

Manipulation of Cell Spreading and Cytoskeleton of Stem Cells by Micropatterned Surfaces

Cooper Thome

Chemical Engineering, University of Tennessee at Chattanooga

REU Program: 2017 Cornell NanoScale Science & Technology Facility International Research Experience for Undergraduates (CNF iREU) Program at the National Institute of Material Science (NIMS), Tsukuba, Ibaraki, Japan

CNF iREU Principal Investigator: Dr. Guoping Chen, Tissue Regenerations Group, National Institute for Materials Science

CNF iREU Mentor: Yingjun Yang, Tissue Regenerations Group, National Institute for Materials Science

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Contact: gwf532@mocs.utc.edu, guoping.chen@nims.go.jp, yang.yingjun@nims.go.jp

Website: http://www.cnf.cornell.edu/cnf_2017reu.html

Abstract:

This study aimed to further explore the manipulation of cell spreading and cytoskeleton of mesenchymal stem cells (MSCs) by use of easily fabricated micropatterned surfaces designed for single-cell culture and precise control of cell spreading area. Micropatterned surfaces were fabricated by a UV photolithographic process using azidophenyl-derivatized PVA (AzPhPVA), a photoreactive and non-cell-adhesive polymer. AzPhPVA and micropatterned surfaces were characterized using assorted techniques. General MSC cytoskeletal structure and morphology were analyzed using fluorescence and confocal microscopy. Significant cytoskeletal and morphological differences were observed between cells with small and large spreading areas.

Introduction:

Mesenchymal stem cells (MSCs) are undifferentiated cells that exhibit multipotency, the ability to differentiate into a variety of types of cells, as well as the potential for long-term self-renewal. MSCs have a variety of therapeutic applications and are widely studied in tissue engineering [1]. It has been shown that MSC structure and behavior are dependent on the cellular microenvironment, which has both biochemical and biophysical components. The cell spreading area, a biophysical microenvironmental component, has been shown to affect MSC cytoskeletal formation and differentiation, amongst other cell behaviors [2,3].

Our goal was to fabricate micropatterned surfaces designed to precisely control cell spreading of single cells to further analyze cell morphology and cytoskeletal structure of MSCs. Specifically, actin structures of MSCs at varied spreading areas were examined.

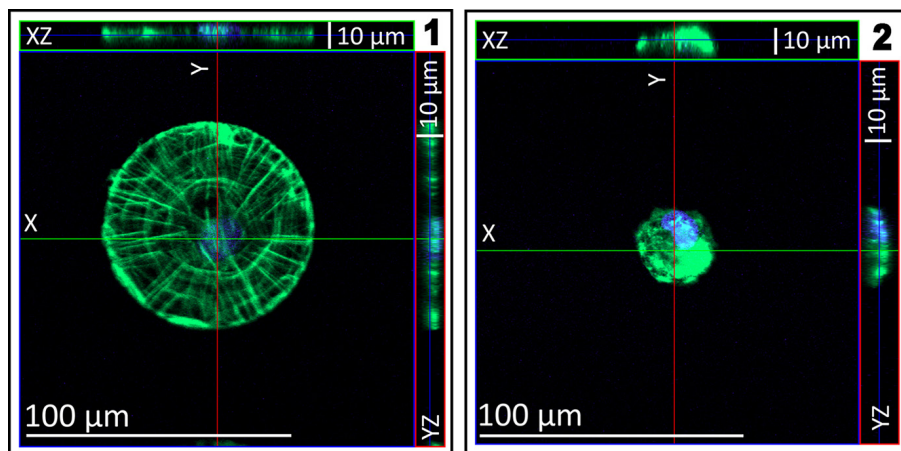


Figure 1, left: Confocal microscope image of MSC cultured on 80 μm micropattern and stained for F-actin. Note the defined actin filaments in the large image and the flat morphology shown in the XZ and YZ cross-sections. Figure 2, right: Confocal microscope image of MSC cultured on 30 μm micropattern and stained for F-actin. Note the randomly oriented cortex actin in the large image and the rounded morphology shown in the XZ and YZ cross-sections.

Experimental Procedure:

Azidophenyl-derivatized poly(vinyl alcohol) (AzPhPVA), a photoreactive and non-cell-adhesive polymer, was synthesized from poly(vinyl alcohol) (PVA) and 4-azidobenzoic acid and purified according to a previous work [2]. The resulting AzPhPVA solution was analyzed using ultraviolet-visible spectroscopy, which indicated successful grafting of the azidophenyl-derived group to the PVA. Using $^1\text{H-NMR}$ spectroscopy, the grafting ratio was calculated to be 2.5%.

To fabricate micropatterned surfaces, cell-adhesive polystyrene substrates were cut from a vacuum gas plasma treated tissue culture flask. Substrates were then coated with 200 μl of AzPhPVA using a drop coat method. The AzPhPVA was dried at 25°C in the dark. After drying, the AzPhPVA coated substrates were UV irradiated through a photomask containing six matrices of microdots with designed diameters of 10, 20, 30, 40, 60, and 80 μm . After UV irradiation, samples were placed in an ultrasonic bath in Milli-Q water in order to remove the excess and unexposed AzPhPVA, revealing patterns of polystyrene microdots in the AzPhPVA layer. The resultant micropatterned surfaces were characterized using both optical microscopy and atomic force microscopy (AFM). 3D images of the microdots collected from AFM were used to determine micropattern diameter and thickness. Examples of standard 3D AFM images are seen in Figures 1 and 2. The diameters of the micropatterned microdots were nearly identical to those of the microdots on the photomask. The thickness of the micropatterns varied from 50.56 to 90.76 nm, which is suitable for single-cell adhesion and culture.

After micropattern characterization, human MSCs were cultured on the micropatterns. After a few hours, cells adhered and spread on microdots, which controlled cell spreading area and morphology. Cell adhesion was significantly lower for micropatterns with diameters of 10 and 20 μm when compared to larger diameter micropatterns. After spreading, MSCs were stained for F-actin filaments which were observed by fluorescence and confocal microscopy.

Results and Discussion:

The UV photolithography micropatterning technique was successful for fabricating micropatterns for single-cell culture and precise control of cell spreading. Fluorescence microscopy revealed significant cytoskeletal and morphological differences between cells with varied spreading areas. Cells with larger spreading areas, such as on the 80 μm microdots, exhibited highly ordered actin structures characterized by defined

filaments organized in both the radial and concentric fashion. Cells with smaller spreading area, mostly 30 μm and below, did not exhibit defined filaments but instead showed randomly oriented cortex actin. Additionally, the morphology of the MSCs at varied cell spreading areas was quite different. As seen in confocal microscope images in Figures 3 and 4, cells with larger cell spreading areas had a flat morphology, while cells with smaller spreading areas became more rounded.

Future Work:

Future work includes further cytoskeletal analysis of MSCs with varied cell spreading, as well as the study of the influence of cell spreading area on MSC behavior, possibly in relation to biochemical microenvironmental components. The UV photolithographic technique also allows for the creation of a variety of micropattern geometries which may be further explored.

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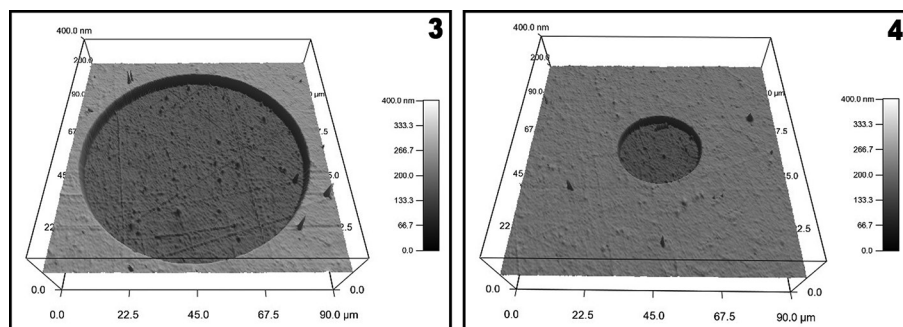


Figure 3, left: Typical 3D atomic force microscopy image of 80 μm microdot. The bottom of the microdot is cell-adhesive polystyrene, while the walls and rest of the upper surface are non-cell-adhesive AzPhPVA. **Figure 4, right:** Typical 3D atomic force microscopy image of a 30 μm microdot. The bottom of the microdot is cell-adhesive polystyrene, while the walls and rest of the upper surface are non-cell-adhesive AzPhPVA.