

3.051J/BE.340 Problem Set 5
due 4/13/06

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1) (20 pts) Surfaces that guide the growth of neuronal axons are of interest for reconstructing severed nerve tissues, and for the fabrication of neuronal networks on microelectrode arrays to study biological response to toxins and pharmaceuticals. In a recent effort, Beebe and coworkers (Z. Zhang et al., *Biomaterials* **2005**, 26, 47) studied neurite outgrowth on glass surfaces treated with different surface modifications.

In one surface modification, the glass surface was first reacted with 3-mercaptopropyl trimethoxysilane (MTS) and subsequently with N- γ -maleimidobutyryloxy succinimide ester (GMBS), to allow coupling of peptides and proteins, as shown in the scheme below.

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See Fig. 1(a) in Zhang, Zhanping, Raphael Yoo, Matthew Wells, Thomas P. Beebe, Jr., Roy Biran, and Patrick Tresco. "Neurite outgrowth on well-characterized surfaces: preparation and characterization of chemically and spatially controlled fibronectin and RGD substrates with good bioactivity." *Biomaterials* 26 (2005): 47-61.

XPS was performed on the surface after each step of the surface modification. High resolution C_{1s} spectra were deconvoluted into contributions from different bond configurations. For the MTS-modified surface, 2 peaks were found with binding energies of 284.6 eV (58%) and 285.7 eV (42%). After modification with GMBS, four C_{1s} peak contributions were resolved, at energies 284.6 eV (28%), 285.7 eV (52%), 287.0 eV (13%) and 289.1 eV (7%).

a) What chemical groups contribute to each of the peaks observed in the XPS data for these samples?

b) Compare the observed peak ratio for the MTS-modified surface with the ratio you would expect from the chemical scheme proposed. What might explain the discrepancies?

c) For the GMBS-modified surface, does the XPS spectrum support the proposed chemical scheme? Explain.

d) Static SIMS measurements were also performed on the GMBS-modified surface. A portion of the positive ion spectrum is shown below. From the reported surface

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chemistry, provide all possible chemical fragments that might contribute to the 6 highest peaks of the spectra (assume a +1 charge).

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See Fig. 3(a), second image, in Zhang, Zhanping, Raphael Yoo, Matthew Wells, Thomas P. Beebe, Jr., Roy Biran, and Patrick Tresco. "Neurite outgrowth on well-characterized surfaces: preparation and characterization of chemically and spatially controlled fibronectin and RGD substrates with good bioactivity." *Biomaterials* 26 (2005): 47-61.

e) Following reaction with GMBS, the peptide GRGDSY or the protein fibronectin were covalently coupled to the surface. Neurite outgrowth on these surfaces was compared to that of the GMBS-modified surface alone. Data for neurite length after 72 h in serum free media is shown in the figure below. Outgrowth on the RGD-coupled surface in the presence of soluble RGD was also measured.

i) Neurite outgrowth was more prominent on the fibronectin-coupled surface compared with the RGD-coupled surface. What might explain this result?

ii) When soluble RGD peptide was added to the medium of neurons seeded on the RGD-coupled surface, the neurite length was reduced to a value comparable to the GMBS-modified surface. Explain this finding.

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See Fig. 7 in Zhang, Zhanping, Raphael Yoo, Matthew Wells, Thomas P. Beebe, Jr., Roy Biran, and Patrick Tresco. "Neurite outgrowth on well-characterized surfaces: preparation and characterization of chemically and spatially controlled fibronectin and RGD substrates with good bioactivity." *Biomaterials* 26 (2005): 47-61.

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2. (12 pts) Venous catheters are used in patients who require long-term IV therapy, such as chemotherapy or hemodialysis. Such catheters are highly susceptible to clot formation. In an attempt to reduce risk of clot formation, Byun and coworkers (O.D. Krishna et al., *Biomaterials* **2005**, *26*, 7115) covalently grafted a phospholipid layer onto a silicone catheter and performed platelet adhesion studies on the unmodified and modified catheters. Grafting was achieved by first performing plasma polymerization of allyl alcohol ($\text{H}_2\text{C}=\text{CHCH}_2\text{OH}$), followed by reaction of resulting $-\text{OH}$ surface groups with acryloyl chloride in 5% solution in tetrahydrofuran for 7 h to create vinyl groups on the surface. Finally, monoacrylated phospholipids (1stearoyl-2-[12-(acryloyloxy) dodecanoyl]-sn-glycero-3-phosphocholine) were covalently bonded to the surface by assembling vesicles of the acrylated phospholipid on the surface and heating at 80°C for 15 min. A schematic of the surface modification procedure is shown below.

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See Fig. 1 in Krishna, Ohm D., Kwangmeyung Kim, and Youngro Byun. "Covalently grafted phospholipid monolayer on silicone catheter surface for reduction in platelet adhesion." *Biomaterials* 26 (2005): 7115-7123.

XPS was performed on the initial silicone surface, and the surface after each step of the modification. The table below provides atomic concentrations determined from the low resolution spectra for samples measured at a take-off angle of 45° using Mg K_α radiation ($E=1254$ eV). (Si 2p, C 1s, O 1s and N1s electrons).

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See Table 1 in Krishna, Ohm D., Kwangmeyung Kim, and Youngro Byun. "Covalently grafted phospholipid monolayer on silicone catheter surface for reduction in platelet adhesion." *Biomaterials* 26 (2005): 7115-7123.

a) The Si concentration is first observed to decrease after plasma polymerization of allyl alcohol (Sil-Al) and then to increase after reaction with acryloyl chloride (Sil-Al-Ac). Provide a likely explanation for these results.

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b) Using the data provided, estimate the thickness of the grafted phospholipid (PC) layer.

c) Based on your result from (b), does the PC layer grafted on the silicone catheter have a comparable packing density to lipid layers within a cell membrane, as suggested in the schematic?

3. (8 pts) As a novel strategy for nanoscale patterning of proteins on SAM surfaces, G.Y. Liu developed an approach utilizing the AFM (K. Wadu-Mesthrige et al., *Biophysical J.* **2001**, *80*, 1891). In an aqueous medium containing 1 mM mercaptopropanoic acid (MPA) ($\text{HSCH}_2\text{CH}_2\text{COOH}$), a large constant force (10-30 nN) was applied as the tip rastered across a region of specified dimension of a decanethiol ($\text{HS}(\text{CH}_2)_9\text{CH}_3$) SAM. The high force disrupted the gold-thiol bonds, causing decanethiol molecules to be released from the region. The exposed gold regions were subsequently coated by mercaptopropanoic acid molecules in the medium. Finally, the surface was exposed to 10 $\mu\text{g}/\text{ml}$ solution of lysozyme (LYZ), a positively charged protein at pH 7 with approximate dimensions $4.5 \times 3 \times 3 \text{ nm}^3$, to obtain the surface shown in (C) below.

a) In this strategy, why are the released decanethiol molecules displaced by MPA, rather than re-adsorbing to the exposed Au surface?

b) Why are the lysozyme molecules observed to adsorb only to the regions patterned with MPA molecules?

c) LYZ molecules adsorbed to the patterned line appear to adopt a different orientation than those adsorbed in the rectangular region. What could account for this difference?

d) Do the LYZ molecules appear to be denatured on the MPA surface? Explain your reasoning.

Images removed for copyright reasons.

See Fig. 2 in Wadu-Mesthrige, Kapila, Nabil A. Amro, Jayne C. Garno, Song Xu, and Gang-yu Liu. "Fabrication of Nanometer-Sized Protein Patterns Using Atomic Force Microscopy and Selective Immobilization." *Biophysical Journal* 80 (2001): 1891-1899.

A) $400 \times 400 \text{ nm}^2$ image of initial decanethiol SAM; B) a $10 \times 150 \text{ nm}^2$ line and $100 \times 150 \text{ nm}^2$ rectangle of MPA written into decanethiol SAM; C) region after exposure to LYZ solution; D) cross-section profiles taken from the white lines on images (B) and (C). The y-origin represents the Au surface.