Regulation of Stem Cell Density and Functions by Micropatterned Surfaces

Alexa M. Espinoza

2019 NNCI iREU Intern

Intern Affiliation: Chemical Engineering, University of Florida

Program: 2019 National Nanotechnology Coordinated Infrastructure International Research Experience for Undergraduates (NNCI iREU) Program at the National Institute of Material Science (NIMS), Tsukuba, Ibaraki, Japan

NNCI iREU Principal Investigator: Dr. Guoping Chen, National Institute for Materials Science, Tsukuba, Japan

NNCI iREU Mentor: Yongtao Wang, National Institute for Materials Science, Tsukuba, Japan

Primary Sources of 2019 NNCI iREU Funding: National Science Foundation via awards ECCS-1626153 (NNCI Coordinating Office at Georgia Tech) and NNCI-1542081 (Cornell NanoScale Facility)

Contact: guoping.chen@nims.go.jp

Website: http://cnf.cornell.edu/education/reu/2019

Abstract:

Cell density plays a vital role in regulating cell functions, such as adhesion and spreading, proliferation, and differentiation. In this study, photo-reactive azidophenyl-derivatized poly(vinyl alcohol) (PhAzPVA) was synthesized by introducing azidophenyl groups into PVA. The micropatterns with different square density were prepared by PhAzPVA on PS plate using photolithography. Cell density could be controlled by the micropatterns and the influence of cell density on cell functions was investigated. The results showed that the as-prepared microsquares were similar with that of the pre-designed photomask and the cell density could be well adjusted by these micropatterns. Organization of actin filaments and DNA synthesis activity were observed by immunefluorescence staining.

Summary of Research:

Cell density is a very important factor to regulate cell morphology and intercellular signaling in our bodies [1]. However, it remains a great challenge to precisely control cell density *in vitro* by these traditional methods. Currently, many micropatterning techniques including photolithography, microcontact printing, and laser ablation, provide a possible way for precisely controlling cell density *in vitro*. These methods, especially for photolithography play an important role in studying cell biology, biomaterials, and tissue engineering.

As well known, human mesenchymal stem cells (hMSCs) have been used in tissue engineering and repair due to their self-renewability and pluripotency [2]. It has been reported that hMSCs density could affect cell functions, like chondrogenic/osteogenic differentiation. Actin filaments organization and DNA synthesis activity may be also affected by cell density on different microsquares. The cells were seeded in micropatterns and the cell density could be controlled from low to high density. Actin filaments were stained to investigate the organization and orientation. DNA synthesis activity (nuclei staining) was observed by immunefluorescence staining.

Results and Conclusions:

Preparation of Square Micropatterns. Tissue culture polystyrene plates (2.5 × 2.5 cm2) were cut from TCPS flasks and coated with 200 μL of photo-reactive PVA aqueous solution. The coated plates were later air-dried at room temperature in the dark. The PVA-coated plates were later covered with the pre-designed photomask with the density gradient micropatterns and exposed to ultraviolet light at the energy of 0.25 J/cm^2. After UV exposure, the plates were washed with Milli-Q water and ultrasonicated to completely remove the unreacted polymer. Following the washing, the micropatterns were sterilized by immersing them in 70% ethanol and then rinsed with aseptic water. The micropatterned surfaces were observed by a phase-contrast microscope.

Figure 1a shows the images of pre-designed photomask including different square densities. And the ratio of patterns to non-patterns was 1:4, 1:9, 1:25, and 1:50, respectively. According to the pre-designed photomask, the TCPS/PVA micropatterned surfaces prepared through photolithography was successful in controlling the different cell densities of hMSCs needed in Figure 1b, indicating a good controllability of microsquares.

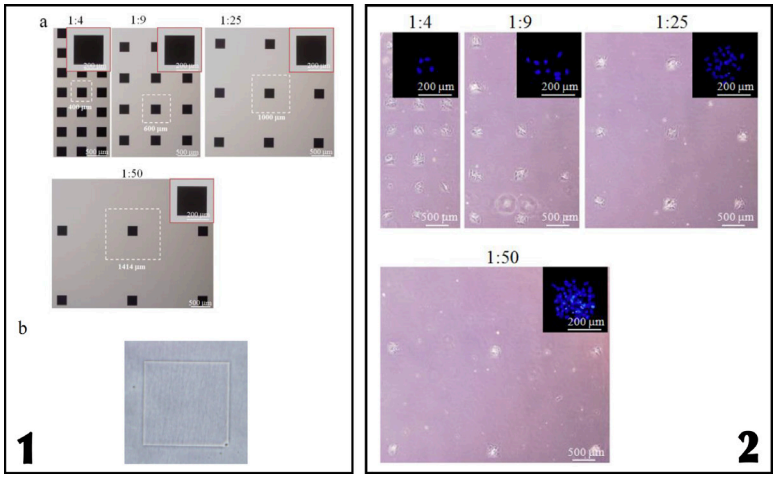

Figure 1, left: *Representative Images of photomask (a) and micropattern (b).* ***Figure 2, right:*** *Representative images of cell morphology on micropatterns. Inserts were the nuclei staining.*

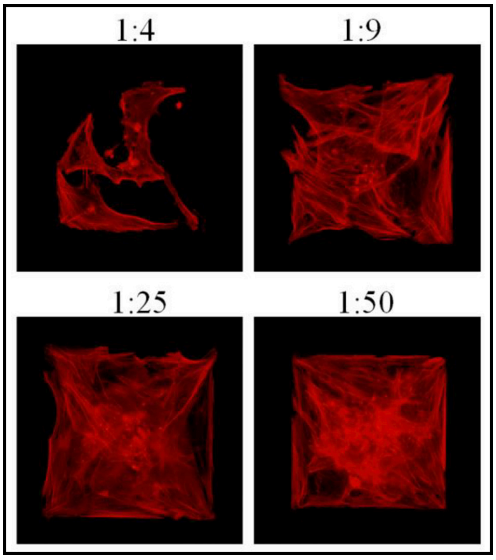

Figure 3: Representative images of fluorescence staining for actin filaments.

Cell Density. After the hMSCs on the micropatterned surface were incubated for 24 hours, cell morphology was observed by photo contrast microscope. Further, nuclei were stained by Hoechst 33258 at room temperature to calculate cell density.

Figure 2 showed the cell morphology on the microsquares, and the cells only migrated into the micropattern part but not in non-pattern parts. Moreover, cell density was also observed by nuclei staining in the insert figures. The results showed that cell density was controlled from low to high.

Cell Functions. The micropatterned surface was used to investigate the relationship between cell density and cell functions. Actin filaments were re-organized in different density microsquares in Figure 3. DNA synthesis activity (BrdU staining) was regulated by the micropatterns with different density in Figure 4. The staining results showed that DNA synthesis activity decreased as cell density increased.

Future Works:

Gene transfection is known as a gene-modified technique that has become well developed over the years and it has broad applications in the bioengineering field. It has been shown that cell density has an influence on several cell functions including proliferation and differentiation. However, its influence on gene transfection is unclear. Therefore, cell density may affect gene transfection of stem cells due to different nuclei activity. In the next work, the relationship between cell density and gene transfection will be investigated.

Acknowledgments:

Dr. Gouping Chen, Yongtao Wang, Dr. Lynn Rathbun, NIMS staff and members, Cornell University, and National Science Foundation funding via awards ECCS-1626153 (NNCI Coordinating Office at Georgia Tech) and NNCI-1542081 (Cornell NanoScale Facility).

References:

[1] W. Song, H. Lu, N. Kawazoe, and G. Chen, "Gradient patterning and differentiation of mesenchymal stem cells on micropatterned polymer surface," Journal of Bioactive and Compatible Polymers, vol. 26, no. 3, pp. 242-256, 2011.

[2] Y. Yang, X. Wang, X. Hu, N. Kawazoe, Y. Yang, and G. Chen, "Influence of Cell Morphology on Mesenchymal Stem Cell Transfection," ACS Applied Materials & Interfaces, vol. 11, no. 2, pp. 1932-1941, 2018.

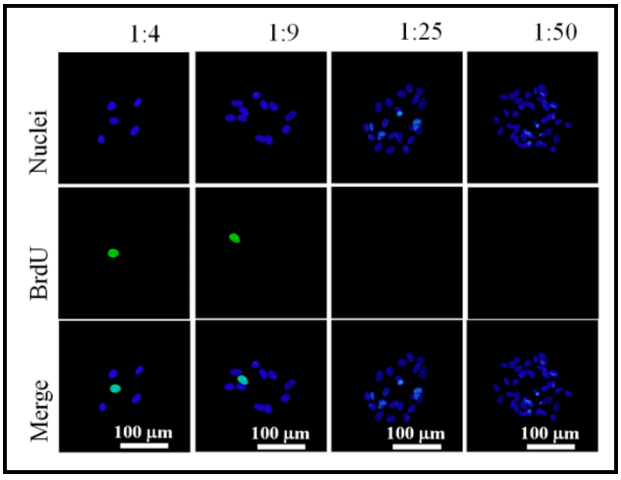

Figure 4: Representative images of BrdU positive cells.