

Single Cell Studies on Patterned, Sculptured Thin Films

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Abstract:

In this experiment, cell studies were conducted on a patterned, sculptured thin film (STF) surface. STFs are a bionanomaterial that features a tightly-packed field of chiral, polymeric projections. This unique structure exhibits a large surface area to volume ratio; giving it significant and valuable properties as a growth medium for cells. Use of this material in transplant and biomedical events might require cells to be grown in a specific pattern. To study that phenomenon, a silicon wafer was patterned using lithography techniques, and future research will involve the transplantation of cells onto the surface of this patterned wafer.

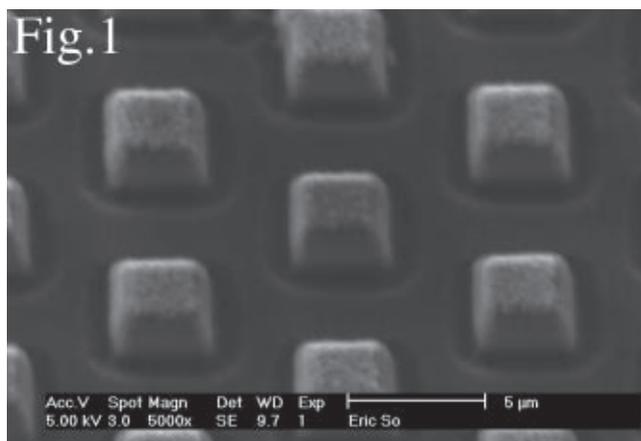
Introduction:

Sculptured thin films (STFs) came into existence around 1994 [1]. The films are often made with a parylene derivative (typically di-chloro-di-p-xylylene) due to the well characterized biological and chemical properties of the polymer [2,3]. The basic structure of an STF is a field of chiral, polymeric projections that resemble corkscrews at the nanoscopic level. This structure, while very simplistic, allows for unique properties as a biological growth medium. The surface of the film is not smooth, presenting a large surface to volume ratio which is ideal for cell growth. The nature of the structure is also conducive to the binding of the extracellular matrix.

These unique properties of STFs make them an ideal candidate for research related to transplants and synthetic tissues. As a precursor to that research, experimentation must be conducted to study the growth patterns of cells on a patterned STF surface. The aspects of the growth that should be studied include the spacing of the pattern, growth on and off of the patterned film, and changes from normal cell growth-rates on the patterned surface versus the flat, non-patterned surface.

Methods and Materials:

1. The project was begun by acquiring silicon wafers, polished on one side and cut into approximately 1" squares.



2. The wafers were cleaned by acetone, isopropyl alcohol and water to remove salts and organic residues.
3. The wafers were dried with nitrogen and spun with an adhesive at 4000 rpm.
4. The wafers were all then spun in 1813 positive photoresist and baked at 115°C on a hot plate for one minute.
5. A soda-lime mask prepared by a laser writer to show squares of 30 μm on a side spaced 30 μm apart was used to expose the wafers to intense UV radiation.
6. The patterned wafers were developed with MSCD26 and blow-dried with nitrogen.
7. The wafers were cleaned in an oxygen plasma furnace and stored for later use.
8. (Future work) As the wafers are needed, they will be placed on a 2-axial motor setup, held in place by tape.
9. The wafer will then be placed in a parylene deposition system that has been loaded with 0.70g of parylene C.
10. The deposition process will then be initiated. For the deposition to take place, the furnace must be at 690°C, the chamber gauge at 135, the vaporizer at 175 and the vacuum at 32. The last 3 numbers are given without units since they are not necessarily accurate on the instrument, but an arbitrary standard set well-beyond the actual value.

11. Once the deposition has finished, the wafer will be removed, cleaned by acetone, washed with water, dried and characterized. A not-to-scale example of the final product's pattern is shown in Figure 1, courtesy of Eric So (graduate student in Dr. Demirel's Lab).
12. Once the wafers have been sufficiently characterized, they will be shipped off to Hershey, PA, for transplanting of HEK-293 cells. The cells will then be studied for approximately 10 days. Their cell counts will be taken using fluorescence imaging and data will be collected about the location and frequency of the cells.

Results:

Results were achieved using a scanning electron microscope (SEM). Figure 2 shows a flat film. This film was produced by an improper distance (approximately 1.5 inches from substrate to funnel). This problem was fixed by reducing the distance to 0.5 inches. In Figure 3 you can see that there are columnar features. In an endeavor to minimize variables, the distance between the substrate and the funnel was reduced, but no rotation was employed-yielding a columnar surface. In Figure 4 the appropriate distance of 0.5 inches was utilized and a rotation of 0.1 revolutions per second. In Figure 2 the divide between the light spaces is a gap between the substrate and the film that resulted from a partial peeling of the film. Figures 3 and 4 do not show a clear substrate since the images were taken of a section of film that was protruding over the edge and showed a better image of the overall film.

Conclusion:

Due to several equipment failures during the time frame of the research, the full objectives of the project

could not be attained. Progress was made in showing this researcher how to prepare an STF and how to operate a variety of equipment including the scanning electron microscope, lithography tools, deposition machines, and an oxygen plasma furnace. The researcher was also able to develop analytical cleaning techniques and expand his interests in the field of biology.

Future Directions:

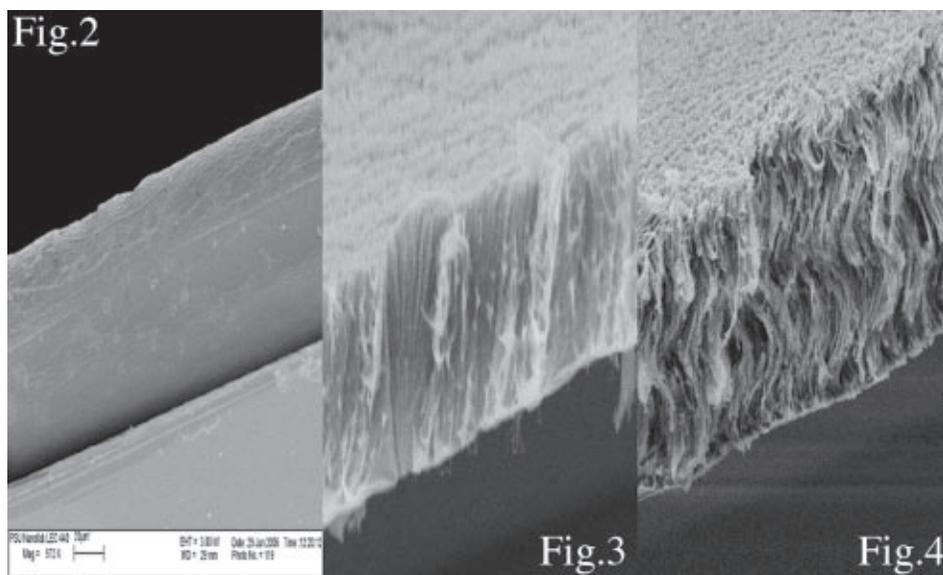
The future directions for this project include finishing the existing project, as well as testing other variables in the scope of this project. The distance between squares where the cells will be transplanted must be analyzed and the data has a likelihood of being cell-dependent. This dependency of the cells and the distance are other avenues of research which might be productive for transplant and synthetic tissue technologies.

Acknowledgements:

Dr. Melik Demirel and Eric So for their help with this project. The staff of the Penn State Nanofabrication Facility. The Cornell NanoScale Facility for their hospitality at the final symposium. The National Science Foundation in conjunction with the National Nanotechnology Infrastructure Network for their funding and support.

References:

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STFs produced at different funnel distances. Figure 2: 1.5 inches. Figure 3: 0.5 inches. Figure 4: 0.5 inches with a rotation of 0.1 revolutions per second.