco's modified Eagle medium (DMEM, Gibco). Following the step of centrifuging, the supernatant was discarded, and the accumulated sediment was re-suspended in low-glucose DMEM supplemented with 10% fetal bovine serum (FBS, Gibco). MSCs of the second passage were used in the later experiments of cell adhesion and differentiation.

2.3. Staining of cells and morphological observations of nuclei

The PLGA films were sterilized by 75% alcohol for 45 min, fully rinsed by phosphate buffered saline (PBS) and then placed into 12-well tissue culture plates (TCPs). MSCs were seeded on the smooth and micropillared films at the density of about 2.6×10^3 cells per cm^2. After 24 h culture, cells on the PLGA films were rinsed gently with warm PBS twice, fixed with 4% paraformaldehyde for 10 min, and then permeabilized with 0.1% Triton X-100 in PBS for 10 min. The fixed MSCs were treated with 1 μg/ml phalloidin-tetramethyl rhodamine B isothiocyanate (phalloidin-TRITC, Sigma) for 30 min to label filamentous actins (F-actins) followed with 4′,6-diamidino-2-phenylindole (DAPI, Sigma) staining for 5 min to label nuclei. At last the samples were rinsed with Milli-Q water. The nuclei on micropillar arrays were observed under an inverted fluorescence microscope (Axiovert 200, Zeiss) equipped with a charge coupled device (CCD, AxioCam HRC, Zeiss).

Besides the examination of the deformed nuclei in the inverted microscope, we also reconstructed the vertical image stacks using a confocal microscope (LSM 710, Zeiss). In order to show both cell nuclei and micropillar arrays, we captured images using both standard detector (fluorescence or reflected mode) and transmitted detector (transmission mode).

2.4. Quantification of nuclear shape and statistics of its distribution

Shape index (SI) was introduced to quantify the shape of a nucleus. SI was defined as $4\pi S/l^2$, where the area (S) and perimeter (l) of each nucleus were measured using the software ImageJ 1.48v. Nuclei of at least 150 cells were measured on each group of micropillars.

In order to show distribution of SI, the probabilities of SI of cell nuclei with a step value of 0.05 were calculated from 0 to 1. According to the distribution profiles, we defined SI < 0.8 as the criterion of a deformed cell nucleus.

The fractions of deformed nuclei f were plotted as a function of micropillar height h. The data was fitted by a Boltzmann sigmoid. The equation can be, by adaptation from our previous determination of critical micelle concentration [50], written as

$$f = \frac{f_0 - f_\infty}{1 + e^{(h - h_{\text{critical}})/\delta}} + f_\infty$$

Here h_{critical} is the center of the Boltzmann sigmoid, giving the critical micropillar height for the deformation transition of cell nuclei; f_0 and f_∞ represent the asymptotic values of f at infinitely small and large h, respectively; δ reflects the deviation of h around h_{critical}.

The slope at the critical micropillar height was derived as

$$\left.\frac{df}{dh}\right|_{h=h_{\text{critical}}} = \frac{f_\infty - f_0}{4\delta}$$

Therefore, the transition zone in the Boltzmann sigmoid has a