Interfacial adsorption of fibrinogen and its inhibition by RGD peptide: a combined physical study

2004 J. Phys.: Condens. Matter 16 S2483

(http://iopscience.iop.org/0953-8984/16/26/022)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **16** (2004) S2483–S2491 PII: S0953-8984(04)80639-8

Interfacial adsorption of fibrinogen and its inhibition by RGD peptide: a combined physical study

Johanna Armstrong[1], Henryk J Salacinski[2], Qingshan Mu[1], Alex M Seifalian[2], Louise Peel[3], Neville Freeman[3], Cathy M Holt[4] and Jian R Lu[1,5]

[1] Biological Physics Group, Department of Physics, UMIST, PO Box 88, Manchester M60 1QD, UK
[2] Biomaterial and Tissue Engineering Centre, Department of Surgery, Royal Free and University College Medical School, UCL, Pond Street, London, NW3 2QG, UK
[3] Farfield Sensors Ltd, Salford University Business Park, Salford, M6 6AJ, UK
[4] Cardiac Physiology Unit, University of Manchester, The Manchester Medical School, Stopford Building, Oxford Road, Manchester, M13 9PT, UK

E-mail: j.lu@umist.ac.uk

Received 5 May 2004
Published 18 June 2004
Online at stacks.iop.org/JPhysCM/16/S2483
doi:10.1088/0953-8984/16/26/022

Abstract

The Arg–Gly–Asp (RGD) peptide sequence is known as a cell recognition site for numerous adhesive proteins present in the extracellular matrix (ECM) and in blood. Whilst surface immobilized RGD groups enhance cell attachment, RGD components present in solution can effectively inhibit cell attachment by competing with endogenous ligands for the same recognition site. In contrast to the widely reported binding to cell integrin, this study demonstrates a new RGD feature: its inhibitive effect on fibrinogen adsorption. Through a combined analysis of spectroscopic ellipsometry, neutron reflection and dual polarization interferometry, we show that the kinetic process of fibrinogen adsorption as a model pro-coagulant at the silica/solution interface and in the absence of any cells can be substantially reduced by the addition of RGD in solution and that the extent of the reduction is dependent on the relative concentration of RGD.

1. Introduction

Almost immediately following the implantation of a biomaterial within the body, proteins adsorb to the biomaterial surface [1, 2]. This adsorption stimulates further biological responses such as cell adhesion [3], activation of inflammatory cells [1], and complement activation [4]. Successful implant deployment requires a well corroborated mechanistic theory of interfacial events at the molecular and cellular levels to predict these interrelated processes, thereby

[5] Author to whom any correspondence should be addressed.

devising effective approaches to promote the functional integration of the implant into the local tissue environment with long-lasting performance.

Fibrinogen is a soluble cell adhesion protein whose interfacial adsorption plays a key role in underlining the initial interfacial events in a complex manner. Because of this, fibrinogen has been widely used as a model pro-coagulant in assessing surface biocompatibility [5]. In blood, plasma fibrinogen can be cleaved to form fibrin monomers by thrombin, a highly specific enzyme generated at sites of either vascular lesions or implant deployment. The cleaved strands in turn self-associate to form a homopolymeric structure, leading to the formation of insoluble fibrin clots [6–8]. Although it is widely accepted that fibrinogen has a strong affinity to a wide range of biomaterial surfaces and that its surface deposition initiates platelet adhesion onto thrombogenic surfaces through binding to a platelet thrombus, there is a severe lack of direct information about the molecular mechanistic processes at the interface. In spite of extensive research (for example see [3–5]), it is still difficult to ascertain how the amount and structural conformation of fibrinogen adsorbed affects platelet adhesion and how the different surface chemistry of biomaterials affects fibrinogen adsorption.

The Arg–Gly–Asp (RGD) peptide sequence is well known as a cell recognition site for many adhesive proteins present in extracellular matrices (ECM) and in blood, including fibrinogen [9]. Many studies have reported that polymeric biomaterial surfaces pre-conditioned with RGD peptides (usually through chemical bonding) show accelerated healing and endothelialization [10–12]. In contrast, the addition of soluble RGD peptide into a cell culture can inhibit cell surface attachment and adhesion. These observations have been well explained by the competitive recognition of synthetic RGD peptides for cell integrins. This theory, however, does not offer an explicit explanation for the selective promotion of RGD peptides to the surface attachment of different cell types. For example, a number of recent studies have reported that polymeric biomaterial surfaces pre-conditioned with RGD peptides showed accelerated healing and endothelialization [13–15], indicating that the synthetic RGD sequence inhibits platelet receptors and reduces platelet aggregation whist enhancing endothelial cell adhesion. We hypothesize that the RGD discrimination to cellular responses arises from their intervening effect on fibrinogen adsorption and the subsequent fibrin layer formation on biomaterial surfaces.

In spite of the knowledge of the RGD mediation on cell recognition, its intervening process on fibrinogen adsorption is so far little known. The aim of this work is to demonstrate the effect of RGD peptide on fibrinogen adsorption. Our results have shown that the addition of RGD peptide into aqueous solution can indeed slow down the dynamic process of fibrinogen adsorption and reduce the equilibrated amount of adsorption as well. Since our experiment has been done in the absence of cells, the effect of RGD peptide on fibrinogen adsorption is clearly different from its established role in cell recognition. Our results are well supported by the work of Olivieri and Tweden [14] that indicates that surface immobilized RGD peptides may impose their influence by altering the structural conformations of ECM proteins.

Because of the complex roles played by fibrinogen in eliciting a cascade of blood coagulation and thrombosis, it is necessary to examine its interaction with a model substrate with and without RGD peptide. In this work the interfacial adsorption of fibrinogen has been examined on the well-characterized model surface silicon oxide. This surface allows neutron reflectivity (NR), spectroscopic ellipsometry (SE) and dual polarization interferometry (DPI) biosensor measurements to be performed under solution conditions close to the physiological environment. In comparison with traditional methods of analysing protein deposition onto surfaces, such as radio-labelling and quantitative ELISA, the techniques used in this work offer real-time measurement of surface adsorbed amount (surface excess) as well as the structural conformations of the adsorbed protein layers.

2. Materials and methods

2.1. Physical techniques

Ellipsometric measurements were made using a Jobin-Yvon UVISEL spectroscopic ellipsometer over a typical wavelength range of 300–600 nm [16]. A special liquid cell was constructed to enable the SE measurement at the solid/liquid interface at a fixed incidence angle of 70°. Results were analysed using DeltaPsi I software and the surface adsorbed amount was estimated using the equation developed by De Feitjer *et al* [17].

Neutron reflection was performed on the reflectometer Surf at Rutherford Appleton Laboratory, Chilton, Didcot, UK [18]. The wavelength of neutrons used was between 1 and 6.5 Å. This range of wavelength makes NR inherently sensitive to structural dimensions at the molecular level. In addition, the use of deuterium labelling of the solvent helps to identify the detailed structural distribution within the interfacial layers. Procedures for neutron data analysis are described in [19].

The interferometrical biosensor used was AnaLight® Bio200 (Farfield Sensors). This technique finds the absolute effective refractive index in a waveguide mode by solving the equations of electromagnetism for a system of uniform multiple dielectric layers [20–22]. The sensor chip is an interferometer comprising two vertically integrated slab waveguides, the lower one isolated from the experiment and acting as a reference channel, the upper one providing an evanescent wave for biosensing. Dual polarization (transverse electric (TE), transverse magnetic (TM)) mode excitation is made with face normal incidence injection of the beam. Polarization switching is readily achieved at a rate of 50 Hz, thus giving data updates every 20 ms. The technique is thus effective at real-time measurement of the dynamic process of protein adsorption.

2.2. Materials and samples

Freshly cleaned silicon wafers were immersed in either buffer or human plasma fibrinogen (Sigma) in 0.1 M NaCl with and without RGD before appropriate measurements were made. Fibrinogen solution was always freshly prepared about 1 h before the adsorption measurement. In cases where adsorption was measured from fibrinogen + RGD peptide mixture, freshly prepared fibrinogen solution was poured into a known amount of dry RGD sample and the solution was gently mixed under magnetic stirring. A similar approach was used to incorporate heparin into fibrinogen solution. The solution pH was controlled at 7 using 20 mM phosphate buffer (PBS) and all the measurements were made at 23–25 °C. The concentration of RGD tripeptide (Sigma) was adjusted at either 1 or 2 g l^{-1} and that of heparin (Sigma) was kept at 2 g l^{-1}. The fibrinogen concentrations studied were between 0.01 and 0.5 g l^{-1}. All the glassware and measurement cells were freshly cleaned by soaking in 5% neutral Decon solution (purchased from Decon Laboratory UK), followed by copious rinsing with ultrapure water (UHQ) processed through a PS Elgastat water purification system. D_2O containing 99 + %D was purchased from Sigma and used as supplied. Its surface tension at 25 °C was above 71 mN m^{-1} and was close to the UHQ water freshly processed.

3. Results

3.1. Spectroscopic ellipsometry

In each spectroscopic ellipsometry measurement, the two optical angles, ψ (measuring changes of amplitude of the light before and after reflection) and Δ (measuring changes of phase of the light before and after refection), were recorded against wavelength between 300 and 600 nm.

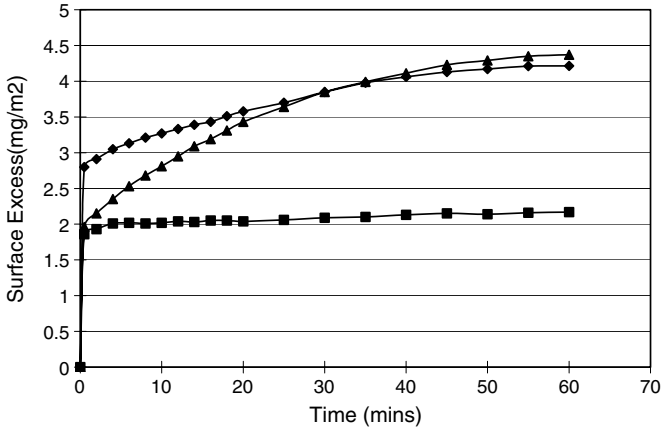

Figure 1. The spectroscopic ellipsometry measurement of fibrinogen adsorption from 0.2 g l^{-1} solution onto silicon oxide measured at the solid/water interface at the RGD peptide concentration of 0 (♦), 1 (▲) and 2 (■) g l^{-1}, pH 7.

Each measurement took about 30 s to complete. Thus, it was possible to record the first point of adsorption about 1 min after fibrinogen solution was placed into the ellipsometry measurement cell.

Information about the amount of protein adsorbed was obtained by performing a uniform layer model fit to each pair of ψ and Δ. We have explained previously that for the formation of an ultrathin interfacial layer, SE is incapable of separating the layer thickness from its volume fraction [23, 24]. To avoid the coupling problem, we have tried to fix the refractive index of the protein layer close to that of the pure protein. We have demonstrated that this approach has little effect on the surface excess [24]. Following the previous work by De Feitjer [17], the surface excess (Γ in mg m^{-2}) can be estimated from the following equation:

$$\Gamma = \frac{\tau(n - n_w)}{a} \tag{1}$$

where τ is the protein layer thickness (in Å), n is the refractive index of the layer and n_w is the refractive index of the aqueous phase. The value of a was related to the change of the solution's refractive index with bulk concentration. To offset the unit conversion the value of a was taken as 1.8 [17, 24]. To guide the view of the trend of surface excess changes with time, continuous lines were drawn through each set of data.

Figure 1 shows that fibrinogen adsorption is time dependent. Within the first 30 min the adsorbed amount is doubled. After this initial period, the adsorption reaches its saturation limit. The addition of 1 g l^{-1} RGD peptide reduces the initial adsorption by some 30–50% on incubation within the first 10 min, but then the adsorbed amount reaches 100% of fibrinogen alone adsorption by 30 min. However, 2 g l^{-1} RGD reduces fibrinogen adsorption by some 50 %. Interestingly, this level of reduction is maintained for the full 60 min period of measurement, clearly showing the increasing effect of RGD in inhibiting fibrinogen adsorption onto the model SiO_2 surface. It should be noted however that SE is only capable of updating the time-dependent adsorption over the timescale of min. Also note that although the measurements offered a progressive change of layer thickness with time, its absolute value was not meaningful in the ellipsometry measurement because of the assumption of the corresponding layer refractive index as indicated previously. Finally, the RGD peptide alone had no measurable adsorption over the concentration of 1–2 g l^{-1} under the same solution conditions.

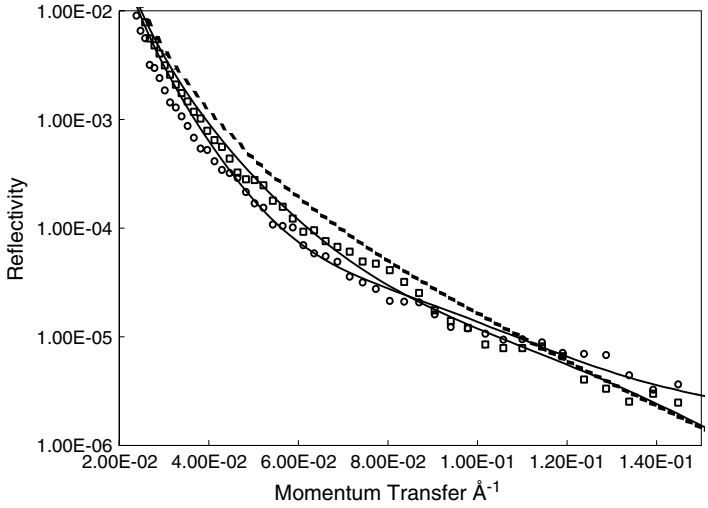

Figure 2. The neutron reflection measurement of fibrinogen adsorption at the hydrophilic SiO_2/water interface, at fibrinogen concentrations of 0 (dashed curve), 0.01 g l^{-1} (□) and 0.2 g l^{-1} (○). The continuous curves represent the best fits with the layer structures described in the text.

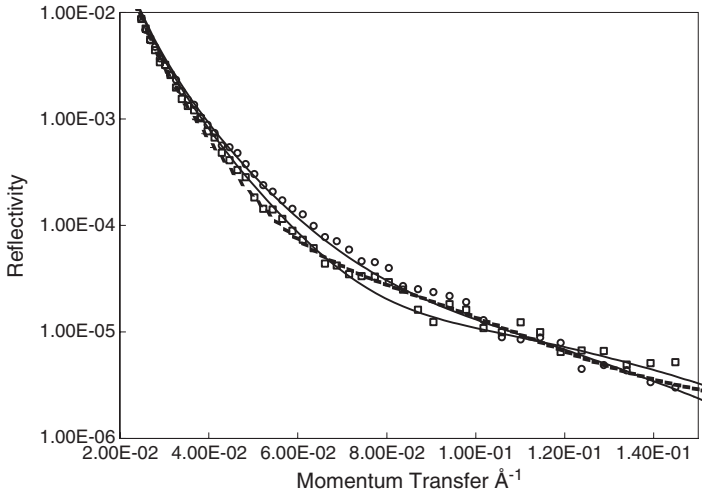

Figure 3. The neutron reflection measurements to show the effect of RGD on fibrinogen adsorption at RGD concentration of 0 (dashed curve), 1 g l^{-1} (□) and 2 g l^{-1} (○). The continuous curves represent the best fits.

3.2. Neutron reflection

Neutron reflectivity profiles were measured at the silicon oxide/D_2O interface under otherwise similar solution conditions to those used for the SE measurements. As changes in reflectivity occur mainly below 10^{-3}, we have expanded the measured data shown in figures 2 and 3 to indicate the changes over this range. This means that the usual signatory occurrence of the critical angle of neutron reflectivity at the momentum transfer around 0.014 $Å^{-1}$ is not shown here.

Figure 2 compares reflectivity profiles measured at the silicon oxide/D_2O interface with and without fibrinogen. The differences clearly indicate fibrinogen adsorption. Neutron reflectivity allows us to reveal the amount of fibrinogen adsorption and the layer thickness simultaneously. Such information was also obtained through model fitting to the reflectivity profiles measured as in the case of the ellipsometry data analysis outlined previously. A single layer model of 40 Å was found to be adequate for describing the adsorption at a fibrinogen concentration of 0.01 g l^{-1}, indicating that there was no inhomogeneity across the interface layer.

However, the single uniform layer model was found to be inadequate to describe the layer structure adsorbed at a higher concentration of 0.2 g l^{-1}. The reflectivity profile was best fitted using a three layer model with a total thickness of 60 Å. This model consisted of a middle sublayer of 22 Å with high protein volume fraction and two low volume fraction sublayers of some 20 Å each at the SiO_2 side and the bulk solution side. This indicated that upon increasing surface adsorption, the structure of the protein layer also changed. With the formation of a dense polypeptide layer, its polypeptide fragments did not collapse onto the solid surface fully. Instead, the protein layer retained a 20 Å gap that had a depletion of polypeptide distribution. It should also be noted that the fit shown in figure 2 does not model the measured data at low momentum transfer range well. The misfit over this range could be improved by incorporating a further loose layer of some 30 Å but containing some 5% polypeptide on the bulk solution side. This again indicates the sensitivity of NR to the fragment distribution of the protein across the interface. The *in situ* structural conformation may have a direct implication to cell attachment and spreading. A similar layer structure was found at the highest concentration of 0.5 g l^{-1} except that under this concentration the outer polypeptide sublayer on the solution side was more diffuse and broadly distributed.

In the case of co-adsorption with RGD, little reduction in the total amount of fibrinogen adsorption was seen at 1 g l^{-1} RGD. This observation was broadly consistent with the ellipsometry data, bearing in mind that it took about 1 h to complete the NR measurement. The data in the case of neutron reflectivity represented a time-averaged measurement over this period. However, as the concentration of RGD increased to 2 g l^{-1}, fibrinogen adsorption was reduced by some 50%, again in good agreement with the ellipsometry data (figure 3).

3.3. DPI biosensor measurements

Although neutron reflectivity gave more specific information about the volume fraction distribution of the adsorbed protein layer, each neutron reflectivity measurement took some 30–60 min to complete. Thus the structural conformation represented the time-averaged outcome. Because the DPI biosensor updates its measurement on a timescale of seconds, it provides a better real-time update on protein adsorption. Figure 4 shows a steady increase of fibrinogen adsorption with time over the first 5 min, and then it tends to a plateau around 1 mg m^{-2}. Note that this adsorption occurred at the much lower fibrinogen concentration of 0.01 g l^{-1} and that the biosensor chip surface contained silicon oxynitride. Given the differences in surface chemistry and protein concentration, the timescale of the dynamic adsorption is broadly consistent with the ellipsometry results shown in figure 1.

When RGD is mixed with the fibrinogen solution, the total amount of adsorption is substantially reduced. This is again similar to the trend observed from the ellipsometry work as already described in figure 1. The biosensor data, however, show more variation of the adsorbed amount with time over the initial stage of interfacial adsorption.

It is useful to note that heparin has been widely used in clinical medicine as an anti-coagulant. Its role is to inhibit fibrin formation, thereby inhibiting blood coagulation. For

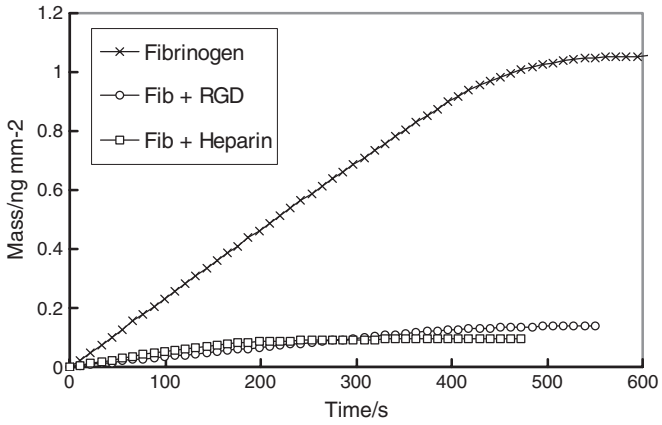

Figure 4. The DPI biosensor real-time variations of fibrinogen adsorption and its comparison with the solutions containing 2 g l^{-1} RGD and 2 g l^{-1} heparin. The fib concentration was fixed at 0.01 g l^{-1}.

comparison, we also show in figure 4 that the addition of heparin into fibrinogen solution can reduce fibrinogen adsorption onto the silica surface in a manner similar to the action of RGD peptide. This raises a query as to whether the molecular mechanistic process imposed by the two model anti-coagulants is similar. A useful experiment for unravelling this issue is to probe the possible binding of anti-coagulant onto fibrinogen pre-adsorbed onto the substrate surface by neutron reflection. Such an experiment is in progress.

4. Discussion

The combined measurements from the three surface sensitive techniques offer molecular details about the real-time variation of the adsorbed amount (SE, DPI) and the structural conformations (NR), all at the substrate/solution interface. Both SE and DPI show a convincing time-dependent adsorption of fibrinogen over the period of the first 20–30 min. The difference in the time-dependent process as shown in figures 1 and 4 for pure fibrinogen adsorption arises from different protein concentration and the chemical nature of the silicon oxide surfaces. Although NR measurement requires a much longer time to accomplish, it has revealed the unique structural feature of the polypeptide distribution along the surface normal. The structural distribution for the fibrinogen layer was found to be distinctly different from those obtained for other proteins such as lysozyme and albumins in our previous work [25, 26]. Note that the isoelectric point (IP) for fibrinogen is around 5.5. At pH 7, both the protein and the silicon oxide surface are negatively charged. The charge repulsion may contribute to the depletion of the polypeptide within the first 20 Å closest to the oxide surface. Adjacent to the inner depleted region is the middle dense layer of some 20 Å containing some 80% polypeptide, followed by an outer diffuse layer of 30–60 Å into the bulk solution. The main structural characteristic was retained for the adsorbed fibrinogen layers over the high concentration range. However, when the concentration was low, there was insufficient sensitivity in the reflectivity data to justify such a layer distribution other than the fitting of a uniform model.

All three techniques are capable of revealing the effects of regulating factors on fibrinogen adsorption at the silicon oxide/solution interface. RGD is effective at reducing fibrinogen adsorption at concentrations around 1–2 g l^{-1}. The inhibition caused by RGD clearly shows that the time-dependent fibrinogen adsorption is manifested through RGD binding between

incoming fibrinogen from solution and the ones already immobilized on the silicon oxide surface. It is interesting to note that there is a clear difference in the adsorbed amount at the starting point (after about 1 min of solution in contact with the surface) with and without RGD, as is evident from figure 1. In the absence of RGD, the surface excess is about 2.8 mg m^{-2}, as compared with 1.4 mg m^{-2} in the presence of RGD. The difference indicates possible RGD-specific molecular recognition between fibrinogen molecules and the competitive process upon addition of the soluble synthetic RGD peptide. At 1 g l^{-1} RGD the inhibitive effect is time dependent, indicating the dynamic replacement of RGD peptides by surface binding entities from incoming fibrinogen molecules. After some 30 min the protein surface excess eventually reaches the same value as in the absence of RGD peptide, suggesting the complete displacement of pre-engaged RGD peptides. At 2 g l^{-1} RGD there is little sign of variation of any further fibrinogen adsorption with time, indicating a strong inhibitive effect from the RGD peptides present in the solution. These SE observations are entirely consistent with the DPI data shown in figure 4 and the results together reveal the competitive process between RGD blocking and fibrinogen self-recognition, a molecular process implicating the initial stage of interfacial coagulation leading to thrombosis.

In addition, the DPI measurement shows that addition of heparin results in similar inhibition to the RGD peptide. As heparin is well known for its anti-coagulating effect, the reproduction of its inhibitive action to fibrinogen adsorption might imply a possible resemblance between the two regulating factors. These experiments demonstrate that the molecular mechanistic processes involved in fibrinogen deposition onto the surface of biomaterials, and the regulation by therapeutic molecules, can be effectively screened by these surface-sensitive techniques.

References

[1] Tang L and Eaton J W 1994 Mechanism of acute inflammatory response to biomaterials *Cells Mater.* **4** 429–36

[2] Wesslen B, Kober M, Freij-Larrson C, Ljunghi A and Paulsson M 1994 Protein adsorption of poly(etherurethane) surfaces modified by amphiphilic and hydrophilic polymers *Biomaterials* **15** 278–84

[3] Remes A and Williams D F 1992 Immune response in biocompatibility *Biomaterials* **13** 731–43

[4] Dekker A and Beugeling T 1991 Protein adsorption and the interaction of human endothelial cells with polymer surfaces *Modern Aspects of Protein Adsorption on Biomaterials* ed Y F Missirlis and W Lemm (Amsterdam: Kluwer–Academic) pp 25–8

[5] Holmlin R E, Chen X, Chapman R G, Takayama S and Whitesides G M 2001 Zwitterionic SAMs that resist nonspecific adsorption of protein from aqueous buffer *Langmuir* **17** 2841

[6] Blomback B 1996 Fibrinogen and fibrin–proteins with complex roles in hemostasis and thrombosis *Thromb Res.* **83** 1–75

[7] Plow E F, Pierschbacher M D, Ruoslahti E, Marguerie G A and Ginsberg M H 1985 The effect of Arg–Gly–Asp-containing peptides on fibrinogen and von Willebrand factor binding to platelets *Proc. Natl Acad. Sci. USA* **82** 8057–61

[8] Tranqui L, Andrieux A, Hudry-Clergeon G, Ryckewaert J J, Soyez S, Chapel A, Ginsberg M H, Plow E F and Marguerie G 1989 Differential structural requirements for fibrinogen binding to platelets and to endothelial cells *J. Cell Biol.* **108** 2519–27

[9] Pierschbacher M D and Ruoslahti E 1984 Cell attachment activity of fibrinogen can be duplicated by small synthetic fragments of the molecule *Nature* **309** 30–3

[10] Hersel U, Dahmen C and Kessler H 2003 RGd modified polymers: biomaterials for stimulated cell adhesion and beyond *Biomaterials* **24** 4385–415

[11] Hubbell J A 2003 Materials as morphogenetic guides in tissue engineering *Curr. Opin. Biotechnol.* **14** 551–8

[12] Shakesheff K M, Cannizzaro S M and Langer R 1998 Creating biomimetic micro-environments with synthetic polymer-peptide hybrid molecules *J. Biomater. Sci. Polym. Edn* **9** 507–18

[13] Tweden K S, Harasaki H, Jones M, Blevitt J M, Craig W S, Pierschbacher M and Helmus M N 1995 Accelerated healing of cardiovascular textiles promoted by RGD peptide *J. Heart Valve Dis.* **4** (Suppl 1) S90–7

[14] Olivieri M P and Tweden K S 1999 Human serum albumin and fibrinogen interactions with an adsorbed RGD-containing peptide *J. Biomed. Mater. Res.* **46** 355–9

[15] Chang M C, Wang B R and Huang T F 1995 Characterisation of endothelial cell differential attachment to fibrin and fibrinogen and its inhibition by RGD peptides *Thromb Haemost.* **74** 764–9
[16] Drevillon B, Perrin J, Marbot R, Violet A and Dalby J L 1982 Fast polarization modulated ellipsometer using a microprocessor system for digital Fourier analysis *Rev. Sci. Instrum.* **53** 969–77
[17] De Feitjer J A, Benjamins J and Veer F A 1978 Ellipsometry as a tool to study the adsorption behavior of synthetic and biopolymers at the air–water interface *Biopolymers* **17** 1759–72
[18] Hayter J B, Penfold J, Highfield R R, Pullman B J, Thomas R K and McMullen A I 1981 A new technique for investigating interfacial phenomenon *J. Chem. Soc. Faraday Trans.* I **77** 1437–48
[19] Lu J R and Thomas R K 1998 Neutron reflection from wet interfaces *J. Chem. Soc. Faraday Trans* **94** 995–1018
[20] Cross G H, Ren Y and Freeman N J 1999 Young's fringes from vertically integrated slab waveguides: applications to humidity sensing *J. Appl. Phys.* **86** 6483–8
[21] Cross G H, Reeves A, Brand S, Swann M J, Peel L L, Freeman N J and Lu J R 2004 *J. Phys. D: Appl. Phys.* **37** 74–9
[22] Lu J R, Swann M J, Peel L L and Freeman N J 2004 Lysozyme adsorption studies at the silica/water interface using dual polarisation interferometry *Langmuir* **20** 1827–32
[23] Gilchrist G A, Lu J R, Keddie J L, Staples E and Garrett P 2000 The adsorption of pentaethylene glycol monododecyl ether at the solid poly(methyl methacrylate)–water interface: a spectroscopic ellipsometry study *Langmuir* **16** 740–8
[24] Murphy E, Kiddie J L, Lu J R, Brewer J and Russell J 1999 The adsorption of model proteins onto phosphorylcholine incorporated polymers studied by spectroscopic ellipsometry *Biomaterials* **20** 1501–11
[25] Su T J, Lu J R, Thomas R K, Cui Z F and Penfold J 1998 The effect of pH on the adsorption of lysozyme at the hydrophilic silicon oxide–water interface, a neutron reflection study *Langmuir* **14** 438–45
[26] Su T J, Lu J R, Thomas R K, Cui Z F and Penfold J 1998 The conformational structure of bovine serum albumin layers adsorbed at the silica–water interface *J. Phys. Chem.* B **102** 8100–8