

## Solutions

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- $\gamma$ -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) (5 pts) What chemical groups contribute to each of the peaks observed in the XPS data for these samples?

**For the MTS-modified surface:**

**284.6 eV: SiCH<sub>2</sub>CH<sub>2</sub>C**

**285.7 eV: OCH<sub>3</sub>, CCH<sub>2</sub>S**

**For the GMBS-modified surface:**

**284.6 eV: SiCH<sub>2</sub>CH<sub>2</sub>C, CCH(CO) (ring), NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>COO, CCH<sub>2</sub>CH<sub>2</sub>C (ring)**

**285.7 eV: OCH<sub>3</sub>, CCH<sub>2</sub>S, SCCH(CO) (ring), NCH<sub>2</sub>CH<sub>2</sub>**

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287.0 eV: CCON (rings)

289.1 eV: CCOO

b) (3 pts) 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?

**For the MTS-modified surface, the expected ratio 284.6 eV: 285.7 eV is 2:3 based on stoichiometry, while the observed is closer to 3:2. The deviation may indicate hydrocarbon surface contamination or loss of  $-OCH_3$  side groups of the silane, e.g., through formation of an Si-O-Si linkage with a neighboring coupled MTS group.**

c) (2 pts) For the GMBS-modified surface, does the XPS spectrum support the proposed chemical scheme? Explain.

**For the GMBS-modified surface the expected ratio 284.6 eV: 285.7 eV: 287.0 eV: 289.1 eV is 7:5:4:1 based on the proposed chemistry, while the observed ratio is 4:7.5:2:1. The large deviation in the expected and observed peak ratios suggest the surface modification did not yield the proposed chemical structure. The 2:1 ratio of CON to COO groups suggests that the ester group may have hydrolyzed to form a terminal COOH.**

d) (6 pts) 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 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.

77 amu:  $C_5NH_3$

79 amu:  $C_4NOH$ ,  $C_5NH_5$

81 amu:  $C_4O_2H$ ,  $C_4NOH_3$ ,  $SiOC_3H$ ,  $SC_4H$

83 amu:  $C_4O_2H_3$ ,  $C_4NOH_5$ ,  $SiOC_3H_3$ ,  $SC_4H_3$

91 amu:  $C_6NH_5$ ,  $C_5NOH$

95 amu:  $C_4O_2NH$ ,  $C_5NOH_5$ ,  $SC_4NH$

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

**Fibronectin has other adhesion sites besides the RGD sequence that could promote axon outgrowth.**

ii) (2 pts) 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.

**Soluble RGD competes with coupled RGD in complexing with neuron integrins, causing bonds to the surface to be reduced, and thereby limiting axon outgrowth.**

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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.

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  $80^\circ\text{C}$  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  $45^\circ$  using Mg  $K_\alpha$  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) (3 pts) The Si concentration is first observed to decrease after plasma polymerization of allyl alcohol (SiI-Al) and then to increase after reaction with acryloyl chloride (SiI-Al-Ac). Provide a likely explanation for these results.

**The reduction in Si signal is due to the polymerized allyl alcohol layer (Al) created on the silicone surface. This layer decreases the probability that Si 2p electrons are able to escape from the surface to be detected. The increase in Si concentration after reaction with acryloyl chloride suggests that the thickness of the Al layer has decreased during the reaction. This is possible because plasma polymerization often leads to unbound polymer molecules intermixed with grafted molecules. The reaction with acryloyl chloride was performed in organic solvent (THF) over an**

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extended period, which allowed for the dissolution of polymers in the Al layer that were not covalently grafted.

b) (7 pts) Using the data provided, estimate the thickness of the grafted phospholipid (PC) layer.

To obtain a measure of the PC layer thickness, we can model the Sil-Al-Ac and Sil-Al-AcPC samples as 2 layers (Sil (2) and Al-Ac (1) or Sil (2) and Al-AcPC (1)) to determine the thickness of the Al-Ac and Al-AcPC layers. Taking the difference of these thicknesses then provides an estimate for the PC layer thickness.

We can perform this calculation using the Si 2p signal, which arises solely from the bottom silicone layer:

$$I_{Si2p} = I_{Si2p,\infty}^2 \exp\left(\frac{-d}{\lambda_{e,1} \sin \theta}\right)$$

Rearranging this expression:  $d = -\lambda_{e,1} \sin \theta \ln \frac{I_{Si2p}}{I_{Si2p,\infty}^{(2)}}$

for polymers:  $\lambda_{e,1}(nm) = \rho^{-1}(49E_{kin}^{-2} + 0.11E_{kin}^{.5})$

$E_B = hv - E_{kin}$  for MgK $_{\alpha}$  x-rays:  $hv = 1254 eV$

$E_{B,Si2p} = 102 eV$

$\rightarrow E_{kin} = 1152 eV$

$\lambda_{e,1}(nm) = 3.73\rho^{-1}$

If we assume a polymer density of 1.1 g/cm<sup>3</sup> then  $\lambda_{e,1} = 3.4 nm$

For the Si-Al-Ac sample:  $d = -3.4 \sin 45 \ln \frac{13}{21.9} = 1.3 nm$

For the Si-Al-AcPC sample:  $d = -3.4 \sin 45 \ln \frac{10.5}{21.9} = 1.8 nm$

The PC layer thickness is therefore estimated to be 0.5 nm thick.

c) (2 pts) 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?

The phospholipids are much longer than 0.5 nm when the tails are extended, as would be expected in a dense-packed configuration. The grafted layer appears to

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have significantly lower packing density than a cell membrane. A typical cell membrane phospholipid bilayer is ~ 5-10 nm thick, so a monolayer would be ~ 2.5-5 nm thick.

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-Mesthridge et al., *Biophysical J.* **2001**, 80, 1891). In an aqueous medium containing 1 mM mercaptopropionic 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 mercaptopropionic acid molecules in the medium. Finally, the surface was exposed to 10  $\mu\text{g/ml}$  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) (2 pts) In this strategy, why are the released decanethiol molecules displaced by MPA, rather than re-adsorbing to the exposed Au surface?

**The MPA molecules are in much higher concentration in solution than the decanethiol molecules, and also able to diffuse faster, due to their smaller size.**

b) (2 pts) Why are the lysozyme molecules observed to adsorb only to the regions patterned with MPA molecules?

**The MPA regions are terminated with  $-\text{COOH}$  groups. At pH 7, the MPA regions exhibit a partial negative charge. Positively charged LYZ shows a high affinity for these regions due to electrostatic attraction. The hydrophobic decanethiol regions of the surface would also be susceptible to protein adsorption, but apparently with lower affinity than to the MPA surface. The concentration of LYZ in solution (10  $\mu\text{g/ml}$ ) was too low to observe any molecules adsorbed on the decanethiol SAM within the  $400 \times 400 \text{ nm}^2$  image. At higher solution concentrations, adsorption on the hydrophobic regions would be expected.**

c) (2 pts) 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?

**The patterned line has a width of 10 nm, on the same scale as the LYZ dimensions. Therefore, LYZ molecules adsorbed in the line are likely to interact with the hydrophobic regions of the decanethiol molecules that make up the “walls” of the patterned groove. Interactions with hydrophobic regions may favor a vertical orientation of the proteins.**

d) (2 pts) Do the LYZ molecules appear to be denatured on the MPA surface? Explain your reasoning.

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**The two feature heights observed in (D) (3.0 nm and 4.3 nm) are consistent with the reported globular dimensions of LYZ, which suggests the molecules are adsorbed substantially intact.**

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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.