Experimental and computational examination of protein-surface interactions

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Abstract: Using a combination of experimental and computational approaches, the interaction between anastellin, a recombinant fragment of fibronectin, and representative biomaterial surfaces has been examined. The molecular interaction was directly quantified by atomic force microscope (AFM) based force spectroscopy, complemented by adsorption
measurements using quartz crystal microbalance (QCM). It was found that the anastellin molecules facilitates a stronger adhesion on polyurethane films (72.0 pN nm\(^{-1}\)) than on poly (methyl methacrylate) films (68.6 pN nm\(^{-1}\)). This is consistent with the adsorption measurements of anastellin on the two polymeric surfaces, observed by QCM. Molecular dynamics simulations of the behaviour of anastellin on polyurethane in water solution were performed to rationalise the experimental data, and show that anastellin is capable of rapid adsorption to PU while its secondary structure is stable upon adsorption in water.

INTRODUCTION

In order to design medical devices that are exposed to physiological environments with prolonged service life and enhanced biocompatibility, it is vital to understand how biological objects interact with the surface of the engineered component, and the underlying biophysical mechanisms. For the development of biomaterials, the predominant mechanisms include the adsorption of proteins which is the initial stage for cell adhesion.\(^1\) One of the major proteins that significantly affects the compatibility of biomaterials used intracorporeally is fibronectin (Fn). This is an important extracellular protein that exists in a variety of forms (e.g. circulating plasma, tissue and cellular).\(^2\) It is made up of three types of domain (I, II and III). Types I and II are stabilised with disulphide bonds while type III fibronectin domains (FnIII) are capable of unfolding under tension. This can expose hidden, cryptic binding sites such as the Arg-Gly-Asp (RGD) sequence found in the tenth fibronectin type III domain (FnIII10) and the Pro-His-Ser-Arg-Asn (PHSRN) sequence in the ninth fibronectin type III domain (FnIII9). The RGD sequence allows binding to cell-bound integrin receptors, such as α5β1, to enable adhesion of cells to the extracellular matrix while the PHSRN sequence has a synergistic effect on this binding.\(^3\) The RGD peptide sequence has been incorporated onto
biomaterials, in its linear form, or cyclic forms, or as part of a larger fragment in order to improve aspects of biocompatibility such as osseointegration. Use of a protein fragment offers advantages over whole Fn such as improved stability and increased density of desired sequences on the surface.

Another area of Fn that has been shown to elicit potentially useful interactions is the C-terminal fragment of the first FnIII domain (FnIII1c), known as anastellin, which has 76 amino acid residues. The FnIII1 domain has been identified as a region associated with matrix formation by Fn-Fn interactions. When mixed with whole Fn molecules, anastellin can create a different form of Fn known as superfibronectin which is adept at supporting cell adhesion and spreading. Anastellin and superfibronectin have been reported to contribute towards anti-tumour, anti-metastatic, and anti-angiogenic performance through a mechanism that involves extracellular signal-regulated kinases (ERK) and a decrease in cyclin D1, cyclin Al, and cyclin-dependent kinase 4 (cdk4).

Upon adsorption to solid surfaces, the conformation of Fn at the interface appears to depend largely on the characteristics of the surface. Proteins tend to adopt an extended conformation on hydrophilic surface but a compact, globular conformation on hydrophobic surfaces. Furthermore, other factors such as surface chemistry and topography have been found to play an important role. Hydrophobic surfaces adsorb more Fn than hydrophilic surfaces, the globular conformation adapted could accommodate more molecules and hence a greater packing density. The change of conformation to an elongated form is important since it can expose the aforementioned binding sites such as RGD and PHSRN and allow matrix formation and cell attachment. Interaction between protein and solid surface and the consequent confirmation can be examined with a wide range of experimental approaches including atomic force microscopy (AFM), quartz crystal microbalance, fluorescence
resonance energy transfer (FRET), measuring the adsorption amount of labelled Fn, and enzyme-linked immunosorbent assays (ELISA). 19,20,24,25

With the readily controlled molecular structure, chain length, and functional groups, polymers have been widely used as biomaterials. Examples include polyurethane (PU) for ureteral catheters, 26 poly (methyl methacrylate) (PMMA) as bone cement, 27 polytetrafluoroethylene (PTFE) as artificial heart valves and vascular grafts, 28 and ultra-high density polyethylene in joint replacement implants. Palacio et al. 29,30 have investigated the adhesion of whole Fn, bovine serum albumin and collagen to PMMA surfaces as well as di- and triblock copolymers of PMMA with poly (2-hydroxyethyl methacrylate) (PHEMA) and/or poly (acrylic acid) (PAA). They used AFM in force-volume mode with probes coated with silane and protein, and reported that the PMMA regions of the polymer had lower adhesion than PAA regions due to weaker interactions between the polymer and hydrophobic regions of Fn. The adhesive force between Fn and PMMA was reduced from 1.0 nN to 0.7 nN as the pH of the surrounding medium is decreased from 7.4 to 6.2. It is possible that the protein is less negatively charged at lower pH so that the electrostatic repulsion from the acid groups of the polymer is reduced. Hydrophobicity ought to be an indicator of adhesion since it would affect the repulsive force between the protein and the polymer.

For a number of years, a molecular simulation approach has been used to investigate protein-surface adsorption, 31 giving significant insight into the mechanism of adsorption and the factors that drive this. Due to its relevance to biomaterials 1 the adsorption of a number of fibronectin modules onto surfaces have been investigated. 32-34 These have shown that fibronectin adsorption is relatively non-specific, with it readily adsorbing onto organic, 33 inorganic, 34 and graphite surfaces. 32
In the present work, adhesion and adsorption of anastellin on to two polymeric films, PMMA and PU, were measured by AFM and QCM. Influences of both chemical composition and hydrophobicity of the polymeric surfaces on the protein-surface interaction were examined. The experimental results were then compared with the results of molecular dynamics simulations.

MATERIALS AND METHODS

Materials

Poly (methyl methacrylate) (PMMA; MW 94,600) was purchased from Acros Organics. Polyurethane (PU) was purchased from Fluka. The FnIII1c protein fragment, phosphate buffered saline (PBS; pH 7.4) tablets, tetrahydrofuran (THF; ≥ 99.9 %), ethylene glycol (EG; 99.8 %), diiodomethane (DIM; 99 %) and ethanol (≥ 99.8 %) were purchased from Sigma.

Spin Coating

Silica wafer (SW) was cleaned with deionised water, followed by 70 % ethanol and finally with THF in order to sterilise the surface and remove both water-soluble and organic contaminants. Polymers were dissolved in THF (2 % w/v). Consequently, the polymer solution was spin coated onto silica wafer at 2,000 rpm for 30 s with a Laurell WS-400-6NPP spin coater as per Mallinson et al. 35

Contact Angle Goniometry

The advancing contact angles ($\theta_A$) of three solvents (deionised water (DW), ethylene glycol (EG) and diiodomethane (DIM)) on the two polymer surfaces were measured in order to calculate the surface energy ($\gamma_s$) and surface energy components of the surfaces. As per Mallinson et al. 35 small drops of DW (18.2 MΩ, surface tension ($\gamma_L$) 72.8 mN m$^{-1}$ at 20 °C),
EG ($\gamma_L$ 48.0 mN m$^{-1}$ at 20 °C) and DIM ($\gamma_L$ 50.8 mN m$^{-1}$ at 20 °C) were placed on the surface with a needle, followed by measuring both the left and right contact angles with a Krüss DSA30B contact angle goniometer (CAG). At least 2 repeats were made for each surface with 3 drops per sample, resulting in at least 12 measurements per sample. Using these contact angle values, the surface energies were calculated using a Visual Basic application developed by Lamprou et al.,$^{36}$ based on the formula proposed by Good and Oss.$^{37}$

**Circular Dichroism**

Circular dichroism (CD) was performed in order to determine whether the protein fragment retained the expected structure when reconstituted. The protein sample was dissolved in PBS solution (pH 7.4) at 200 µg mL$^{-1}$. Spectra were read with a Chirascan CD spectrophotometer (Applied Photophysics) in the wavelength range 190-280 nm, with step size of 1 nm and bandwidth of 1 nm, using a quartz cuvette with a path length of 0.1 mm (Hellma). Three spectra were averaged and data were processed with Chirascan Viewer and with Microsoft Excel.

**Atomic force microscopy**

AFM measurements were performed in ambient using a Bruker Multimode 8 AFM equipped with a Nanoscope Controller V and SNL-10 probes (cantilever C: nominal spring constant 0.24 N m$^{-1}$ and nominal resonant frequency 56 kHz). Cantilevers were silanised to allow protein functionalisation by submerging in 2 mM APTES in toluene for 5 min as per Couston et al.$^{38}$ The cantilevers were subsequently washed with toluene and deionised water in order to remove unbound silane. The FnIII1c protein fragment was added by incubating the tip in a drop of 1 mg mL$^{-1}$ protein solution (in PBS pH 7.4) for 10 min followed by a thorough rinsing with deionised water to remove unbound protein. Each polymer surface was scanned 4 times with each AFM probe before and after protein functionalisation. This was done with
two different probes. For each of the AFM images approximately 160 curves were chosen at random from each of the force-volume images (20 curves x 4 areas x 2 probes). Deflection sensitivity was calibrated with a sapphire reference sample and the tip radius was determined with a PA01 spiked reference sample (Mikromasch). Ramps were made over 1 × 1 µm squares.

Surface roughness was determined using images captured using unfunctionalised probes in PeakForce-Quantitative Nanomechanical (PF-QNM) mode. Images were analysed with Bruker Nanoscope Analysis version 1.5 to view force curves and calculate surface roughness. Adhesion values were extracted from the ramps using an in-house Python script. Adhesive forces were then normalised for the effect of tip radius by dividing by tip radius in accordance with equation 1 by Sugawara et al. 39

\[ A = 4\pi RT \]  
(Eq.1)

where \( A \) is the corrected adhesion, \( R \) is tip radius and \( T \) is surface tension of the medium.

**Quartz Crystal Microbalance**

Quartz crystal microbalance (QCM) is a technique that uses a mass sensor 40-42 to measure the adsorption of biopolymers 43,44 or synthetic electrolytes 45 from liquid and allows observation of not only the adsorption kinetics and adsorbed mass but also of the viscoelastic properties of adsorbed polymer layers at the solid liquid interface. 23,46,47 The technique relies upon the resonant frequency of a quartz crystal. A frequency shift (\( \Delta f \)) will be induced by any change in adsorbed mass, as \( \Delta f \) is related to the adsorbed mass per unit surface \( \Delta m \), by a linear relationship known as the Sauerbrey equation (Eq. 2).

\[ \Delta m = \frac{C \Delta f}{n} \]  
(Eq. 2)
where \( n \) is the overtone number (\( n = 1 \) in the present case) and \( C \) is a constant that describes the sensitivity of the device the changes in mass. Additionally, the exponential decay of the oscillation amplitude, \( D \), is recorded which can reveal information about the viscoelastic properties of the adsorbed layer.

PU and PMMA thin films were formed on gold coated AT-cut quartz crystals by spin-coating, by the same protocol as on the Si wafers, and a SiO\(_2\) crystal was used to replicate the silica wafer surface. All crystals were purchased from Testbourne Ltd. The crystals were placed in a home-built quartz crystal microbalance and all measurement were taken at room temperature. For adsorption measurements, the QCM chamber was flooded with HPLC-grade water solution and left until the frequency stabilises to an equilibrium state at the liquid/solid interface may be achieved. The protein fragment was initially dissolved in PBS buffer (pH 7.4) with a concentration of 1.0 mg mL\(^{-1}\), and further diluted by HPLC-grade water to a concentration of 0.025 mg mL\(^{-1}\). The HPLC-water in the QCM chamber was then replaced by the protein solution while the change in frequency and dissipation was recorded. A representative QCM adsorption result is presented in Fig. 1 where changes in frequency and corresponding adsorbed amount are shown.

Fig. 1 Second axis graphs with frequency and mass.

**Molecular dynamics**

The simulated system consisted of a single anastellin molecule, a slab of PU, water and ions. The PU surface was constructed from slab of crystalline PU, consisting of 48 chains (three layers of sixteen), with each chain containing two monomer units. The slab was constructed
using the crystal structure for the trans-trans-dicyclohexylmethane 4,4’-diisocynate (HMDI) monomer determined by Nigar et al.\textsuperscript{48} An energy minimisation followed by short (20 ps) NVT and NpT simulations of the slab in contact with a vacuum were performed. The remainder of the simulation box was filled with water and an energy minimization and short NVT run were performed on the resulting system. The structure of the surface was then held fixed for the remainder of the simulations.

The structure of anastellin was taken from RCSB (accession code: 1Q38). Initially the protein centre-of-mass is placed at $z = 40\,\text{Å}$, approximately $20\,\text{Å}$ from the PU surface. Four different initial orientations were used: Arg-down where the cluster of arginine residues was orientated towards the surface; Arg-up where the cluster of arginine residues orientated away from the surface; N-down where the N-terminus pointed towards the surface and C-down where the C-terminus pointed towards the surface. Cl\textsuperscript{−} and Na\textsuperscript{+} ions are added to neutralise the $+5e$ charge on the protein and simulate different salt conditions. The system is periodic in the $x$ and $y$ directions and has repulsive Lennard-Jones walls in the $z$-direction.

The protein was modelled using the Charmm27 force field (with CMAP corrections),\textsuperscript{49} the Charmm Generalised Force Field\textsuperscript{50} was used to model the PU surface, and water was modelled using the Charmm-variant of TIP3P water (with van der Waals interaction sites on the hydrogen atoms). All simulations were performed at 298 K, with the velocity rescaling algorithm of Bussi et al.\textsuperscript{51} used to control the temperature. For each starting orientation and salt concentration simulations of 100 ns were performed, with a timestep of 2 fs. Bonds involving hydrogen atoms were constrained using the LINCS algorithm\textsuperscript{52} and the water geometry was held fixed using the SETTLE algorithm.\textsuperscript{53} Long-range electrostatics were modelled using particle-mesh Ewald summation\textsuperscript{54} with a real space cut-off of 10 Å and a reciprocal space grid spacing of 0.16 Å\textsuperscript{-1}. Van der Waals interactions were truncated at 10 Å.
The simulations were performed using the Gromacs MD package, version 4.6.3. Standard Gromacs tools were used to set up and analyse the simulations.

**Statistical Analysis**

Statistical analysis was performed in Microsoft Excel, Python and Minitab 17. A significance level of 5 % was chosen. Significance between adhesive forces under different conditions were determined with a one-way ANOVA with a Tukey test.

**RESULTS AND DISCUSSION**

**Contact Angle Goniometry and Surface Energies**

The contact angles of water on all substrates used are presented in Table 1 and agree well with the literature values for PMMA (74 °, 56 and 69 °, 29), PU (85.1 °, 35) and silica wafer (57.9 °, 35). From the chemical structures of PU and PMMA, it can be estimated that PMMA would be more hydrophobic since displays a greater frequency of lone electron pairs. The surface energies and surface energy components for all the surfaces are shown in Table 2. The Ra values (Table 2), based on the surface topography images collected with AFM, show that the films are smooth – 2.0 ± 0.1 nm and 3.1 ± 0.3 nm for PMMA and PU respectively. This suggests that the adsorption of Fn and water contact angle are not affected by surface roughness.

Table 1 Advancing contact angles of SW, PMMA and PU surfaces, n = 12.

Table 2 Surface energy components by CAG and surface roughness by AFM of SW, PMMA, and PU surfaces.
Circular Dichroism

The negative maximum at 218 nm (Fig. 2) suggests anti-parallel beta sheets as is expected for the protein fragment in its native conformation as imaged by Briknarova et al with NMR spectroscopy.

![Circular dichroism (CD) spectrum of FnIII1c.](image)

Atomic Force Microscopy

Anastellin functionalisation increases adhesion on all surfaces tested (Fig. 3). This may be partly due to the protein functionalisation process possibly adding a small layer of crystals from the PBS as well as the intended protein fragment. Such increases in tip radius do not appear to be the only factor since the differences between the PMMA (16.8 % increase) and PU (12.5 % increase) values are greater than those between the silica values (48.2 % increase). Since tip radius is accounted for, the difference between the polymers and the APTES-functionalised and protein-ATPES-functionalised probes is likely due to hydrophilic-hydrophobic interactions between protein fragment and elements of the polymer chains as found by Palacio et al. with the interactions between Fn and PMMA and poly (acrylic acid) (PAA). It appears that the adhesion of the anastellin-functionalised probes to the PU films was greater than to PMMA films (data not shown), contrary to previous work. This could be at least partly due to the fact that while Palacio et al.’s experiments were performed under liquid these were performed in air at ambient humidity reducing the role of hydrophilic-hydrophobic interactions.
Fig. 3 Difference in adhesion force by surface between silanised probes (APTES) and protein-functionalised probes (anastellin). All differences are significant.

Quartz crystal microbalance

Fig. 4 Density of anastellin on SiO$_2$, PU and PMMA surfaces.

Fig. 4a shows a representative measurement of protein adsorption on PMMA, in which both frequency and the corresponding mass change are presented as a function of time. It is clear that upon the introduction of protein solution, the mass at the already equilibrated polymer-buffer solution interface is increased, which confirms the adsorption of protein on the polymeric film. And the adsorption amounts of the protein (the changed frequency) on three different surfaces are compared in Fig. 4b. It was found that protein adsorbed the most on the silica surface, which is consistent with the AFM measurements where strong adhesion between protein and silica surface was observed. The PU surface shows a higher adsorption than the PMMA albeit the relative broad error bar, which agrees with the adhesion results. The increase in experimental error between silica and the polymer coated surfaces can be attributed to the increased surface roughness on the polymer thin films present as proven in the contact angle experiments.
Molecular dynamics

The adsorption mechanism and adhesion of anastellin to the polymer surfaces required further investigation by molecular dynamics. Due to high adhesion in the AFM studies and high adsorption in the QCM studies PU was selected for these further studies.

Independent of starting orientation the protein adsorbs onto PU surface within 100 ns, however, the equilibrium protein-surface separation and orientation depend on the initial configuration (Fig. 5). Starting from the Arg-down configuration the protein adsorbs rapidly onto the surface with little change in the orientation (the protein lies parallel to the surface across the entire simulation). In this case the final protein centre of mass position is ~32 Å, which is comparable to the width of the PU layer plus half the protein width. For the other starting configurations, adsorption typically takes longer and often involves transient contacts between the surface and protein before permanent adsorption. Additionally, the final separation between the protein centre-of-mass and surface is substantially larger, suggesting that in these cases the bulk of the protein lies further from the surface. This can be seen through the protein orientation. In particular, when starting from the Arg-up configuration the protein reorients so the N-terminus is towards the surface, so the final orientation of the protein in this case is close to that found form starting in the N-down conformation. For the C-down starting conformation, for most of the simulation the protein lies normal to the surface with the C-terminus pointing down (θ ~180 °) but slow reorientations of the protein are evident, notably towards the end of the simulation.

Fig. 5 Protein centre-of-mass position (top) and orientation (bottom) for no salt simulations. Simulations starting in the Arg-down, Arg-up, C-down, and N-down conformations are denoted by black, red, green, and blue lines respectively.
Fig. 6 Simulation snapshots showing adsorption of anastellin on to polyurethane surface. (a) Protein in Arg-down starting configuration at (left to right) t = 0 ns, 13 ns, 25 ns, 47 ns, 50 ns, and 100 ns. (b) Protein in Arg-up starting configuration at (left to right) t = 0 ns, 5 ns, 10 ns, 48 ns, 55 ns, and 100 ns. (c) Protein in N-down starting configuration at (left to right) t = 0 ns, 1 ns, 15 ns, 20 ns, 50 ns, and 100 ns. (d) Protein in C-down starting configuration at (left to right) t = 0 ns, 1 ns, 10 ns, 27.5 ns, 47 ns, and 100 ns. Residues involved in adsorption (see text) are highlighted.

Qualitative information on protein adsorption may be found from viewing simulation snapshots. For the Arg-down conformation (Fig. 6), these show the rapid adsorption of the protein onto the PU surface, with little subsequent change to either the protein structure or orientation. From the Arg-up conformation, the protein initially reorients itself in solution so that the N-terminus is directed towards the surface (t = 5 ns), followed by attachment to the surface through this region. While adsorbed on the surface, the protein can slowly reorient. Similarly, when the protein is initially placed in the N-down conformation, the protein’s N-terminus rapidly adsorbs onto the surface (t = 1 ns). For the C-down conformation the protein attaches through the C-terminus (t = 10 ns) but more slowly than for N-down conformation (whereas the N-down conformation attached to the surface after only 1 ns the C-down conformation is still in bulk water at this time). Compared to the N-down conformation the protein appears to have greater orientational freedom, which may also be seen from the variation in protein angle over time. While the initial attachment is through the residues at the extreme end of the C-terminus, this changes towards the end of the simulation, with residues at the C-terminus end of the beta-sheet coming into contact with the surface.
Fig. 7 Residue centre-of-mass positions for protein starting in (top to bottom) Arg-down, Arg-up, C-down, and N-down conformations.

The different starting orientations lead to attachment through different regions of the protein. This may be seen through considering the centre-of-mass positions of the individual residues (Fig. 7). Apart from the Arg-down conformation, in which most of the residues lie close to the surface, only small numbers of residues are typically in contact with the surface. For both Arg-up and N-down these are the N-terminus and the loop joining the third and fourth beta-strands (around residue 61). This second region contains a number of residues with hydrophilic side-chains, which are capable of forming hydrogen bonds with the polar groups in the PU surface. For the C-down orientation while the initial contact is through the C-terminal end at the end of the simulation this has detached from the surface with a loop containing residues 22 to 26 (joining the first and second beta-strands). Again this is a predominately hydrophilic region, which is consistent with the highest adsorption being found for hydrophilic surfaces. The residues that are in contact with the surface (taken to be those with separation between the surface and centres-of-mass less than 5 Å) are shown in Table 3.

Table 3 Residues in contact with surface

![Graph](image)

Fig. 8 (a) Secondary structure amounts for (top to bottom) Arg-down, Arg-up, C-down, and N-down starting conformations. Black, red, and green lines denote turn, beta-strand, and random coil respectively. (b) Secondary structure distributions for (top to bottom) Arg-down, Arg-up, C-down, and N-down starting conformations.
Surface adsorption has little effect on the structure of the protein. Shown in Fig. 8a are the secondary structure compositions for the different starting structures. In all cases the structure remains predominately beta-strand, with turn and random coil. Apart from N-down, there is a slight increase in the amount of random coil when the protein adsorbs, with the amount of turn decreasing. For N-down the amount of random coil and turn remains largely unchanged across the simulation, in agreement with the CD measurements. The distribution of the different secondary structure motifs is also largely unchanged during the simulations (Fig. 8b).

From consideration of all of the simulations it may be seen that three particular regions of the protein are especially important in mediating attachment; these are the loop joining the first and second beta-strands (around residue 23), the loop joining the third and fourth beta-strands, and the C-terminus. These contain predominately hydrophilic residues, with attachment being driven by hydrogen bonding between these groups and the N-H and C=O groups in the PU. Adsorption through flexible regions suggests that these play a role in mediating surface adsorption, similar to the fly-casting mechanism exhibited by some DNA-binding proteins. ⁶⁰

The use of silanisation to functionalise the AFM probe means that the protein attaches to the probe via the N-terminal ⁶¹ since this allows covalent bonding. Protein that is not covalently bonded to the AFM probe ought to be removed by the washing stages. This method for protein functionalisation has been used previously for antibodies ³⁸ and for Fn. Attachment of protein by the N-terminal would suggest that the C-down, Arg-down and Arg-up orientations used in the MD simulations are most relevant to the situation in the AFM experiments while all orientations are relevant to the QCM experiments.
CONCLUSION

In this paper the adsorption of anastellin, a C-terminal fragment of the fibronectin type III domain, onto biomaterial surfaces was investigated using a combination of experimental and theoretical methods. Understanding the adsorption of proteins onto synthetic surfaces is of interest in the development of new materials for medical applications. By combining a number of different methods, this work provides a picture of the adsorption ranging from the molecular to macroscopic levels.

Agreement between adhesion data and adsorption results confirm that there is greater interaction between anastellin and PU than between anastellin and PMMA. There appears to be agreement between the circular dichroism data and the molecular dynamics simulations of anastellin which both indicate a stable secondary structure. Molecular dynamics simulations of anastellin on polyurethane show that adsorption is strong and occurs rapidly. The simulations also show that the secondary structure of anastellin is stable upon adsorption to polyurethane in water and remains mostly stable even in saline solutions. Analysis of the simulations suggest that adsorption onto polyurethane is mediated by hydrophilic amino acids, due to hydrogen bonding with C=O and N-H groups in the polymer backbone, and residues in flexible regions of the protein.

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to AFM and contact angle measurements was provided by the EPSRC Centre in Continuous Manufacturing and Crystallisation (CMAC).
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**Figure captions**

Fig. 1 Second axis graphs with frequency and mass.

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<table>
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<th>Surface</th>
<th>DW</th>
<th>DIM</th>
<th>EG</th>
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<td>56.6 ± 1.3</td>
<td>42.2 ± 2.6</td>
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<tr>
<td>PMMA</td>
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<tr>
<td>PU</td>
<td>85.9 ± 12.2</td>
<td>42.1 ± 7.5</td>
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Table 2 Surface energy components by CAG and surface roughness by AFM of SW, PMMA, and PU surfaces.

<table>
<thead>
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<th>Surface</th>
<th>$\gamma_s^{lw}$</th>
<th>$\gamma_s'$</th>
<th>$\gamma_s$</th>
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<td>SW</td>
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<td>PMMA</td>
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<td>PU</td>
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<td>4.07</td>
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Table 3 Residues in contact with surface

<table>
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<th>Conformation</th>
<th>Residues in Contact</th>
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<tr>
<td>Arg-down</td>
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<tr>
<td>Arg-up</td>
<td>P7, Y61</td>
</tr>
<tr>
<td>C-down</td>
<td>N21, V23</td>
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<tr>
<td>N-down</td>
<td>A6, P7, Q8, Q60, Y61, G62</td>
</tr>
</tbody>
</table>
Equations

Equation 1
\[ A = 4\pi RT \]

Equation 2 Sauerbrey equation
\[ \Delta m = \frac{C \Delta f n}{n} \]