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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) (2 pts) 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 $(\text{OCH}_2\text{CH}_2)\text{OH}$ ($-\text{EG}_6$) terminated alkane thiols adsorbed from ethanol solutions. Explain what drives the formation of a self-assembled monolayer on gold.

A SAM is formed on gold by the favorable exothermic reaction of thiol groups with gold atoms on the surface, according to:



van der Waal's interactions favor close-packing of the alkyl chains on the surface, with molecules adopting a tilt angle of 30° from the direction normal to the Au surface.

b) (5 pts) 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.

For the $-\text{OH}$ and $-\text{COOH}$ mixed SAMs, there is a negative deviation from linearity of $\cos \theta$ values observed at low fractions of hydrophilic thiol. This might be explained by a slight adsorption preference of $-\text{CH}_3$ terminated alkane thiols over $-\text{OH}$ and $-\text{COOH}$ terminated thiols.

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OH or –COOH terminated thiols, which might occur due to higher solubility of the hydrophilic thiols in ethanol, thereby reducing their relative surface affinity. For the EG₆ terminated SAMs, a positive deviation from linearity is observed. For this system, the EG₆-terminated thiols are substantially longer than the –CH₃ terminated thiols, and consequently will overcoat the –CH₃ end groups even at low surface fractions, resulting in increased wettability and the positive deviation from linearity. At concentrations above 30% EG₆ thiol, the –CH₃ thiols are fully covered by the EG chains and the contact angle remains constant.

c) (3 pts) 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 –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.

For the protein to transfer, the work of adhesion of the protein to the PDMS stamp surface must be overcome, i.e., the work of adhesion must be higher between the SAM surface and the protein. The –CH₃ terminated SAMs would have a low work of adhesion with IgG because their secondary bonds form through weak dispersive forces. By incorporating polar groups –COOH, –OH, and ether oxygens (EG₆), hydrogen-bonding between the protein and SAM can occur, resulting in a sufficiently high work of adhesion with the protein to overcome its adhesion to PDMS.

d) (4 pts) 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 –COOH, –OH, and –EG₆ 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?

Work of adhesion of water to the substrate is calculated as:

$$W_{adh} = \gamma_w (1 + \cos \theta)$$

where γ_w is the surface tension of water (73 dyn/cm), and θ is the contact angle of water on the substrate. Using the $\cos \theta$ values provided in the article, $W_{adh} = 70, 78$ and 67 mJ/m^2 for the $-\text{COOH}$ (50%), $-\text{OH}$ (55%) and $-\text{EG}_6$ (2.5%) SAM systems, respectively. These values are similar, suggesting a critical level of hydrogen bonding. By comparison, the work of adhesion of water on the PDMS stamp is 46 mJ/m^2 , based on contact angle measurement. Since water bonds to polar surfaces through similar mechanisms as proteins, W_{adh} values for water can be a useful metric for assessing the whether protein transfer will take place on a given surface.

e) (5 pts) 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.

Air is a gaseous mixture of N_2 , O_2 , and in lesser amounts CO_2 , H_2 , and inert gases. The surface of PDMS can thus be expected to exhibit diverse chemical groups after plasma oxidation, including $-\text{Si-OH}$ groups formed by reactions with the PDMS chains. Trichlorosilanes and trialkoxysilanes readily react with hydroxyl groups:



f) (3 pts) 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.

For fluorinated stamps, the work of adhesion of the protein to the stamp surface is likely to be lower than for PDMS. The protein can thus form stronger secondary bonds with the $-\text{CH}_3$ SAM surface, allowing transfer even in the absence of more polar groups. For the amine terminated surfaces, the opposite is true. Proteins can form stronger secondary bonds with the stamp, so that a larger number of polar groups on the SAM surface is required to effectively overcome the higher work of adhesion to the stamp.

The fluorinated stamp has a work of adhesion with water of 36.5 J/cm^3 . For the 100% $-\text{CH}_3$ terminated SAM, the work of adhesion of water is calculated to be 45 mJ/m^2 , suggesting that the $-\text{CH}_3$ SAM interface would also have stronger bonding with the protein. For the $-\text{NH}_2$ modified stamp, $W_{adh,\text{H}_2\text{O}} = 137 \text{ mJ/m}^2$, while the

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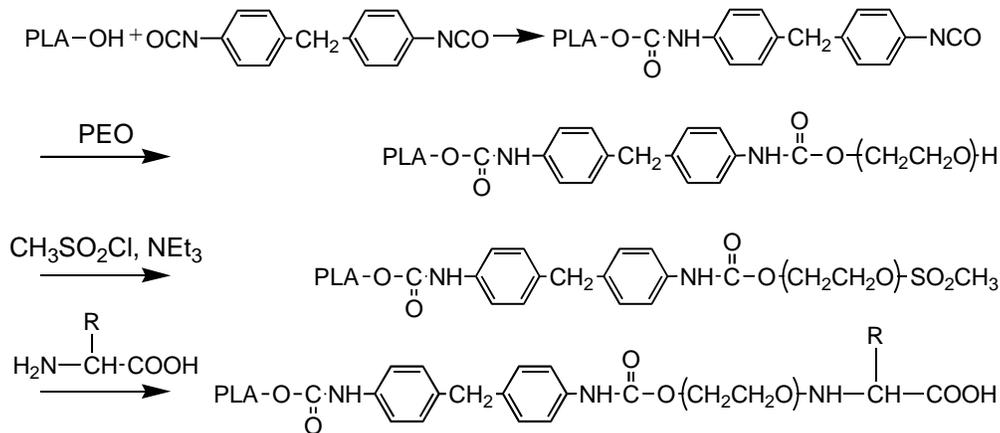
80% -COOH mixed SAM has a $W_{\text{adh,H}_2\text{O}} = 117 \text{ mJ/m}^2$, which is lower but in the same range.

g) (3 pts) Would you expect the $-\text{CF}_3$ and $-\text{NH}_2$ modified stamp surfaces to be stable in air? Why or why not?

For the fluorinated stamp, the surface should be stable in air to reconstruction because the fluorocarbons exhibit a lower surface energy than PDMS and are thus preferred at the surface. For the $-\text{NH}_2$ modified surfaces, the surface energy is higher and one might expect the surface to reconstruct over time in air, burying the higher energy amines.

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).

a) (3 pts) In the synthesis of the block copolymer (PLE), the $-\text{OH}$ terminus of PLA (0.83 mmol eq.) was first coupled with 4,4'-methylenediphenyl diisocyanate (MDI, 0.89 mmol) and subsequently reacted with 0.37 mmol of PEG (PEO) which had $-\text{OH}$ terminal groups at each end. What undesirable product(s) might be obtained in the reaction with this stoichiometry?

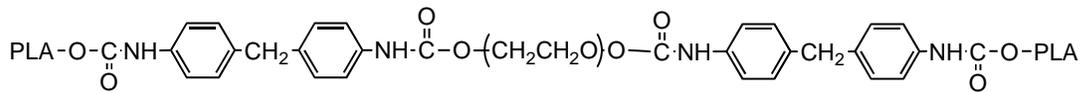


Scheme 1. Synthetic pathways of peptide-bearing PLA-*b*-PEO

Since the molar quantity of PEG was about half that of PLA, both $-\text{OH}$ ends of PEG could react with the isocyanate groups from MDI to form PLA-PEG-PLA triblock copolymer, in addition to the PLA-PEG diblocks desired for surface modification.

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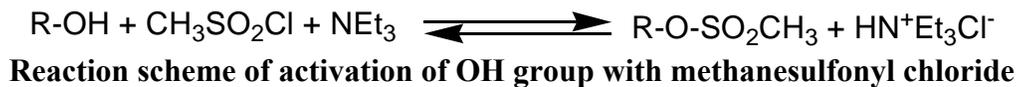
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Chemical structure of PLA-PEO-PLA triblock copolymer

b) (4 pts) To couple amino acid(s) onto the hydroxyl group of the PEO chain end, the -OH should first be activated, and methanesulfonyl chloride was employed with triethylamine (NEt_3) in this research. Explain the role of NEt_3 in this reaction. Is there an alternative reagent to enhance the OH activation?

The by-product of this reaction is HCl. To increase the forward reaction rate, the HCl concentration should be decreased. NEt_3 can trap HCl with the formation of an organic salt. Tresyl chloride ($\text{CF}_3\text{CH}_2\text{SO}_2\text{Cl}$) can be used as an alternative with higher activity.



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) (3 pts) 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.

The results suggest that the film surface is not coated with the PEG component of the PLE block copolymer. If the surface were predominantly PEG (PEO), a significantly lower level of cell attachment would be expected, since PEO resists adsorption of proteins that facilitate cell attachment to surfaces. This could be the case because PEO has a higher surface energy than PLA, and the films were dried in air.

d) (3 pts) 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?

Arg and Lys both have positively charged side groups while Asp has a negatively charged side group. Since the many adhesion proteins as well as cell surfaces are negatively charged, the positively charged PLE62 additives would lead to an additional electrostatic attraction of proteins or cells to the PLA film. These single amino acid chain ends would not bond specifically with cell integrins, whose ligands are typically several amino acid units long.

e) (2 pts) Only the PLA modified with PLE62 RGD demonstrated measurable cell proliferation (values >100%). Provide an explanation for this finding.

Proliferation requires cells to first adhere and spread on a surface. The peptide sequence RGD is a well known ligand for cell integrins, which mediate cell attachment. The increased cell adhesion to this surface further implies that attaching RGD to PEG facilitates localization of the PEG block at the surface.