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Original investigation

Assessment of Salivary Pellicle on the Surface of Dental Alloys, *in vivo*

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ABSTRACT

Objectives: The molecular composition of the salivary pellicle adsorbed on the surface of dental alloys is determined by the chemical structure of the alloy. The purpose of this study is to compare the composition of salivary pellicle on two dental alloys, chrome cobalt (Co-Cr) and nickel chromium (Ni-Cr) surfaces.

Materials and Methods: Protein profile of 2 hour salivary pellicle on customized Co-Cr and Ni-Cr palatal plates were kept in the mouth of healthy volunteers for two hours. Pellicle was used to compare the parotid and the whole saliva using SDS-PAGE (sodiumdodecylsulphate-poyacrylamide gel electrophoresis) and Western blot analysis.

Results: Only a limited number of salivary proteins was observed in both Co-Cr and Ni-Cr pellicle. In addition, there were differences in proteins observed Co-Cr pellicle vs. Ni-Cr pellicle.

Conclusion: The result of this study suggest there is selectivity in salivary protein adsorption to Co-Cr and Ni-Cr dental prostheses.

Keywords: dental alloys, dental pellicle, saliva

INTRODUCTION

Although the mechanical properties of dental materials are studied in the field of prosthodontics, studies on the biological influences of these materials on oral tissues are limited. In order to determine the appropriate materials that will be used in dental restorations it is important to know about the dynamic change that occurs between tissues and materials. Since the components of salivary pellicle mediate the interaction of the dental material with the physiological environment, defining the molecular composition of the salivary interface between the dental material and oral environment, i.e. salivary pellicle could play a key role in the biocompatibility of the dental materials. Dental pellicle was first described by Manly in 1943 as an organic layer that develops in a very short time after the eruption of teeth (1).

The surfaces of dental materials are covered with a layer of saliva (salivary pellicle) within a very short period of time. Salivary proteins play a key role in the formation of the salivary pellicle, which consists primarily of salivary proteins and glycoproteins and called "*acquired pellicle*". The composition of the pellicle is determined by the composition of saliva as well as the chemistry of the surface it adsorbs (2–4). Studies also demonstrated that there is a unique selectivity in the molecular composition of acquired enamel pellicle as compared to the molecular composition of saliva (5).

The selective adsorption of the proteins in the salivary pellicle is determined by the type and physical characteristics of the dental material. Studies have shown that salivary pellicle modulates bacterial colonization on the tooth and mucosal surfaces of the oral cavity (6), it represents an interface between the oral environment and the tooth and mucosal surface. Therefore, understanding the molecular composition of the pellicle forming on dental materials will facilitate understanding the biocompatibility of dental materials and their roles in modulating the microbial flora in the oral cavity.

The purpose of this study to examine the molecular composition of the pellicle formed on the surfaces of two non-precious metals, namely, Co-Cr and Ni-Cr dental alloys both of which are frequently used in prosthodontics treatment.

MATERIAL AND METHOD

Study population

Pellicle and saliva samples were collected from healthy volunteers who had no oral or systemic diseases, and had good oral hygiene and cooperation. Induviduals with dental restorations or tooth decay and/or health problems were excluded from the study group. Before the study was started, approval was received from the Ethics Board. The study was conducted in accordance with the principles of the Helsinki Declaration.

Preparation of the Samples

To prepare the nickel-chromium (Ni-Cr) (Wiron 99) and chrome-cobalt (Co-Cr) (Argeloy NP) alloy samples, two impressions were taken from the upper jaws of the volunteers with an irreversible hydrocolloid impression material (CA37, Cavex, Harlem, Holland); and palatal plate samples were prepared in the casting device (SchützDent, Frankfurt, Germany) according to the manufacturer's recommendations (Figure 1).

After the preparation of samples ultrasonic cleaning was performed in distilled water for 10 minutes, and the samples were sterilized under a pressure of 2 kg/cm³ at 120°C for 20 minutes.

Collection of Saliva and Pellicle Samples

For each pellicle collection, the palatal plates were kept in the mouth for 2 hours while the subject refrained from food or drink. At the end of 2 hours, the plate was removed from the mouth and rinsed gently with distilled water. The pellicle formed on the prosthesis was collected on a glass wool plug soaked with 2% sodium dodecyl sulfate (SDS) in Tris-buffered saline (hydroxymethyl) amin-omethane (0.05 M Tris and 0.15 M sodium chloride). The collected material was stored at -50°C until used. The adsorbed material was eluted by treating the glass wool plugs with 2% SDS in Tris-buffered saline from each volunteer three times at different days. Totally, 42 pellicle samples 6 for each volunteer were collected. This was followed by centrifugation at x 4500 g for 15 minutes at 4°C. The supernatant was then collected and lyophilized.

Unstimulated whole saliva collection of each volunteer was performed at 9 o'clock after brushing by spitting into a tube chilled on ice for two minutes, then centrifuged at 5000 g for 15 minutes at 4°C, as previously described (7, 8).

The parotid salivary gland is mostly preferred for direct saliva collection (9, 10) as it is pure, lacks the components that might mix in the mouth; and especially, it is free from bacteria. In our study, the parotid saliva was collected by using the modified "Carlson-Crittenden" Saliva Collection Device following tooth brushing at 9 am. by stimulating with 2% citric acid at 30 seconds interval (5, 11, 12).

The saliva samples were centrifuged at 4°C for 15 minutes to remove the debris. Both whole and parotid saliva samples were lyophized and stored at +4°C. For analysis the lyophilized samples were resuspended with double distilled water equivelent to the original volume of the collected saliva sample.

The total amount of protein was determined on 30 µl using the BCA (Bicinchoninic Acid) (13) and the remaining samples were lyophilized and resuspended into distilled water at 1 µg/µl.

SDS-PAGE

The SDS-PAGE (sodiumdodecylsulphate-polyacrylamide gel electrophoresis) was used for separating the proteins and determining the molecular weights of the proteins; and 10 ml of separation gel was prepared at 12.5% concentration (5 ml of separation buffer, 4.17 acrylamide, 0.78 double distilled water, 0.015 Temed, 0.05 ammonium persulfate); and 5 ml loading gel was prepared at 5% concentration (2.1 loading buffer, 0.84 acrylamide, 2 Dd water, 0.007 Temed, 0.025 persulfate).

In a pre-marked order, the samples were then placed in wells with 20 µg of saliva samples and 60 µg of pellicle samples. The Low-Molecular-Weight Protein Standard (LMW PS) was used as the standard (161–0305, Bio-Rad, Hercules, CA, the USA).

The electrophoresis tank was filled with the electrode buffer that was diluted with double distilled water at a rate of 1/5 (25 mM Tris, 192 mM Glycine, 1% SDS), the lid was closed, connected to power supply (Biorad, Hercules, CA, the USA) and were made to run at first for 1 hour at 75 Volts, then at 150 Volts until the bands reached the end of the gel. The gels were kept overnight on a shaker, which included coomassie blue (Biorad, Hercules, CA, the USA), and which was prepared previously for the staining process (Lab-Line Instruments, Melrose Park ILL, the USA). Destaining was performed to the gel in fixation buffur consisting of 10% glacial acetic acid, 40% ethanol and 50% double distilled water until the bands became visible and the gel became clear.

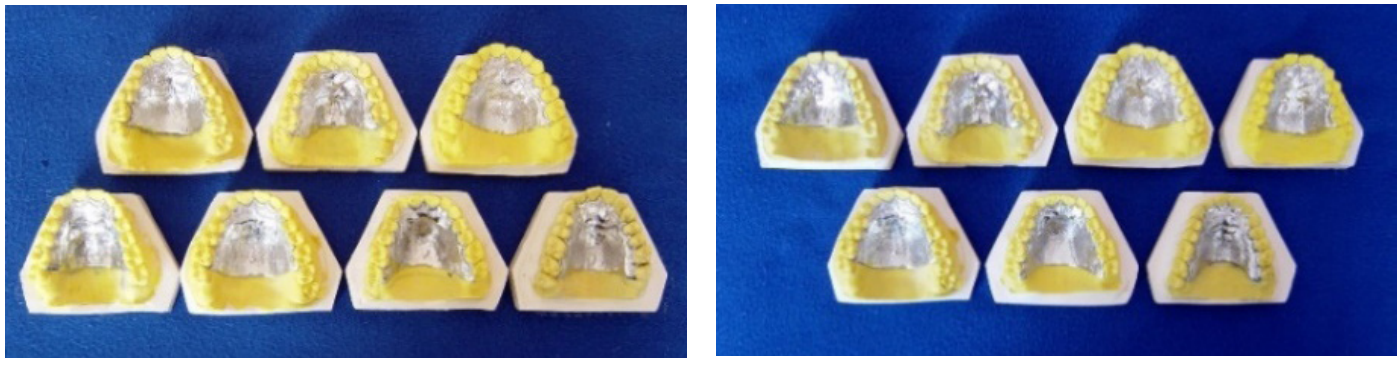

Figure 1. Ni-Cr and Co-Cr palatal plate samples.

Western Blotting

For the western blotting process initially, the pellicle samples were collected together, then the gel prepared with the SDS-PAGE method from saliva samples of the 7th volunteer was dublicated. Low molecular weighted (161-0305, Bio-Rad, Hercules, CA, USA) and high molecular weighted (161-0309, Bio-Rad, Hercules, CA, USA) protein standarts were used.

The proteins, which were separated by the SDS-PAGE method, were transferred to nitrocellulose membranes (Schleicher & Schuell, Keene; NH, the USA). The prepared transfer buffer [25 mM Tris (Biorad, Hercules, CA, the USA) + 192 mM Glycine (Biorad, Hercules, CA, the USA) + 20% Methanol (Fisher Scientific, Pittsburg, PA, the USA)] was placed in an electrophoretic transfer device. The device was then connected to a power supply (Biorad, Hercules, CA, the USA) and run overnight at +4°C at 20 volts.

To prevent non-spesific binding,the gel that non-specific binding, the gel that were transferred onto the nitrocellulose membranes were kept in TBS (Tris buffer saline) with 5% Tween 20 Solution (Sigma, St. Louis, Mo., the USA) on a laboratory shaker for 2 hours at room temperature.

The blotted membrane was then incubated with primary antibody that were diluted with 1/500 TBST solution {25 mM Tris-HCl pH: 7.5 (Biorad, Hercules, CA, the USA) + 0.5 M NaCl (Sigma, St. Louis, MO, the USA) + 0.05 20% Tween 20 (Sigma, St Louis, MO, the USA)} + 2 mg/ml BSA (Sigma St Louis; MO; the USA) +0.1% sodium azide (Sigma St Louis, MO, the USA) in mixer for 2 hours at room temperature. The primer antibody was rabbit anti-human whole serum (Sigma, H 8765, lot 011H4869, St Louis, MO, the USA, diluted 1:500 in TBST+2 mg/ml BSA+0.1% azide) and rabbit anti-human HPS (prepared in lab, diluted 1:500 in TBST+2 mg/ml BSA+0.1% azide). The nitrocellulosemembrane was then washed with TBST three times for 15 minutes each, and incubated with secondary antibody [diluted with1/5000 TBST, Goat Anti Rabbit Alkaline Phosphatase (Calbiochem #401312 lot B20368)+2 mg/ml BSA (Calbiochem, the USA)] and were kept on a laboratory shaker at room temperature for two hours, followed by 3 times wash with TBST, 15 minutes each.

Then the membrane was incubated in color developing buffer that consisted of 10ml Alkaline phosphatase buffer [100 mM Tris-HCl (Sigma, St Louis, MO, the USA) + 100 mM NaCl (Sigma, St. Louis, MO, the USA) + 5 mM MgCl2 (Sigma, StLouis, MO, the USA)+ 33 µl BCIP+ 44µl NBT] until color developed. The reaction was stopped by washing with double distilled water and allowed to dry at room temperature.

Determination of Molecular Weights of the Proteins

System for the seperation of total protein in the pellicle samples was determined using the BCA methods. Subsequently, a 20 µg protein of each pellicle sample, human whole saliva, and human parotid saliva were subjected to 12.5% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) along with low and high molecular weight standards. Protein profiles of Ni-Cr and Co-Cr saliva samples were compared to that of parotid and whole saliva. The relative mobility (Rf) of proteins migration within the gel was estimated using acalibration curve based on the relative mobility of the standard proteins.

Statistical Evaluations

The Wilcoxon Test in matched samples was employed to compare the protein band numbers that were seen in the saliva and pellicle samples; and the Spearman Correlation Coefficient was used to evaluate whether there was a relation between the protein band numbers that were determined in the saliva and pellicle samples.

Since equal number of protein band count is not sufficient to assume the profile as similar,the similarity coefficient was calculated. The similarity coefficient of the protein profiles of the pellicle layer, which was formed on the surfaces of the two alloys, was calculated by the "SM (Ni-Cr, Co-Cr) = a + b + c + d" formula. In this formula:

a. The number of the bands in the pellicle surfaces of each alloys
b. The number of the bands that exist on Ni-Cr surface, but that does not exist on Co-Cr pellicle
c. The number of the bands that exist on Co-Cr surface, but that does not exist on Ni-Cr pellicle
d. The number of the bands that do not exist on either of the alloys.

The individuals with similarity coefficients over 71% were accepted as similar, those with similarity coefficients 51% and below were accepted as different, and those with similarity coefficients between 51-71% were accepted as inconclusive.

RESULTS

Seven healthy volunteers participated in the study, five were males and two females. Their age ranged between 21-29 years, and the mean age was 25 years. The protein profiles of the pellicle on the dental alloy surface showed similarity, specifically in terms of number of protein bands in the pellicle as compared to that observed in whole and parotid saliva. However, there were variations in the number of protein bands observed in the pellicle of Ni-Cr versus Co-Cr alloys.

All salivary proteins were not present in the pellicle samples of both alloys. The molecular weights of proteins on both saliva and alloy surfaces were 76, 68, 66, 24 kDa. Proteins with molecular weight of 100, 92, 80, 53, 47 kDa seen in saliva samples were not present within the pellicle samples. It was determined that the molecular weights of the proteins that adsorbed only on Ni-Cr surface were 56, 49, 45 kDa; the molecular weights of those that adsorbed only to Co-Cr surface were 42 and 37 kDa. The protein that originated from parotid saliva and had 33 kDa weight was detected on both alloy surfaces. The proteins detected in Co-Cr pellicle with 42 and 37 kDa molecular weight were serum-originated proteins that did not exist in the saliva (Figure 2). The relation between the band numbers was not found to be statistically significant according to the Spearman Correlation Coefficient evaluation. According to

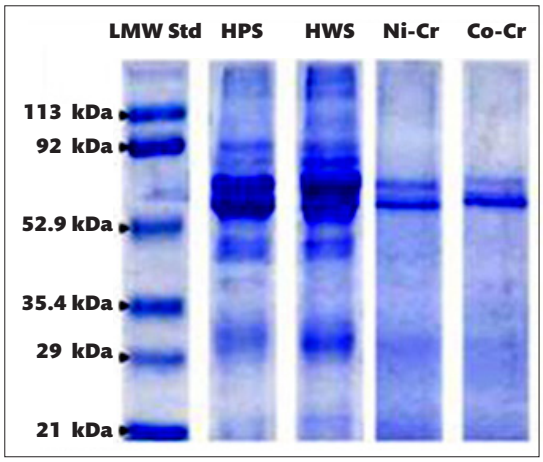

Figure 2. Gel electrophoresis (12% SDS-PAGE) stained with amido black sain: (kDa) Kilodalton (LMW std) molecular weight of the Standard, (HPS) Human parotid saliva, (HWS) human whole saliva, (Ni-Cr) nickel chromium pellicle, (Co-Cr) Cobalt-chromium pellicle.

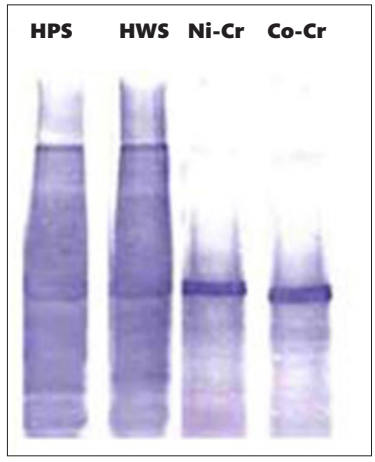

Figure 3. Western Blot analyses using antihuman HPS. (HPS) Human parotid saliva, (HWS) human whole saliva, (Ni-Cr) nickel chromium pellicle, (Co-Cr) cobalt chromium pellicle.

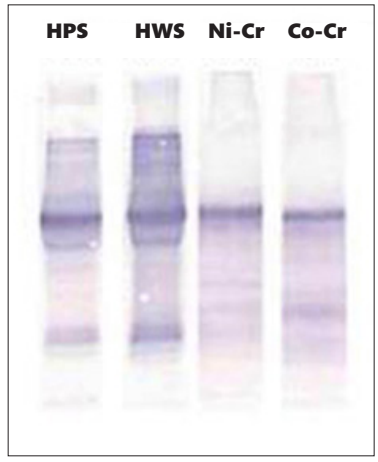

Figure 4. Western Blot analyses using antihuman whole serum. (HPS) Human parotid saliva, (HWS) Human whole saliva, (Ni-Cr) nickel chromium pellicle, (Co-Cr) cobalt chromium pellicle.

the similarity coefficient rates, the protein profile of 1 volunteer in two alloys (Co-Cr and Ni-Cr) was accepted as different; the protein profiles of 2 volunteers were accepted as similar, and the protein profiles of 4 volunteers were accepted as suspicious. In Western blot analysis incubated with Antihuman saliva antiserum, proteins of saliva origin with 67, 63, 60, 48, 44, 40, 32, 29 kDa were found to be seen on both alloy surfaces. Proteins with 58 kDA molecular weight adsorbed on adsorbed on both Ni-Cr and Co-Cr alloys surfaces saliva-derived proteins of 53, 50 and 25 kDA weight were observed on the Co-Cr alloy surface and 35 kDa-weighted saliva-derived protein was observed on the Ni-Cr alloy surface (Figure 3) .In western blot analysis which incubated with anti-human whole serum, weighing 70, 60, 37 kDa proteins of saliva origin were found to be seen on both alloy surfaces. The serum originated protein weighing 50 kDa was adsorbed on both alloy surfaces (Figure 4).

The similarity coefficient of the two alloy proteins in the gel that was obtained from the samples collected to form the control gel for Western Blot was determined as 50% ; and the relation in the profiles was accepted as suspicious. The similarity coefficient rates differing between individuals were consistent with the finding claiming that saliva protein adsorption shows individual characteristics. In statistical terms, the similarity coefficient rates show that the two pellicle protein profiles were not completely similar for all the volunteers. The Western Blot Analysis should be performed with antibodies that are more specific for the purpose of determining to which protein the different bands belong on the alloy surfaces.

DISCUSSION

The biological importance of pellicle, the presence on different surfaces and the relation with biofilm formation have been reported by many authors (12, 14–19). However the similarities and differences of the pellicle layer forming on the surfaces of non-precious Co-Cr and Ni-Cr dental alloys, and evaluate the origin of the proteins are not identified well.

In previous histochemical studies, it was shown that this organic film layer is composed of mostly saliva-originated proteins (20). Hay conducted studies; and determined that the *Acquired Pellicle* was formed with the selected adsorption of the saliva and serum components on the surface of the teeth (21, 22). The presence of only part of the salivary proteins in the pellicle layer shows that the pellicle is formed as a result of selective adsorption of the surrounding macromolecules (23). It was reported that the thickness and composition of the pellicle layer showed individual differences which might be influential on susceptibility to oral diseases (24).

Several studies were conducted to evaluate the pellicle layer forming on auto-polymerizing resins (12), PMMA (24), and on titanium (25). When the literature was reviewed, the data on the pellicle layer, which forms on the surfaces of frequently used dental alloys in prosthetic treatment were limited.

When the protein profiles of 7 volunteers were separated with the SDS-PAGE and evaluated, it was determined that there were differences in the protein profiles of the pellicle layer forming on the two alloy surfaces of each volunteer; and the difference varied between individuals. The protein profiles of the pellicle layer forming on the same alloy of different volunteers showed similarities, but the protein profile of the pellicle layer forming on the two alloy surfaces were different.

The molecular weights of the proteins that were detected in both saliva samples but not in the pellicle samples were 100, 92, 80, 53, 47 kDa. The failure in the adsorption of all salivary proteins on alloy surfaces supports the finding that protein adsorption is selective (8, 23).

The proteins weighing only 40.33 kDa, which are only detected in parotid saliva, and the proteins weighing 43–31 kDa, which are only detected in whole saliva, show that the protein profiles of the saliva samples taken from different volunteers gland, and the protein profiles of the whole saliva may differ. This finding,shows parallelism with the previous studies that examined saliva samples taken from diffrerent gland mouths (26,27).

Özden et al. (8) compared the composition of the Ni-Cr pellicle formed *in vivo* with the parotid and whole saliva profiles; and determined that the Ni-Cr pellicle did not adsorb all the proteins found in saliva; in other words, the adsorption had a selective characteristic, which is similar to our study. However, they also determined that Ni-Cr pellicle contained some proteins that were not found in both saliva samples. Authors of previous studies determined that the secretory IgA whose presence in enamel pellicle and prosthetic pellicle was determined (28) as a result of Western Blot process and salivary amylase participated in the structure of Ni-Cr pellicle. When it is considered that amylase and sIgA show antimicrobial effect and that they modulate the bacterial colonization on the prosthetic surfaces (5, 29), their regulation of bacterial colonization on the Ni-Cr surface was found to be possible. It was also determined in the same study that lysozyme was not a component of Ni-Cr pellicle.

Parallel to these data, in our study, we determined that the protein weighing 45 kDa in the Ni-Cr pellicle was of whole saliva origin. The proteins weighing 56.49 kDa seen in both saliva samples and in Ni-Cr pellicle were not detected in Co-Cr pellicle; and the proteins weighing 42 and 37 kDa that were not detected in saliva samples were adsorbed only on the Co-Cr surface. These data suggest that the adsorption mechanism is different on each different material surface. The lack of adsorption of all salivary proteins to the surface shows that the adsorption has a selective characteristic.

The determination of the presence of the pellicle layer forming on the material surfaces-just like the proteins of serum origin on the surface of the teeth-shows once again that the protein profile of the pellicle layer is formed by both saliva and serum-originated proteins.

Adhesion of bacteria to oral tissue surfaces occurs with the electrostatic and hydrophobic forces. The adhesion process is also affected by the specific interaction among the receptors in the salivary pellicles and the attachment areas on the microbial cell surfaces. For this reason, the data on the composition of saliva pellicles forming on certain surfaces will help us to understand the microbial colonization on these surfaces (4,12). The researchers, who believe that prosthetic-originating stomatitis is associated with the increase in the number of *Candida albicans* and other microorganisms on the prosthetic surface, stated that prosthetic pellicle played an important role in colonization. They claimed that the pathogenic plaque formation might be reduced by changing the acrylic resin surface of the prosthesis chemically (28). Carlen et al. (30) conducted a study in which they examined the bacteria-adhesive plasma proteins in the pellicle layer forming on the *in vitro* hydroxyapatite and *in vivo* tooth surfaces; and reported that bacterial adhesion depended not only on the species of the bacteria but also on the pellicle composition forming of different saliva and plasma proteins.

Singh et al. (31), Wang et al. (32) conducted studies in which the chemical composition of the materials are changed to reduce the biofilm formation. In these studies, different strategies such as changing the surface characteristics, reducing the viability of the bacteria adhering kept by chemical components, and reducing the initial bacteria adhesion to reduce the biofilm formation were employed (33).

When all these data are evaluated, one of the points that should not be forgotten in dental restorations is that the success of the biological response to the material used in such restorations depend on not only to the correct material selection, but also to the biological responce.

Future results that will be obtained by examination of the biological effects of the materials will make significant progression in prosthodontics.

Since the pellicle layer serves as a dynamic mediator or an impressive system regulating the interaction between the biomaterials with the surrounding tissues, it is believed that knowing the composition of the pellicle layer may shed light on designing new biomaterials and on the modification of the existing materials. There is a need for new studies that will be conducted on pellicle formation on the surfaces of commonly used materials like titanium and ceramics and on special biological molecules that will attach to the surfaces. These studies will ensure the long-term success of implants; and bring new data on bacterial adhesion on the surface and improvements in prosthetic restorative materials like ceramics and acrylic.

We believe that when the mechanical properties of materials are improved, the biological requirements should not be ignored and biological responce to biomaterials should be the main subject of more detailed studies. Also conducting further studies with different materials to confirm the results of our study will make contribution to the literature.

Informed Consent: Written informed consent was obtained from the volunteer who participated in the study.

Compliance with Ethical Standards: The study was conducted in accordance with the Helsinki Declaration principles. Approval was obtained from the local ethics committee decision dated 2004

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - FB; Design - NÖ; Supervision - IA; Fundings - NÖ; Materials - FB; Data Collection and/or Processing - FB, NÖ; Analysis and/or Interpretation - FB, NH, IA; Literature Search - FB, NÖ; Writing Manuscript - FB, IA; Critical Review - IA

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Glickman I. Clinical Periodontology. Clinical P. Philadelphia-London: W. B. Saunders Co; 1972. 290–311 p.
2. Ericson T MK. Saliva, formation, composition and possible role. In: Thylstrup A, Fejerskov O, editors. Textbook of Cariology. Copenhagen: Munksgaard; 1986. 28–32 p.
3. Edgerton M, Tabak LA, Levine MJ. Saliva: a significant factor in removable prosthodontic treatment. J Prosthet Dent 1987;57:57–66. [CrossRef]
4. Edgerton M, Levine MJ. Biocompatibility: its future in prosthodontic research. J Prosthet Dent 1993;69:406–415. [CrossRef]
5. Al-Hashimi I, Levine MJ. Characterization of in vivo salivary-derived enamel pellicle. Arch Oral Biol 1989;34:289–295. [CrossRef]
6. Gibbons RJ. Bacterial adhesion to oral tissues: a model for infectious diseases. J Dent Res 1989;68:750–760. [CrossRef]
7. Ueda A. Adsorption of salivary proteins and serum proteins to titanium. J Osaka Dent Univ 1998;32:59–65.
8. Ozden AN, Haghighat N, Al-Hashimi I. Preliminary evaluation of salivary pellicle on nickel-chromium alloy in vivo. Quintessence Int 2002;33:731–735.
9. Olsson J, van der Heijde Y, Holmberg K. Plaque formation in vivo and bacterial attachment in vitro on permanently hydrophobic and hydrophilic surfaces. Caries Res 1992;26:428–433. [CrossRef]
10. Shahal Y, Steinberg D, Hirschfeld Z, Bronshteyn M, Kopolovic K. In vitro bacterial adherence onto pellicle-coated aesthetic restorative materials. J Oral Rehabil 1998;25:52–58. [CrossRef]
11. Lee SJ, Kho HS, Lee SW, Yang WS. Experimental salivary pellicles on the surface of orthodontic materials. Am J Orthod Dentofacial Orthop 2001;119:59–66. [CrossRef]
12. Yoo J-H, Kho H-S, Kim Y-K, Lee S-W, Chung S-C. Experimental salivary pellicles formed on the surface of self-curing resin. J Oral Rehabil 2003;30:251–259. [CrossRef]
13. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85. [CrossRef]
14. Steinberg D, Mor C, Dogan H, Zacks B, Rotstein I. Effect of salivary biofilm on the adherence of oral bacteria to bleached and non-bleached restorative material. Dent Mater 1999;15:14–20. [CrossRef]
15. Steinberg D, Eyal S. Early formation of Streptococcus sobrinus biofilm on various dental restorative materials. J Dent 2002;30:47–51. [CrossRef]
16. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 2006;17 Suppl 2:68–81. [CrossRef]
17. Zhang N, Melo M, Weir M, Reynolds M, Bai Y, Xu H. Do Dental Resin Composites Accumulate More Oral Biofilms and Plaque than Amalgam and Glass Ionomer Materials? Mater (Basel, Switzerland) 2016;9:888. [CrossRef]
18. Mutahar M, O'Toole S, Carpenter G, Bartlett D, Andiappan M, Moazzez R. Reduced statherin in acquired enamel pellicle on eroded teeth compared to healthy teeth in the same subjects: An in-vivo study. PLoS One 2017;12:e0183660. [CrossRef]
19. Ventura TM da S, Cassiano L de PS, Souza E Silva CM de, et al. The proteomic profile of the acquired enamel pellicle according to its location in the dental arches. Arch Oral Biol 2017;79:20–29. [CrossRef]
20. Meckel AH. The formation and properties of organic films on teeth. Arch Oral Biol 1965;10:585–598. [CrossRef]
21. Hay DI. The isolation from human parotid saliva of a tyrosine-rich acidic peptide which exhibits high affinity for hydroxyapatite surfaces. Arch Oral Biol 1973;18:1531–1541. [CrossRef]
22. Hay DI. Fractionation of human parotid salivary proteins and the isolation of an histidine-rich acidic peptide which shows high affinity for hydroxyapatite surfaces. Arch Oral Biol 1975;20:553–558. [CrossRef]
23. Hannig C, Hannig M. The oral cavity--a key system to understand substratum-dependent bioadhesion on solid surfaces in man. Clin Oral Investig 2009;13:123–139. [CrossRef]
24. Göcke R, Gerath F, von Schwanewede H. Quantitative determination of salivary components in the pellicle on PMMA denture base material. Clin Oral Investig 2002;6:227–235. [CrossRef]
25. Kohavi D, Klinger A, Steinberg D, Sela MN. Adsorption of salivary proteins onto prosthetic titanium components. J Prosthet Dent 1995;74:531–534. [CrossRef]
26. Amerongen N, Oderkerk CH, Driessen AA. Role of mucins from human whole saliva in the protection of tooth enamel against demineralization in vitro. Caries Res 1987;21:297–309. [CrossRef]
27. Denny P, Hagen FK, Hardt M, et al. The proteomes of human parotid and submandibular/sublingual gland salivas collected as the ductal secretions. J Proteome Res 2008;7:1994–2006. [CrossRef]
28. Edgerton M, Levine MJ. Characterization of acquired denture pellicle from healthy and stomatitis patients. J Prosthet Dent 1992;68:683–691. [CrossRef]
29. Scannapieco FA, Torres GI, Levine MJ. Salivary amylase promotes adhesion of oral streptococci to hydroxyapatite. J Dent Res 1995;74:1360–1366. [CrossRef]
30. Carlén A, Rüdiger SG, Loggner I, Olsson J. Bacteria-binding plasma proteins in pellicles formed on hydroxyapatite in vitro and on teeth in vivo. Oral Microbiol Immunol 2003;18:203–207. [CrossRef]
31. Singh AV, Vyas V, Patil R, et al. Quantitative characterization of the influence of the nanoscale morphology of nanostructured surfaces on bacterial adhesion and biofilm formation. PLoS One 2011;6:e25029. [CrossRef]
32. Wang Z, Shen Y, Haapasalo M. Dental materials with antibiofilm properties. Dent Mater 2014;30;e1–e16. [CrossRef]
33. Hasan J, Crawford RJ, Ivanova EP. Antibacterial surfaces: the quest for a new generation of biomaterials. Trends Biotechnol. 2013;31:295–304. [CrossRef]