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Saliva and Serum Protein Adsorption on Chemically Modified Silica Surfaces

J. Lehnfeld[1], Y. Dukashin[2*], J. Mark[2*], G.D. White[2*], S. Wu[2*],
V. Katzur[1], R. Müller[1] (iD), and S. Ruhl[2] (iD)

Abstract

Biomaterials, once inserted in the oral cavity, become immediately covered by a layer of adsorbed proteins that consists mostly of salivary proteins but also of plasma proteins if the biomaterial is placed close to the gingival margin or if it becomes implanted into tissue and bone. It is often this protein layer, rather than the pristine biomaterial surface, that is subsequently encountered by colonizing bacteria or attaching tissue cells. Thus, to study this important initial protein adsorption from human saliva and serum and how it might be influenced through chemical modification of the biomaterial surface, we have measured the amount of protein adsorbed and analyzed the composition of the adsorbed protein layer using gel electrophoresis and western blotting. Here, we have developed an in vitro model system based on silica surfaces, chemically modified with 7 silane-based self-assembled monolayers that span a broad range of physicochemical properties, from hydrophilic to hydrophobic surfaces (water contact angles from 15° to 115°), low to high surface free energy (12 to 57 mN/m), and negative to positive surface charge (zeta potentials from −120 to +40 mV at physiologic pH). We found that the chemical surface functionalities exerted a substantial effect on the total amounts of proteins adsorbed; however, no linear correlation of the adsorbed amounts with the physicochemical surface parameters was observed. Only the adsorption behavior of a few singular protein components, from which physicochemical data are available, seems to follow physicochemical expectations. Examples are albumin in serum and lysozyme in saliva; in both, adsorption was favored on countercharged surfaces. We conclude from these findings that in complex biofluids such as saliva and serum, adsorption behavior is dominated by the overall protein-binding capacity of the surface rather than by specific physicochemical interactions of single protein entities with the surface.

Keywords: biomaterials, surface properties, self-assembled monolayers, pellicle, protein amount, protein composition

Introduction

Any surface that comes into contact with a protein-containing solution will become rapidly coated with a film of adsorbed proteins, which frequently determines further biological processes, such as the attachment of microorganisms, plant, or animal cells (Wilson et al. 2005; Banerjee et al. 2011; Sterzenbach et al. 2020). In many instances, this is an undesired process called *biofouling*, causing negative effects in the marine and food industry, bioanalytics, and, most important, the medical field (Norde 2008; Banerjee et al. 2011). Within the medical discipline, dentistry uses a great variety of biomaterials. In the mouth, these materials become exposed to the proteins of saliva; in the case of dental implants, to blood plasma; or at the gingival margin, to a mixture of both biofluids. Exposure to saliva leads to the formation of the so-called salivary pellicle, a thin acellular film consisting mostly of salivary proteins and glycoproteins that have adsorbed to the biomaterial surface and serve as substrate for subsequent microbial colonization (Siqueira et al. 2012; Lindh et al. 2014). Exposure to serum on the other side, especially extracellular adhesion proteins, influences cell adhesion and thus tissue integration of an implant (Wilson et al. 2005).

It is therefore important to study the composition of this adsorbed protein layer and to determine how it can be modulated through chemical modification of the biomaterial surface such that undesired negative outcomes can be prevented. Numerous studies have investigated the adsorption of proteins from purified protein solutions, finding that their behavior depends on the properties of the protein, characteristics of the material surface, and environmental conditions (Michiardi

[1]Institute of Physical and Theoretical Chemistry, University of Regensburg, Regensburg, Germany
[2]Department of Oral Biology, University at Buffalo, The State University of New York, Buffalo, NY, USA
*Authors contributing equally to this study

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Corresponding Authors:
R. Müller, Institute of Physical and Theoretical Chemistry, University of Regensburg, Universitätsstraße 31, Regensburg, 93053, Germany.
Email: rainer.mueller@chemie.uni-regensburg.de
S. Ruhl, Department of Oral Biology, University at Buffalo, The State University of New York, 3435 Main Street, Foster Hall, Room 213A, Buffalo, NY 14214, USA.
Email: shruhl@buffalo.edu

et al. 2007; Patil et al. 2007; Norde 2008; Guo et al. 2016). However, less is known about the adsorption behavior of complex biofluids. Most studies there focus mainly on the quantification of protein adsorption (Lindh et al. 1999; Comelles et al. 2010). Valuable investigations of qualitative protein adsorption have been performed on hydroxyapatite as a model for tooth enamel (Heller et al. 2017) and on actual clinical biomaterials either in situ or in vitro (Kohavi et al. 1995; Lee et al. 2001; Fischer and Aparicio 2021). Due to the complex influence of the poorly controllable surface properties of those materials in terms of surface roughness, hydrophilicity, and charge, it is not possible to reliably predict the impact of single surface properties on protein adsorption. A limited number of studies have used model systems but were restricted to only a few surface functionalizations (Aroonsang et al. 2014; Gibbins et al. 2014; Visalakshan et al. 2019). In vivo studies also suffer from a variety of disadvantages, such as interindividual variability in environmental conditions and saliva composition (Schipper et al. 2007), which complicate comparing the results for different surfaces. Besides, small amounts of adsorbed proteins require elaborate sample preparation before analysis (Kohavi et al. 1995).

To take a step back and study the influence of basic physicochemical parameters on adsorption of proteins from complex biofluids, we chose a model system in which we were able to control environmental conditions and ensure an invariant composition of the protein solutions such that results obtained for different surface modifications can be compared. Silica beads were chosen as model substrates due to their high specific surface area, resulting in sufficient amounts of proteins conducive to analytic tests. Silica allowed the functionalization with silane-based self-assembled monolayers (SAMs) of varying functional groups. This way, we could obtain stable coatings with reproducible surface properties that cover a broad range of surface hydrophilicities, surface free energies (SFEs), and zeta potentials, allowing us to test the influence of those characteristics on quantitative and qualitative biofluid protein adsorption in detail. Ample amounts of adsorbed proteins allowed us to study protein layer composition and identify its components by SDS-PAGE and immunoblot analysis. Taken together, our model system enables us to fill an important gap in knowledge by comprehensively investigating the qualitative adsorption of proteins from complex biofluids on a variety of surfaces with precisely tailored and well-characterized physicochemical properties.

Materials and Methods

Materials

As model surfaces for x-ray photoelectron spectroscopy (XPS), static contact angle, as well as streaming current measurements, single-side polished silicon wafers (n-type, phosphor doped; thickness, 650 to 700 μm; resistivity, 5 to 25 Ω·cm) were obtained from Si-Mat Silicon Materials and cut into specimens (10 × 10 mm) by Disco Hi-Tec Europe GmbH. For infrared spectroscopy, monodisperse silica spheres (diameter, 1 μm) were obtained from Micromod Partikeltechnologie GmbH. Protein adsorption experiments were performed with silica beads (diameter, 250 to 315 μm; specific surface area, 0.19 m²/g) obtained from Brace GmbH. All silanes were purchased from abcr GmbH; solvents, probe liquids, and further reagents were obtained from Sigma-Aldrich Chemie GmbH.

Biofluids

Saliva was obtained from consenting donors via a procedure approved by the University at Buffalo Human Subjects Institutional Review Board (030-505616). For the quantification of protein adsorption, mechanically stimulated whole saliva was obtained from 4 individuals via paraffin wax chewing and expectoration into a polypropylene vial. Samples were cooled on ice and sequentially filtered with low–protein binding syringe filters of decreasing pore size (5 to 0.2 μm; Acrodisc with Supor polyethersulfone membrane). Finally, saliva was pooled, aliquoted, and stored at −18 °C.

To determine qualitative protein adsorption, saliva was obtained from donors without stimulus. Collected saliva was centrifuged at 7,000 rpm for 15 min at 4 °C, and the clarified supernatant was used for adsorption experiments.

Human AB serum was obtained from Valley Biomedical. Aliquots were stored at −30 °C until use for adsorption experiments.

Substrate Functionalization and Analysis of Physicochemical Properties

Synthesis routes to obtain surface coatings with 7 terminal functions, as well as all methods to verify the synthesis success and characterize the physicochemical properties, are summarized in the Appendix.

Quantification of Adsorbed Proteins

The bicinchoninic acid assay was used to determine the protein concentration in the investigated biofluids (Sigma-Aldrich Chemie GmbH). A total protein content of 0.91 mg/mL (0.86 to 0.96; median [interquartile range]) was determined for pooled saliva. The protein concentration of saliva from individual donors (n = 9) used for gel electrophoresis was 1.08 mg/mL (0.90 to 1.30). A protein content of 48.3 mg/mL (45.1 to 55.2) was obtained for serum. The bicinchoninic acid assay was also applied to quantify the amount of adsorbed proteins. In the first way, we determined the protein amount directly on the silica beads after adsorption at 25 °C using a procedure that we previously published (Müller et al. 2006; Eichler et al. 2011; Staehlke et al. 2019; see Appendix). In the second way, protein adsorption was performed at 37 °C to mimic the ambient conditions in the oral cavity, and the protein amount was determined after detachment from the surfaces by heating the beads in anionic surfactant solution (SDS; see Appendix).

Gel Electrophoresis, Staining of Proteins and Glycans, and Immunoblotting

SDS-PAGE and immunoblotting were performed following standard procedures recently described by our group (Thamadilok et al. 2020). Details and antibodies are provided in the Appendix.

Results

Verification of Successful Surface Modification

The chemical structures of the silane-based SAMs carrying different terminal functions are depicted in Figure 1A. Five modifications have been developed and characterized during studies of our group (Müller et al. 2006; Schweikl et al. 2007; Katzur et al. 2012), and the modifications with SO_3H and Py were newly developed and are used here for the first time. Successful surface functionalization could be proven by XPS, infrared spectroscopy, and water contact angle measurements.

Through XPS analysis, successful surface modification is confirmed by a decrease in the silicon content and an increase in the carbon content as compared with uncoated SiO_2. Furthermore, the incorporation of new elements, such as nitrogen, sulfur, bromine, and fluorine, are characteristic for the introduction of specific chemical functions.

In the infrared spectra (Fig. 1B), the negative band at $3,740$ cm^{-1} is caused by the reduced number of surface silanol groups after silanization. In addition, bands in the range of $3,000$ to $2,800$ cm^{-1} can be attributed to C–H stretching vibrations of the CH_2 units of the SAM backbone. For Py, several bands characteristic for pyridinium can be detected in the range of $1,635$ to $1,465$ cm^{-1}, while for NH_2, characteristic bands of N−H stretching vibrations are found at $1,585$ and $1,460$ cm^{-1}. The presence of COOH groups was confirmed by the appearance of the C=O stretching vibration at $1,725$ cm^{-1}.

The results of the water contact angle measurements (Fig. 1C) show that silanization increased surface hydrophobicity in comparison with SiO_2, which correlated well with the new terminal functional group. Nonpolar functions such as CH_3 and CF_3 resulted in high water contact angles, and polar and ionizable functions in low contact angles. Additionally, the hydrophilicity of final modifications of multiple-step reactions differed significantly from the results obtained for the previous intermediate SAMs and agreed well with our previously published and other literature data (Liu et al. 2002; Shyue et al. 2004; Janssen et al. 2006; Müller et al. 2006; Müller et al. 2007; Schweikl et al. 2007; Müller et al. 2009; Katzur et al. 2012).

Physicochemical Properties of the Modified Surfaces

The SFEs for the different surface coatings—as calculated from static contact angle measurements with water, formamide,
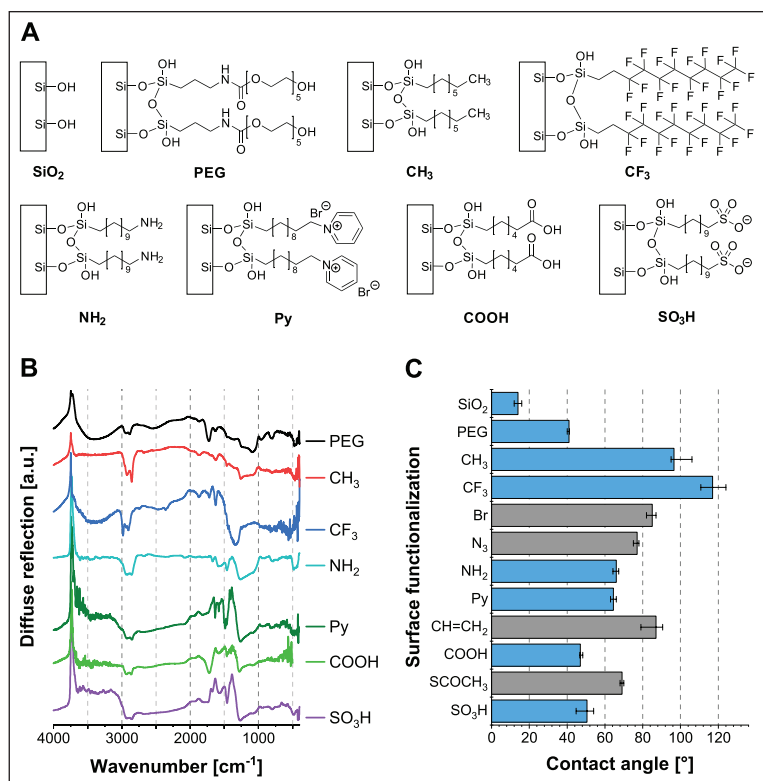

Figure 1. Realized surface coatings. (**A**) Overview of the examined self-assembled monolayers with different functional groups. The modifications NH_2, pyridinium (Py), and SO_3H are synthesized via a bromine monolayer (Br), which is substituted by azide (N_3) and reduced to NH_2, substituted by Py or thioacetate ($SCOCH_3$), and oxidized to SO_3H. Carboxylic acid groups (COOH) are generated via oxidation of a monolayer with terminal vinylic groups (CH=CH₂). (**B**) Infrared spectra of SAMs recorded via DRIFT spectroscopy. (**C**) Water contact angles of final modifications (blue) as well as their intermediate SAMs (gray). Median ± interquartile range ($n \geq 8$). SAM, self-assembled monolayer.

and diiodomethane—vary in a broad range (Fig. 2A), from low SFEs of ~25 and ~12 mN/m for CH_3 and CF_3, respectively, to the highest total SFE for SiO_2 (~56 mN/m). The coatings with acidic groups (COOH and SO_3H) and SiO_2 possessed the largest polar components (13.6 to 15 mN/m). These results are in good accordance with our previously published and other literature data (Janssen et al. 2006; Katzur et al. 2012).

The isoelectric points (IEPs) and zeta potentials (at pH 7.4) of the surface coatings were obtained from streaming current measurements (Fig. 2B, C). Results have been published in part, and extracted values are provided in the Appendix (Eichler et al. 2011; Katzur et al. 2012). An IEP of 2.8 and a strongly negative zeta potential under physiologic conditions (<100 mV) were observed for SiO_2 in accordance with literature data (Shyue et al. 2004). The surface modifications without ionizable groups (PEG, CH_3, and CF_3) are characterized by their similar IEPs in the range pH 3 to 4 and their negative zeta potential at pH 7.4, similar to literature data (Chan et al. 2003; Shyue et al. 2004). When compared with these coatings, the pH-dependent protonation behavior of the primary amine or carboxylic acid groups in the modifications NH_2 and COOH causes a shift in the IEP. However, for COOH, the zeta potential approaches 0 mV at pH 3, and the IEP of NH_2 is shifted to
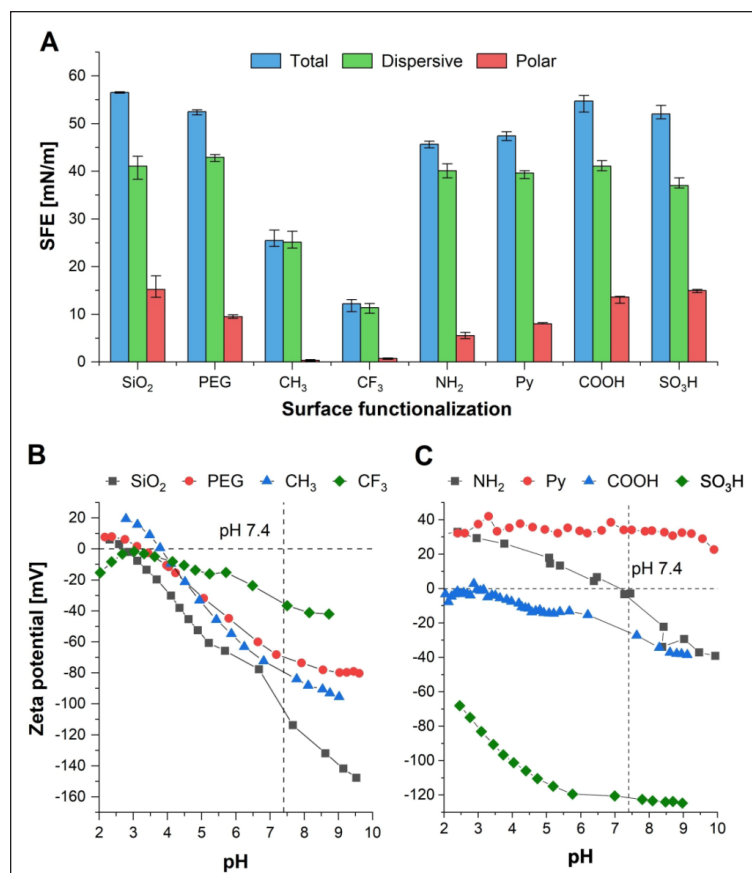
Figure 2. Physicochemical properties of surface coatings. (**A**) Surface free energy (SFE) as well as its polar and dispersive component of self-assembled monolayers with different functional groups, calculated from static contact angles with water, formamide, and diiodomethane according to the Lifshitz-van der Waals/acid-base approach. Median ± interquartile range (n ≥ 8). (**B**, **C**) Zeta potential of functionalized substrates, obtained from streaming current measurements.

linear correlation was obtained between the amounts of adsorbed proteins and hydrophilicity, SFE, or zeta potential (Appendix Figs. 3–5). For PEG, considered a *nonfouling* surface coating, significant differences were observed between methods of protein measurement. Specifically, small amounts were detected directly on the surface, and considerably larger protein amounts were found after protein desorption.

For serum proteins, larger amounts of adsorbed proteins were measured, ranging from <50 ng/cm^2 to ~400 ng/cm^2, likely due to the at least 40-fold higher protein concentration of human AB serum when compared with saliva. For CH_3, CF_3, and COOH, very small amounts of adsorbed proteins were determined with both quantification methods. In contrast, for NH_2, Py, and SO_3H, the quantification method based on desorption provided considerably lower amounts as compared with measurements directly on the surface. Again, no linear correlation was obtained between the amounts of adsorbed proteins and hydrophilicity, SFE, or zeta potential (Appendix Figs. 3–5). For PEG, a situation similar to the result with salivary proteins was obtained. In terms of the amount of serum protein, no statistically significant correlation was observed between the methods ($R^2 = 0.215$, $r = 0.464$, $P > 0.05$; Appendix Fig. 1).

When serum and salivary proteins were compared, data were positively correlated if quantification was performed directly on the surface ($R^2 = 0.761$, $r = 0.873$, $P < 0.01$; Appendix Fig. 2). No such correlation could be observed by comparing the protein amounts obtained after protein desorption ($R^2 = 0.240$, $r = 0.490$, $P > 0.05$).

pH 7.1, leading to an almost electrically neutral coating at pH 7.4. In contrast to that, strongly charged surfaces without an IEP in the measured pH range are obtained for Py (~+35 mV) and SO_3H (<−60 mV) as previously reported (Shyue et al. 2004).

Quantitative Protein Adsorption

The amounts of adsorbed proteins were quantified on the surfaces as well as from the eluates after protein desorption (Fig. 3). Preliminary experiments showed that maximum protein adsorption occurred after 1 h for saliva and after 10 min for the much higher concentrated serum (Appendix Fig. 6).

For saliva, a statistically significant positive correlation is obtained for the amounts of proteins measured by both methods (excluding PEG: $R^2 = 0.958$, Pearson correlation coefficient $r = 0.979$, $P < 0.01$; Appendix Fig. 1). Clearly, the different surface modifications resulted in different amounts of adsorbed proteins, ranging from as low as <20 ng/cm^2 for CH_3 and CF_3 to 40 to 50 ng/cm^2 for NH_2 and SO_3H. However, no

Qualitative Protein Adsorption

SDS-PAGE and western blotting revealed a complex pattern of adsorbed proteins that depended on the surface modification and the biofluid analyzed (Figs. 4, 5). Initial time kinetics of adsorption showed that the relative pattern of protein bands did not change substantially between short incubation times (up to 5 min) and longer incubation times (up to 3 h). At time points beyond 3 h, protein degradation became apparent. Optimal band intensity was reached after 1 h for saliva and 10 min for the 40-fold higher concentrated serum (Appendix Fig. 6).

Among the salivary proteins identified, lysozyme C stood out because, being a positively charged protein, it adsorbed on the negatively charged SiO_2 substrates as well as on the COOH and SO_3H modification. However, larger glycoproteins—including the negatively charged salivary agglutinin (DMBT1), mucin 7 (MUC7), and zinc-α_2-glycoprotein (ZAG)—exhibited a more complex adsorption pattern: they also bound to the negatively charged SiO_2 substrates, the nonfouling PEG, and the positively ionizable NH_2 modifications.
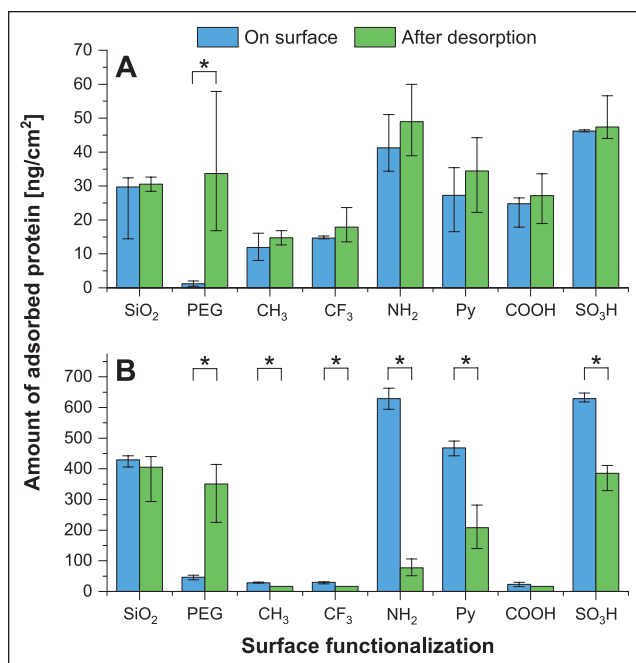
Figure 3. Quantitative protein adsorption. Amounts of adsorbed protein, determined via bicinchoninic acid assay directly on the surface or after protein desorption in the eluates. Median ± interquartile range. Adsorption on functionalized surfaces was performed from (**A**) whole human saliva or (**B**) human AB serum. On each surface, differences in the amounts of adsorbed protein obtained with the applied quantification methods were evaluated with the nonparametric Mann-Whitney U test, and statistically significant differences (*$P < 0.05$) are indicated. Further comparison of the 2 quantification methods as well as time kinetics of protein adsorption from saliva and serum are provided in the Appendix.

For serum, mainly 2 groups of surface coatings can be differentiated: protein-repelling ones (CH_3, CF_3, and COOH) and protein-adhesive ones (SiO_2, PEG, NH_2, Py, and SO_3H). Among the protein-adhesive surfaces, no large differences were seen in the overall pattern of adsorbed proteins. Albumin, the most abundant protein in serum and carrying a slightly negative charge, adsorbed preferentially on PEG and the positively ionizable functions NH_2 and Py. Fibronectin, which plays an important role in cell adhesion and is slightly negatively charged, also adsorbed on SiO_2 substrates as well as PEG, NH_2, and Py, but in contrast to albumin on the negatively charged SO_3H modification.

Discussion

We could demonstrate that our silica-based model system can serve as a platform to study the basic principles of protein adsorption from complex biofluids on a broad array of surfaces with precisely tailored physicochemical properties under controllable environmental conditions. We could show here that quantitative adsorption of proteins from complex physiologic
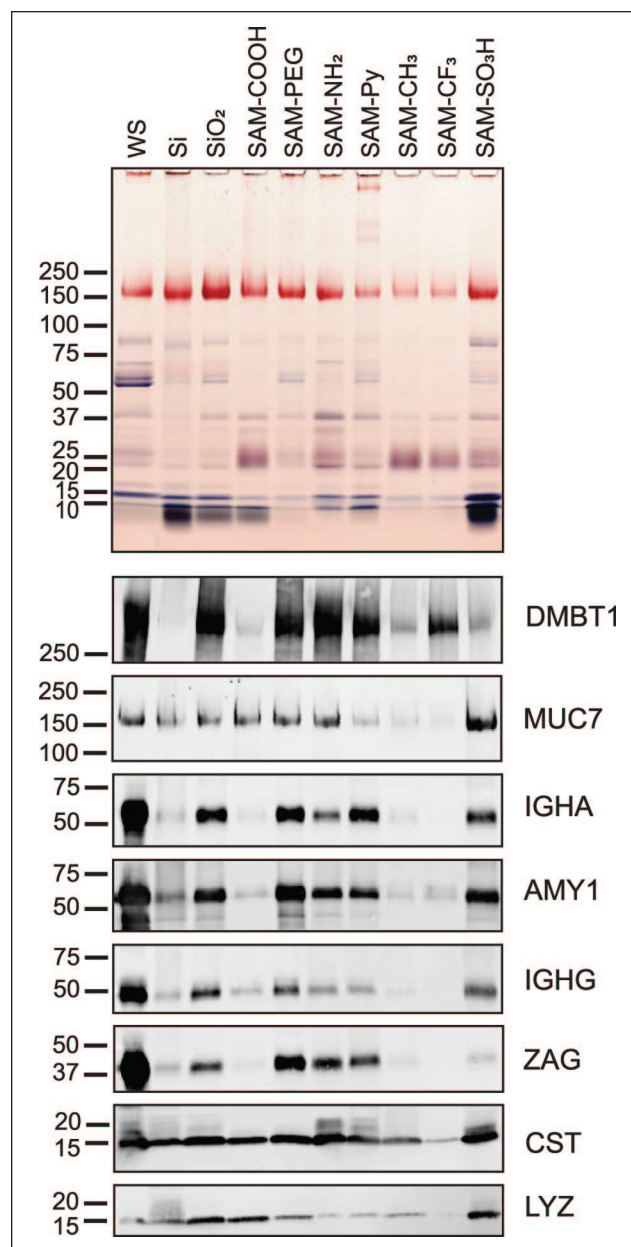

Figure 4. SDS-PAGE and immunoblot analysis of proteins from saliva adsorbed to chemically modified Si beads. Salivary proteins adsorbed to untreated (Si) and oxidized silica beads (SiO_2) as well as to beads modified with 7 chemical functionalities were eluted from the silica bead surface by heating in SDS. Optimal time points for complete protein adsorption were obtained in kinetic studies (Appendix Fig. 6). Equal volumes of eluates were separated by SDS-PAGE. The original whole saliva (WS) that was used for adsorption was run for comparison (first lane). Proteins in the gel were stained with Coomassie blue, and glycans on glycoproteins were revealed by periodic acid–Schiff stain (pink bands). Nitrocellulose transfers from gel replicates were probed with antibodies against salivary agglutinin (DMBT1), mucin 7 (MUC7), immunoglobulin A heavy chain (IGHA), salivary α-amylase (AMY1), immunoglobulin G heavy chain Fc portion (IGHG), zinc-α2-glycoprotein (ZAG), cystatins (CST), and lysozyme (LYZ). Bound primary antibodies were detected with Alexa Fluor 488–tagged IgG secondary antisera.
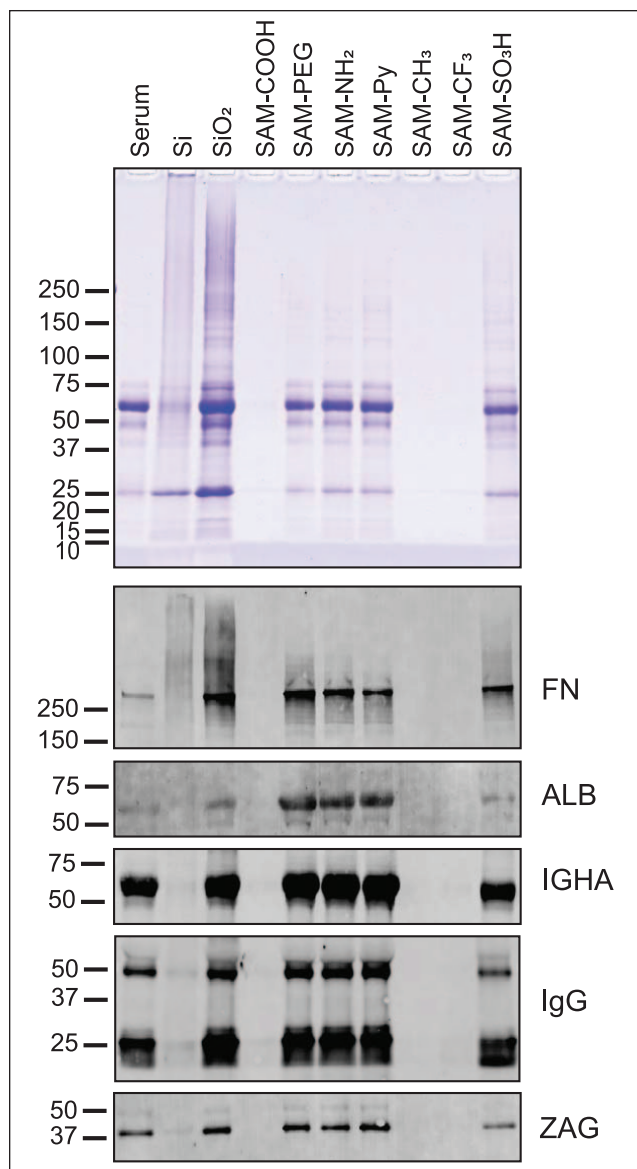
Figure 5. SDS-PAGE and immunoblot analysis of proteins from serum adsorbed to chemically modified Si beads. Serum proteins adsorbed to untreated (Si) and oxidized silica beads (SiO$_2$) as well as to beads modified with 7 chemical functionalities were eluted from the silica bead surface by heating in SDS. Equal volumes of eluates were separated by SDS-PAGE. The original serum that was used for adsorption was run for comparison (first lane). Proteins in the gel were stained with Coomassie blue. Pink bands, generated by the staining of glycoproteins with periodic acid–Schiff stain, were not visible in the gel due to a general lack of densely glycosylated proteins in serum. Nitrocellulose transfers from gel replicates were probed with antibodies against fibronectin (FN), albumin (ALB), immunoglobulin A heavy chain (IGHA), immunoglobulin G F(ab)2 portion (IgG), and zinc-α2-glycoprotein (ZAG). Bound primary antibodies were detected with Alexa Fluor 488–tagged IgG secondary antisera.

fluids is mainly determined by the chemical surface modification. However, no direct correlation was observed between the adsorbed protein amounts and particular physicochemical surface properties, such as hydrophilicity, SFE, and zeta potential.

Our data, obtained with saliva and serum, are thus not fully in agreement with postulated principles of protein adsorption deduced from studies with solutions containing only 1 type of protein (Wertz and Santore 2001; Michiardi et al. 2007; Patil et al. 2007; Norde 2008; Guo et al. 2016). Yet, even in these simplified experimental systems, conflicting results have been obtained. One such example is the adsorption of proteins to hydrophobic surfaces (Wertz and Santore 2001; Norde 2008). These discrepancies among results suggest that protein adsorption occurs as a phenomenon influenced by a multitude of factors, much more complex than previously assumed.

While in our study no overall correlation of quantitative protein adsorption with the measured surface characteristics could be delineated, certain observations agree with what has been reported on the adsorption behavior of complex protein solutions. Regarding the effect of surface wettability, the lowest protein adsorption was measured in the present study for the most hydrophobic coatings CH$_3$ and CF$_3$, agreeing with our results (Müller et al. 2007) and those of Wertz and Santore (2001) but contradicting the study of Norde (2008). With respect to the effect of SFE, a report studying the protein adsorption from fetal bovine serum found the lowest amounts of proteins adsorbed on surfaces with the highest SFE, while the highest amounts were observed on surfaces with medium SFE, but overall no clear correlation became apparent (Comelles et al. 2010). As far as the zeta potential is concerned, we found less serum protein on modifications carrying moderately negative surface charges, an observation that had been made (El-Ghannam et al. 2001). One particular puzzling finding was the difference in protein amounts adsorbed on the PEG-coated surface when measured directly on the surface versus when measured in the eluate after desorption from the surface. A possible explanation could be based on the different temperatures for the 2 protein measurements during the adsorption process. Enhanced protein adsorption at elevated temperatures would be in accordance with research showing a loss of PEG's protein-repelling behavior with increasing temperature (Leckband et al. 1999; Efremova et al. 2001). All these partially conflicting reports and observations illustrate the complexity of protein adsorption and dependence from multiple factors in addition to the physicochemical properties of the surface.

Regarding qualitative protein adsorption, 2 main findings can be stated. First, protein adsorption from saliva appears to be more complex than adsorption from serum. Large variations in adsorption among the surface modifications can be observed for salivary proteins. In contrast to that, adsorption of serum proteins is more uniform and seems to be largely influenced by the abundance of the protein in the biofluid rather than by the widely varying surface properties studied. Thus, adsorption of the majority of the examined proteins cannot directly be linked to the physicochemical properties of the surface modifications. Second, the adsorption pattern of a few proteins, with the positively charged lysozyme (IEP, 11.1) as the most prominent example, is nevertheless governed by characteristics of the surface coating despite the complexity of the biofluid composition

(Norde and Lyklema 1991). Accumulation of this protein on negatively charged surfaces is therefore clearly ruled by attractive electrostatic interactions between the surface and the protein. This rule also accounts for albumin (IEP, 5.5 to 5.7) from serum (Walz et al. 2006) in that, according to the immunoblot, it seems to be accumulated on positively charged surfaces more than on surfaces carrying negative charges. When compared with lysozyme, however, the charge-dependent adsorption behavior is significantly less pronounced than for albumin, as it can also adsorb on likewise charged hydrophilic surfaces due to its low internal stability, being a *soft protein* (Norde and Lyklema 1991; Norde 2008).

Adsorption of many other proteins from these complex biofluids, examined in this study, did not directly and predictably follow physicochemical expectations. In saliva, the glycoproteins MUC7 and ZAG are negatively charged due to extensive glycosylation and sialylation (IEP, 3.1 or 5.2 to 5.4, respectively; Walz et al. 2006). Nevertheless, they adsorb not only to modifications with positively charged groups (NH_2, Py) but also to SiO_2 and PEG surfaces. The tendency of mucins to adsorb more on hydrophilic surfaces has been reported by Aroonsang et al. (2014). The adsorption behavior of, for example, amylase and immunoglobulins did not follow strict electrostatic interactions as well. Taken together, these observations can possibly be explained by the unique and complex interactions of salivary proteins that involve salivary micelles (Soares et al. 2004). Those globular complexes are characterized by an accumulation of MUC7, lactoferrin, and immunoglobulin A (IgA), whereas lysozyme mainly occurs as a single component (Soares et al. 2004). Micelles possibly adsorb via a multitude of physicochemical interactions and may explain the simultaneous adsorption of diverse saliva proteins.

Given the observed complexity of protein adsorption from biofluids, more research will be needed to delineate rules for predicting the adsorption of certain protein components of interest. For future custom design of material surface coatings for clinical dental applications, it will be of importance to learn more about the adsorption of particular proteins that are known to either foster the attachment of tissue cells or serve as adhesion substrates for colonizing oral bacteria. Since some proteins playing a key role in that context have been described (such as fibronectin from plasma; Wilson et al. 2005) as well as MUC7, DMBT1, IgA, and proline-rich proteins from saliva (Lee et al. 2001; Ligtenberg et al. 2010; Sterzenbach et al. 2020), the next logical experimental step will be to compare biomaterial surface modifications that show substantial differences in the adsorption of such proteins and to study how subsequent attachment of cells or adhesion of bacteria will be influenced by their presence or absence on a given material surface. Equally important will be to get a clearer idea about protein complexes in saliva by determining their molecular structure and studying how they adsorb to surfaces. For clinical dental materials research, SAMs are a valuable tool for in vitro studies as well as for clinical applications. Silane-based SAMs can be designed as functional coatings of passivating metals and oxidic ceramic materials in the interproximal spaces and at the gingival margin of dental restorations or implants where they are not exposed to attritive wear. Fundamental research must be continued for the development of new chemical surface modifications in dentistry.

Author Contributions

J. Lehnfeld, contributed to data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; Y. Dukashin, J. Mark, G.D. White, S. Wu, V. Katzur, contributed to data acquisition and analysis, and critically revised the manuscript; R. Müller, S. Ruhl, contributed to conception, design, and data interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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Declaration of Conflicting Interests

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ORCID iDs

R. Müller [iD] https://orcid.org/0000-0001-9641-7780

S. Ruhl [iD] https://orcid.org/0000-0003-3888-4908

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