Time-Concentration Equivalence of Protein Adsorption Kinetics onto surfaces of Metallic Biomaterials

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INTRODUCTION: Proteins adsorb onto a biomaterial surface from the surrounding fluid phase within seconds (at physiological concentrations) and govern the subsequent response of the body to the biomaterial. It is difficult to measure the kinetics of protein adsorption and the conformation of individual molecules at physiological concentrations.[1] Adsorption of proteins onto surfaces out of single protein solutions at lower concentrations may be equivalent to adsorption at physiological concentrations, however at different time scales, especially if the desorption rate is near zero. It may be possible to equate a long-time/low-concentration adsorption experiment with a short-time/high-concentration one. Fibrinogen (Fb) is a major protein found on the surfaces of implanted biomaterials and plays a vital role in thrombus formation and inflammatory response. Ti-6Al-4V was selected for this study owing to its high biocompatibility and importance in medical device applications. Thus the goal of the current study is to elucidate the time-concentration equivalence of protein adsorption kinetics onto biomaterial surfaces.

METHODS: Fraction I, type I-S Fibrinogen from bovine plasma (Sigma-Aldrich) was suspended in 0.154M phosphate buffered saline (PBS), pH 7.4 at concentrations of 100, 5, 2 and 0.5 μg mL^{-1}. Ti-6Al-4V samples (n=3) were polished using a mechanical-chemical polishing technique as described in a previous paper.[2] A Digital Instruments multi-mode AFM-2 with nanoscope IIIa scanning probe microscopy controller (Veeco Instruments) was used for imaging. Ex situ experiments[2] were performed with sample immersion time varying from 0 to 180 minutes. For each concentration used, at least three images were obtained for each immersion time for each of the three samples (n_{total}=9 for any given combination of immersion time and solution concentration). AFM bearing and section analysis software was used to determine area fraction covered by Fb and height of Fb, respectively. The data was analyzed using a Langmuir adsorption kinetics model; (i) $\theta(t) = \theta(\infty)[1-\exp(-t/\tau)]$ (ii) $\tau = (k_0C+k_1)^{-1}$; where t is the time in min., $\theta(t)$ is the fraction area covered by Fb in time t, $\theta(\infty)$ is the equilibrium fraction area coverage, τ the is time constant, C is protein concentration in μg/ml, k_0 is adsorption rate constant and k_1 is desorption rate constant.

RESULTS: The kinetics of Fb adsorption onto Ti-6Al-4V was measured and modeled. Figure 1 shows a plot of fraction area covered by Fb molecules as a function of time and concentration. A network-like assembly pattern of Fb molecules was observed to form over time for each concentration used. The time required to attain equilibrium coverage varied for each concentration. Figure 2 shows a series of height mode images of Fb adsorption at equilibrium for the four concentrations used. The height of a monolayer of Fb was found to be 2.5 ± 0.5 nm over the concentration range used. No desorption events were observed indicating an irreversible adsorption process, a condition necessary to attain equivalent area coverage and similar assembly pattern. The adsorption rate constant (k_0) was found to be 1.85 [± 0.05] x 10^{-4} (ml/μg•s).

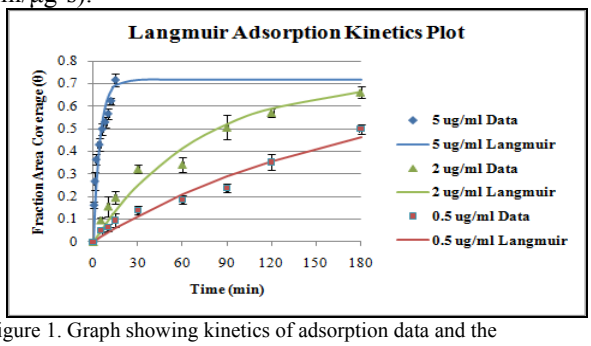
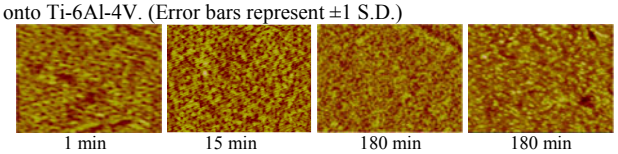


Figure 1. Graph showing kinetics of adsorption data and the corresponding Langmuir Curve fits for 5, 2 and 0.5 μg/ml Fb adsorption onto Ti-6Al-4V. (Error bars represent ±1 S.D.)



1 min	15 min	180 min	180 min
100 μg/ml	5 μg/ml	2 μg/ml	0.5 μg/ml

Figure 2. AFM height mode images of Fb adsorbed onto Ti-6Al-4V. Notice that there is a lack of network-like assembly pattern for the 0.5 μg/ml concentration indicating that equilibrium coverage was not attained even after 180 minutes of immersion. Scan size: 1 μm^2. Scan height: 8 nm.

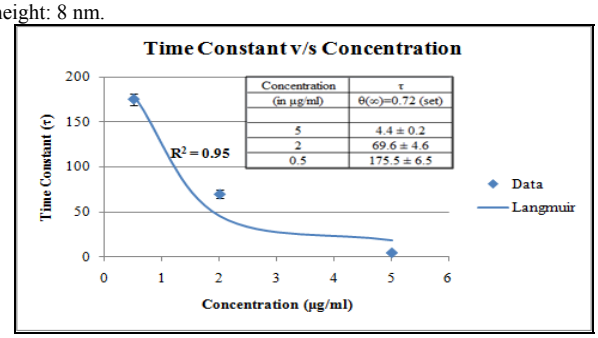


Figure 3. Graph showing Langmuir adsorption time constant (τ) versus Fb concentration. Each data point represents the Langmuir adsorption time constant (τ) calculated from kinetics experiments for a fixed $\theta(\infty)$=0.72. The Langmuir trend line shown was obtained by regression analysis of the equation $\tau = (k_0C+k_1)^{-1}$.

CONCLUSION: Time-Concentration equivalence of Fb adsorption was observed on the surface of Ti-6Al-4V. The equilibrium fraction area coverage for each concentration used was set at 0.72 for modeling purposes. For any concentration used, the equilibrium fraction area coverage will converge to this value. Results from this study clearly show that slowing the kinetics of Fb adsorption by lowering the concentration does not affect the formation of a network-like assembly pattern and the height of adsorbed Fb monolayer.

REFERENCES: 1. Castner DG, Surf. Sci. 2002; 500(1-3): 28-60 2. Jawrani, NT, Gilbert, JL Trans. of SFB 2009