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BME 590-03

HW 1

Homework 1: Read the attached article (Mrksich et al.). Take notes as you read (#1) and then answer the remaining questions. You can have a general discussion of the paper with others in class, but you must answer the questions independently.

1. **As you read the assigned article, take notes using the template below. Notes do not have to be in complete sentences. Getting in the habit of taking notes as you read scientific papers will help you maximize your understanding and your retention of knowledge. (6 pt)**

Key words (5-10): Microcontact printing (μCP), Self-assembled monolayer (SAM), alkanethiol, fibronectin, cell patterning, gold, silver, adsorption, nonlithographic

Subject: Developing novel and simple methods of patterning SAMs in order to control the locations of cell growth on a surface

Hypothesis: A procedure known as microcontact printing can be utilized to transfer alkanethiolates to gold / silver surfaces, which will selectively adsorb fibronectin depending on the composition of the terminal groups.

Methodology:

Thin films of titanium and gold / silver were first added to glass coverslips by evaporation. A set of slides in which the SAM simply coated the surface (no pattern) were then prepared by immersing the slides in solutions of alkanethiol in ethanol. Next, substrates in which the SAM displayed patterns were created using microcontact printing. This process involves first using photolithography to prepare molds which can be used to produce PDMS "stamps." These stamps are then inked with alkanethiol and pressed upon the slide, transferring alkanethiol to the slide according to pattern on the PDMS. This leaves areas with one type of SAM and areas of bare gold / silver. The slide can then be immersed in a solution containing another alkanethiol, which bears groups resist protein adsorption. This fills in the spaces which did not received a SAM during the microstamping process. This results in a plate in which the SAM which was stamped on will adsorb protein but those which fill in the gaps between these regions will resist adsorption. These plates were then placed in solutions containing fibronectin in PBS buffer to allow for protein adsorption. The plates were then transferred to solutions containing endothelial cells, which attached to the regions with fibronectin. The cell microfilaments were then stained with fluorescein-labelled phalloidin in order to study their arrangement.

Key Results: The overall findings indicate that the μCP process described is a suitable means of generating patterned SAMs. These SAMs were found to be stable on gold for 5-7 days, but degraded in temperatures greater than 100^{o} C or when exposed to UV radiation. The patterned SAMs on silver were found to only remain stable for 2-3 days and the cells attached to fibronectin were found to invade the regions of tri(ethylene glycol)-tipped SAMs, which were supposed to resist protein adsorption and subsequent cell attachment. The SAMs attached to gold were found to stand at roughly 30^{o} to the normal, while the SAMs attached to silver were found to stand perpendicular to the surface.

Significance to the field: This method of patterning SAMs is far more convenient than the previously used method, which was dependent on photolithography. Microcontact printing can be used to control the shape and positions of cells attached to surfaces. If SAMs are tailored so that their terminal groups are ligands which elicit a cellular response, this technology could be highly useful in varying cellular metabolic activity based off a cells location on a surface.

2. **Describe the process of microcontact printing and how it works. (3 pt)**

   The process begins with creating a PDMS stamp from a master pattern. The stamped is then "inked" with an alkenethiol and then brought into contact with the surface of interest. This transfers the alkanethiolates to the surface to form a SAM only in the regions where the stamp touches the surface, creating a pattern. The bare regions not touched by the stamp can then be given a different type of SAM by immersing the surface in a solution containing a different alkanethiol.

3. **Ellipsometry was used to measure protein adsorption. Investigate and describe briefly how this technique works. What information does it provide? How does this relate to the other techniques for assessing protein adsorption that were discussed in class? (6 pt)**

   In general, ellipsometry works by emitting linearly-polarized light onto a surface then recording the polarization of the elliptically-polarized beam reflected off the surface. The difference in polarization between the incident light and reflected light can be used to determine the thickness and optical properties of a film on a surface. This difference in polarization occurs due to interference between light reflecting off the surface and light passing through the film. The

other two major methods of characterizing a surface film are SPR and QCM. SPR is also an optical analytical method, although it functions by measuring the change in refractive index of a surface. QCM is an acoustic method that measures change in the resonance frequency when the quartz crystal is subject to an oscillating electrical field. This frequency changes as the mass increases due to protein adsorption. Ellipsometry is cheaper and faster than both of these methods however it can only be conducted on flat surfaces with homogeneously absorbed layers. The other two methods also tend to have a slightly higher sensitivity.

4. Explain the results presented in figure 3. (3 pt)

Figure 3 displays the results of an experiment in which surfaces containing a SAM of hexadecanethioloate were placed in solutions containing varying concentrations of fibronectin for 24 hrs. The resulting thickness of adsorbed protein was recorded for each surface. It was determined that letting the slides sit for 2 hours in a solution with a fibronectin concentration of 25 μg / mL provided an adequately dense protein layer while minimizing protein waste. For all concentrations greater than 0.5 μg / mL, the thickness of the of adsorbed protein was found to either decrease or remain the same after 2 hours. Looking at the 2 hour mark, increasing the concentration from 0.5 μg / mL to 5 μg / mL was found to produce almost a ten-fold increase in thickness. Increasing the concentration from 5 μg / mL to 25 μg / mL resulted in an increase of around 10 angstroms. Increasing the concentration from 25 μg / mL to 50 μg / mL was found to result in an increase of a few angstroms and increasing the concentration from 50 to 500 μg / mL was found to result in a negligible increase in thickness.

5. Explain why cells grow in the patterns shown in figure 6. (3 pt)

The SAMs have been attached to the slide such that there is a pattern of a methyl-terminated SAM, with the space in between being filled in by a tri(ethylene glycol)-terminated SAM. The methyl-terminated SAM promotes protein adsorption while the tri(ethylene glycol)-terminated SAM resists it. Therefore, after immersion in the protein solution there exists a pattern of adsorbed fibronectin on the slides. The cells attach to and grow along these lines of adsorbed fibronectin. In images C and D, however, the cells seem to slightly protrude into the tri(ethylene glycol)-terminated SAM region at the edges of the pattern. The authors note that this is likely due to the cell layer extending off of itself rather than the cells actually attaching to the tri(ethylene glycol)-terminated SAM regions.