

**3.051J/20.340J Problem Set 4**  
**due 3/23/06**

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1) (25 pts) There is wide interest in the development of microarrays of antibodies that would enable high-throughput analysis of proteins for diagnostic applications (proteomics). Microcontact printing of proteins has been considered as a potential method for inexpensive and rapid fabrication of such structures. In a recent article, Chen and coworkers (J.L. Tan et al., *Langmuir* **2002**, *18*, 519) investigated how the surface chemistry of the substrate and stamp influences the ability to print proteins. Download and read the article, then address the following questions.

a) In this work, gold surfaces were first coated with mixed self-assembled monolayers (SAMs) having different ratios of  $-\text{CH}_3$  terminated and  $-\text{COOH}$ ,  $-\text{OH}$ , or  $(\text{OCH}_2\text{CH}_2)_6\text{OH}$  ( $-\text{EG}_6$ ) terminated alkane thiols adsorbed from ethanol solutions. Explain what drives the formation of a self-assembled monolayer on gold.

b) Advancing water contact angles were measured as a function of the fraction of hydrophilic thiol used in the ethanol solution, as shown in the figure below. Provide an explanation for the deviations from linearity observed for each mixed monolayer system.

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See Fig. 3(d) in Tan, J. L., J. Tien, and C. S. Chen. "Microcontact Printing of Proteins on Mixed Self-Assembled Monolayers." *Langmuir* 18 (2002): 519-523.

c) To transfer proteins onto the mixed SAM surfaces in patterns, a PDMS stamp was fabricated and fluorescently-labeled immunoglobulin G (IgG) was adsorbed onto the stamp surface. Upon subsequently contacting the stamp to the SAM surfaces, they found that protein transfer only took place above a critical hydrophilic thiol content. Results for SAMs incorporating  $-\text{COOH}$  terminated thiols are shown in the figure below. Provide an explanation for why protein transfer occurred only as the surfaces were made more hydrophilic.

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See Fig. 2 in Tan, J. L., J. Tien, and C. S. Chen. "Microcontact Printing of Proteins on Mixed Self-Assembled Monolayers." *Langmuir* 18 (2002): 519-523.

d) For the three mixed SAM systems studied, the lowest fraction of hydrophilic thiol at which protein transfer began to take place was 50%, 55% and 2.5% for  $-\text{COOH}$ ,  $-\text{OH}$ , and  $-\text{EG}_6$  thiols, respectively. Use the contact angle data to calculate the work of adhesion of water to the mixed SAM surface at these compositions, and compare these values to the work of adhesion for water on the PDMS stamp. Why might work of adhesion of water be correlated with protein transfer, as suggested by the authors?

e) The authors next modified the surface of the PDMS stamp, to understand the effects of stamp chemistry on protein transfer. To do so, the PDMS stamp was first oxidized using an air plasma. After plasma treatment, the stamps were treated with (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane vapor or aminopropyl trimethoxysilane and 50 mmol HCl in ethanol (see Materials and Methods). Provide the expected reactions that would result in  $-\text{CF}_3$  and  $-\text{NH}_2$  modified surfaces, respectively.

f) Using the modified stamps, the fraction of  $-\text{COOH}$  thiols required for protein transfer changed to 0% for  $-\text{CF}_3$  modified stamps and 80% for  $-\text{NH}_2$  modified stamps (compared with 55% for unmodified stamps). Provide an explanation for these findings.

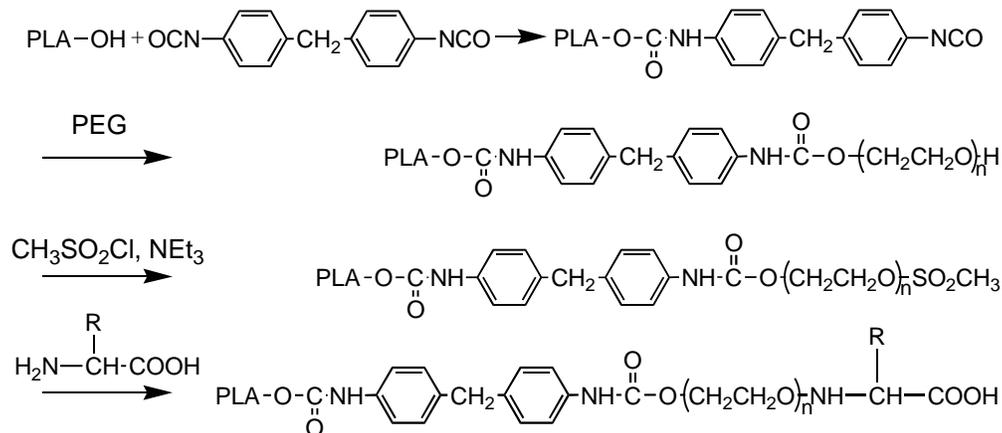
g) Would you expect the  $-\text{CF}_3$  and  $-\text{NH}_2$  modified stamp surfaces to be stable in air? Why or why not?

2. (15 pts) Polylactide (PLA) is used in biomedical applications due to its physicochemical properties and degradability. For tissue engineering applications, however, cell adhesion and proliferation on PLA is generally poor. To enhance cartilage cell adhesion to PLA scaffolds, Yu et al. (*J. Biomed. Res. Part B: Appl. Biomater*, **76B**, 64, 2006) developed a block copolymer of PLA and poly(ethylene glycol) (PEG) to be used as an additive in PLA. To induce adhesion of chondrocytes, various amino acids or the peptide RGD were coupled to the free PEG chain end of the PLA-PEG block copolymer (referred to as PLE).

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a) In the synthesis of the block copolymer (PLE), the -OH terminus of PLA (0.83 mmol eq.) was first coupled with 4,4'-methylenebisphenyl diisocyanate (MDI, 0.89 mmol) and subsequently reacted with 0.37 mmol of PEG which had -OH terminal groups at each end. What undesirable product(s) might be obtained in the reaction with this stoichiometry?



Scheme 1. Synthetic pathways of peptide-bearing PLA-PEG diblock copolymer

b) To couple amino acid(s) onto the hydroxyl group of the PEG chain end, the -OH should first be activated, and methanesulfonyl chloride was employed with triethylamine ( $\text{NEt}_3$ ) in this research. Explain the role of  $\text{NEt}_3$  in this reaction. Is there an alternative reagent to enhance the OH activation?

The figure below shows chondrocyte proliferation (percentage of cells originally seeded adhered to surface after 72 h) on films of neat PLA and PLA modified by adding a fraction of PLE62 (6000 g/mol PLA, 2000 g/mol PEG) or a PLE62 derivative to the film casting solution. Films were made by casting polymer solutions into a round glass mold and allowing the solvent (methyl chloride) to evaporate in air over 24 h. PLE62 derivatives employed as additives included PLE62 end-modified with the amino acid(s) *L*-aspartic acid (PLE62asp), *L*-arginine (PLE62arg), or *L*-lysine (PLE62lys), or the peptide sequence RGD (PLE62 RGD). As a control, chondrocytes were also seeded on tissue culture polystyrene (TCPS), known to promote cell attachment.

c) From the data below, films of PLA incorporating PLE62 showed similar levels of cell attachment/proliferation as PLA. Provide an explanation for these results.

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See Fig. 10(b) in Yu, Guanhua, Jian Ji, Huiguang Zhu, and Jiacong Shen.

"Poly(D,L-lactic acid)-block-(ligand-tethered poly(ethylene glycol)) Copolymers as Surface Additives for Promoting Chondrocyte Attachment and Growth." *J Biomed Mat Res Part B: Appl Biomater* 76B (2006): 64-75.

d) The data also show that films modified with PLE62arg and PLE62lys exhibited higher cell adhesion/proliferation than films modified with PLE62asp. Explain why this might be the case. Would these systems form bonds with cell surface integrins?

e) Only PLA modified with PLE62 RGD demonstrated measurable cell proliferation (values >100%). Why might this system exhibit improved proliferation of chondrocytes?