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Control of cell nucleus shapes via micropillar patterns

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ABSTRACT

We herein report a material technique to control the shapes of cell nuclei by the design of the micro-topography of substrates to which the cells adhere. Poly(D,L-lactide-*co*-glycolide) (PLGA) micropillars or micropits of a series of height or depth were fabricated, and some surprising self deformation of the nuclei of bone marrow stromal cells (BMSCs) was found in the case of micropillars with a sufficient height. Despite severe nucleus deformation, BMSCs kept the ability of proliferation and differentiation. We further demonstrated that the shapes of cell nuclei could be regulated by the appropriate micropillar patterns. Besides circular and elliptoid shapes, some unusual nucleus shapes of BMSCs have been achieved, such as square, cross, dumbbell, and asymmetric sphere-protrusion.

1. Introduction

Cell–material interaction is one of the basic topics in the fields of biomaterials and regenerative medicine. To date, several chemical and physical properties of materials have been recognized as cues to regulate cell responses [1–7]. The simplest physical cue might be surface topography [8–16]. While much progress has been made about cell control by surface patterning [17–34], relatively little concerns the regulation of cell nuclei.

Some mild self deformation of cell nuclei by surface patterns or significant nucleus elongation by micropipette aspiration had been reported in the literature [35–40]. Recently, a severe deformation of the nuclei of cells by a material factor, especially tumor cells, was observed on polydimethylsiloxane micropillars by Anselme et al. [41,42]. While this self deformation phenomenon is surprising and stimulating, a part of key questions are open. For instance, as the experimental conditions are concerned, is a critical micropillar height necessary for a severe nucleus distortion? Is gravity responsible for the deformation? Can micropits also enable the deformation of cell nuclei?

In this paper, poly(D,L-lactide-*co*-glycolide) (PLGA) micropillars of a series of heights were fabricated by us, molded by micropit templates of silicon, as schematically presented in Fig. 1. PLGA micropits were also prepared by molding silicon micropillars. We revealed that only micropillars with a sufficient height could lead to a severe self deformation of cell nuclei in our research. What's more, this paper is focused upon nucleus deformation of stem cells, with bone marrow stromal cells (BMSCs) as demonstration. The highlight is that we tried to control cell nuclei by altering micropillar patterns. Several interesting shapes of cell nuclei were observed, which are extraordinary as usual, yet highly probable if the cells were seeded on our well-designed micropillar patterns.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) with a molar ratio of lactide and glycolide 85:15 (PLGA85/15) in a granular form was obtained from Purac Inc. (Netherlands). The number average molecular weight was 3.59×10^5 and the polydispersity index was 1.72.

2.2. Preparation of PLGA micropillars and micropits

The micropatterned silicon wafers were fabricated by Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences. The PLGA micropillar array was molded by the silicon micropit template. Briefly, the template was poured over by a 5 wt% PLGA solution in dichloromethane. After drying in the air for 48 h, the PLGA film was peeled off the silicon template and cut into pieces for future use. PLGA micropits were obtained using a micropillar template. A smooth PLGA film, as a control, was also prepared by pouring the polymer solution on a smooth silicon wafer.

2.3. Characterization of PLGA films

Scanning electronic microscopy (SEM) was used to observe those smooth and rough PLGA films. The samples were coated with gold prior to observation. The electric voltage was set as 20 kV in the electronic microscope.

2.4. Isolation and culture of BMSCs

Neonatal Sprague Dawley (SD) rats were used to isolate BMSCs. The marrow was flushed out, centrifuged, and resuspended in low-glucose Dulbecco's modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco).

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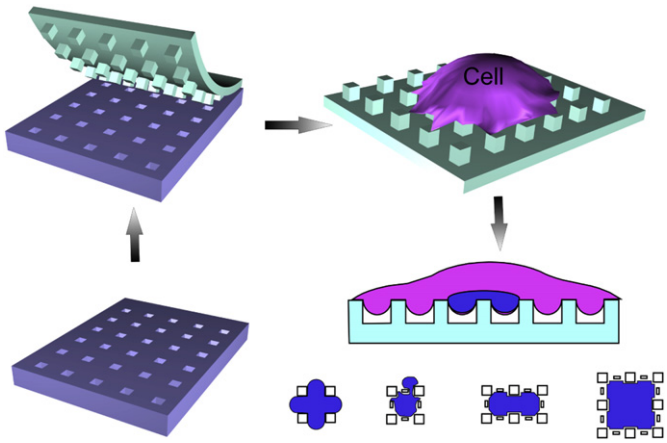

Fig. 1. Schematic presentation of the fabrication of PLGA micropillars and the possible deformation of nuclei of cells on the micropillars. The PLGA micropillars were molded from a silicon template of micropits. Significant nucleus deformation might happen under appropriate conditions. Some preferred shapes of cell nuclei could be achieved via design of micropillar patterns.

Cell culture was carried out in a humidified incubator at 37 °C with a 5% CO_2 atmosphere. BMSCs of the early passage were used for later experiments.

Prior to use, the PLGA films were sterilized by 75% alcohol, exhaustively rinsed by phosphate buffered saline (PBS) solution. BMSCs in the growth medium were seeded on the PLGA films with 1×10^4 cells into one well of 12-well tissue culture plates (TCPs).

2.5. Cell staining and observations

After 6 h culture on the PLGA films, cells were rinsed carefully with a warm PBS to remove non-adherent cells. All samples were fixed with 4% paraformaldehyde in PBS for 10 min and then permeabilized with a 0.1% v/v Triton X-100 for 10 min. For fluorescence staining, the cells were incubated with 1 μg/ml Phalloidin-TRITC (Sigma) for 1 h at room temperature to label the filamentous actins. After thoroughly rinsed, nuclei were labeled with 5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI, Sigma) for 10 min. All the stained samples were rinsed extensively with Milli-Q water, prior to observation under an inverted microscope (Axiovert 200, Zeiss) mounted with CCD (AxioCam HRC, Zeiss).

2.6. MTT assay

The viability of cells was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 1 ml of culture medium containing 0.5 mg/ml MTT was added into each well of TCPs, followed by incubation at 37 °C for 4 h. Then the MTT-containing medium was replaced by dimethyl sulfoxide (DMSO). For the control group in TCPs, the culture medium was discarded, and the same quantity of DMSO was added to each well. The optical density (OD) was measured at wavelength 492 nm by using Multiskan (MK3, Thermo labsystems).

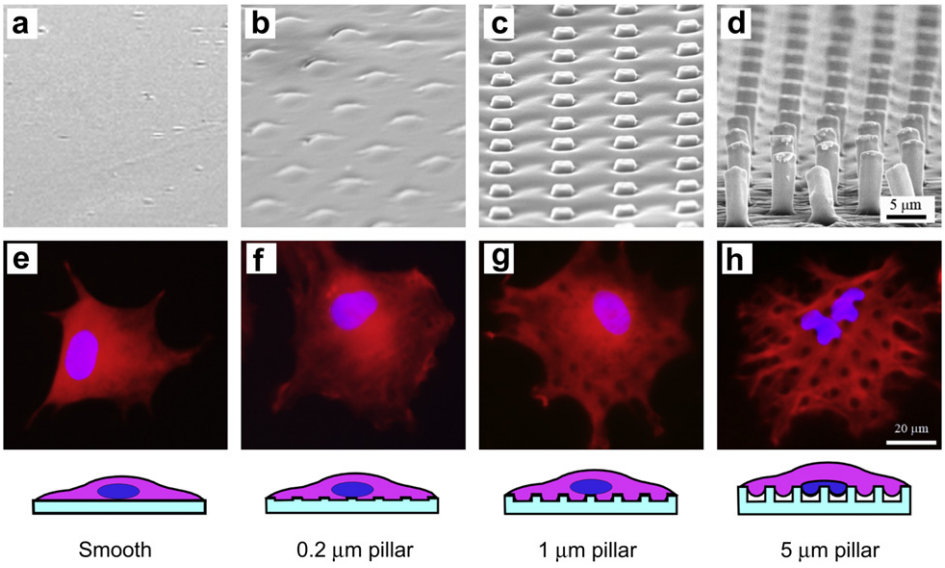

Fig. 2. SEM micrographs of smooth and rough PLGA films of the marked heights of micropillars (a–d) and fluorescent micrographs of BMSCs on the corresponding films after 6 h culture (e–h). The cells were stained to label the nuclei (blue) and filamentous actins (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

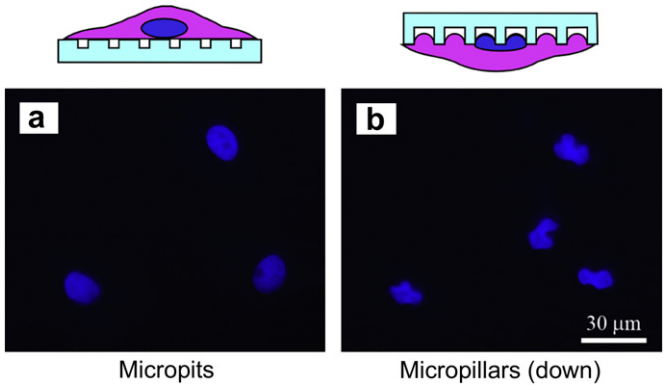

Fig. 3. Fluorescent optical micrographs of nuclei of BMSCs after 6 h culture on the PLGA micropits of 5 μm depth (a) and on the PLGA micropillars of 5 μm height (b). In (b), the micropillared film was overturned after seeding cells for 0.5 h.