

RESEARCH ARTICLE

Is the proteomic composition of the salivary pellicle dependent on the substrate material?

Simone Trautmann¹  | Nicolas Künzel² | Claudia Fecher-Trost³ | Ahmad Barghash^{2,4} | Johanna Dudek¹ | Veit Flockerzi³ | Volkhard Helms² | Matthias Hannig¹

¹Clinic of Operative Dentistry, Periodontology and Preventive Dentistry, University Hospital, Saarland University, Homburg, Germany

²Center for Bioinformatics, Saarland Informatics Campus, Saarland University, Saarbruecken, Germany

³Department of Experimental and Clinical Pharmacology and Toxicology, PZMS, Saarland University, Homburg, Germany

⁴Department of Computer Science, German Jordanian University, Amman, Jordan

Correspondence

Simone Trautmann, Clinic of Operative Dentistry, Periodontology and Preventive Dentistry, University Hospital|Saarland University, Kirrberger Strasse building 80.2, 66421 Homburg, Germany.
Email: simone.trautmann@uks.eu

Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Numbers: SFB512, SFB1027; HOMFOR

Abstract

Purpose: The use of dental restorative materials is a routine task in clinical dentistry. Upon exposure to the oral cavity, continuous adsorption of salivary proteins and other macromolecules to all surfaces occurs, representing the first step in dental biofilm formation. Different physico-chemical properties of substrate materials potentially influence the composition of the initial biofilm, termed pellicle. This study aimed at characterizing and comparing the individual proteomic composition of the 3-min pellicle formed on bovine enamel and six restorative materials.

Experimental Design: After chemical elution, pellicle proteins were identified by nano-LC-HR-MS/MS. Proteomic profiles were analyzed in terms of molecular weights, isoelectric points, molecular functions and compared to saliva to reveal substrate material-specific adsorption patterns.

Results: A total of 1348 different pellicle proteins were identified, with 187–686 proteins in individual 3-min pellicles. Unexpectedly, this yielded quite similar distribution patterns independent of the substrate materials. Furthermore, overall similar fold changes were obtained for the major part of commonly enriched or depleted proteins in the pellicles.

Conclusions and Clinical Relevance: The current results point to a minor role of the substrate material on the proteomic composition of the 3-min pellicle and represent core data for understanding the complex surface interactions in the oral cavity.

KEYWORDS

dental materials, individual 3-min pellicle proteome, nano-LC-HR-MS/MS, quantitative proteomic comparisons, salivary protein enrichment/depletion

1 | INTRODUCTION

The use of dental restorative and implant materials is a daily routine in dentistry. Different materials are used depending on the individual treatment needs for restorations/fillings/implants and each material

has different applications, benefits, lifetimes, and costs. However, the materials also have different physico-chemical properties concerning their surface free energy, hydrophobicity, charge, microstructure, and binding capacities which can be expected to influence biofilm formation, mainly by affecting initial protein adsorption, pellicle formation, and subsequent bacterial adherence. After all, one prefers dental materials that combine beneficial mechanical and physico-chemical properties and, at the same time, minimize bacterial adhesion.

Abbreviations: BSE, bovine spongiform encephalopathy; emPAI, exponentially modified protein abundance index; fc, fold change; MF, molecular function; MW, molecular weight; PMMA, polymethyl methacrylate

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. Proteomics – Clinical Applications published by Wiley-VCH GmbH.

Under *in vivo* conditions, all orally exposed surfaces are covered by the acquired salivary pellicle, which represents the base for the development of the oral biofilm [1–3]. The continuous formation of the acquired salivary pellicle, termed simply pellicle for the sake of convenience, starts within seconds after oral hygiene and is due to the adsorption of proteins and other biomolecules from the surrounding oral fluids [4, 5]. It predominantly consists of salivary proteins and fulfills a protective role by acting as a semipermeable membrane protecting the tooth surface against demineralization processes and mechanical damages [1, 6–8].

The proteinaceous composition of the pellicle layer reflects a complex interplay between adsorption and desorption of different proteins, their conformational changes, and enzymatic composition [9]. The very initial step in pellicle formation, taking place within the first few minutes after oral hygiene, is due to the adsorption of salivary proteins to all exposed surfaces [4]. As the pellicle matures, additional salivary biomolecules such as lipids, carbohydrates, nucleic acids, and protein aggregates adsorb, leading to the formation of the so-called pellicle matrix, a meshwork of a densely structured basal layer and an outer layer of globular appearance [10, 11]. The continuously growing outer layer provides more and more binding sites for bacterial adhesions, resulting in the formation of a bacterial biofilm. It has been concluded from previous *in vitro* and *in vivo* studies that pellicle formation is a highly selective process [4, 12, 13], whereby the physico-chemical properties of the surface were supposed to determine the adsorption of salivary proteins to it [2].

Many studies investigated the influences of different substrate materials on the bacterial biofilm formation and the antibiofilm properties of diverse dental materials [14–17]. However, an important prerequisite for bacterial adhesion and biofilm formation is the presence of the acquired salivary pellicle, which forms immediately after oral hygiene [4].

The work of Fischer and Aparicio summarizes the rather small body of studies investigating the formation and characteristics of the salivary pellicle on dental materials [18].

Although protein adsorption is the first biological response at the interface between a substrate surface and a biological fluid, a systematical elucidation of the effect of different substrate materials on the precise proteomic composition of the *in situ* acquired short-term pellicle is still missing [2]. Supplemental Table S1 summarizes the available literature related to this topic, including the respective limitations of the single studies due to the study design, methodology, their *in vitro* character, or the restricted extent. Among them, there are studies investigating the ultrastructure [3], the amino acid composition [19], or the physico-chemical properties of salivary pellicles formed on different substrate materials [20]. Other works focused on the enzymatic activity or the adsorption of single salivary proteins [21–24], whereat these results should not be transferred unrestrictedly to the adsorption kinetics of the full salivary proteome. More recent studies investigated the salivary pellicle formed on different substrate materials using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [25] or two-dimensional gel electrophoresis (2-DE) [26, 27]. Based on these results, the authors proposed differences in the proteomic composition of the salivary pellicle on the different

Clinical Relevance

The characterization and comparison of the initial pellicle proteome formed on seven different dental materials showed unexpected high similarities among all substrates. These results suggest a minor relevance of the respective substrate material properties on the proteomic composition of the individual 3-min pellicle. Most likely this can be ascribed to a prompt shielding of the physico-chemical substrate properties upon salivary exposure. The proteomic data of the present study impart the first detailed insight in the process of pellicle formation on different dental materials under oral conditions. Far-sighted, the current results can contribute to a deeper understanding of the process of protein adsorption at any interface between a complex biological fluid and solid surfaces.

substrate materials. However, those studies were conducted *in vitro* and simply resulted in protein patterns on the SDS-PAGE/2-DE-gels or the detection of single proteins by immunoblotting. In several recent studies, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used as up to date-method to elucidate the pellicle proteome on dental materials in detail [26, 28–31]. Yet likewise, these studies were conducted *in vitro* and focused on the differences in the proteomic pellicle profiles formed on variably pretreated titanium specimens or differences between titanium and feldspar ceramics. To the best of our knowledge, the only studies elucidating the detailed proteomic composition of the salivary pellicle formed on a dental material *in situ* with a contemporary method are Delius et al. 2017 and Trautmann et al. 2019 [12, 32]. Therein, the individual 3-min *in situ*-pellicle formed on ceramics was characterized using nano-liquid chromatography-high resolution-tandem mass spectrometry (nano-LC-HR-MS/MS). Till this day, a comprehensive analysis and comparison of the proteomic composition of the *in situ* pellicle formed on different dental materials with an up to date methodology enabling reliable protein identifications of its entire proteome is missing.

As the initial pellicle represents the base for all subsequent adsorption processes and bacterial adherence, its proteomic composition is likely to influence those subsequent steps. Therefore, the aim of the current study was to comprehensively elucidate the individual proteomic composition of the 3-min *in situ* pellicle formed on seven different dental materials. In a second step, substrate material-specific adsorption patterns were analyzed by comparing the proteomic profiles of the 3-min pellicle to the profile of the corresponding saliva.

2 | MATERIALS AND METHODS

The current study follows up on previous work described in Trautmann et al. 2020 [13] and uses essentially the same methodology. For completeness, it is summarized here again.

2.1 | Human subjects

The 3-min pellicles were collected from five nonsmoking healthy members of the laboratory staff or students (aged 22–44 years, three female, two male). Subjects did not consume any antibiotics, antimicrobial, or anti-inflammatory drugs during the 6 months prior to this study and had not been treated by radiotherapy. Visual oral examination was carried out by an experienced dentist; the subjects did not exhibit active dental carries, gingivitis, periodontal disease, nor any other dental disease potentially affecting oral fluid composition. The pellicle collection protocols were approved by the medical ethics committee of the Medical Association of Saarland, Germany (proposal # 238/03, 2009, 2012) and subjects gave their informed written consent to participate in this study.

All experiments started at 9.00 am to avoid circadian effects on the salivary composition. Subjects refrained from any food or beverages 2.5 h before starting and conducted individual oral hygiene with the respective standard hygiene products to guarantee individual day-to-day conditions. To avoid influences caused by any ingredients of the hygiene products, subjects conducted oral hygiene by the use of dental silk and tooth brushing without the use of any tooth paste 30 min before saliva collection and intraoral exposure. Over a fixed time period of maximum 20 min on ice, 10 mL of unstimulated saliva were collected.

2.2 | Pellicle collection

The enamel slabs were derived from the labial surfaces of bovine incisors (2-year-old cattle, tested bovine spongiform encephalopathy [BSE]-negative). For the individual pellicle formation, six pure enamel specimens with a total surface area of 8 cm² were polished stepwise by wet grinding with abrasive paper increasing the grit size from 120 to 4000 (Buehler). After removal of dentin, the resulting rectangular specimens of pure enamel (thickness of ≈0.3 mm) were polished from both sides as well as edges up to 4000 grit size. Slabs were purified, washed, and rehydrated in sterile water for minimum 12 h before exposure to the oral cavity. Additionally, six dental materials used in dentistry were applied: dental gold alloy (BioHerador, 86.2% gold, 11.5% platinum, Heraeus-Kulzer), titan (Frios, 99.3% titanium, 0.3% iron, 0.25% oxygen, Friadent), composite (Herculite, hybrid composite with quartz and silicon dioxide, Kerr), ceramics (VitaMark II, feldspath, Vita), polymethyl methacrylate (PMMA; Palapress, Heraeus-Kulzer), and polytetrafluoroethylene (PTFE/Teflon). 8 cm² (six to eight specimens) of each dental material slabs were polished as described above, ultrasonicated in 70% isopropanol and air-dried.

For individual pellicle formation in each subject, two specimens of the respective substrate material were placed consecutively in the buccal sulcus of the lower jaw in the region of the premolar and molar teeth for 3 min and for three to four follow-up rounds. To eliminate residual saliva and nonadsorbed epithelial cells or microorganisms, slabs were individually rinsed by 20 mL water from a pressure cylinder (Buerkle) and air-dried after removal from the oral cavity. Single slabs were incubated successively in Triton X-100 (1% v/v Triton X-100 in TRIS-HCl

buffer (0.02 M TRIS, 0.15 M NaCl, pH 7.5)) on ice followed by ultrasonication in RIPA-buffer (Cell Signaling Technology) at 4°C to elute the adsorbed pellicle components. Both solutions contained EDTA-free protease inhibitor mix (Complete, Roche). Probes were frequently vortexed to increase protein relief during the elution-steps.

2.3 | Precipitation of pellicle proteins

The pools of eluted proteins from the different dental substrate materials and enamel specimens of each subject were precipitated with trichloroacetic acid, washed twice with acetone, and air dried. The protein pellets were denatured for subsequent gel electrophoresis.

2.4 | Saliva collection

Over a fixed time period of maximum 20 min, 10 mL of unstimulated saliva was collected on ice, sterile-filtered (5 μm filter unit, Whatman), and centrifuged. Salivary flow rates of the subjects were in the norm of healthy individuals (0.5 mL/min for unstimulated saliva). Ninety micrograms of each sample were denatured for subsequent gel electrophoresis.

2.5 | Gel electrophoresis of proteins and preparation for mass spectrometry

Eluted proteins were dissolved in NuPAGE LDS (lithium dodecyl sulfate)-sample buffer, denatured for 20 min at 60°C, separated for 10 min on NuPAGE 4%–12% Bis-Tris gradient gels to remove the detergent interfering the subsequent mass spectrometry, fixed and visualized with colloidal Coomassie stain (20% v/v methanol, 10% v/v phosphoric acid, 10% w/v ammonium sulfate, 0.12% w/v Coomassie G-250). Salivary proteins were separated completely on NuPAGE 4%–12% Bis-Tris gradient gels, fixed and visualized as described above. Stained areas of the pellicle samples were cut in three pieces, stained areas of salivary samples were cut in 15 pieces and alternately washed twice with solution A (50 mM NH₄HCO₃) and solution B (50 mM NH₄HCO₃ and 50% v/v acetonitrile). As controls, an empty lane of each electrophoresis gel was cut analogously and analyzed in parallel. After reduction and carbamidomethylation of the proteins, gel pieces were washed twice alternating with solution A and B and dried in a vacuum centrifuge. After overnight in-gel trypsination (10 ng/μL porcine trypsin, Promega), resulting peptides were extracted twice in aqueous extraction buffer (2.5% formic acid, 50% acetonitrile), concentrated in a vacuum centrifuge and resuspended in 21 μL of 0.1% formic acid.

2.6 | Nano-LC-HR-MS/MS

Six microliters of the digested peptides were automatically transferred to a nanoflow liquid chromatography system (Ultimate

3000 RSLC, ThermoFisher Scientific, TF Scientific). Tryptic peptides were consecutively loaded on a trap (100 $\mu\text{m} \times 2$ cm, Acclaim PepMap100C18, 5 μm , TF Scientific) and a separation column (Acclaim PepMap column, C18; 2 μm ; 75 $\mu\text{m} \times 25$ cm, both TF Scientific) at a flow rate of 200 nL/min using buffer A (water and 0.1% formic acid) and B (90% acetonitrile and 0.1% formic acid). Peptides were eluted using a gradient from 4% to 30% buffer B in 100 min, 30% to 55% B in 20 min, 55% to 90% B in 7 min, constant 90% B for 2 min, 90% to 50% B in 1 min, constant 50% B for 3 min, 50% to 90% B in 1 min, constant 90% B for 4 min, 90% to 4% B in 1 min, and constant 4% B for 20 min. Eluted peptides were directly sprayed through a coated silica electrospray emitter (PicoTipEmitter, New Objective) into the LTQ Orbitrap Velos Pro mass spectrometer (TF Scientific). Spectra were acquired in a data-dependent mode as described before [13], whereas full scan MS spectra (m/z 300–1700) were acquired in the Orbitrap analyzer using a target value of 10^6 , peptide ions with charge states $> +2$ were fragmented in the high-pressure linear ion trap by low-energy CID with normalized collision energy of 35% using the TOP 10 method [33].

2.7 | Raw LC-MS data analysis

Fragmented peptide masses were initially analyzed by using the MASCOT algorithm and TF Proteome Discoverer 1.4 software. Peptides were matched to tandem mass spectra by Mascot version 2.4.0 (Matrix Science) by searching the SwissProt database (version 2018_03, number of protein sequences 557,992 containing 20,253 human sequences) against human proteins. Spectra were matched with a mass tolerance of 7 ppm for precursor masses and 0.5 Da for fragment ions. We used tryptic digestion and allowed for up to two missed cleavage sites. Cysteine carbamidomethylation was set as a fixed modification and deamidation of asparagine and glutamine, acetylation of lysine and oxidation of methionine were set as variable modifications. The MASCOT output files were uploaded in the program Scaffold (Version 4.8.9, Proteome Software Inc.). Peptide identifications were accepted if they had a probability greater than 95.0% (peptide FDR \leq 0.38% decoy) by the Scaffold Local FDR algorithm. Protein identifications were accepted if they had a probability greater than 90.0% (protein FDR \leq 1.6% decoy) and contained at least two unique peptides/protein [13].

2.8 | Bioinformatics analysis of mass spectrometrical data

2.8.1 | Qualitative analysis

The Biopython [34–36] and GOATOOLS [37, 38] Python libraries and modules were used to obtain the gene IDs and corresponding Gene Ontology (GO) terms for the proteins in the samples, to cluster them into groups sharing similar molecular functions (MFs), and to perform GO term enrichment analyses.

2.8.2 | Quantitative analysis

The exponentially modified protein abundance index (emPAI)-values of each protein were used to calculate the protein content, that is, the percentage of molar amount of substance, using the formula

$$\text{content protein } i \text{ (mol\%)} = \frac{\text{emPAI}_i}{\sum_n \text{emPAI}_n} \times 100$$

in order to obtain a value comparable between the samples [39].

The fold change (fc; here: measure of degree of change in protein content from the saliva to the pellicle) was considered to be enriched when $fc > 2$ and depleted when $fc < 0.5$ as described in Delius et al. 2017 [12]. Distribution patterns of molecular weight (MW) and isoelectric point were compared using the one-sided Mann–Whitney U rank test. Isoelectric points were taken from the isoelectric point database [40].

All analyses were performed in Python using the packages SciPy, NumPy, IPython notebooks, Matplotlib, Seaborn, and Pandas in addition to the already mentioned packages [41–46].

3 | RESULTS AND DISCUSSION

3.1 | Individual proteomic composition of the saliva and 3-min pellicle formed on different dental materials

In this study, the initial pellicle formed on different dental restorative materials, namely ceramics, gold, titan, PMMA, composite, and polytetrafluoroethylene (PTFE/Teflon) as most hydrophobic control, was characterized and compared to the one formed on bovine enamel. Within the initial phase of pellicle formation, taking place within a matter of seconds up to a few minutes after oral hygiene, mainly salivary proteins adsorb to the substrate surface [4, 47]. These adsorptions represent the very first interactions between the exposed surfaces and the components of the surrounding fluids. If there are any compositional differences dependent on the substrate material, these differences are most likely present in the initial pellicle. Up to date, there is no extensive information about the proteomic composition of the short-term pellicle based on the lack of practicable techniques for the analysis of this very thin layer. As the composition of this initial pellicle is likely to influence all subsequent interactions/adsorption processes between the surface and the biomolecules as well as microorganisms present in the oral liquid phase, the current study focused on the identification of the 3-min pellicle proteome. In order to identify potential substrate material-specific differences in this basal layer resulting in compositionally different pellicles, the current study focused on the proteomic composition of the 3-min pellicle formed on the different substrate materials. To the aim of identifying selective adsorption patterns of salivary proteins occurring on the different substrates in detail on individual level, the 3-min in situ pellicle and the corresponding saliva of five subjects were analyzed and compared separately (workflow depicted in Supplemental Scheme 1) (proteomic data of saliva and 3-min

TABLE 1 Number of identified proteins and overlaps in the individual 3-min pellicle on different dental substrate materials, bovine enamel, and saliva

Identified proteins		Subject					Diversity	Overlapping proteins
		I	II	III	IV	V		
Saliva		1009	875	855	1032	760	1435	525
3-min pellicle	Enamel	490	244	488	479	296	772	147
	Ceramics	262	187	286	491	234	613	82
	Composite	400	345	384	517	343	706	181
	Gold	251	282	298	391	214	565	95
	Titan	327	269	276	479	307	624	141
	PMMA	427	460	467	671	513	873	241
	PTFE	554	475	418	686	503	953	251
Overlap pellicle		174	108	163	255	124	323	105
Overlap saliva and pellicle		146	107	139	247	116	312	60
Diversity pellicle		815	686	782	994	747	1348	566
Diversity saliva and pellicle		1362	1136	1193	1446	1070	1946	971

PMMA, polymethyl methacrylate; PTFE, polytetrafluoroethylene.

pellicle on bovine enamel were taken from Trautmann et al. 2020 [13]). To ensure a high identification quality of the individual samples, a chemical elution followed by an elaborate nano-mass spectrometric protocol already described in Trautmann et al. 2019 [32] was applied. A virtually complete elution of the 3-min pellicles from the different substrate materials was verified by transmission electron microscopy analyses conducted before and after elution (Supplemental Figure S1). The corresponding saliva samples of the five subjects were analyzed in parallel to enable the comparison of the individual proteomic profiles and elucidate the enrichment or depletion of the proteins detected in the 3-min pellicle.

The applied protocol resulted in the identification of between 187 and 686 different proteins in the individual 3-min in situ pellicles and of 760 to 1032 different proteins in the corresponding saliva of the five subjects (Table 1). Therein, 82–251 proteins belonged to the overlap of all subjects on a single substrate. Diversities of 565 to 953 different proteins identified among the individual pellicle samples of the five subjects underpinned the previously stated individual fingerprint of the pellicle proteome [12, 13, 32] (Supplemental Figure S7). A total of 108 to 255 proteins were identified in the pellicle-samples of the single subjects on all substrates, with 107 to 247 being present in the intersection of the pellicles and the corresponding saliva as well. In total, this study was able to identify 1946 different proteins, including 1348 proteins in the 3-min pellicle and 1435 proteins found in the saliva of the five subjects (Supplemental Table S2).

When forming the overlap of all substrate materials, 105 proteins were identified in the 3-min pellicle of all subjects on all substrate materials, including all previously stated proteins of the pellicle-base proteome [32]. Intersecting these 105 proteins with the saliva gave 60 proteins that were identified in the 3-min pellicle on all substrate materials as well as in the saliva of all subjects (exclusively present proteins on single substrate materials listed in Supplemental Table S3).

The current results represent the so far largest number of 1348 uniquely identified pellicle proteins. This is a significant increase over the 772 proteins identified earlier in a previous analysis that was included in the current study [13]. There, the individual 3-min pellicle of the same five subjects formed on bovine enamel was characterized and compared to saliva in terms of qualitative and quantitative aspects. The almost two-fold increase is likely due to the use of the identical chemical elution protocol combined with an elaborate nano-MS procedure and the additional use of six dental substrate materials. The different numbers of identified proteins on the various substrate materials might, on the one hand, be due to different adsorption velocities relying on the respective physico-chemical properties of the substrate materials. A second conceivable reason for these differences might be the use of a standardized elution protocol for the elution of the 3-min pellicle from each substrate material. Even though the efficiency of the chemical elution protocol was checked by electron microscopy, the presence of some closely bound residual proteins on the different substrate materials cannot be excluded entirely. In correlation to the applied mass spectrometric methodology, there are some potential limitations of the experimental design based on differences in the accessibility of tryptic cleavage sites of single proteins or on the presence of such a broad range of different protein abundances. Still, the huge and diverse list of protein identifications argues for veritable results and an efficient experimental setup. Overall, in the course of the current study, the standardized protocol was effectively used to identify the so far largest number of pellicle proteins even on an individual level.

3.2 | Bioinformatics analyses of the identified proteins

Based on the list of identified proteins, a systematic qualitative as well as quantitative bioinformatics evaluation was conducted. The

qualitative analysis bases upon the pure identification of the proteins whereas the quantitative analysis rests upon the emPAI-values of the single proteins obtained during individual mass spectrometry. The individual fingerprint of the pellicle proteome and the high diversity of proteins present in the saliva and the 3-min pellicles formed on the different substrate materials resulted in high interindividual differences between the single subjects. To extract higher-order meaning from the broad range of individual results, the mean over the five subjects were drawn throughout the analyses.

3.2.1 | Physico-chemical properties of the identified proteins

To the aim of elucidating differences in the proteomic composition of the 3-min pellicle formed on the different substrate materials, the basic physico-chemical properties of the identified proteins were considered. At first, the MWs were analyzed systematically based on the single subjects and on single substrate materials to search for specific adsorption patterns. This analysis yielded a broad range of MWs between 5 and 250 kDa with highest abundances in the small- and middle-sized MW range between 10 and 60 kDa. By taking a closer look at the MW range between 0 and 100 kDa in the qualitative background, a rather uniform distribution over the MW spectrum with constantly decreasing probabilities from about 60 toward 100 kDa was depicted for all substrate materials and the saliva (Supplemental Figure S2). Overall, the MWs of the pellicle proteomes were significantly smaller than the ones of the saliva, except for ceramics and PMMA that showed no significant differences on qualitative level. While the *p*-value is a measure for the significance of the difference between two distributions, it cannot provide information about the actual size of this difference. To measure how different two distributions are, effect sizes are calculated. The appropriate effect size measure for the Mann-Whitney *U* test, which was used in this study to compare the weight distributions, is the rank-biserial correlation. A value of 0 means both distributions are completely equal, a correlation of 1 that the second distribution is completely different from the first and has higher values, -1 that the second distribution is completely different from the first but has lower values. The effect size values within the qualitative evaluation were ≤ 0.085 , pointing to highly similar MWs in the pellicle proteomes on all substrate materials and the corresponding saliva.

The quantitative analysis revealed a more manageable amount of MWs between 5 and 100 kDa depicting again similar distribution patterns between the different substrate materials (Figure 1/ Supplemental Figure S3 for individual distribution patterns). They showed highest abundances in the range of 10–20 kDa, followed by medium abundances between 50 and 70 kDa. In general, mostly small MW proteins were expected to be identified in the 3-min pellicle formed on the different substrate materials, due to their higher mobility and hence higher adsorption velocity during the initial phase of pellicle formation. This expectation was already underpinned by the results of Trautmann et al. 2020 [13], where the individual 3-min pellicle formed on bovine enamel was characterized and compared to

saliva. Therein, mostly small MW proteins were identified to adsorb to the initial pellicle. This study gave very similar results for all substrate materials with the largest part of the adsorbed proteins possessing MWs between 10 and 20 kDa. When comparing the pellicle proteomes to the salivary proteome, the proteins adsorbed to PTFE, PMMA, and gold had significantly higher MWs. However, the effect size of the differences between the salivary proteome and the proteins adsorbed to PTFE, PMMA, and gold was rather small (rank-biserial correlation = 0.002–0.106). One may speculate that large proteins may be able to undergo larger conformational changes than smaller, more stable ones. In the case of PTFE and PMMA, such large proteins would undergo conformational changes to expose their hydrophobic groups and interact with these hydrophobic surfaces. In the case of gold, thiol bonds could be formed between protein side chains having a thiol group and the surface material. Likewise, the proteins may undergo conformational changes to expose the thiol groups and form the bridges [48]. Those interactions would be stronger than the ones formed by small proteins, and therefore the larger proteins might repel those loosely bound smaller proteins from the respective substrate surface. A second conceivable explanation for the higher MWs on PTFE and PMMA is the observation of Brash and Lyman [49] concerning the Vroman effect on hydrophobic surfaces. They suggested that proteins adsorb proportionally to their surface collision frequency or concentration without a preferential, surface-selective adsorption of one protein over another on hydrophobic surfaces.

Overall, the current results depict very similar MW distribution patterns with highest abundances in the range of 10–20 kDa, followed by medium abundances between 50 and 70 kDa for the pellicle proteins identified on all substrate materials. This points to a similar composition of the 3-min pellicle proteome independent of the respective substrate material.

Subsequently, the isoelectric points (*pI*s) of the identified proteins were analyzed. The proteins were assorted to three *pI*-ranges: with a *pH* below 6.3 (negatively charged in the oral cavity), in the physiological *pH* of the oral cavity ranging from 6.3 to 7.6 (neutral) and with a *pH* higher than 7.6 (positively charged in the oral cavity) [50]. The qualitative analysis disclosed a broad coverage of *pI*s between *pH* 4 and 11 on all substrate materials and in the saliva. The quantitative evaluation showed the distribution patterns of the salivary proteins to possess a broad spectrum of isoelectric points with mostly rather low probabilities of presence (Figure 2/Supplemental Figure S4 for individual distribution patterns). Therein, the largest part (47%) was located in the *pH* range below 6.3. Only 17% of the salivary proteins possessed isoelectric points in the *pH* range above 7.6, exhibiting positive net charges. The pellicle proteins found on the different substrate materials had highly similar distribution patterns of the isoelectric points. For the most part, these patterns were merely differing in the probabilities of presence of the single isoelectric points. Compared to the saliva with only 17%, a clearly increased fraction of proteins had isoelectric points above 7.6 with 24% on PTFE up to 40% on composite. Overall, these analyses revealed similar distribution patterns of the isoelectric points on all substrate materials, relying most likely on the adsorption of the same salivary proteins. The comparison of the distribution

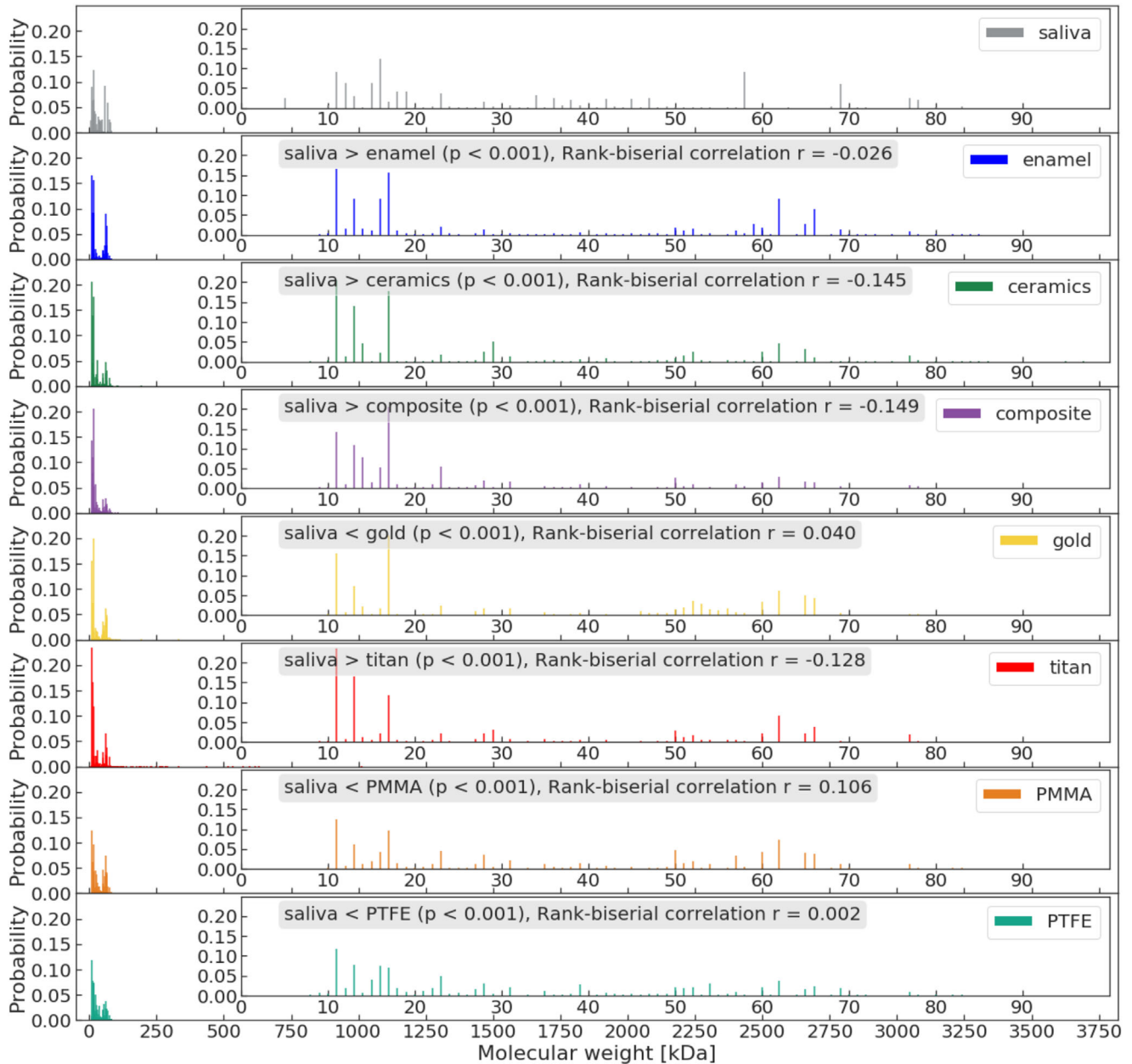


FIGURE 1 Quantitative molecular weight distribution patterns of proteins identified in the saliva and 3-min pellicle on seven substrate materials, averaged over five subjects, based on exponentially modified protein abundance index (emPAI)-values. Occurrence probabilities are plotted against the molecular weight of the identified proteins. Outer histograms depict the distribution patterns on the whole molecular weight spectrum of all proteins identified. Inner histograms depict a magnification of the low to medium molecular weight range from 0 to 100 kDa. Data of saliva and enamel were taken from Trautmann et al. 2020 [13], copyright permission obtained

patterns from saliva and the 3-min pellicles revealed a favored adsorption of positively charged proteins on all substrate materials.

Taken together, the current results regarding the physico-chemical properties of the identified proteins point to a favored adsorption of proteins with low MWs and preferentially positively charged proteins during the initial stage of pellicle formation on all substrate materials. These results are in line with the findings of a previous study on the 3-min pellicle on ceramics. There, the adsorbed proteins that were significantly enriched in the 3-min pellicle had on average higher isoelectric points than the ones in the liquid saliva phase [12].

The current results point to a similar composition of the 3-min pellicle proteome independent of the respective substrate material. Those findings deviate from previous literature that reported clear differences in the pellicle composition between dental enamel and the dental materials titanium and PMMA [27]. Those earlier analyses were based on a 2D gelectrophoresis of the residual saliva remaining after a 2 h-exposure to the different substrate materials, depicting the respective protein spots within the different *pI*- and *kDa*-ranges. The authors identified different protein patterns on the 2D gels and suggested an influence of the underlying substrate material on the salivary film

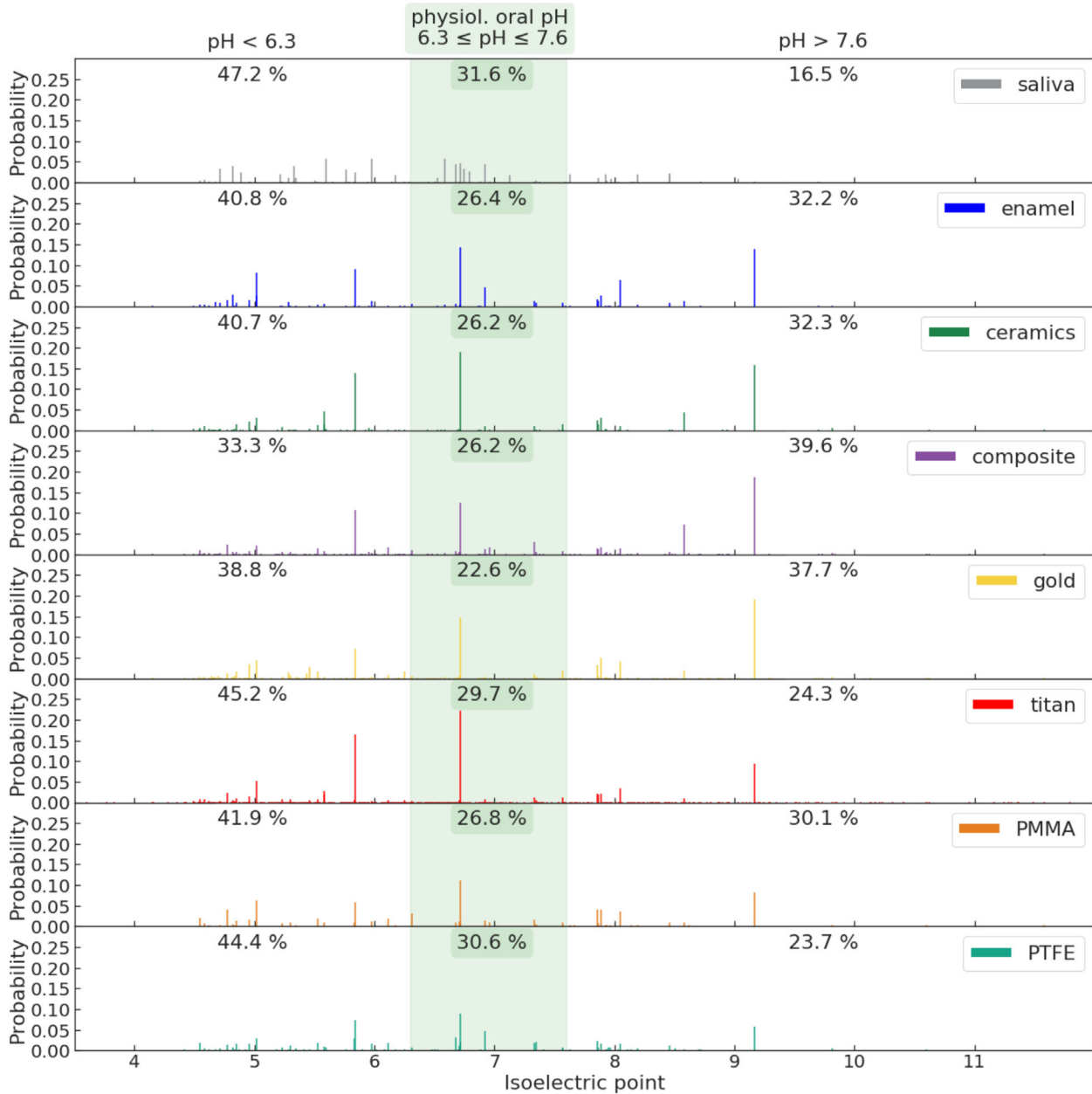


FIGURE 2 Quantitative isoelectric point distribution patterns of proteins identified in the saliva and 3-min pellicle on seven substrate materials, averaged over five subjects. Occurrence probabilities based on exponentially modified protein abundance index (emPAI)-values are plotted against the isoelectric points of the identified proteins. Green areas depict the physiological pH-range of 6.3–7.6 in the oral cavity. Percentages of proteins possessing pIs below, within, or higher than the physiological oral pH-range are shown in boxes. Basic data of saliva and enamel were taken from Trautmann et al. 2020 [13], copyright permission obtained

composition. Even though the current results show slight differences in the probabilities of presence of single MWs and isoelectric points, the overall interpretation points to a minor relevance of the substrate material on the composition of the 3-min pellicle.

3.2.2 | Molecular functions of the identified proteins

Next, it was analyzed whether the adsorption patterns of specific substrate materials could be associated with particular MFs of the iden-

tified proteins. For this, the MFs annotated in the Gene Ontology (GO) for the 1946 identified proteins were analyzed on qualitative and quantitative level [37]. For this comparison, the GO terms (database version 01/07/2019) of the proteins in the individual salivary and 3-min pellicle proteomes were assorted to 14 precast categories representing the highest level of the MF branch below the root term. Figure 3 depicts the mean result of the five subjects. On a global level, the distribution patterns for individual substrate materials and the saliva are highly similar to each other. At closer inspection, one detects slight differences both on the qualitative as well as on the quantitative level. For example,

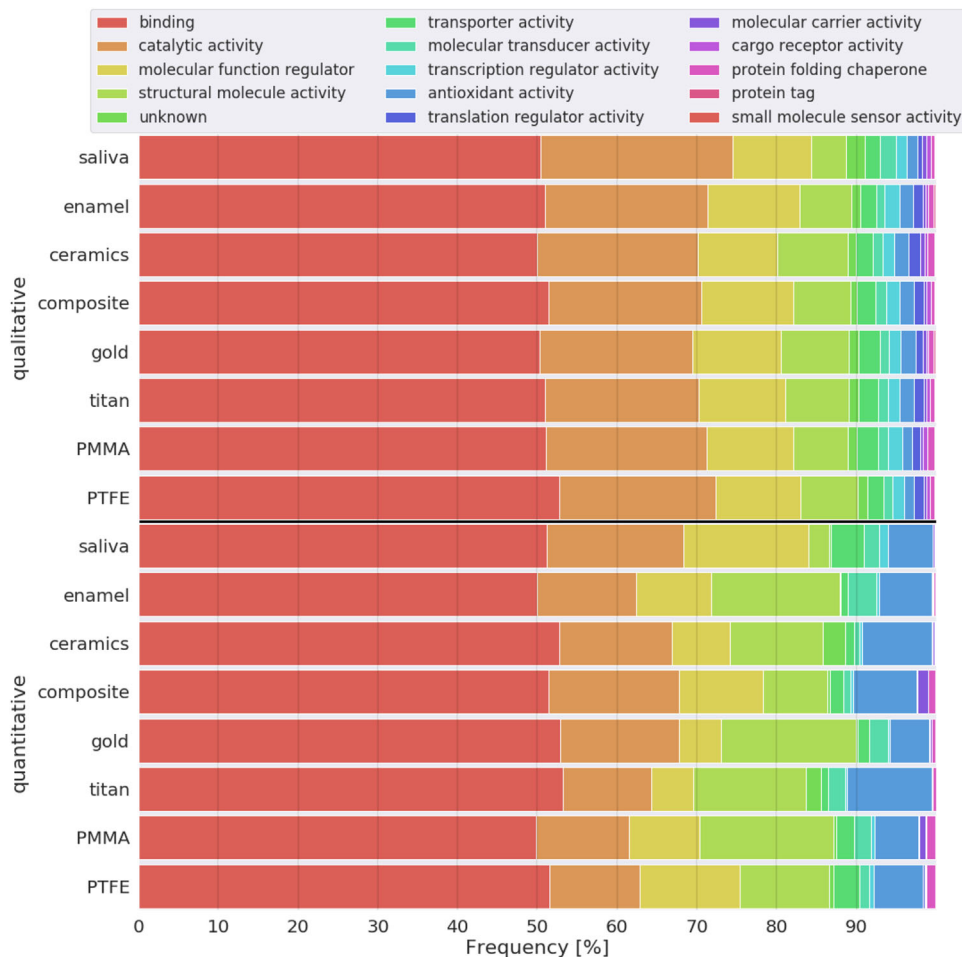


FIGURE 3 Molecular function distribution patterns of proteins identified in the saliva and 3-min pellicle on seven substrate materials, averaged over five subjects. Quantitative evaluation based on relative protein content values (mol%) depicted in the upper half, qualitative evaluation based on protein presence shown in the lower half. Frequencies of molecular functions assorted to fourteen precast categories are depicted in different colors

saliva contains the largest fraction of proteins having catalytic activity (colored orange in Figure 3) but the smallest fraction of proteins having structural molecule activity (light green). While the frequency of proteins belonging to the category MF regulator (yellow) was represented rather equally throughout all dental materials and the saliva on the qualitative level, a clearly increased frequency was detected in the saliva on the quantitative level. As mentioned, the amount of proteins with MFs being assorted to the category structural molecule activity was elevated in the pellicle formed on all substrate materials, both on qualitative and quantitative level with an even higher increase on quantitative level. This elevated presence might be in line with a potential higher demand for structural proteins providing manifold binding sites for the adsorption of further proteins, other macromolecules, or later bacteria in the process of biofilm formation.

In general, the adsorption of salivary proteins most likely relies largely on their physico-chemical properties. Still the selective adsorption of proteins sharing specific MFs is conceivable. Indeed, such a selective adsorption of proteins possessing specific MFs would rather rely on similar binding motifs or functional domains within the protein

structure facilitating their interaction with the respective substrate material or the initial pellicle than on their MF itself.

Enrichment analysis of identified molecular functions

To extract biological meaning from the list of annotated MFs, an enrichment analysis against the full genomic background was performed. Hereby, an enriched MF reflects a significantly elevated presence of such proteins in the adsorbed proteome. Figure 4 depicts the distribution pattern of the commonly enriched MFs identified in the salivary and pellicle proteomes of all subjects as well as their overlap on the different substrate materials (see Supplemental Figure S5 for individual distribution patterns). Basically, the distribution patterns are rather similar, whereby the categories protein binding and enzymatic activity are most frequent, whereas all other categories are rarely or even not present on the different substrate materials. An exception are the pellicle proteomes formed on the substrate materials gold and ceramics, which contain no proteins with exclusively enriched MFs in the category protein binding. For gold and titan, no MF was exclusively enriched in the category enzymatic activity. All other substrate

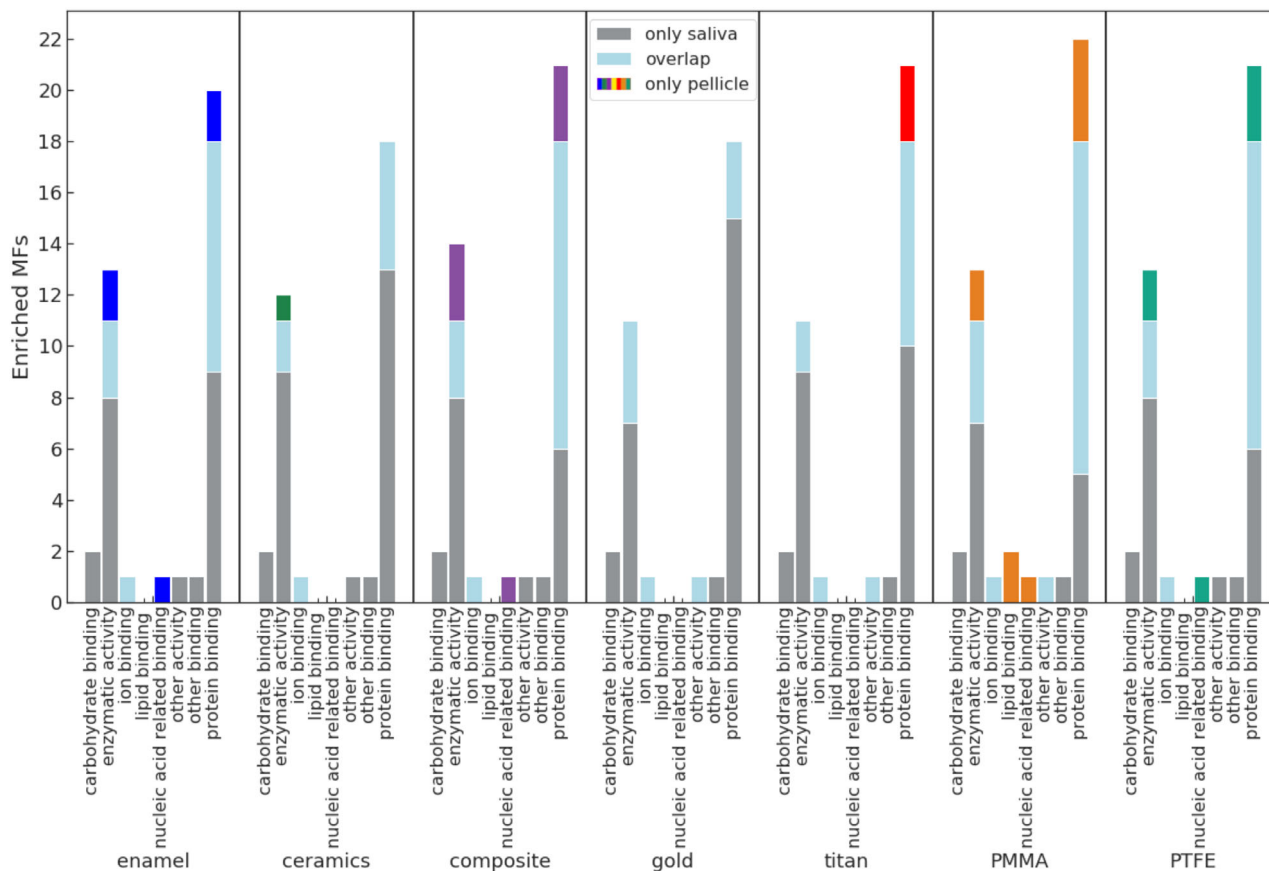


FIGURE 4 Number of commonly enriched molecular functions identified for proteins in saliva (gray), both in saliva and in 3-min pellicle (light blue) or exclusively in 3-min pellicle (different colors), averaged over five subjects. Data for saliva and enamel were taken from Trautmann et al. 2020 [13], copyright permission obtained

materials possess one up to six exclusively enriched MFs in those two categories, pointing to selective adsorption processes of proteins having these MFs on the respective substrate material. Even though these adsorption processes are most likely based on specific binding motifs or functional domains of these protein structures, the presence of these proteins sharing specific MFs might improve the functionality of the initial pellicle. For example, proteins sharing MFs of the category catalytic activity might impart protective properties against bacterial adhesions/metabolites on the respective substrate material. A further example is given by a recent *in vitro* study focusing on profiling the immunological roles of pellicle proteins on the conventional implant abutment materials titan and feldspathic ceramics [30]. Based on ontological networks, the authors proposed putative immunological prospects of surface-adsorbed salivary proteins. In line with the present results, such findings might favor the use of one substrate material over the other ones in the dental clinic and should be further investigated in future studies.

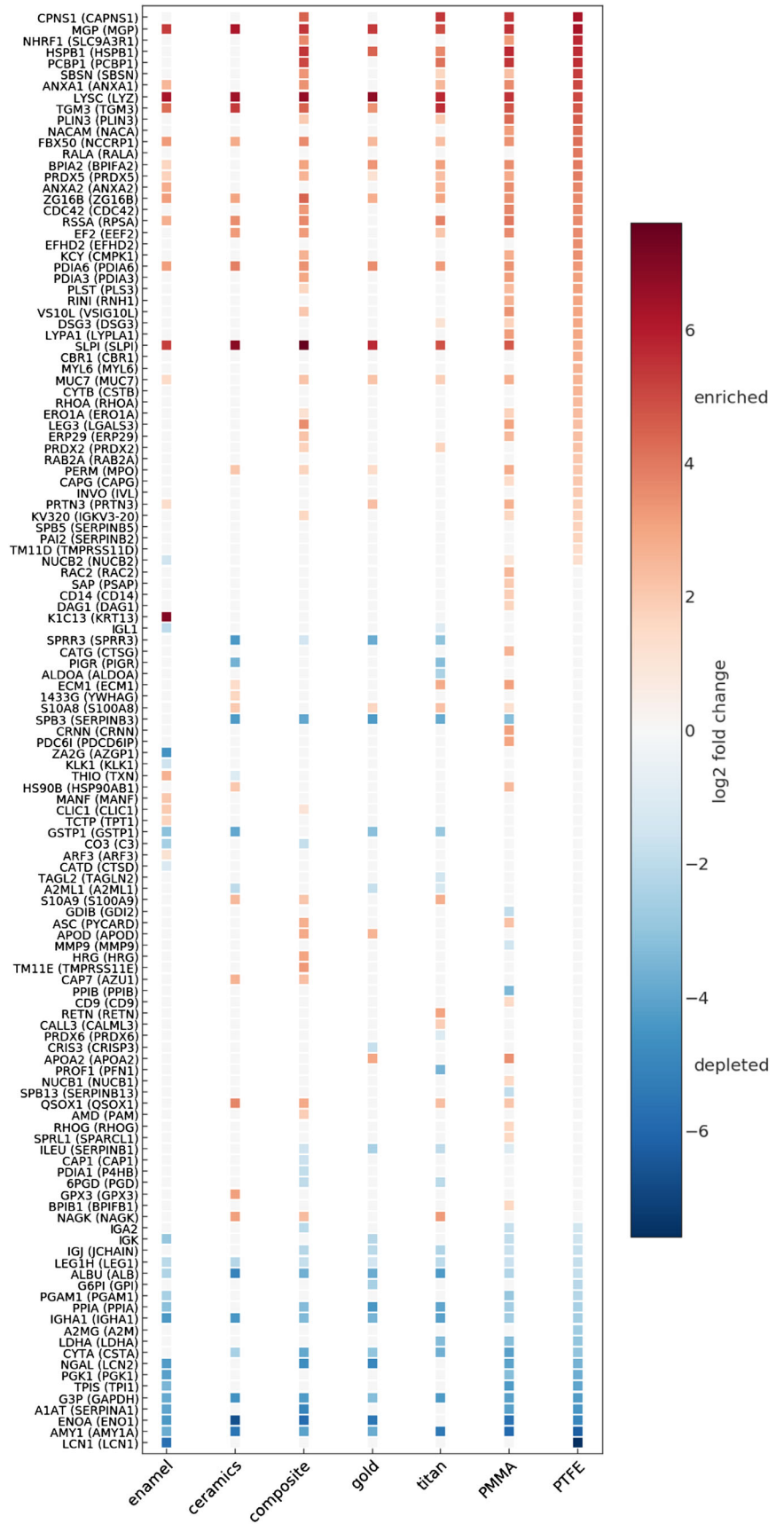
Taken together, the enrichment analyses showed slight differences in the exclusively enriched MFs for the proteins adsorbed on the different substrate materials. Still, the overall interpretation of the data points to a rather minor relevance of the substrate material on the distribution pattern of the enriched MFs and hence, the composition of the 3-min pellicle.

3.2.3 | Selective adsorption of distinct salivary proteins

In the next step, the selective adsorption of salivary proteins in the 3-min pellicle was analyzed on the basis of the mole fractions (mole%) of the identified proteins for the different subjects and substrate materials relative to the saliva proteome (Supplemental Figure S6). A protein was considered to be enriched on a substrate material for an $f_c > 2.0$ and depleted for an $f_c < 0.5$ [12]. In this analysis, we focused on the proteins that showed the same trend (enriched or depleted) for one of the substrate materials tested on all subjects. We found that 127 proteins were commonly enriched or depleted in the 3-min pellicle of all subjects on at least one up to seven substrate materials. The averaged distribution patterns of these 127 proteins are depicted in Figure 5.

Several proteins were found to be enriched or depleted exclusively in the pellicle formed on a single substrate material. On the other hand, seven proteins (MGP, LYSC, TGM3, FBX50, ZG16B, PDIA6, and SLP1) were enriched on all substrate materials, sharing similar f_c s and pointing to an analogous adsorption kinetics. Five proteins (LEG1H, ALBU1, IGHA1, G3P, and AMY1) were identified to be depleted on all substrate materials, pointing to rather inert protein structures or repulsive properties.

FIGURE 5 Fold changes of proteins being commonly enriched or depleted in all subjects in 3-min pellicle on one up to seven substrate materials. Enriched proteins are shown in red shades, depleted proteins are shown in blue shades, proteins present in 3-min pellicle without significant fold change compared to saliva are shown in light gray. Different substrate materials are plotted against the accession numbers (and gene names) of the proteins



Virtually all of the enriched or depleted proteins detected in the 3-min pellicle showed similar fcs on at least two or even more substrate materials. Only the two proteins nucleobindin-2 (NUCB2) and Thioredoxin (THIO) were found to be enriched on one substrate material and depleted on another. Thereby, the calcium-binding protein NUCB2 was depleted in the 3-min pellicle formed on bovine enamel and enriched on PMMA. THIO is a protein participating in various redox reactions and was enriched in the 3-min pellicle formed on bovine enamel, whereas it was depleted in the pellicle on ceramics. The physiological background of the enriched or depleted presence of those proteins on the different substrate materials remains open. The similar, substrate material independent binding behavior of all other enriched or depleted proteins points to a negligible role of the respective substrate material in this context.

4 | CONCLUDING REMARKS

Taken together, the overall interpretation of the current results suggests a rather minor relevance of the respective substrate material properties on the proteomic composition of the individual 3-min pellicle. Furthermore, the data underpins the hypothesis of a pellicle layer which is physiologically functional even after a few minutes of formation time [4, 12]. Presumably, the surface of the substrate materials is promptly covered with salivary proteins adsorbing upon exposure to the oral cavity, thereby shielding in part its respective physico-chemical properties and forming a uniform basal pellicle layer. This scenario is in line with the highly meaningful pellicle formation-phenomenon in acting as physiological mediator by instantly covering all orally exposed particles and masking the physico-chemical surface properties of different substrate materials.

The proteomic data of the present study not only contribute to a much more detailed explanation of the process of pellicle formation on dental materials under oral conditions. The current results are also of high relevance for a deeper understanding of the process of protein adsorption at any interface between a complex biological fluid and solid surfaces.

ACKNOWLEDGMENTS

This study was funded by the German Research Foundation (DFG SFB1027, projects B3 and C3). Claudia Fecher-Trost was supported by HOMFOR and by DFG SFB512 to Veit Flockerzi.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD031449 and <https://doi.org/10.6019/PXD031449>, PXD031452 and <https://doi.org/10.6019/PXD031452>, PXD031454 and <https://doi.org/10.6019/PXD031454>, PXD031464 and <https://doi.org/10.6019/PXD031464> [51–53].

ORCID

Simone Trautmann  <https://orcid.org/0000-0003-0570-7382>

REFERENCES

- Hannig, M., Fiebiger, M., Güntzer, M., Döbert, A., Zimehl, R., & Nekrashevych, Y. (2004). Protective effect of the in situ formed short-term salivary pellicle. *Archives of Oral Biology*, 49(11), 903–910. <https://doi.org/10.1016/j.archoralbio.2004.05.008>.
- Hannig, C., & Hannig, M. (2009). The oral cavity—a key system to understand substratum-dependent bioadhesion on solid surfaces in man. *Clinical Oral Investigations*, 13(2), 123–139. <https://doi.org/10.1007/s00784-008-0243-3>.
- Hannig, M. (1997). Transmission electron microscopic study of in vivo pellicle formation on dental restorative materials. *European Journal of Oral Sciences*, 105(5 Pt 1), 422–433. <https://doi.org/10.1111/j.1600-0722.1997.tb02139.x>.
- Hannig, M., & Joiner, A. (2006). The structure, function and properties of the acquired pellicle. *Monographs in Oral Science*, 19, 29–64. <https://doi.org/10.1159/000090585>.
- Lendenmann, U., Grogan, J., & Oppenheim, F. G. (2001). Saliva and dental pellicle – a review. *Advances in Dental Research*, 14, 22–28. <https://doi.org/10.1177/08959374000140010301>.
- Hannig, M., & Balz, M. (2001). Protective properties of salivary pellicles from two different intraoral sites on enamel erosion. *Caries Research*, 35(2), 142–148. <https://doi.org/10.1159/000047446>.
- Buzalaf, M. A. R., Hannas, A. R., & Kato, M. T. (2012). Saliva and dental erosion. *Journal of Applied Oral Science*, 20(5), 493–502. <https://doi.org/10.1590/s1678-77572012000500001>.
- Siqueira, W. L., Custodio, W., & McDonald, E. E. (2012). New insights into the composition and functions of the acquired enamel pellicle. *Journal of Dental Research*, 91(12), 1110–1118. <https://doi.org/10.1177/0022034512462578>.
- Hannig, C., Hannig, M., & Attin, T. (2005). Enzymes in the acquired enamel pellicle. *European Journal of Oral Sciences*, 113(1), 2–13. <https://doi.org/10.1111/j.1600-0722.2004.00180.x>.
- Hannig, M. (1999). Ultrastructural investigation of pellicle morphogenesis at two different intraoral sites during a 24-h period. *Clinical Oral Investigations*, 3(2), 88–95. <https://doi.org/10.1007/s0078400500084>.
- Lie, T. (1977). Scanning and transmission electron microscope study of pellicle morphogenesis. *Scandinavian Journal of Dental Research*, 85(4), 217–231. <https://doi.org/10.1111/j.1600-0722.1977.tb00556.x>.
- Delius, J., Trautmann, S., Médard, G., Kuster, B., Hannig, M., & Hofmann, T. (2017). Label-free quantitative proteome analysis of the surface-bound salivary pellicle. *Colloids and Surfaces B: Biointerfaces*, 152, 68–76. <https://doi.org/10.1016/j.colsurfb.2017.01.005>.
- Trautmann, S., Künzel, N., Fecher-Trost, C., Barghash, A., Schalkowsky, P., Dudek, J., Delius, J., Helms, V., & Hannig, M. (2020). Deep proteomic insights into the individual short-term pellicle formation on enamel—an in situ pilot study. *Proteomics Clinical Applications*, 14(3), 1900090. <https://doi.org/10.1002/prca.201900090>.
- Teughels, W., Van Assche, N., Sliepen, I., & Quirynen, M. (2006). Effect of material characteristics and/or surface topography on biofilm development. *Clinical Oral Implants Research*, 17 Suppl 2, 68–81. <https://doi.org/10.1111/j.1600-0501.2006.01353>.
- Busscher, H. J., Rinastiti, M., Siswomihardjo, W., & Van Der Mei, H. C. (2010). Biofilm formation on dental restorative and implant materials. *Journal of Dental Research*, 89(7), 657–665. <https://doi.org/10.1177/0022034510368644>.
- Wang, Z., Shen, Y., & Haapasalo, M. (2014). Dental materials with antibiofilm properties. *Dental Materials*, 30(2), e1–e16. <https://doi.org/10.1016/j.dental.2013.12.001>.
- Renner, L. D., & Weibel, D. B. (2011). Physicochemical regulation of biofilm formation. *Materials Research Society Bulletin*, 36(5), 347–355. <https://doi.org/10.1557/mrs.2011.65>.

18. Fischer, N. G., & Aparicio, C. (2021). The salivary pellicle on dental biomaterials. *Colloids and Surfaces B: Biointerfaces*, 200, 111570. <https://doi.org/10.1016/j.colsurfb.2021.111570>.
19. Sönju, T., & Glantz, P.-O. (1975). Chemical composition of salivary integuments formed in vivo on solids with some established surface characteristics. *Archives of Oral Biology*, 20(10), 687–691. [https://doi.org/10.1016/0003-9969\(75\)90138-7](https://doi.org/10.1016/0003-9969(75)90138-7).
20. Baier, R. E., & Glantz, P.-O. (1978). Characterization of oral in vivo films formed on different types of solid surfaces. *Acta Odontologica Scandinavica*, 36(5), 289–301. <https://doi.org/10.3109/00016357809029079>.
21. Hannig, C., Wasser, M., Becker, K., Hannig, M., Huber, K., & Attin, T. (2006). Influence of different restorative materials on lysozyme and amylase activity of the salivary pellicle in situ. *Journal of Biomedical Materials Research Part A*, 78(4), 755–761. <https://doi.org/10.1002/jbm.a.30758>.
22. Hannig, C., Huber, K., Lambrichts, I., Gräser, J., D'haen, J., & Hannig, M. (2007). Detection of salivary alpha-amylase and lysozyme exposed on the pellicle formed in situ on different materials. *Journal of Biomedical Materials Research Part A*, 83(1), 98–103. <https://doi.org/10.1002/jbm.a.31313>.
23. Yoshida, E., & Hayakawa, T. (2013). Adsorption study of pellicle proteins to gold, silica and titanium by quartz crystal microbalance method. *Dental Materials Journal*, 32(6), 883–887. <https://doi.org/10.4012/dmj.2013-136>.
24. Aroonsang, W., Sotres, J., El-Schich, Z., Arnebrant, T., & Lindh, L. (2014). Influence of substratum hydrophobicity on salivary pellicles: Organization or composition? *Biofouling*, 30(9), 1123–1132. <https://doi.org/10.1080/08927014.2014.974155>.
25. Lima, E. M. C. X., Koo, H., Vacca Smith, A. M., Rosalen, P. L., & Del Bel Cury, A. A. (2008). Adsorption of salivary and serum proteins, and bacterial adherence on titanium and zirconia ceramic surfaces. *Clinical Oral Implants Research*, 19(8), 780–785. <https://doi.org/10.1111/j.1600-0501.2008.01524.x>.
26. Dorkhan, M., Svensäter, G., & Davies, J. R. (2013). Salivary pellicles on titanium and their effect on metabolic activity in *Streptococcus oralis*. *BioMed Central Oral Health*, 13, 32. <https://doi.org/10.1186/1472-6831-13-32>.
27. Svendsen, I. E., & Lindh, L. (2009). The composition of enamel salivary films is different from the ones formed on dental materials. *Biofouling*, 25(3), 255–261. <https://doi.org/10.1080/08927010802712861>.
28. Pantaroto, H. N., Amorim, K. P., Matozinho Cordeiro, J., Souza, J. G. S., Ricomini-Filho, A. P., Rangel, E. C., Ribeiro, A. L. R., Vaz, L. G., & Barão, V. A. R. (2019). Proteome analysis of the salivary pellicle formed on titanium alloys containing niobium and zirconium. *Biofouling*, 35(2), 173–186. <https://doi.org/10.1080/08927014.2019.1580360>.
29. Cavalcanti, I. M. G., Ricomini Filho, A. P., Lucena-Ferreira, S. C., Da Silva, W. J., Paes Leme, A. F., Senna, P. M., & Del Bel Cury, A. A. (2014). Salivary pellicle composition and multispecies biofilm developed on titanium nitrided by cold plasma. *Archives of Oral Biology*, 59(7), 695–703. <https://doi.org/10.1016/j.archoralbio.2014.04.001>.
30. Wei, C.-X., Burrow, M. F., Botelho, M. G., Lam, H., & Leung, W. K. (2020). In vitro salivary protein adsorption profile on titanium and ceramic surfaces and the corresponding putative immunological implications. *International Journal of Molecular Sciences*, 21(9), 3083. <https://doi.org/10.3390/ijms21093083>.
31. Souza, J. G. S., Bertolini, M., Costa, R. C., Lima, C. V., & Barão, V. A. R. (2020). Proteomic profile of the saliva and plasma protein layer adsorbed on Ti-Zr alloy: The effect of sandblasted and acid-etched surface treatment. *Biofouling*, 36(4), 428–441. <https://doi.org/10.1080/08927014.2020.1769613>.
32. Trautmann, S., Barghash, A., Fecher-Trost, C., Schalkowsky, P., Hannig, C., Kirsch, J., Rupf, S., Keller, A., Helms, V., & Hannig, M. (2019). Proteomic analysis of the initial oral pellicle in caries-active and caries-free individuals. *Proteomics Clinical Applications*, 13(4), 1800143. <https://doi.org/10.1002/prca.201800143>.
33. Fecher-Trost, C., Wissenbach, U., Beck, A., Schalkowsky, P., Stoerger, C., Doerr, J., Dembek, A., Simon-Thomas, M., Weber, A., Wollenberg, P., Ruppert, T., Middendorff, R., Maurer, H. H., & Flockerzi, V. (2013). The in vivo TRPV6 protein starts at a non-AUG triplet, decoded as methionine, upstream of canonical initiation at AUG. *The Journal of Biological Chemistry*, 288(23), 16629–16644. <https://doi.org/10.1074/jbc.M113.469726>.
34. Cock, P. J. A., Antao, T., Chang, J. T., Chapman, B. A., Cox, C. J., Dalke, A., Friedberg, I., Hamelryck, T., Kauff, F., Wilczynski, B., & De Hoon, M. J. L. (2009). Biopython: Freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics*, 25(11), 1422–1423. <https://doi.org/10.1093/bioinformatics/btp163>.
35. Schuler, G. D., Epstein, J. A., Ohkawa, H., & Kans, J. A. (1996). Entrez: Molecular biology database and retrieval system. *Methods in Enzymology*, 266, 141–162. [https://doi.org/10.1016/s0076-6879\(96\)66012-1](https://doi.org/10.1016/s0076-6879(96)66012-1).
36. Sayers, E. W., Beck, J., Brister, J. R., Bolton, E. E., Canese, K., Comeau, D. C., Funk, K., Ketter, A., Kim, S., Kimchi, A., Kitts, P. A., Kuznetsov, A., Lathrop, S., Lu, Z., Mcgarvey, K., Madden, T. L., Murphy, T. D., O'leary, N., Phan, L., ... Ostell, J. (2019). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 47(D1), D9–D28. <https://doi.org/10.1093/nar/gkz899>.
37. Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., & Sherlock, G. (2000). Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics*, 25(1), 25–29. <https://doi.org/10.1038/75556>.
38. Tang, H., Klopfenstein, D., Pedersen, B., Flick, P., Sato, K., Ramirez, F., & Mungall, C. (2015). GOATOOLS: Tools for gene ontology. *Zenodo*, <https://doi.org/10.5281/zenodo.31628>.
39. Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J., & Mann, M. (2005). Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Molecular & Cellular Proteomics*, 4, 1265–1272. <https://doi.org/10.1074/mcp.M500061-MCP200>.
40. Kozłowski, L. P. (2017). Proteome-pI: Proteome isoelectric point database. *Nucleic Acids Research*, 45(D1), D1112–D1116. <https://doi.org/10.1093/nar/gkw978>.
41. Jones, E., Oliphant, T., & Peterson, P. (2001). *SciPy: Open Source Scientific Tools for Python*.
42. Walt, S., Colbert, S. C., & Varoquaux, G. (2011). The NumPy array: A structure for efficient numerical computation. *Computing in Science & Engineering*, 13(2), 22–30. <https://doi.org/10.1109/mcse.2011.37>.
43. Perez, F., & Granger, B. E. (2007). IPython: A system for interactive scientific computing. *Computing in Science & Engineering*, 9(3), 21–29. <https://doi.org/10.1109/mcse.2007.53>.
44. Hunter, J. D. (2007). Matplotlib: A 2D graphics environment. *Computing in Science & Engineering*, 9(3), 90–95. <https://doi.org/10.1109/mcse.2007.55>.
45. Waskom, M., Botvinnik, O., O'Kane, D., Hobson, P., Ostblom, J., Lukauskas, S., & Qalieh, A. (2018). *mwaskom/seaborn: v0.9.0* (July 2018). Zenodo. <https://doi.org/10.5281/zenodo.1313201>.
46. McKinney, W. (2010). *Data Structures for Statistical Computing in Python*.
47. Lindh, L., Aroonsang, W., Sotres, J., & Arnebrant, T. (2014). Salivary pellicles. *Monographs in Oral Science*, 24, 30–39. <https://doi.org/10.1159/000358782>.
48. Müller, C., Lüders, A., Hoth-Hannig, W., Hannig, M., & Ziegler, C. (2010). Initial bioadhesion on dental materials as a function of contact time, pH, surface wettability, and isoelectric point. *Langmuir*, 26(6), 4136–4141. <https://doi.org/10.1021/la903299y>.

49. Brash, J. L., & Lyman, D. J. (1969). Adsorption of plasma proteins in solution to uncharged, hydrophobic polymer surfaces. *Journal of Biomedical Materials Research*, 3(1), 175–189. <https://doi.org/10.1002/jbm.820030114>.
50. Baliga, S., Muglikar, S., & Kale, R. (2013). Salivary pH: A diagnostic biomarker. *Journal of Indian Society of Periodontology*, 17(4), 461–465. <https://doi.org/10.4103/0972-124x.118317>.
51. Perez-Riverol, Y., Xu, Q.-W., Wang, R., Uszkoreit, J., Griss, J., Sanchez, A., Reisinger, F., Csordas, A., Ternent, T., Del-Toro, N., Dianes, J. A., Eisenacher, M., Hermjakob, H., & Vizcaíno, J. A. (2016). PRIDE Inspector Toolsuite: Moving toward a universal visualization tool for proteomics data standard formats and quality assessment of ProteomeXchange datasets. *Molecular & Cellular Proteomics*, 15(1), 305–317. <https://doi.org/10.1074/mcp.O115.050229>.
52. Deutsch, E. W., Bandeira, N., Sharma, V., Perez-Riverol, Y., Carver, J. J., Kundu, D. J., García-Seisdedos, D., Jarnuczak, A. F., Hewapathirana, S., Pullman, B. S., Wertz, J., Sun, Z., Kawano, S., Okuda, S., Watanabe, Y., Hermjakob, H., Maclean, B., Maccoss, M. J., Zhu, Y., ... Vizcaíno, J. A. (2020). The ProteomeXchange consortium in 2020: Enabling 'big data' approaches in proteomics. *Nucleic Acids Research*, 48(D1), D1145–D1152. <https://doi.org/10.1093/nar/gkz984>.
53. Perez-Riverol, Y., Bai, J., Bandla, C., García-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D. J., Prakash,

A., Frericks-Zipper, A., Eisenacher, M., Walzer, M., Wang, S., Brazma, A., & Vizcaíno, J. A. (2022). The PRIDE database resources in 2022: A hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Research*, 50(D1), D543–D552. <https://doi.org/10.1093/nar/gkab1038>.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Trautmann, S., Künzel, N., Fecher-Trost, C., Barghash, A., Dudek, J., Flockerzi, V., Helms, V., & Hannig, M. (2022). Is the proteomic composition of the salivary pellicle dependent on the substrate material? *Proteomics - Clinical Applications*, 16, e2100109. <https://doi.org/10.1002/prca.202100109>