3.051J/20.340J   Problem Set 6
Solutions

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.

Image removed for copyright reasons.
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.

a) (8 pts) 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.?

To obtain data that could be fitted to the persistent random walk model, the cell trajectory was broken into steps of size jΔt, where Δt=0.5 min. The mean squared distance traveled for j=1-6 was plotted against time (minimum 3 data points per avg.). The line of best fit for the longer time intervals (2-3 min) was then used to calculate the persistence time (P) and the speed (S).
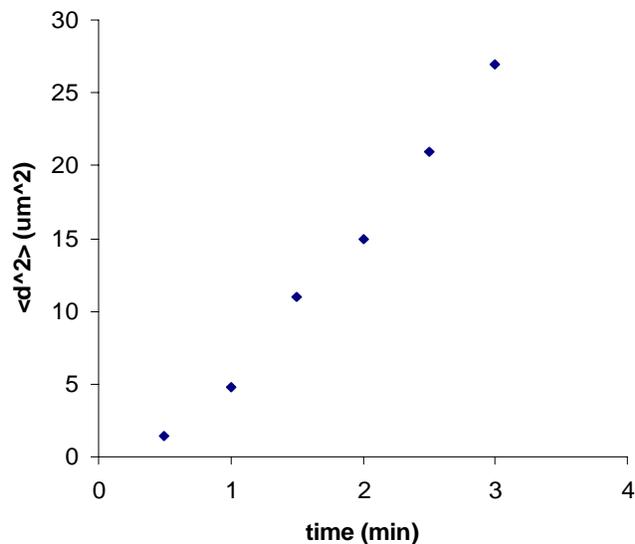
For long times, t>>P:

$$\langle d(t)^2 \rangle = nS^2Pt - nS^2P^2$$

$$\langle d(t)^2 \rangle = 12t - 9 \ (\mu m^2)$$

$$\frac{\text{-intercept}}{\text{slope}} = P = \frac{9}{12} = 0.75 \ \text{min}$$

$$S = \sqrt{\text{slope}/2P} = \sqrt{12/(2 \times 0.75)} = 2.8 \mu m / \text{min}$$

	M=20, t=0.5	M=10, t=1	M=6, t=1.5	M=5, t=2	M=4, t=2.5	M=3, t=3
$\langle d^2(t) \rangle$ (µm²)	1.4	4.8	11.	33	45	51

The obtained values for S and P by this method are both somewhat higher than values reported in the text, but of the same magnitude.

b) (2 pts) 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.

Cell migration occurs by lamellipod extension, and the transfer of cell contractile forces to the substrate through "focal contacts" formed in the lamellipod. Focal contacts are made from the clustering of cell integrins as they bond with adhesion proteins on the surface. In media containing plasma proteins, biomaterials surfaces will typically adsorb adhesion proteins, greatly facilitating focal contact formation.

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) (2 pts) Could the observed debris be the result of damage by the AFM tip? Why or why not?

Since the measurements were done in tapping mode, significant shear forces are not encountered by the cells. The debris is therefore not likely to be an artifact of the experimental technique.

ii) (2 pts) Explain why the OTS surface might show less debris accumulation.

The OTS surface is hydrocarbon in nature, and thus can only form weak van der Waals' bonds with the cell surface, compared with the PU and glass surfaces, which can interact with polar groups on the surface of cells. The cells will thus detach more readily from OTS.

d) (5 pts) 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?

Image removed for copyright reasons.

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.

For the estimate, the cell outlines can be approximated as circles or ellipses. The spreading areas are then $\pi D^2/4$ and $\pi D_1 D_2/4$, respectively, where D is the circle diameter and D_1 and D_2 are the major and minor axes of the ellipse. For the three surfaces, the estimated areas obtained from the figure are: 73±18 μm (glass); 45±7 μm (PU); 116±25 μm (OTS).

The higher cell spreading areas on OTS likely reflect enhanced protein deposition and/or denaturing on this surface due to its hydrophobic nature. Higher cell spreading areas are generally correlated with higher adhesion strength, indicating that neutrophils are more strongly adhered to the OTS surface.

e) (2 pts) Why do the images in these figures exhibit fluorescence in two different colors in different regions of the cell?

For these experiments two different fluorescent staining agents were used: ethidium bromide, which stains DNA in the nucleus, resulting in red fluorescence, and FITC-phalloidin, which stains F-actin in the cell body and yields a green fluorescence.

f) Zhou et al. compared neutrophil spreading areas on the 3 surfaces under 4 different conditions (Table 1).

Figure removed for copyright reasons.
 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) (5 pts) 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)

To determine whether the spreading areas are statistically different, we can subject the data to two sample t-tests. We will assume that N=30 for all surfaces, since the paper only specifies that the sample number is greater than 30.

For glass vs. PU:

$$\sigma_p^{\,2} = \frac{(N-1)S_x^{\,2} + (N'-1)S_{x'}^2}{N+N'-2} = \frac{29(11)^2 + 29(7)^2}{58} = 85.$$

$$t = \frac{\langle x \rangle - \langle x' \rangle}{\sigma_p \sqrt{\dfrac{1}{N} + \dfrac{1}{N'}}} = \frac{79 - 88}{(85)^{1/2}(0.0667)^{1/2}} = -3.78$$

Performing similar calculations for

Glass vs. OTS: $\sigma_p^{\,2}$ = 68.5; t = -1.87

PU vs. OTS: $\sigma_p^{\,2}$ = 32.5; t = -3.39

Assuming 95% confidence level, we hypothesize:

$$-t_{0.975} < t < t_{0.975} \qquad \Rightarrow \mu_x = \mu_{x'}$$

Here υ=58 for the pooled population, so that $t_{0.975}$ = 2.00.   Therefore we test:

$$-2.00 < t < 2.00$$

Our t values for glass vs. PU and glass vs. OTS fall outside of this range, indicating a statistically significant difference (95% confidence) between the surfaces. Glass vs. OTS, however, falls inside this range, indicating the universal means of the sample distributions are indistinguishable.

ii) (3 pts) Provide the 95% confidence interval for the universal mean for neutrophil spreading on PU in the presence of both fMLP and plasma proteins.

A confidence interval for small datasets can be computed from:

$$\left(\langle x \rangle - t_{\frac{1+P}{2}} S_m \right) < \mu < \left(\langle x \rangle + t_{\frac{1+P}{2}} S_m \right) \quad \textbf{where} \quad S_m = \frac{S}{\sqrt{N}}$$

For neutrophils on PU with plasma proteins and fMLP in the medium,

$$\left(112 - 2.05 \times \frac{3}{\sqrt{30}} \right) < \mu < \left(112 + 2.05 \times \frac{3}{\sqrt{30}} \right)$$

$$111 < \mu < 113$$

where the number of degrees of freedom ν=29.

iii) (2 pts) Which of the 4 conditions tested would you expect to be most relevant to *in vivo* conditions? Explain.

The experimental condition most relevant to the *in vivo* situation is when the medium contains both plasma proteins and fMLP. The inclusion of fMLP simulates the activation of neutrophils that would occur naturally by molecules released at the site of injury. Plasma proteins will also be abundant at the site of injury.

g) (2 pts) Fig. 6 of the paper shows that neutrophil speed decreases linearly with increasing spreading area for activated neutrophils. Explain this finding.

Cell mobility decreases with increasing cell spreading area because cells that are more spread are more tightly adhered to the surface. In order to migrate, more/stronger secondary bonds between the surface and cell must first be broken.

h) (2 pts) Based on the results of this paper, what surface chemistries are optimal for reducing risk of bacterial infection?

This work suggests that neutrophil migration will be faster on biomaterials surfaces having more polar chemistries, to which neutrophils are less strongly adhered.