Acta Biomaterialia 27 (2015) 13–20

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Micropillar arrays as potential drug screens: Inhibition of micropillar-mediated activation of the FAK–Src–paxillin signaling pathway by the CK2 inhibitor CX-4945

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ARTICLE INFO

Article history:
Received 12 February 2015
Received in revised form 24 August 2015
Accepted 25 August 2015
Available online 28 August 2015

Keywords:
Micropillar array
Epithelial-to-mesenchymal transition (EMT)
Focal adhesion
Drug screening

ABSTRACT

Here, we demonstrate the possible applications of micropillar arrays in screening anti-metastasis drugs. Human lung adenocarcinoma A549 cells incubated in multiwell plates containing micropillars exhibited markedly different physical/biochemical behavior depending on pillar dimensions. In particular, A549 cells grown in plates containing 2-μm diameter, 16-μm pitched pillar arrays showed epithelial-to-mesenchymal transition (EMT)-like behavior; cell body elongation, and highly increased activation of the focal adhesion kinase (FAK)–Src–paxillin signaling cascade. FAK is the most prominent kinase involved in dynamic regulation of the actin cytoskeleton and cell adhesion, migration, and invasion. Activation of FAK, a hallmark of cancer cell adhesion and migration, is normally induced by various growth factors, such as transforming growth factor-β (TGF-β). Here, we found that pillar-mediated activation of signaling molecules mimicked that induced by TGF-β. Notably, micropillar arrays with specific dimensions accelerated the elongation of cells, an effect linked to the activation of signaling molecules related to EMT. Micropillar-induced FAK activation could be arrested by the casein kinase-2 (CK2) inhibitor CX-4945, a drug candidate with activity against TGF-β-induced cancer cell metastasis, demonstrating the possibility of using inorganic microstructures for cell-based drug screening.

Statement of Significance

In this work, we have fabricated flexible substrates with regular arrays of micrometersized pillars, and used them to grow A549 human lung adenocarcinoma cells. Cells exhibit dramatically different behavior depending on the intervals of pillars. Especially, cells grown in certain pillar structures show epithelial-to mesenchmal transition (EMT)-like morphology and related molecules, which is similar to the activation obtained using expensive cytokine TGF-β. Based on the fact that pillar arrays may activate EMT like transition, screening of anti-cancer drug using pillar arrays have demonstrated as well in our work. Our study confirms that mechanical stimulation may exert similar effects with chemical stimulation, and such mechanical structures could be used as a large-scale drug screening platforms.
Cell morphogenesis on engineered substrate is not new, but the present work could be distinguished with its unique fabrication process that can mass produce the structures and it could be applied for high-throughput drug screening. Also, we suggest the formation of focal adhesions on pillar structures and consequent strain as the possible mechanism behind the observed EMT-like transition. Currently, we are working on full-scale profiling of metabolomics and proteomics of cells grown in large-scale pillar arrays as well.

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http://dx.doi.org/10.1016/j.actbio.2015.08.041
1742-7061/

1. Introduction

The epidemiology of a disease can reflect a wide range of potential causes. Cancer, in particular, is a complex disease with a multitude of possible causative factors, encompassing genetic variants, carcinogen exposure, diet and physical activity, UV exposure, and infectious diseases. Currently ongoing cancer studies at the cellular level provide essential insight into cancer dynamics and cellular responses towards possible carcinogens.

It is well recognized that chemical interference (carcinogens) is not the only factor that influences cell fate; the physical environment may also have a significant impact. As early as the 1980s, morphological changes were observed in cells grown on fibrous substrates. With advances in microfabrication and nanofabrication techniques, synthetic substrates with long-range ordered structures came to be employed as cellular substrates by the late 1990s [1]. Cells grown on substrates with artificial topographical features may exhibit dramatic changes in cell morphology and genetic expression. The interactions of cells with substrate topography are well summarized in recent reviews by Bettinger et al. [2], Stevens et al. [3] and MacQueen et al. [4]. These authors have shown that substrates with a synthetic topography can influence cell morphology, adhesion, migration, and proliferation. In short, artificial physical (mechanical) environments may permanently alter cellular interactions as well as cell fate. These observations suggest that synthetic morphological substrates can be used to discover new cellular functions and cellular signaling pathways. In application, substrates can be engineered to enhance the controlled adhesion and alignment of cells for use in implants, artificial organs, and prosthetic devices.

Nano- or micron-sized pillar arrays are among the most widely studied substrate morphologies for examining cell adhesion and alignment. A variety of cell types has been investigated on pillar substrates, including osteosarcoma [5], epithelial cells [6], stem cells [7,8], fibroblasts [9], and cancer cells [10]. Although these previous studies have employed different cell types, pillar dimensions and substrate materials, each research group concluded that pillar dimensions that approximate cell size strongly affect cell morphology and migration, and that focal adhesion kinase (FAK) plays a central role in these processes [5,11,12]. However, the precise mechanism by which substrate topography activates cells and cellular networks is still a matter of debate, and further applications of substrate-induced tailoring of cells and cellular networks have yet to be developed.

In the present study, topographic substrates (micropillar arrays) were fabricated and employed to stimulate epithelial-to-mesenchymal transition (EMT) in various cell lines, including human lung adenocarcinoma A549 cells. A combination of standard lithography and soft lithography enabled the large-scale fabrication of micron-sized patterns. The present work represents the first realization of 96-well plates and 10-cm petri dish covered with micropillar arrays. Such large-scale topographic substrates could be employed to study various aspects of cellular behavior. As proof of principle, we here investigated stimulation of EMT and subsequent inhibition of EMT by drug candidates.

2. Materials and methods

2.1. Fabrication of micropillar arrays

2.1.1. Fabrication of silicon masters

Silicon masters were patterned on 8-inch silicon wafers by photolithography and dry etching (National Nanofab Center, Korea). Pillar dimensions used in this study were 2 μm in diameter and 10 μm in height; pitch (distance between pillars) was varied from 4 μm to 20 μm.

2.1.2. Fabrication of polymer masters

Master pillar arrays were fabricated using SU8-2010 (Microchem, MA, USA) negative photoresist. SU8-2010 photoresist was spun onto 4-inch silicon wafers, and a two-step soft-bake process (65 °C for 2 min and 95 °C for 5 min) was performed. Patterns were exposed with mask aligner (Midas Systems, Korea) and then baked post-exposure at 65 °C for 1 min and at 95 °C for 2 min. After developing in SU-developer (Microchem), master pillar arrays were hard-baked up to 230 °C with a gradual increase in temperature (~1 °C/min) to ensure the mechanical stability of the pillars.

2.1.3. Intermediate mold and final micropillar arrays

The surface of silicon masters was functionalized with self-assembled monolayers (SAM) of trichloro(1*H*,2*H*,2*H*-perfluorooctyl)silane, which served to increase the surface hydrophobicity for more facile detachment of intermediate polydimethylsiloxane (PDMS) molds (not necessary for pillar arrays made with SU8-2010). The intermediate PDMS molds were synthesized by mixing PDMS precursor with curing agent at a 10:1 ratio and curing the molds directly on the SAM-functionalized silicon masters (SU8-2010 masters) at 80 °C for 4 h. PDMS molds provide convenient release of final polymer structures, and their use is economically advantageous compared with silicon masters, since the intermediate PDMS molds can be employed more than ten times without significant feature degradation. Micropillar-array substrates were cast from UV-curable NOA63 resin (Norland Products, NJ, USA) by gently pressing the intermediate PDMS mold onto target PET substrates (96-well plates, 10-cm petri dishes) wetted with a film of NOA63 precursor, which was then cured by UV exposure for 1 min. NOA63 is a clear, colorless photopolymer that can be cured with 350–380 nm UV light (https://www.norlandprod.com/adhesives/noa%2063.html). After curing NOA63, the intermediate PDMS mold was detached from the sample, releasing the NOA63 micropillar arrays deposited on PET substrates. PET substrates were then cut to fit conventional 6-well plates. Fig. 1 shows a schematic diagram of the fabrication process and images of substrates with pillars. Formation of such hierarchical molding-based structures has been reported by several research groups [13,14], and these structures have been successfully employed to stimulate cell behaviors [15,16]. However, to our knowledge, large-scale substrates (96-well plates, 10-cm petri dishes) filled with micrometer-scale features have not been previously realized.

2.1.4. Characteristics of pillars

Pillar dimensions were uniform for each sample, but were varied between samples to explore the relationship between pillar spacing and cellular response. The characteristic dimensions of the micropillar arrays are summarized in Supplementary Information, Fig. S1. In this study, pillar dimensions are defined by pillar diameter and pitch (distance between neighboring pillars). For example, (2.8) denotes micropillar arrays with 2 μm pillar diameter and 8 μm pitch. The percent area (coverage) between top and bottom areas decreases as the pitch increases (Supplementary Information, Fig. S1(d)).

2.2. Cell growth on micropillar arrays

Fabricated micropillar arrays were sterilized by washing with acetone and isopropyl alcohol. A549 human lung epithelial adenocarcinoma cells, HeLa human cervical cancer cells, and CCD-18Lu human lung fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM; Hyclone, UT, USA) containing 10%