Experimental report

28/08/2015

Proposal: 9-13-550 **Council:** 4/2014

Title: Adsorption of fibrinogen onto stainless steel.

Research area: Chemistry

This proposal is a new proposal

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Samples: steel substrate
fibrinogen

Instrument	Requested days	Allocated days	From	To
D17 He3 Spin Filter	2	2	06/10/2014	08/10/2014

Abstract:

With a view to better understanding the interactions between biomedical implants and the body, we propose a study into the surface structure of a key protein, fibrinogen. The aim is to determine the effect of pH, concentration and ionic strength on the adsorption affinity and structural conformation of the protein onto stainless steel. This should aid in understanding the reasons why fibrinogen often denatures on these surfaces and how therefore to prevent internal blood clotting.

Protein Adsorption on Stainless Steel - Experimental Report
D17 October 2014, Experiment no. 9-13-550

Aim

Despite the far-reaching importance of stainless steel surfaces, they are yet to be studied using neutron reflectometry, to the best of our knowledge. The austenitic grade 316L is used widely as a biomaterial, for example in structural implants such as screws. Understanding protein adsorption to biomaterial surfaces is a crucial step in determining the likelihood of their success or failure; proteins cover the surface within seconds of implantation, and their behaviour thereupon has a significant effect on subsequent cell adhesion. The aim of this work was to study the adsorption of two blood plasma proteins - fibrinogen and serum albumin (Figure 1) - on a stainless steel surface, as well as a chromium surface, since chromium oxide is believed to constitute the majority of the oxide layer that renders the steels 'stainless'.

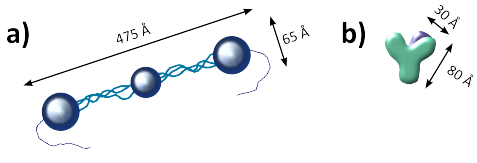

Figure 1: Extremely simplified schematics (not to-scale) of a) fibrinogen and b) HSA.

Results

Fabrication of a stainless steel surface suitable for neutron reflectometry proved distinctly challenging; the high absorption cross-section of iron prevented the use of a block of polished steel, and so an electron beam deposition method was used, whereby pieces of 316L stainless steel were evaporated onto a silicon substrate. Although the final film showed some significant inhomogeneity as a function of depth, with a 5-layer model required to fit the reflectometry data (Figure 2), XPS and TOF-SIMS both suggested the surface itself was a satisfactory model for 316L stainless steel. Additionally, the SLD of the surface layer ('e') was a value close to that predicted for a chromium oxide with some iron present, as expected.

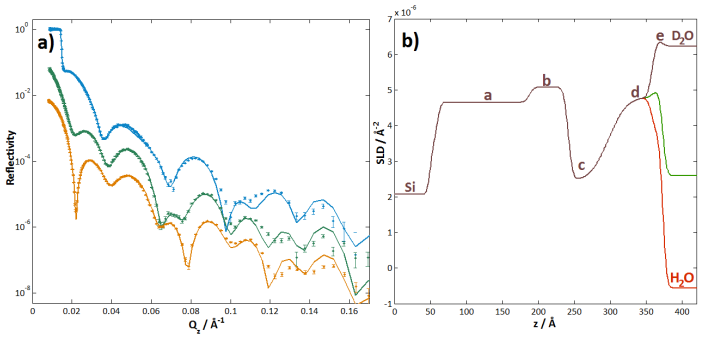

Figure 2: a) Reflectivity profiles for the steel film under D_2O, H_2O and a 50:50 mixture in descending order (profiles staggered by a factor of 10 for clarity). Data is shown as points with models as solid lines. b) SLD profiles for the model fits. The steel film is divided into 5 separate layers, labelled a-e.

Concentration / ppm		Thickness / Å	Roughness / Å	% hydration
400	F1	21.0	7.0	95
	F2	38.7	6.0	80
	F3	72.0	7.0	95
4000	F1	21.0	4.5	92
	F2	37.4	6.0	78
	F3	124.0	2.0	89
	F4	100.0	8.0	95

Table 1: Fitted parameters for the steel surface with 400 and 4000 ppm fibrinogen, assuming block models, where fibrinogen layers are denoted F1, F2 &c.

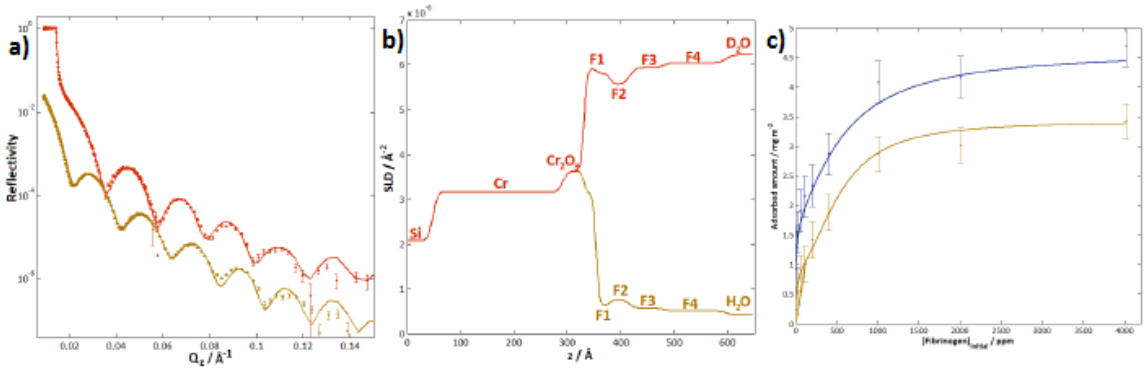

Figure 3: a) Reflectivity profiles for Cr with 4000 ppm fibrinogen in either D_2O or H_2O (profiles staggered by a factor of 10 for clarity). Data is shown as points with models as solid lines. b) SLD profiles for the model fits. c)Comparison of the fibrinogen isotherm on stainless steel (blue) and on chromium (yellow) with two-step model fits shown as the solid lines.

Fibrinogen in PBS was added to the surface in increasing concentrations (kept at 37°C throughout), up to the physiological concentration (∼4000 ppm). A three-layer block model was found to give the best fit to the data, with the middle layer having the lowest hydration (and hence highest protein concentration). At higher concentrations, a further diffuse layer was necessary to fully describe the data. The fit parameters for 400 and 4000 ppm are summarised in Table 1. From the fitted coverage values, an isotherm was calculated, to which the two-step isotherm model gave the best fit, in good agreement with data subsequently acquired using a quartz crystal microbalance (QCM). Fibrinogen showed a very similar adsorption behaviour on the chromium surface (Figure 3), although the generated isotherm revealed a lower overall coverage, as shown in Figure 3c. The fitted structure of the adsorbed fibrinogen suggested some denaturation was occurring, as the layer parameters were incompatible with adsorption of fully extended protein molecules in any conformation. Denaturation of fibrinogen upon adsorption to a biomaterial is potentially dangerous, as it could lead to undesirable clotting and thrombosis.

Adsorption of human serum albumin (HSA) on one of the stainless steel film surfaces at the physiological concentration of 30,000 ppm showed considerably less coverage, with fitted

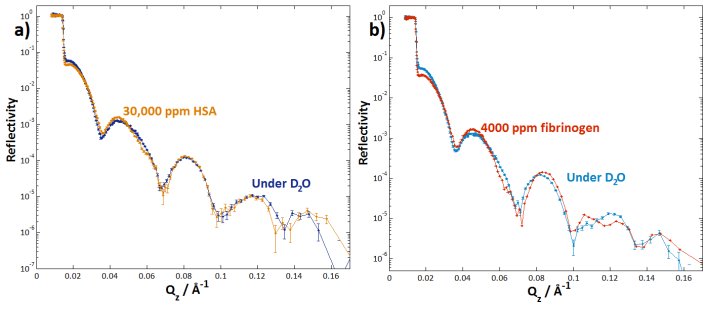

Figure 4: Comparison of experimental data (no model fits shown) for the D_2O contrasts and highest protein concentrations for a) the HSA/steel system b) the fibrinogen/steel system.

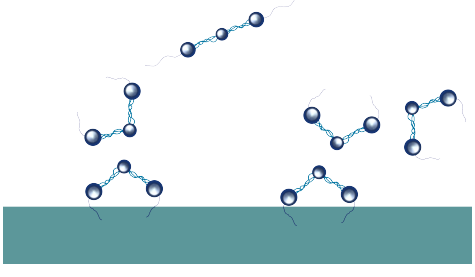

Figure 5: Simplified schematic of a possible adsorption model for fibrinogen on 316L stainless steel and chromium surfaces..

hydration values of >90 %, suggesting the protein had little affinity for the surface (Comparisons of the unfitted neutron data for the physiological concentrations of fibrinogen and HSA in D_2O are shown in Figure 4). As albumin is a globular hydrophobic protein that does not generally unfold upon adsorption, this is unsurprising - fibrinogen is more likely to change its conformation in order to adsorb, such that the sections with a greater binding affinity to the surface are exposed.

Conclusions

The adsorption behaviour of fibrinogen and HSA to 316L stainless steel and chromium surfaces were successfully studied; the fibrinogen protein adsorbed to a greater extent than the HSA, although the adsorbed layers were still highly hydrated, and the structural fits suggested protein unfolding, with a possible adsorption model depicted in Figure 5. Possibly the most significant result of this work was the development of a method by which stainless steel surfaces may be studied using neutron reflectometry; this is currently being refined in order to create more homogeneous layers.