

3.051J/20.340J Problem Set 6
due 4/20/06

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1) (35 pts) Implant-related infection is an important contributor to device failure. Such infections can develop when bacteria such as *Staphylococcus epidermis* resides on the biomaterial surface and is introduced during surgical implantation. In the inflammatory response to tissue injury, the body's natural first line of defense against such bacteria is neutrophils, which arrive at the site of injury via chemotaxis and eliminate invading bacteria by phagocytosis. The ability of neutrophils to migrate across the biomaterial surface may thus be important for control of bacterial infection associated with implants.

To further examine this issue, Marchant and coworkers studied the migration of neutrophils on three model surfaces: a polyurethane (PU) commonly used in cardiovascular devices, a glass slide and a self-assembled monolayer of octadecyltrichlorosilane (OTS) on glass (Y. Zhou et al., *J. Biomed. Mater. Res.* **2004**, *69A*, 611).

Download and read the article, then address the following questions.

The data below shows the trajectory of a single neutrophil on the polyurethane surface in the absence of serum proteins and the neutrophil activator N-formylmethionyl-leucyl-phenylalanine (fMLP), determined from time-lapse optical microscopy. Data was collected in 30 sec intervals over 10 minutes.

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See Fig. 1(a) in Zhou, Yue, Claire M. Doerschuk, James M. Anderson, and Roger E. Marchant. "Biomaterial Surface-dependent Neutrophil Mobility." *Journal of Biomedical Materials Research* *69A* (2004): 611-620.

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- a) From the cell data provided, determine the speed and persistence time of the neutrophils on PU, assuming the persistent random walk model. Give a detailed description of your methodology. How do your results compare with those reported by Zhou et al.?
- b) Neutrophils were found to exhibit substantially higher speeds on all 3 surfaces in the presence of plasma proteins. Explain why this might be the case.
- c) AFM measurements were performed on living neutrophils moving on the 3 surfaces in buffer solution (no plasma proteins or fMLP in the medium) (Figure 7). For the PU and glass surfaces, the trailing end of cells (uropods) were long and left significant cell “debris”, while neutrophils on the OTS surface showed cleaner detachment.
- i) Could the observed debris be the result of damage by the AFM tip? Why or why not?
- ii) Explain why the OTS surface might show less debris accumulation.
- d) From the fluorescence microscopy images from Figure 5d-f, estimate the cell spreading area on the 3 surfaces in the presence of plasma proteins and fMLP. Explain the methodology you use. Why might neutrophils on the OTS surface exhibit higher spreading area? What do the results suggest about the relative adhesion strengths for neutrophils on these surfaces?

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See Fig. 5(d), (e), and (f) in Zhou, Yue, Claire M. Doerschuk, James M. Anderson, and Roger E. Marchant. "Biomaterial surface-dependent neutrophil mobility. *Journal of Biomedical Materials Research* 69A (2004): 611-620.

- e) Why do the images in these figures exhibit fluorescence in two different colors in different regions of the cell?
- f) Zhou et al. compared neutrophil spreading areas on the 3 surfaces under 4 different conditions (Table 1).

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See Table 1 in Zhou, Yue, Claire M. Doerschuk, James M. Anderson, and Roger E. Marchant. "Biomaterial Surface-dependent Neutrophil Mobility." *Journal of Biomedical Materials Research* 69A (2004): 611-620.

- i) Is there a statistically significant difference between measured spreading areas on any of the surfaces in the presence of proteins but without fMLP added? (show your analysis)
- ii) Provide the 95% confidence interval for the universal mean for neutrophil spreading on PU in the presence of both fMLP and plasma proteins.
- iii) Which of the 4 conditions tested would you expect to be most relevant to *in vivo* conditions? Explain.
- g) Fig. 6 of the paper shows that neutrophil speed decreases linearly with increasing spreading area for activated neutrophils. Explain this finding.
- h) Based on the results of this paper, what surface chemistries are optimal for reducing risk of bacterial infection?