Published in final edited form as:

J Biomed Mater Res A. 2011 July ; 98(1): 7–18. doi:10.1002/jbm.a.33084.

IMMUNOBLOT ANALYSIS OF PROTEINS ASSOCIATED WITH SELF-ASSEMBLED MONOLAYER SURFACES OF DEFINED CHEMISTRIES

Rena M. Cornelius^{1,*}, Sucharita P. Shankar^{2,*}, John L. Brash¹, and Julia E. Babensee²

¹Department of Chemical Engineering and School of Biomedical Engineering, McMaster University, Hamilton, ON, Canada

²Wallace H. Coulter Department of Biomedical Engineering Georgia Institute of Technology and Emory University 313 Ferst Drive Atlanta, GA 30332 USA

Abstract

Intact and fragmented proteins, eluted from self assembled monolayer (SAM) surfaces of alkanethiols of different chemistries (-CH₃, -OH, -COOH, -NH₂), following exposure to human plasma (HP) or human serum (HS), were examined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting techniques. The SAM surfaces were incubated for 1 hour with 10% (v/v) sterile-filtered heat-inactivated (h.i.) HS or 1% (v/v) sterile-filtered h.i. HP preparations [both in phosphate buffered saline (PBS)]. Adsorbed proteins were eluted using 10% SDS/2.3% dithioerythritol for characterization of protein profiles. The type of incubating medium may be an important determinant of adsorbed protein profiles, since some variations were observed in eluates from filtered versus control unfiltered h.i. 10% HS or 1% HP. Albumin and apolipoprotein A1 were consistently detected in both filtered h.i. 10% HS and 1% HP eluates from all SAM surfaces and from control tissue culture-treated polystyrene (TCPS). Interestingly, Factor H and Factor I, antithrombin, prothrombin, high molecular weight kininogen (HMWK) and IgG were present in eluates from OH, COOH and NH₂ SAM surfaces and in eluates from TCPS, but not in eluates from CH₃ SAM surfaces, following exposure to filtered h.i. 10% HS. These results suggest that CH₃ SAM surfaces were the least pro-inflammatory of all SAM surfaces. Overall, similar trends were observed in the profiles of proteins eluted from surfaces exposed to filtered 10% HS or 1% HP. However the unique profiles of adsorbed proteins on different SAM surface chemistries may be related to their differential interactions with cells, including immune/inflammatory cells.

Keywords

self-assembled monolayers; inflammation/immune response; plasma/serum proteins; SDS-PAGE; immunoblot

INTRODUCTION

Protein adsorption is the initial event in mediating cellular interactions to direct the host response^{1,2}. Protein adsorption to biomaterial surfaces can be influenced by surface

To whom the correspondence should be addressed: Julia E. Babensee, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 313 Ferst Drive, Atlanta, GA 30332, Phone: (404) 385-0130, Fax: (404)-894-4243, julia.babensee@bme.gatech.edu.

*Both authors contributed equally towards the completion of this work.

properties including chemistry, hydrophobicity/hydrophilicity, and charge. In this work, a focus was placed on examining the profiles and compositions of adsorbed serum or plasma proteins eluted from the surfaces of model self-assembled monolayers (SAM) of alkanethiols that presented different endgroup chemistries; a robust, well-described system with controlled surface properties^{3–14}. The types of adsorbed proteins probed for in the biomaterial eluates included proteins which support or inhibit immune/inflammatory cell adhesion, survival and differentiation and hence influence the ensuing host response^{15–21}. Furthermore, the presence of distinctly pro- or anti-inflammatory proteins may respectively activate or passivate adherent cells and hence indirectly control recruitment of other cells^{22–24}.

In tissue engineering (TE) applications, the biomaterial component of the combination products is crucial in regulating cell adhesion, proliferation, differentiation, survival, and migration^{25–27}, as directed by the adsorbed protein layer²⁸. Acceptance of a biomaterial *in vivo* depends on its biocompatibility and integration with the host immune system^{29–32}. Biomaterial components of combination products may elicit non-specific inflammatory responses^{15,22,23,29} mediated through instantaneous host protein adsorption¹⁶, recruitment of phagocytic cells and antigen presenting cells (APCs) such as macrophages, resulting in wound healing and fibrosis^{33–40}. In addition, biomaterials can modulate the specific immune responses generated against the biological components of combination products²⁸, for example, acting as an adjuvant, due to maturation of APCs such as dendritic cells (DCs) and hence facilitate DC-orchestrated host responses^{28,41,42}.

In previous studies conducted by the Babensee laboratory, alkanethiol SAM surfaces having defined and distinct chemistries, charge and hydrophobicity/hydrophilicity presenting -CH_3 , -OH , -COOH or -NH_2 endgroups were utilized to examine the hypothesis that surface properties direct distinct phenotypic outcomes in immune cells. It was observed that, CH_3 SAM elicited lowest DC maturation and higher T cell tolerization compared to all other SAM chemistries⁴³. Furthermore, the Babensee laboratory has also shown with the identical set of four SAM chemistries, that different patterns of DC receptor glycan ligands associated with adsorbed serum/plasma proteins were observed as a function of surface chemistry⁴⁴. To further develop this research, this study examined the profiles of adsorbed proteins on the identical set of SAM surfaces to gain insight into the presence/absence of specific pro-/anti-inflammatory proteins and the low DC maturation and high associated autologous T cell immunosuppressive apoptosis observed earlier on CH_3 SAM. In this specific context, the hypothesis connecting these previous observations was that differential chemistries directed differential protein adsorption and hence differential DC-protein interactions, thereby causing varying DC activation levels. Towards further elucidation of mechanism, the goal in this study was to recreate the DC culture conditions directing serum/plasma protein adsorption on SAMs for elucidation of probable biomaterial-associated DC ligands. Specifically, the different alkanethiol SAM chemistries were incubated *in vitro* with either 10% (v/v) filtered heat inactivated (h.i.) human serum (HS) or 1% (v/v) filtered h.i. human plasma (HP) for 1 hour. Adsorbed proteins were eluted and analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting for a profile of adsorbed proteins. The types of serum/plasma preparations used in this study are equivalent to those used to culture human DCs with biomaterials, to hence mimic which adsorbed proteins these DCs may interact with on biomaterial surfaces to affect their resultant phenotype, as previously studied⁴³. The proteins probed for included cell-adhesive proteins (e.g., fibrinogen, fibronectin, vitronectin), complement proteins [e.g. complement 3 (C3), complement Factor B], fibrinolytic proteins (e.g. plasminogen), pro-inflammatory proteins and those present in abundance such as albumin or immunoglobulin G (IgG)⁴⁵.

From this comprehensive analysis of a wide range of adsorbed proteins, a snapshot in time emerges of likely protein candidates that may govern early (within 1 hour) interactions with cells and direct their subsequent responses. Within this one hour time, Vroman effect displacement of adsorbed proteins with others likely occurs but the time course of these changes is not within the scope of this study. Differential protein profiles were observed in eluates from the different SAM surfaces following exposure to filtered h.i. HS or HP (or to control unfiltered h.i. HS or HP). Specifically, Factor H and Factor I, antithrombin, prothrombin, high molecular weight kininogen (HMWK) and IgG although present in eluates from OH, COOH and NH₂ SAM surfaces or from tissue culture-treated polystyrene (TCPS) could not be detected in eluates from CH₃ SAM surfaces, following exposure to filtered h.i. 10% HS. Additionally, in experiments with assessing adsorption of radiolabelled fibrinogen from buffer, it was found that adsorption was lower on the CH₃ SAM surface than on the other SAM surfaces. Finally, the composition of the protein medium (plasma, serum), its concentration (1%, 10%) and whether filtered or not, affected the profiles of proteins eluted from surfaces having different SAM chemistries. We have previously demonstrated that the lowest DC maturation and highest T cell tolerization was observed on CH₃ SAM surfaces⁴³. The protein profiles in CH₃ SAM eluates observed in this study support the anti-inflammatory nature of CH₃ SAM surfaces and explain the modest DC maturation supported by all other SAM surfaces⁴³.

METHODS

Self-Assembled Monolayer Preparation

Self-assembled monolayer surfaces were assembled at Georgia Institute of Technology on 35 mm X 10 mm sterile TCPS dishes (Corning, Corning, NY)¹² and used for HS or HP adsorption and elution experiments. Alternatively, alkanethiol monolayers were assembled on both sides of 9 mm X 9 mm glass coverslips (Bellco Glass, Inc., Vineland, NJ) and used for protein quantification experiments utilizing radiolabelled protein. Clean dishes or coverslips were coated sequentially with 50 Å Ti followed by 150 Å Au films using an electron beam evaporator (CVC Products/Veeco, Rochester, NY) on the bases of the dishes as described in¹², or on both sides of the coverslips and stored under vacuum at room temperature (RT) for two weeks until used. The following alkanethiols were used as received from commercial sources (1 mM in absolute ethanol): 1-dodecanethiol (SH-(CH₂)₁₁-CH₃) (CH₃ SAM), 11-mercapto-undecanol (HS-(CH₂)₁₁OH) (OH SAM), 11-mercaptoundecanoic acid (HS-(CH₂)₁₀-COOH) (COOH SAM), (Aldrich Chemical, Milwaukee, WI) and 11-amino-1-undecanethiol, hydrochloride (C₁₁H₂₆ClNS) (NH₂ SAM) (Dojindo Laboratories, Gaithersburg, MD). The SAM surfaces were allowed to assemble by 12 hr incubation at RT by immersing the Ti/Au-coated dishes in alkanethiol solutions. Following this, the dishes were washed with 95% ethanol (Sigma, St. Louis, MO) dried with N₂ gas (Airgas South, Chamblee, GA) for 10 minutes in a fume hood, equilibrated with Phosphate Buffered Saline (PBS) (Gibco, Grand Island, NY) for 5 minutes and used fresh. For coating both sides of the coverslips in alkanethiol solutions, the coverslips were immersed in polypropylene vials having outer diameter of 12.5 mm (VWR International, San Diego, CA) containing 2 ml of 1 mM alkanethiol solution each, such that both Au/Ti-coated sides were constantly exposed to solution and the edges were in contact with the vial walls. Each vial containing coated coverslips was sealed with parafilm (VWR) individually and shipped as such (continuously in respective alkanethiol solutions, under which conditions the SAMs remain completely stable for 24–48 hours) at RT to Dr. John Brash's laboratory at McMaster University and used immediately for radiolabeled fibrinogen adsorption and elution experiments as described below. Upon removal of the coverslips coated on both sides from the coating SAM solutions, the coverslips were air-dried for 5 minutes, equilibrated with PBS at RT for 5 minutes in separate vials and then placed in PBS

in 24-well plates for 10 minutes at RT. Advancing water contact angles, performed at Georgia Institute of Technology, (measured for $n=3$ deionized water droplets in ambient air per SAM surface, calculating angles on two sides per droplet, values presented as average and standard deviations) for the CH_3 , OH, COOH and NH_2 SAM surfaces were $108 \pm 2^\circ$, $24 \pm 5^\circ$, $31 \pm 3^\circ$ and $35 \pm 4^\circ\text{C}$, respectively⁴⁴. Unmodified surfaces of TCPS were used as controls, with a typical contact angles of 71.8° ⁴⁶.

Radiolabeling of fibrinogen

A solution of human fibrinogen (Enzyme Research Laboratories Inc, South Bend, IN, USA) in isotonic Tris-buffered saline (TBS) (50 mM Tris base, 150 mM NaCl; pH 7.4) was prepared, aliquotted and stored at -70°C . The fibrinogen was labeled with ^{125}I (McMaster Nuclear Reactor, McMaster University, Hamilton, Ontario, Canada), using the iodine monochloride method. Following this, radiolabeled fibrinogen was passed through an anion exchange column packed with AG-1-X4 resin (BioRad, Richmond, CA, USA) for removal of unbound radioactive iodide. The amount of unbound radioactive iodide that still remained in the labeled protein solution was measured to be less than 1%. This quantification was performed on the supernatant obtained following fibrinogen precipitation with trichloroacetic acid (Fisher Scientific, ON, Canada)⁴⁷ solution of 20% w/v in water. In the final protein solution used, the ratio of labeled to unlabeled fibrinogen was maintained at 10%.

Quantification of amounts of adsorbed or eluted fibrinogen

The SAM-coated glass coverslips were first equilibrated in PBS-NaI solutions for 15 minutes for surface hydration and to prevent adsorption of free radiolabeled iodide ion, followed by incubation in 1 mL of ^{125}I -labeled fibrinogen solution (0.5 mg/mL) for 60 minutes at RT and maintained throughout under static conditions^{47,48}. For these adsorption studies, PBS solution containing 5% NaI was used, since the presence of low levels of non-radioactive iodide was observed to reduce non-specific binding of ^{125}I - to Au surfaces. This use of “cold” NaI is important, as Au-I interactions may result in the formation of complex ionic species including AuI_2^- and AuI_4^- , that may lead to erroneously high protein adsorption results.

Following exposure of the SAM-coated coverslips to the fibrinogen solution, the surfaces were rinsed three times for 2 minutes each with 3 mL of PBS-NaI at RT to remove residual non-adsorbed fibrinogen. Subsequently, the surfaces were wicked onto filter paper (BioRad) to remove any remaining buffer. The surface radioactivity was then measured in conjunction with calibration solutions, as necessary. This procedure was followed on four coated coverslips per SAM chemistry, per experiment, with all experiments being carried out in triplicate. The adsorbed proteins were eluted by exposure of the surfaces to a 10% SDS/2.3% dithioerithritol (DTE) (both from Sigma) solution in de-ionized (DI) water, on a shaker at RT for 24 hours. The surfaces were then rinsed three times with PBS to remove traces of the eluting solution. Finally the radioactivity associated with the eluate as well as with the surfaces was determined. Again, four replicates were analyzed per surface chemistry in each experiment and, again with all experiments being performed in triplicate.

Preparation of human plasma (HP) or human serum (HS)

Human blood was obtained from healthy volunteers with informed consent, according to a protocol approved by the Institute Review Board (IRB) # H05012. All subjects enrolled in this research signed an Informed Consent which was approved by the IRB of Georgia Institute of Technology. Peripheral human blood was collected using sterile 60 mL syringes (Becton Dickinson, Franklin Lakes, NJ) and needles (Becton Dickinson) using heparin (333

U/mL blood) (Baxter Healthcare Corporation, Deerfield, IL) as an anticoagulant. The clear yellowish HP layer was separated using lymphocyte separation medium (LSM) (Cellgro MediaTech, Herndon, VA) by differential gradient centrifugation of blood diluted 1:1 with sterile PBS (400 g, 30 minutes, RT) (Thermo Fisher Scientific Inc., Waltham, MA) (Model # 5682, Rotor IEC 216), filtered sterilized (0.22 μ m) (Corning) and heat inactivated for 30 minutes in a water bath pre-warmed to 56°C, aliquotted and stored at -20°C. Alternatively, plasma was h.i. but left unfiltered in order to examine the effects of sterile filtering on the plasma proteins. A stock solution of pooled HP from three donors was used for experiments for either filtered h.i. HP or for control unfiltered HP.

For preparation of HS, peripheral human blood was drawn without heparin anticoagulant and allowed to clot. The non-heparinized blood was centrifuged (3000 rpm, 10 minutes, RT) after which clots were compressed manually with a sterile pipette tip, as needed and maintained at RT for 90 minutes in the TC hood. Resultant HS was collected and cleared by further centrifugations at 3000 rpm for 15 minutes. Again, as necessary, residual precipitates were pressed down and the HS was filter sterilized (0.22 μ m) and h.i. for 30 minutes in a water bath pre-warmed to 56°C, aliquotted and stored at -20°C. Alternatively, serum was h.i. but left unfiltered. A stock solution of pooled HS from three donors was used for experiments for either filtered HS or for control unfiltered h.i. HS. It is noteworthy that heat inactivation of plasma/serum was performed to exactly replicate plasma/serum conditions used in analysis of DC phenotypic responses as directed by SAM chemistry⁴³ which formed the basis for this work. Heat inactivation was performed to minimize high baseline activation of DCs by serum/plasma activating factors, which may mask/interfere with DC responses to the SAM chemistries

Adsorption and elution of HS or HP proteins from biomaterial surfaces

The SAM surfaces were pre-incubated with 3 mL/dish of solutions of 10% (v/v) filtered h.i. HS or with 1% (v/v) filtered h.i. HP in PBS at RT for 1 hr. After this, the HS or HP solutions were removed and the dishes were washed three times for 5 minutes each with PBS at RT to remove residual non-adsorbed HS or HP proteins. To recover surface adsorbed proteins, the SAM-coated dishes were then incubated in 1 mL/dish of 10% SDS/2.3% DTE eluting solution in DI water, on a shaker at RT, for 24 hours. Samples of HS or HP solutions prior to and after incubation of the materials, as well as the protein eluted from each of the SAM surfaces were collected, stored in low retention, 1.7 mL PS vials (VWR) at -20°C at Georgia Institute of Technology and shipped overnight on dry ice to McMaster University and maintained frozen until further analysis.

Polyacrylamide gel electrophoresis and immunoblot analysis of eluates from different biomaterial surfaces

Polyacrylamide gel electrophoresis and immunoblot analyses were performed to characterize the profiles of HS or HP proteins in eluates from different SAM surfaces or from control TCPS surfaces, based on methods described elsewhere^{45,47,48}. Briefly, solutions composed of SDS - proteins from serum, plasma or eluate samples (protein concentration 0.4–0.5 mg/mL; 1 to 150 μ L, depending on the protein concentration) were reduced with β -mercaptoethanol (Bio-Rad) at 95°C for 5 minutes. Then, reduced samples were loaded onto 12% (w/v) polyacrylamide separating gels and a 4% (w/v) stacking gel, prepared based on previously described protocols. Proteins were separated according to size utilizing established SDS-PAGE conditions (200 V, 45–50 minutes), and were subsequently electrophoretically transferred onto an Immobilon polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA, USA). Unbound sites on the PVDF membrane were blocked for 1 hour with 0.3% Tween 20 (Bio-Rad) in TBS and then rinsed with water. The membrane was then exposed, overnight, to a Protogold solution (Bio-cell Research

Laboratories, Cardiff, UK, distributed by Cedarlane Laboratories, Hornby, ON, Canada), resulting in intense staining of protein bands.

Immunoblot analysis was also performed based on previously established protocols^{45,47,48}. As discussed above, proteins were separated according to size based on established SDS-PAGE conditions (200 V, 45–50 minutes), and electrophoretically transferred to a PVDF membrane. The membrane was then cut into 3 mm strips and exposed to a blocking buffer (5% (w/v) nonfat dry milk in TBS) for 1 hour. The strips were exposed to 1:1000 dilutions of the desired primary antibodies (Table 1) in solutions of 1% (w/v) nonfat dry milk and 0.05% (v/v) Tween 20 in TBS for 1 hour, rinsed three times for five minutes each with 0.1% (w/v) nonfat dry milk in TBS, and subsequently exposed to 1:1000 dilutions of the appropriate alkaline-phosphatase (AP) conjugated secondary antibodies (Table 2) in solutions of 1% (w/v) nonfat dry milk and 0.05% (v/v) Tween 20 in TBS for 1 hour. Color development was carried out using 5-bromo-4-chloro-3-indonyl phosphate (BCIP) and nitro-blue tetrazolium (NBT) (both from Bio-Rad). Pre-stained molecular weight standards (Bio-Rad), were used for molecular weight determination of the observed bands. Incubation conditions for antibodies have been carefully established to minimize cross reactivity, but this issue is always possible, and in situations where unexpected bands may be observed, cross reactivity could be a possible explanation. However, unexpected bands were not observed here. All strips, in a given immunoblot figure, were from the same PVDF membrane that were reassembled with proper alignment facilitated by the transfer to the PVDF membrane of a line of red dye, added just before the completion of the gel. Alignment was also facilitated by the two sets of pre-stained molecular weight standards that were run. The entire experiment described herein was run twice and Western and immunoblots were performed at least twice on each sample and a representative blot shown from the same experiment.

Statistical analysis

Statistical analysis was performed using general linear model analysis of variance⁴⁹ with Minitab software (Version 13.20, Minitab Inc., State College, PA) using pairwise comparisons between SAM surfaces and a Tukey post-test. A p value of less than or equal to 0.05 was considered as significant.

RESULTS

In general results from experiments with filtered h.i. 10% HS, “control” unfiltered h.i. 10% HS, filtered h.i. 1% HP and “control” unfiltered h.i. 1% HP were similar. Therefore only data from the filtered h.i. 10% HS are shown, although it should be understood that experiments were done with all four media types. Filtered h.i. 10% HS will be referred to subsequently as “serum” for simplicity.

Gold-stained gels of protein eluates from different SAM surfaces and from control TCPS

The proteins associated with different SAM chemistries and with control TCPS surfaces were detected using gold-stained polyacrylamide gels of serum eluates. A few protein bands were observed in the eluates from all of the biomaterials following incubation with all four media. Data are shown for serum in Figure 1.

Immunoblot analysis of proteins present in eluates from different SAM surfaces and control TCPS incubated with serum

Interestingly, differential profiles of adsorbed proteins were observed in eluates from different SAM surfaces following exposure to serum: CH₃ SAM (Figure 2a), OH SAM (Figure 2b), COOH SAM (Figure 2c) and NH₂ SAM (Figure 2d), as summarized below and

shown in Table 3. Figure 2e shows immunoblots of eluates from control, TCPS. Figure 2f shows immunoblots of serum using the same antibodies as for the eluates. Pre-stained molecular weight standards were used to estimate the molecular weight of the bands. Bands were detected at ~40, 45, 48 kDa [high molecular weight kininogen (HMWK)], ~57, 90 kDa (antithrombin), ~55 kDa⁴⁴, ~150 kDa (Factor H), ~50 kDa (Factor I) and ~40, 80 kDa (prothrombin) in the eluates from OH, COOH and NH₂ SAMs (Figures 2b to 2d, Table 3) and from the TCPS surfaces (Figure 2e), but not in the eluates from CH₃ SAMs (Figure 2a, Table 3). Furthermore, bands for C3, transferrin, albumin, vitronectin, Factor B and apolipoprotein A1 were detected in eluates from most SAM surfaces (Figures 2a to 2d, Table 3), and from control TCPS (Figure 2e, Table 3), while Factor XI, Factor XII, pre-kallikrein, fibrinogen, plasminogen, α_1 -anti-trypsin, fibronectin, β -lipoprotein, α_2 -macroglobulin and hemoglobin were not detected on any SAM surface (Figures 2a to 2d, Table 3). Similar trends were observed for the three other media types (data not shown).

Gold-stained gels of proteins present in filtered or control unfiltered h.i. 10% HS and 1% HP

Differential presence of protein bands was observed between gold-stained gels of solutions of filtered or control unfiltered h.i. 10% HS, or filtered or control unfiltered h.i. 1% HP (data not shown). In general, more protein was seen for control unfiltered h.i. 10% HS or 1% HP compared to filtered h.i. 10% HS or 1% HP, respectively, given that the volume of protein loaded was the same in all cases.

Immunoblot analysis of proteins present in filtered or control unfiltered h.i. 10% HS or 1% HP

As expected, immunoblot analysis of proteins present in filtered or control unfiltered 10% HS or 1% HP samples indicated the presence of bands corresponding to intact proteins or fragments of most of the HS or HP proteins probed for. Proteins detected in the serum include intact or degradation fragments of Factor XI, Factor XII, pre-kallikrein, HMWK, plasminogen, antithrombin, C3, transferrin, α_1 -anti-trypsin, fibronectin, albumin, IgG, α_2 -macroglobulin, vitronectin, Factor B or Factor H, Factor I, apolipoprotein A1, prothrombin or hemoglobin (Figure 2f, Table 3), although other proteins such as β -lipoprotein were not present. In addition, in filtered (Figure 2f) and unfiltered serum, high molecular weight bands were detected for antithrombin III, indicating the formation of antithrombin III-thrombin complex (~90 kDa). Prothrombin bands were seen at ~80 kDa (intact molecule) and at ~40 kDa (cross reactivity with thrombin) (Figure 2f). Certain proteins such as β -lipoprotein or fibrinogen were not detectable (n.d) in either filtered h.i. 10% HS (Figure 2f, Table 3) or control unfiltered h.i. 10% HS (data not shown).

Quantification of adsorbed, eluted and “bound” fibrinogen on different SAM surfaces

To examine the possibility that the differential presence of proteins associated with different SAM chemistries was due to differential elutability of proteins, the amounts of adsorbed, eluted or bound fibrinogen associated with the different surfaces were determined using radiolabeling methods. A significantly higher amount of adsorbed fibrinogen was detected on the COOH or NH₂ SAM surfaces as compared to the CH₃ or OH SAM surfaces. Also, significantly higher amounts of fibrinogen remained on COOH or NH₂ SAM surfaces after elution as compared to CH₃ or OH SAM surfaces (Figure 3a). The percentages of adsorbed fibrinogen eluted from CH₃, OH, COOH, and NH₂ SAM surfaces were $86 \pm 6\%$, $91 \pm 3\%$, $67 \pm 8\%$, and $77 \pm 8\%$, respectively (Figure 3b). Overall, elutability was greater from OH or CH₃ SAM surfaces than from NH₂ or COOH SAM surfaces.

DISCUSSION

SDS-PAGE and immunoblot analysis of proteins eluted from different SAM surfaces and from control TCPS surfaces revealed differential profiles of associated HS or HP proteins. Certain pro-inflammatory proteins were absent from serum eluates of CH₃ SAMs (Figure 2a, Table 3), while being present in serum eluates of OH, COOH, NH₂ SAM or TCPS surfaces (Figures 2b to 2e, Table 3), suggesting that the CH₃ SAM may be the least pro-inflammatory. These results may be related to previous observations of lower DC maturation, measured phenotypically and functionally, and also greater autologous T cell tolerization following culture on CH₃ SAM surfaces treated with serum⁴³.

Immunoblot analysis of biomaterial-associated proteins provides information on potential biomaterial-linked DC ligands. It is important to forge the protein-cell connection to better understand bi-directional immune/inflammatory cell-biomaterial interactions. As professional APCs, DCs represent an important link between innate and adaptive immunity and direct cellular immune responses^{50–55}. Therefore, the acquisition of knowledge of the ability of biomaterials to support DC maturation *in vitro*^{28,41–43} represents a significant initial step towards ultimately examining the potential of the biomaterials to stimulate DC-regulated adjuvant effects under *in vivo* conditions⁵⁶. Biomaterials that are strong adjuvants may be useful in vaccine delivery applications to enhance a beneficial host response. Conversely, minimal biomaterial adjuvant effects may be optimal in combination products for regenerative medicine where a host response needs to be mitigated.

This work is significant in a broader context in that it analyzes protein adsorption profiles as directed by surfaces of distinct, defined chemistries. The SAM surfaces studied were alkanethiols of 10–12 C atoms presenting terminal methyl, hydroxyl, carboxyl and amino groups to give hydrophobic, neutral hydrophilic, anionic hydrophilic and cationic hydrophilic surfaces, respectively^{3,7,9,10,13,57,58,59,60}. Following incubation with filtered serum, as used in primary DC culture media, several serum proteins eluted from TCPS were also found in eluates from the SAM surfaces (Figures 2a to 2e, Table 3). The list comprises proteins such as C3, transferrin, albumin, vitronectin, Factor B and apolipoprotein A1. Additionally, certain clotting factors/and other proteins that were present in eluates from control TCPS were detected only in eluates from some SAMs; HMWK, antithrombin, IgG, Factor H, Factor I and prothrombin were present in the eluates from OH, COOH or NH₂ SAMs (Figures 2b to 2d, Table 3) and from TCPS (Figure 2e, Table 3), but not in eluates from CH₃ SAMs (Figure 2a, Table 3). Furthermore, following exposure of test materials to serum (Figures 2a to 2e, Table 3), vitronectin bands were detected at ~65 kDa and ~75 kDa in the eluates from all SAM surfaces implying that degradation of this protein did not occur. Taken together, these results suggest that the profiles of proteins eluted from some of the SAMs and the hydrophilic TCPS surface were similar, while those from the hydrophobic CH₃ SAMs were different (Figures 2a to 2e, Table 3).

In general, similar trends were observed with filtered h.i. 1% HP as the pre-incubating solution, as used in culture media for an alternate method of primary DC culture (unpublished observations, Shankar, S.P.) and hence investigated in this study. For unfiltered h.i. 1% HP, proteins present on anionic hydrophilic COOH SAM surfaces most closely resembled those present on hydrophilic control TCPS surfaces. As perhaps expected, fibrinogen was present in very small amounts in eluates from CH₃ SAMs that had been exposed to filtered h.i. 1% HP but was undetected in eluates from all surfaces exposed to serum (Table 3). Consistent with these results, a smaller amount of fibrinogen was adsorbed to CH₃ SAMs from buffer than to the other SAM surfaces (Figure 3a). Tegoulia and Cooper also found that high levels of fibrinogen were associated with OH compared to CH₃ SAMs⁹. On the other hand, a decrease in fibrinogen adsorption was observed with increasing OH

content in mixed OH/CH₃ SAMs⁶¹. Furthermore, adsorption of pro-inflammatory C3 was found to be greater on OH than on CH₃ SAMs⁶². In agreement with this finding, lower band intensities for C3 were seen in CH₃ SAM eluates (Figure 2a, Table 3) versus OH SAM (Figure 2b, Table 3). However, C3 was not detected in OH SAM eluates following incubation with filtered h.i. 1% HP or control unfiltered h.i. 1% HP, possibly due to the high affinity of C3b for OH SAM¹ and consequently lower desorption of C3 into the eluate. In a study by Silin et al⁶³ greater amounts of IgG were detected on CH₃ SAMs compared to OH, COOH or NH₂ SAMs contrary to the results presented herein. Interestingly, in the present work, bands for IgG were present in eluates from OH (Figure 2b, Table 3), COOH (Figure 2c, Table 3) or NH₂ SAMs (Figure 2d, Table 3) but not in eluates from control TCPS (Figure 2e, Table 3) or CH₃ SAMs with filtered serum (Figure 2a, Table 3) nor in eluates from COOH or NH₂ SAMs with unfiltered serum. Also, adsorption of anti-inflammatory albumin was found to be higher on CH₃ SAMs than on either OH or COOH SAMs⁶⁴. Finally, bands for albumin and apolipoprotein A1 were detected in eluates from all incubating media. The significance of this finding may be related to lipid and carbohydrate post-translational modifications of apolipoprotein A1^{65,66} that may act as ligands for C-type lectin receptors (CLRs) or scavenger receptors (SRs) in DCs. Table 4 presents a comparison between previous literature and current findings of profiles of key SAM-associated proteins.

The proteins investigated by immunoblotting in this study represent a subset of all the proteins present in serum or plasma. Since it is not feasible to test all serum or plasma proteins, those proteins that are commonly associated with biomaterials and with known significant roles in biological processes associated with biomaterials such as thrombosis and cell adhesion were investigated. Proteins were eluted using SDS as in several other studies⁴⁵. A caveat with this approach is that proteins that are not SDS-elutable are not recovered. However, many of proteins tested for and present in serum (Figure 2f) (or 1% HP) were detected in the serum eluates (Figure 2a to 2e).

With respect to fibrinogen adsorption from buffer, the COOH or NH₂ SAMs adsorbed more than the CH₃ or OH SAMs (Figure 3a). Also, more fibrinogen remained bound to COOH or NH₂ SAMs after elution with SDS/DTE compared to CH₃ or OH SAMs (Figure 3a). These results suggest that the lower intensity of various protein bands in CH₃ SAM blots compared to other SAMs (Figures 2a–2d, Table 3) was not due to lower protein elutability, although it should be acknowledged that proteins other than fibrinogen may be differentially elutable on the different SAMs.

Other factors that may have influenced adsorption include the different media: plasma versus serum; filtration and sterilization causing selective protein removal; the effects of heat inactivation, and concentration (1 versus 10%). At lower concentration, the possibility that highly abundant proteins are more easily detected than less abundant proteins cannot be excluded and may partly be responsible for the non detection of certain proteins especially for 1% HP. However, it should be emphasized that the pre-adsorbing 10% filtered h.i. HS or 1% filtered h.i. HP are identical to serum/plasma protein preparations used in DC culture media. This DC media was used previously to examine DC maturation responses following culture on different SAM surfaces for 10% filtered h.i. HS⁴³, or for 1% filtered h.i. HP (unpublished observations, Shankar, S.P.). Hence, proteins adsorbed from 10% filtered h.i. HS or 1% filtered h.i. HP onto SAM surfaces in this study, are comparable to protein layers that are adsorbed from DC media onto SAM surfaces⁴³ and have been focused upon because they subsequently influence DC responses.

In future work, it is of interest to utilize matrix-associated laser deposition/ionization (MALDI)-time of flight (TOF) advanced analytical methods or other mass spectrometry (MS)-based proteomic tools to study adsorbed and eluted proteins from different SAM

surfaces⁶⁷. Furthermore, enzyme-based cleavage techniques can be used to analyze post-translational modifications of these proteins. Such proteomic analyses require large sample volumes of eluted proteins and high protein concentrations (i.e. large biomaterial surface areas for treatment). While MS has been used extensively to study proteins in solution⁶⁸, transitioning to the analysis of complex adsorbed protein layers directly on biomaterial surfaces^{69,70} would require significant technical development, including detachment of proteins from the surface and their introduction into the analyzer, as a separate study. Furthermore, since cell-biomaterial interactions are recognized as highly important in directing cell responses, future work could include addressing the difficult problem of extrapolating from protein adsorption studies to an effect in functional DC assays. Specifically, the hypothesis suggested by these results is that the mechanism underlying differential DC and associated T lymphocyte activation on CH₃ SAM versus other SAMs is in part due to the decreased adsorbed innate immune components of serum/plasma such as complement (Factor H, I) and coagulation proteins (antithrombin, prothrombin) on CH₃ SAMs. While the authors do not exclude the possibility that a combination of adsorbed proteins may have synergistic effects, the goal would be to identify the initial and crucial adsorbed protein(s) triggering markedly diverse DC responses. One would envision an approach in which one of these “CH₃-SAM missing” proteins is depleted from the serum used to treat one of the other SAM surfaces to see if the level of supported DC maturation would be lowered. Conversely, one may spike via pre-adsorption the CH₃ surface with one of the “CH₃-SAM missing” proteins to see if the level of DC maturation would be increased. We have investigated the relative role of β 1 and β 2-integrins in mediating DC adhesion to serum-coated biomaterials and shown a β 2-integrin-dependence, however, the biomaterial-adsorbed ligand(s) remains to be defined⁷¹. Through such studies, it is expected to inform design/coating of biomaterials that would preferentially allow the formation of biomaterial-associated biopatterns known to induce specific immune cell activation outcomes. A better understanding of the roles of proteins that link biomaterials to immune cells would allow for improved prediction and control of physiological responses. The ultimate goal of this work is to guide the design of biomaterials that have a spectrum of immunomodulatory effects for wide-ranging applications of combination products.

To summarize, we have previously shown that of all SAM chemistries tested, CH₃ SAM elicited lowest DC maturation and triggered increased apoptosis and tolerization of associated autologous T cells⁴³. The important supportive finding from this study is that CH₃ SAM surfaces also exhibited a distinct profile of less pro-inflammatory adsorbed serum/plasma proteins compared to all other SAM surfaces. Notably, certain coagulation and complement proteins were not detected on CH₃ SAM surfaces. While albumin could be detected in the eluates from CH₃ SAM surfaces, lower levels of fibrinogen were measured on CH₃ SAM surfaces as compared to certain SAM surfaces. Taken together, the CH₃ SAM chemistries appear to be less inflammatory to immune cells⁴³, likely due to their unique profile of presented proteins as identified here. Figure 4 is a schematic highlighting potential SAM chemistry-protein-cell interactions. Specifically, this schematic indicates that incubation of CH₃ SAM surfaces with serum/plasma triggers the adsorption of a distinct profile of proteins including albumin, apolipoprotein and low fibrinogen, ultimately leading to lower DC maturation and higher autologous lymphocyte apoptosis⁴³. In contrast, incubation of OH, COOH or NH₂ SAM surfaces in serum/plasma resulted in adsorption of pro-inflammatory complement, coagulation and cell adhesive proteins, finally eliciting higher DC maturation and lower T cell tolerization⁴³.

Acknowledgments

This research has been funded by the National Science Foundation under a CAREER grant (BES-0239152) and by the National Institutes of Health grant 1RO1 EB004633-01A1. The authors thank Timothy Petrie, Jeffrey Capadona and Andres Garcia of Georgia Tech for supplying the Au/Ti-coated substrates for SAM assembly.

References

1. Ratner, BD.; Hoffman, AS.; Schoen, FJ.; Lemons, JE. Biomaterial Science-An introduction to materials in medicine. San Diego: Elsevier Academic Press; 1996.
2. Anderson JM. Biological responses to materials. *Annu Rev Mater Res.* 2001; 31:81–110.
3. Mrksich M, Chen CS, Xia Y, Dike LE, Ingber DE, Whitesides GM. Controlling cell attachment on contoured surfaces with self-assembled monolayers of alkanethiolates on gold. *Proc Natl Acad Sci USA.* 1996; 93(20):10775–10778. [PubMed: 8855256]
4. Lindbald M, Lestelius M, Johansson A, Tengvall P, Thomsen P. Cell and soft tissue interactions with methyl- and hydroxyl-terminated alkane thiols on gold surfaces. *Biomaterials.* 1997; 18(15): 1059–1068. [PubMed: 9239468]
5. Harder P, Grunze M, Dahint R, Whitesides GM, Laibinis PE. Molecular conformation on oligo (ethylene glycol)-terminated self-assembled monolayers on gold and silver surfaces determines their ability to resist protein adsorption. *J Phys Chem B.* 1998; 102(2):426–436.
6. Sigal GB, Mrksich M, Whitesides GM. Effect of surface wettability on the adsorption of proteins and detergents. *J Am Chem Soc.* 1998; 120(14):3464–3473.
7. Kalltorp M, Oblogina S, Jacobsson S, Karlsson A, Tengvall P, Thomsen P. In vivo cell recruitment, cytokine release and chemiluminescence response at gold, and thiol functionalized surfaces. *Biomaterials.* 1999; 20(22):2123–37. [PubMed: 10555080]
8. McClary KB, Ugarova T, Grainger DW. Modulating fibroblast adhesion, spreading and proliferation using self-assembled monolayer films of alkylthiolates on gold. *J Biomed Mater Res.* 2000; 50(3):428–439. [PubMed: 10737886]
9. Tegoulia VA, Cooper SL. Leukocyte adhesion on model surfaces under flow: effects of surface chemistry, protein adsorption, and shear rate. *J Biomed Mater Res.* 2000; 50(3):291–301. [PubMed: 10737870]
10. Shen M, Horbett TA. The effects of surface chemistry and adsorbed proteins on monocyte/macrophage adhesion to chemically modified polystyrene surfaces. *J Biomed Mater Res.* 2001; 57(3):336–345. [PubMed: 11523028]
11. Barbosa JN, Barbosa MA, Aguas AP. Adhesion of human leukocytes to biomaterials: An in vitro study using alkanethiolate monolayers with different chemically functionalized surfaces. *J Biomed Mater Res A.* 2003; 65(4):429–434. [PubMed: 12761831]
12. Keselowsky BG, Collard DM, Garcia AJ. Surface chemistry modulates fibronectin conformation and directs integrin binding and specificity to control cell adhesion. *J Biomed Mater Res A.* 2003; 66(2):247–259. [PubMed: 12888994]
13. Schwendel D, Hayashi T, Dahint R, Pertsin A, Grunze M, Steitz R, Schreiber F. Interaction of water with self-assembled monolayers: neutron reflectivity measurements of the water density in the interface region. *Langmuir.* 2003; 19(6):2284–2293.
14. Dadsetan M, Jones JA, Hiltner A, Anderson JM. Surface chemistry mediates adhesive structure, cytoskeletal organization, and fusion of macrophages. *J Biomed Mater Res A.* 2004; 71(3):439–448. [PubMed: 15476262]
15. Sefton MV, Gemmell CH, Gorbet MB. What really is blood compatibility. *J Biomater Sci Polym Ed.* 2000; 11(11):1165–1182. [PubMed: 11263806]
16. Brash, JL. Protein adsorption at the solid-solution interface in relation to blood-material interactions. Washington, DC: American Chemical Society; 1987.
17. Gorbet MB, Sefton MV. Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials.* 2004; 25(26):5681–5703. [PubMed: 15147815]

18. Collier TO, Jenney CR, DeFife KM, Anderson JM. Protein adsorption on chemically modified surfaces. *Biomed Sci Instrum.* 1997; 33:178–183. [PubMed: 9731356]
19. Kao WJ. Evaluation of protein-modulated macrophage behaviour on biomaterials: designing biomimetic biomaterials for cellular engineering. *Biomaterials.* 1999; 20(23–24):2213–2221. [PubMed: 10614928]
20. Jenney CR, Anderson JM. Adsorbed serum proteins responsible for surface dependent human macrophage behavior. *J Biomater Mater Res.* 2000; 49(4):435–447.
21. Collier TO, Anderson JM. Protein and surface effects on monocyte and macrophage adhesion, maturation and survival. *J Biomater Mater Res.* 2002; 60(3):487–496.
22. Anderson JM. Inflammatory response to implants. *Trans Am Soc Artif Intern Organs.* 1988; 34(2): 101–107.
23. Tang L, Eaton JW. Inflammatory response to biomaterials. *Am J Clin Pathol.* 1995; 103(4):466–471. [PubMed: 7726145]
24. Tang L, Eaton JW. Fibrin(ogen) mediates acute inflammatory responses to biomaterials. *J Exp Med.* 1993; 178(6):2147–2156. [PubMed: 8245787]
25. Peppas NA, Langer R. New challenges in biomaterials. *Science.* 1994; 263(5154):1715–1720. [PubMed: 8134835]
26. Hubbell JA. Biomaterials in tissue engineering. *Nature Biotechnology.* 1995; 13:565–576.
27. Langer R, Tirrell DA. Designing materials for biology and medicine. *Nature.* 2004; 428(6982): 487–492. [PubMed: 15057821]
28. Babensee JE, Anderson JM, McIntire LV, Mikos AG. Host response to tissue engineered devices. *Adv Drug Deliv Rev.* 1998; 33(1–2):111–139. [PubMed: 10837656]
29. Langer R, Vacanti JP. Tissue Engineering. *Science.* 1993; 260(5110):920–926. [PubMed: 8493529]
30. Atala A. Tissue engineering and regenerative medicine: concepts for clinical application. *Rejuvenation Res.* 2004; 7(1):15–31. [PubMed: 15256042]
31. Kulig KM, Vacanti JP. Hepatic tissue engineering. *Transpl Immunol.* 2004; 12(3–4):303–310. [PubMed: 15157923]
32. Lavik E, Langer R. Tissue engineering: current state and perspectives. *Appl Microbiol Biotechnol.* 2004; 65(1):1–8. [PubMed: 15221227]
33. McNally AK, DeFife KM, Anderson JM. Interleukin-4-induced macrophage fusion is prevented by inhibitors of mannose receptor activity. *Am J Clin Pathol.* 1996; 149(3):975–985.
34. Anderson JM. Multinucleated giant cells. *Curr Opin Hematol.* 2000; 7(1):40–47. [PubMed: 10608503]
35. Brodbeck WG, Nakayama Y, Matsuda T, Colton E, Ziats NP, Anderson JM. Biomaterial surface chemistry dictates adherent monocyte/macrophage cytokine expression *in vitro*. *Cytokine.* 2002; 18(6):311–319. [PubMed: 12160519]
36. Xia Z, Triffitt JT. A review on macrophage responses to biomaterials. *Biomed Mater.* 2006; 1(1):R1–R9. [PubMed: 18458376]
37. Kao WJ, Schmidt DR. The interrelated role of fibronectin and interleukin-1 in biomaterial-modulated macrophage function. *Biomaterials.* 2007; 28(3):371–382. [PubMed: 16978691]
38. Van den Beucken JJJP, Walboomers XF, Vos MRJ, Sommerdijk NAJM, Nolte RJMJ, JA. Macrophage behaviour on multilayered DNA-coatings *in vitro*. *J Biomed Mater Res A.* 2007; 80(3):612–620. [PubMed: 17031824]
39. MacEwan MR, Brodbeck WG, Matsuda T, Anderson JM. Monocyte/lymphocyte interactions and the foreign body response: *in vitro* effects of biomaterial surface chemistry. *J Biomed Mater Res A.* 2005; 74(3):285–293. [PubMed: 16124082]
40. Phillips JM, Kao WJ. Macrophage adhesion on gelatin-based interpenetrating networks grafted with PEGylated RGD. *Tissue Engg.* 2005; 11(5–6):964–973.
41. Yoshida M, Babensee JE. Poly(lactic-co-glycolic acid) enhances maturation of human monocyte-derived dendritic cells. *J Biomed Mater Res A.* 2004; 71(1):45–54. [PubMed: 15368253]
42. Babensee JE, Paranjpe A. Differential levels of dendritic cell maturation on different biomaterials used in combination products. *J Biomed Mater Res A.* 2005; 74(4):503–10. [PubMed: 16158496]

43. Shankar SP, Petrie TA, Garcia AJ, Babensee JE. Dendritic cell responses to self-assembled monolayers of defined chemistries. *J Biomater Mater Res A*. 2010; 92A(4):1487–1499.
44. Shankar SP, Chen II, Keselowsky BG, Garcia AJ, Babensee JE. Profiles of carbohydrate ligands associated with adsorbed proteins on self-assembled monolayers of defined chemistries. *J Biomater Mater Res A*. 2010; 92A(4):1329–1342.
45. Babensee JE, Cornelius RM, Brash JL, Sefton MV. Immunoblot analysis of proteins associated with HEMA-MMA microcapsules: Human serum proteins in vitro and rat proteins following implantation. *Biomaterials*. 1998; 19(7–9):839–849. [PubMed: 9663761]
46. Bahulekar R, Tokiwa T, Kano J, Matsumura R, Kojima I, Kodama M. Polyacrylamides containing sugarresidues: synthesis, characterization and hepatocyte attachment studies. *Biotech Tech*. 1998; 12:721–724.
47. Cornelius RM, Brash JL. Identification of proteins adsorbed to hemodialyser membranes from heparinized plasma. *J Biomater Sci Polym Ed*. 1994; 1993(4):291–304.
48. Mulzer SR, Brash JL. Identification of plasma proteins adsorbed to hemodialyzers during clinical use. *J Biomater Mater Res*. 1989; 23:1483–1504.
49. Young SH, Ye J, Frazer DG, Shi X, Castranova V. Molecular mechanism of tumor necrosis factor- α production in 1,3- β -glucan (zymosan)-activated macrophages. *J Biol Chem*. 2001; 276(23):20781–20787. [PubMed: 11259437]
50. Adams S, O'Neill DW, Bhardwaj N. Recent advances in dendritic cell biology. *J Clin Immunol*. 2005; 25(2):87–98. [PubMed: 15821885]
51. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol*. 1991; 9:271–296. [PubMed: 1910679]
52. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature*. 1998; 392(6673):245–252. [PubMed: 9521319]
53. Janeway CAJ, Medzhitov R. Innate Immune Recognition. *Annu Rev Immunol*. 2002; 20:197–216. [PubMed: 11861602]
54. Medzhitov R, Janeway CAJ. Innate Immunity: impact on the adaptive immune response. *Curr Opin Immunol*. 1997; 9(1):4–9. [PubMed: 9039775]
55. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol*. 2000; 18:767–811. [PubMed: 10837075]
56. Singh M, O'Hagan D. Advances in vaccine adjuvants. *Nat Biotechnol*. 1999; 17(11):1075–1081. [PubMed: 10545912]
57. Capadona J, Collard D, Garcia AJ. Fibronectin adsorption and cell adhesion to mixed monolayers of tri(ethylene glycol)- and methyl-terminated alkanethiols. *Langmuir*. 2003; 19(5):1847–1852.
58. Maoz R, Netzer L, Gun J, Sagiv J. Self-assembling monolayers in the construction of planned supramolecular structures and as modifiers of surface properties. *J Chem Phys*. 1988; 85(1985):1059–1064.
59. Whitesides GM, Mathias JP, Seto CT. Molecular self-assembly and nanochemistry: A chemical strategy for the synthesis of nanostructures. *Science*. 1991; 254(5036):1312–1319. [PubMed: 1962191]
60. Barbosa JN, Barbosa MA, Aguas AP. Inflammatory Cell Recruitment and Adhesion to Methyl-Terminated Self-Assembled Monolayers: Effect of Implantation Time. *Microsc Res Tech*. 2005; 66(1):37–42. [PubMed: 15816035]
61. Rodrigues SN, Goncalves IC, Martins MC, Barbosa MA, Ratner BD. Fibrinogen adsorption, platelet adhesion and activation on mixed hydroxyl-/methyl-terminated self-assembled monolayers. *Biomaterials*. 2006; 27(31):5357–5367. [PubMed: 16842847]
62. Hirata I, Hioki Y, Toda M, Kitazawa T, Murakami Y, Kitano E, Kitamura H, Ikada Y, Iwata H. Deposition of complement protein C3b on mixed self-assembled monolayers carrying surface hydroxyl and methyl groups studied by surface plasmon resonance. *J Biomed Mater Res A*. 2003; 66(3):669–76. [PubMed: 12918051]
63. Silin VV, Weetall H, Vanderah DJ. SPR Studies of the Nonspecific Adsorption Kinetics of Human IgG and BSA on Gold Surfaces Modified by Self-Assembled Monolayers (SAMs). *J Colloid Interface Sci*. 1997; 185(1):94–103. [PubMed: 9056309]

64. Martins MC, Fonseca C, Barbosa MA, Ratner BD. Albumin adsorption on alkanethiols self-assembled monolayers on gold electrodes studied by chronopotentiometry. *Biomaterials*. 2003; 24(21):3697–706. [PubMed: 12818541]
65. Dayal B, Ertel NH. ProteinChip technology: a new and facile method for the identification and measurement of high-density lipoproteins apoA-I and apoA-II and their glycosylated products in patients with diabetes and cardiovascular disease. *J Proteome Res*. 2002; 1(4):375–380. [PubMed: 12645894]
66. Zannis VI, Chroni A, Krieger M. Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. *J Mol Med*. 2006; 84(4):276–294. [PubMed: 16501936]
67. McLean, D.; Cornelius, R.; Macri, J.; Malinowski, P.; Brash, JL. Proteomics Inspired Approach to Studying Plasma Protein Adsorption to Biomaterials. 8th World Biomaterials Congress; Amsterdam, The Netherlands. 2008.
68. Miike K, Aoki M, Yamashita R, Takegawa Y, Saya H, Miike T, Yamamura KI. Proteome profiling reveals gender differences in the composition of human serum. *Proteomics*. 2010.1002/pmic.200900496
69. Kim J, Scott EA, Elbert DL. Proteomic analysis of protein adsorption: Serum amyloid P adsorbs to materials and promotes leukocyte adhesion. *J Biomed Mater Res A*. 2005; 75(1):199–209. [PubMed: 16082704]
70. Scott EA, Elbert DL. Mass spectrometric mapping of fibrinogen conformations at poly(ethylene terephthalate) interfaces. *Biomaterials*. 2007; 28 (27):3904–3917. [PubMed: 17582492]
71. Rogers T, Babensee JE. The role of integrins in the recognition of biomaterials by dendritic cells. *Biomaterials*. in press.

Figure 1.
Gold stained SDS-PAGE gels of proteins eluted from control TCPS and from different SAM surfaces following exposure to filtered h.i. 10% human serum.

Figure 2.

Immunoblots of proteins present in the eluates from (a) CH₃ SAM, (b) OH SAM, (c) COOH SAM, (d) NH₂ SAM, (e) control TCPS surfaces following exposure to filtered h.i. 10% HS and (f) filtered h.i. 10% HS. Pre-stained molecular weight standards were used to estimate the molecular weights of the bands. Plasma concentrations, intact molecular weights and intensities of major bands are listed in Table 3.

Figure 3.

(a) Amounts of adsorbed, eluted, and non eluted fibrinogen on different SAMs; (b) elutability (%) of fibrinogen adsorbed on different SAMs. Data are mean $\pm$ S.D, n=12. ‘*’ indicates significantly different levels of adsorbed fibrinogen, ‘#’ indicates significantly different levels of eluted fibrinogen, ‘^’ indicates significantly different levels of non eluted fibrinogen, $p \leq 0.05$.

Peripheral blood monocyte-derived DCs and associated lymphocytes interact *in vitro* with differential adsorbed serum/plasma proteins on different SAM chemistries for 24 hours with distinct cellular activation outcomes ⁽⁴³⁾

Figure 4.

Peripheral blood monocyte-derived DCs and associated lymphocytes interact *in vitro* with differential adsorbed serum/plasma proteins on different SAM chemistries for 24 hours with distinct cellular activation outcomes ⁽⁴³⁾.

Table 1

Proteins probed for on immunoblots along with corresponding hosts and commercial sources of antibodies

Antibody	Host	Source
Factor XI	Goat	Cedarlane Laboratories, Hornby, ON, Canada
Factor XII	Goat	Cedarlane Laboratories, Hornby, ON, Canada
Prekallikrein	Sheep	Cedarlane Laboratories, Hornby, ON, Canada
HMWK	Goat	Cedarlane Laboratories, Hornby, ON, Canada
Fibrinogen	Goat	Cedarlane Laboratories, Hornby, ON, Canada
Plasminogen	Goat	Sigma, St. Louis, MO, USA
ATIII	Sheep	Cedarlane Laboratories, Hornby, ON, Canada
Complement Factor C3	Goat	Calbiochem, Behring Diagnostic, La Jolla, CA, USA
Transferrin	Goat	Sigma, St. Louis, MO, USA
Alpha-1-antitrypsin	Goat	Cedarlane Laboratories, Hornby, Ontario, Canada
Fibronectin	Rabbit	Cedarlane Laboratories, Hornby, Ontario, Canada
Albumin	Goat	Cedarlane Laboratories, Hornby, Ontario, Canada
IgG	Goat	Sigma, St. Louis, MO, USA
Beta-lipoprotein	Goat	Sigma, St. Louis, MO, USA
Alpha-2-macroglobulin	Goat	Sigma, St. Louis, MO, USA
Vitronectin	Sheep	Cedarlane Laboratories, Hornby, Ontario, Canada
Complement Factor B	Goat	Calbiochem, Behring Diagnostic, La Jolla, CA
Complement Factor H	Goat	Calbiochem, Behring Diagnostic, La Jolla, CA
Complement Factor I	Goat	Calbiochem, Behring Diagnostic, La Jolla, CA
Apolipoprotein A1	Goat	ESBE, Markham, ON, Canada
Prothrombin	Sheep	Cedarlane Laboratories, Hornby, Ontario, Canada
Hemoglobin	Rabbit	Sigma, St. Louis, MO, USA

Table 2

Alkaline-phosphate conjugated secondary antibodies used in immunoblotting and corresponding commercial sources

Secondary Antibody	Source
Rabbit Anti Goat IgG Alkaline Phosphatase Conjugate	Sigma Chemical Co., St. Louis, USA
Rabbit Anti Sheep IgG Alkaline Phosphatase Conjugate	Bethyl Laboratories Inc., Montgomery, TX, USA
Goat Anti Rabbit IgG Alkaline Phosphatase Conjugate	Bio-Rad Laboratories, Hercules, CA, USA

Table 3

Concentrations of indicated proteins normally found in plasma, intact molecular weights normally observed on reduced gels, and intensities of major bands on the immunoblots of serum sample or eluates from different SAMs (filtered h.i. 10% HS proteins)

Protein	Plasma Conc.* (ug/mL)	Intact MW** (kD)	Filtered h.i. Serum	Eluate from TCPS	Eluate from CH3	Eluate from OH	Eluate from COOH	Eluate from NH2
Factor XI	5	83	60					
Factor XII	15-45	80	80, 50					
Prekallikrein	35-45	85	85, 55					
HMWK	30-90	120	120-strong	48, 40		45-faint		
Fibrinogen	3000-4000	67,58,47						
Plasminogen	200	94	95-strong, 96-strong					
Antithrombin	150	57	57-v. strong 90-strong			57-faint		90-faint
C3	1100	75,110	100-v. faint 75 Lots of LMW bands present.	75-faint 40-faint	75-v. faint 40-v. faint	75 40	75-faint 40-faint	75-v faint 55-v. faint
Transferrin	2000-3200	80	80- v.strong, LMW bands	80 70	80 70	80-strong 70	80-faint 70	80 70
α -1-antitrypsin	2900	57	57					
Fibronectin	300	200	~200 faint					
Albumin	45000-80000	66	66-v. strong band Num. other bands.	66-strong band	66-thin strong band	66- strong band	66-thin strong band	66- thin strong band
IgG	8000	55, 27	55, 27- strong band, faint other bands.			55	55-faint	55-faint
α -2-macroglobulin	2400	185	185- thin, strong band.					
Vitronectin		75, 65	75, 65 bands LMW bands	75 65	75 65	75, 65 strong	75, 65 strong	75, 65 v. strong
Factor B	200	93	93 thin strong sharp band-many LMW bands	93 - faint	93-faint	93	93	93
Factor H	500	150	150 thin sharp strong band.				150-faint	
Factor I	34	50, 38	50 faint band, 78 faint band.			50	50-faint	
Apolipoprotein A I		28	28 very strong band, some faint HMW bands.	28 v. strong band	28 v. strong band	28 v. strong band	28 v. strong band	28

Protein	Plasma Conc.* (ug/mL)	Intact MW** (kD)	Filtered h.i. Serum	Eluate from TCPS	Eluate from CH3	Eluate from OH	Eluate from COOH	Eluate from NH2
Prothrombin	120	68	78,80 thin strong bands. Faint LMW bands			80		80-strong 40
Hemoglobin	-	64, 32, 16	64, LMW bands					

* Concentration of indicated protein normally found in plasma.

** MW in KD of indicated protein normally found on a reduced gel

Table 4

Comparison of previous literature to current findings: SAM-associated proteins

Protein	Incubating solution	Current findings	Literature
Fibrinogen (Fg)	Filtered h.i. 1% HP Filtered h.i. 10% HS Adsorbed Fg	Fg band: Low in CH3 SAM eluates (Immunoblot) Fg band: Not detected in all SAM eluates (Immunoblot) CH3 SAM < All SAMs (Labeled Fg)	Adsorbed Fg: OH SAM>CH3 SAM (9) Decreased adsorbed Fg with increased OH in mixed OH/CH3 SAM (61)
Complement 3 (C3)	Filtered h.i. 1% HP Filtered h.i. 10% HS	C3 band: Not detected in OH SAM eluates (Immunoblot) C3 band:CH3 SAM < OH SAM eluates (Immunoblot)	Adsorbed C3: OH SAM>CH3 SAM (62)
Immunoglobulin G (IgG)	Filtered h.i. 10% HS	IgG band: Detected in OH, COOH, NH2 SAM Not detected in CH3 SAM, control TCPS	Adsorbed IgG: CH3 SAM> All SAMs (63)
Albumin	Filtered h.i. 1% HP & Filtered h.i. 10% HS	Albumin band: detected in all SAM eluates	Adsorbed albumin: CH3 SAM> OH, COOH SAM (64)