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Research Article

In Vitro Investigation of Surface Roughness and Bacterial Adhesion on Different Dental Porcelains Applying Different Vital Whitening Agents

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In Vitro Investigation of Surface Roughness and Bacterial Adhesion on Different Dental Porcelains Applying Different Vital Whitening Agents

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Abstract

Statement of the problem: Studies on the effects of vital bleaching agents on the surface roughness and bacterial adhesion of different dental porcelain materials are insufficient.

Objective: The aim of this study is to determine the effects of vital bleaching procedures on the surface roughness of different dental porcelains and to investigate whether they facilitate bacterial adhesion.

Materials & Methods: A total of 60 porcelain discs, 12 mm in diameter and 2.2 mm in thickness, were produced from two feldspathic porcelains and one leucite-enriched all-ceramic. The sample groups (n=20) manufactured from Ceramco 3, VMK 95, and Finesse TM were named as C, V, and F respectively. Two bleaching agents were applied to 20 samples belonging to C, V and F groups. The group bleached with 16% carbamide peroxide gel was called the N group. The group bleached with 10% carbamide peroxide gel was called the R group. A total of 6 experimental groups, CN, VN, FN, CR, VR and FR, were formed. Streptococcus mutans strain was used to detect bacterial adhesion before and after bleaching on porcelain surfaces with and without a pellicle layer, and adhesion was determined by colony counting method. The obtained data were analyzed with Kruskal-Wallis test and Spearman's correlation coefficient.

Results: The mean difference in bacterial adhesion colony counts of VN and VR groups was found statistically significant ($P < 0.05$). Application of Nite-White bleaching agent on Finesse porcelain increased the adhesion more ($P < 0.05$). The difference in the number of Streptococcus mutans bacteria adhesion colonies among the other groups was not statistically significant ($P > 0.05$).

Conclusion: The bleaching process could affect the surface roughness of dental porcelain and facilitate bacterial adhesion to the porcelain surface.

Keywords: Dental Porcelains, Vital Bleaching Agents, Surface Roughness, Bacterial Adhesion.

Introduction

Dental aesthetics includes practices that increase the patient's social and psychological self-confidence.1 In patients who have dental restorations, bleaching procedures can also be performed to improve the appearance of the teeth. However, the effects of bleaching processes on restorative materials should be considered.[2]

Among the microbial causes of tooth discoloration, factors such as dental plaque and tartar accumulation, colonization of chromogenic bacteria and fungi are important.[3] Many methods are used in the treatment of these discolorations, such as routine prophylactic procedures, prosthetic and restorative techniques, macroabrasion, microabrasion, and bleaching with oxidizing agents.[4] The active ingredients of the chemicals used in vital bleaching are caustic products with high concentrations of 15-40% hidrogen peroxide (HP) or karbamide peroxide (KP). Despite the use of caustic chemicals, the advantages of in-office vital bleaching are being performed by dental practitioner, protection of the soft tissues, and obtaining quick results. The main disadvantages of home bleaching are not to be under the control of a physician, and the difficulties experienced in the follow-up of the results. However, the effects of the application of chemical bleach on bacterial adhesion to porcelain surfaces are an important issue to be evaluated.

In general, the first step in bacterial infections is the adhesion of bacterial cells to host tissues through the interaction between bacterial adhesion factor, adhesins, and receptors.[5] The first adhesion of the bacteria with the acquired pellicle layer on the tooth surface is realized by the hydrophobic bonding between the molecules. Another effective mechanism in bacterial adhesion is calcium bridges.[6] The positively charged calcium ions from the saliva form this bridge between the negatively charged bacterial cell surface and the negatively charged acquired pellicle layer. As the bacterium gets closer, this bond becomes stronger and permanent adhesion begins. Specific binding between ligands and bacterial adhesions on the tooth surface is the first step in plaque formation[7]. Some streptococci in plaque synthesize extracellular polysaccharides (ECP) using the glucosyltransferase enzyme. These glucans perform bacterial adhesion by hydrogen bonding mechanism. After bacterial adhesion occurs, they synthesize extra glucans.[8]

The acquired pellicle layer is also formed on dental restorations and prostheses. This organic veneer layer is similar to that of a natural tooth, and bacteria are then colonized. There are studies in the literature showing that the pellicle layer formed on dental restorative materials changes the original surface energy of the material and increases the wettability of the materials

to a certain level.[9] The most frequently reported bacterial species in plaque formation is Streptococcus mutans (S. mutans).[10] The attachment of S. mutans to flat surfaces is explained by the electrostatic relationships between the afimbrial adhesins SpaP, Pl, PA and salivar glycoproteins.[11]

The aim of this study was to evaluate and compare the effects of two types of vital bleaching agents on the surface features of three different dental porcelains. In addition, it was investigated whether there is a change in the amount of S. mutans adhesion on porcelain surfaces and the effect of the pellicle layer on the number of colonies on bacterial adhesion.

Materials & Methods

The study was performed in five stages; preparation of the porcelain specimens, measurement of surface roughness values of the samples before and after bleaching, application of the vital bleaching agents on the specimens, detection of the bacterial adhesion, and statistical analysis.-Statistical analysis

-Preparation of porcelain specimens

In order to provide size and surface standardization of metal-ceramic samples, a metal mold from stainless steel was produced with a computer numerical control machine. The metal mold was created with a 12 cm long cylinder and a 12 mm diameter piston precisely placed in the cylinder, and a screw-nut system that can hold the piston at the desired point. In order to produce porcelain samples with standard thicknesses, three marks were prepared with a bur where the distances between the top of the piston and the cylinder were 0.3, 0.4, and 1.4. The produced metal mold was cleaned in an ultrasonic cleaner using 10% ethyl alcohol and distilled water, respectively.

Porcelain specimens were obtained from two feldspathic porcelain and leucite-enhanced all-ceramic materials used in the production of metal-ceramic restorations. Two feldspathic porcelain materials (Ceramco 3, Dentsply Sirona, Germany) (VMK 95, Vita Zahnfabrik H. Rauter GmbH, Germany) were used in the making of metal-ceramic samples. First, metal discs with a diameter of 12 mm and a thickness of 0.3 mm were produced. In order to provide standard dimensions in metal discs, wax samples to be used in casting were produced with the help of metal molds. Casting was carried out using base metal alloy (Remanium® LFC, Dentaurum, Germany).

After leveling, polishing, oxidation and sandblasting, 40 metal discs were covered with a 0.1 mm thick opaque porcelain layer. Veneering with porcelain was performed while the discs were in the metal mold. 1.4 ± 0.1 mm dentin, 0.4 ± 0.1 mm enamel porcelain were applied to each metal disc by condensation technique. Finally, the samples were sanded and glazed with 180, 220 and 320 grit sandpaper, respectively. The samples produced with Ceramco 3 (n = 20) were named as C group, and the samples produced with VMK 95 (n = 20) were named as V group.

Leucite-reinforced all-ceramic specimens were produced as discs with a diameter of 12 mm and a thickness of 2.2 ± 1 mm using FinneseTM All Ceramic (Dentsply, USA) ingots. During the production, the manufacturer's instructions were taken as a guide and the obtained 20 all-ceramic samples were glazed. The samples produced with Finesse (n = 20) were named group F. For color standardization, all porcelain samples were prepared in C3 color tone.

-Measurement of surface roughness values of the samples before and after bleaching

A profilometer (Surftest Analyzer, Mitutoyo, Japan) was used to determine the surface roughness of the samples. The diamond tip measuring force with a diameter of 10 micrometers was set at 4m/N (0.4gf) with a feed rate of 0.5 mm/s. The results were evaluated by making three parallel measurements from the glazed surface of each sample. Ra stands for average surface roughness and is measured in micrometers. The surface roughness measurement values of each sample before bleaching were recorded as SRa1, and the surface roughness values after bleaching were recorded as SRa2. Surface roughness differences resulting from bleaching were calculated with the formula SRa2- SRa1.

-Application of Bleaching Agents

Twenty samples from groups C, V and F were divided into two groups of 10 samples each in order to apply two different bleaching agents. The group to be bleached with Nite-White (Discus Dental, Inc, USA) containing 16% carbamide peroxide gel was called the N group. The groups to be bleached with Rembrant (Dent-Mat Corp, USA) containing 10% carbamide peroxide gel were called the R group. Thus, a total of 6 experimental groups, CN, VN, FN, CR, VR and FR, were formed. The group and number it belongs to was written on the back of each sample with a bur.

Prior to the bleaching process, transparent plaques containing spaces compatible with the diameter of the samples were produced, on which the samples would be placed during bleaching. The bleaching agent was applied to the glazed surface of the samples and left for 8

hours. At the end of each day, the samples were cleaned under running water with a toothbrush. The samples were kept in distilled water at room temperature until the next bleaching procedure. A total of 14 days of bleaching was applied to all samples.

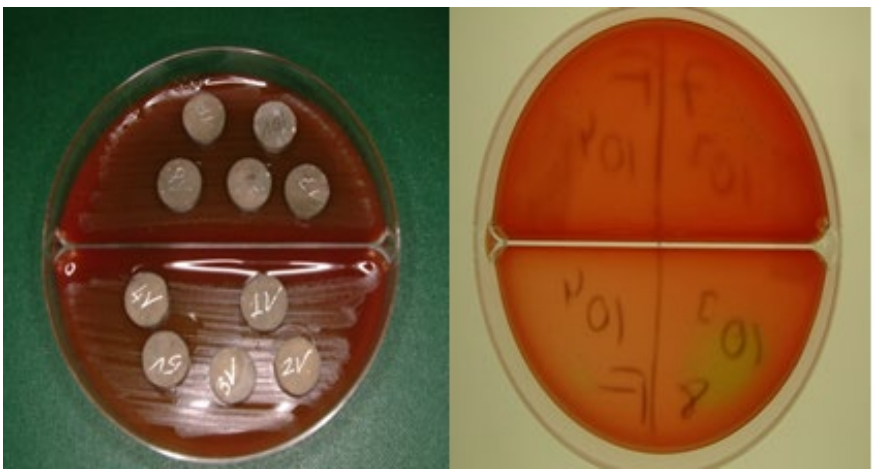
-Detection of Bacterial Adhesion

Saliva samples were collected in a chilled tube from a healthy male individual who was periodontally healthy and had no caries and had not received any medical treatment in the last three months. Saliva collection was achieved by stimulating with paraffin in the morning. The saliva sample was centrifuged twice on the same day at 5000xg at 4ºC to remove debris. It was stored in deepfreeze at -20 ºC to prevent protein denaturation for later use.

"S. mutans" (type A, NTCC 10919) was chosen as the bacterial sample. The stock culture was passaged into blood agar, and after 18 hours of incubation, it was transferred to "Nutrient broth" and the bacteria were suspended. 0.1 ml of the suspension shaken in the vortex was inoculated into six blood agars. Cultures were incubated for 18 hours at 37°C in 5% CO2.

The samples, sterilized in the autoclave before in groups, were placed in the sterile presselle medium glazed surface in contact with the medium (Figure 1). For re-incubation, it was taken into an environment with 5% CO_2 at 37 ºC for 18 hours. At the end of the incubation period, each sample was placed separately in a test tube containing 10 ml sterile saline. It was shaken in a vortex for 1 minute to remove free bacteria. The same procedure was repeated by placing the samples in separate sterile saline test tubes and vortexing them at maximum speed in order to allow the bacteria, which were firmly attached to the surface, to pass into the solution. 1/100 and 1/1000 dilutions were made from this suspension, and 0.1 ml of each was inoculated on blood agar medium and colony counts were made after 18 hours of incubation (Figure 1).

Figure 1. Samples placed on blood agar and detection of adhesion by colony counting method.



The autoclaved samples were immersed in saliva which was thawed at room temperature, for two hours at 37 ºC. Afterwards, the samples were washed under running distilled water for 30 seconds to remove free proteins. The samples were then left to dry at room temperature for 24 hours. Before the experiment, the samples were kept in an oven at 37 ºC for 10 minutes in order to remove water and moisture from the surface.

Samples thought to have formed a pellicle layer were placed on blood agar with bacteria cultivation. After incubation, bacterial adhesion was evaluated by colony counting method. Oral bacterial adhes ion in specimens with and without pellicle layer was repeated after the application of bleaching agents. S. mutans colony count before bleaching was recorded as Sm1, and the number of S. mutans colonies after bleaching was recorded as Sm2. The formula 'Sm difference= Sm2-Sm1' was used to determine the amount of bacterial adhesion change.

The abbreviation Smp1 were used for the number of S. mutans colonies on porcelain pellicle-shaped porcelain surfaces before bleaching, and Smp2 was used for the number of S. mutans colonies on pellicle-shaped porcelain surfaces after bleaching.

If the amount of bacteria adhesion has changed on porcelain surfaces with a pellicle layer, this change was determined by the formula "Smp difference = Smp2-Smp1".

In the statistical evaluation of the study, Kruskal-Wallis test based on the comparison of 6 different groups was applied. The strength of the relationship between the variables was examined with the Spearman's correlation coefficient.

Results

Table 1 shows the descriptive statistical results of S. mutans bacterial colony count values before and after bleaching of porcelain samples without a pellicle layer on the surface.

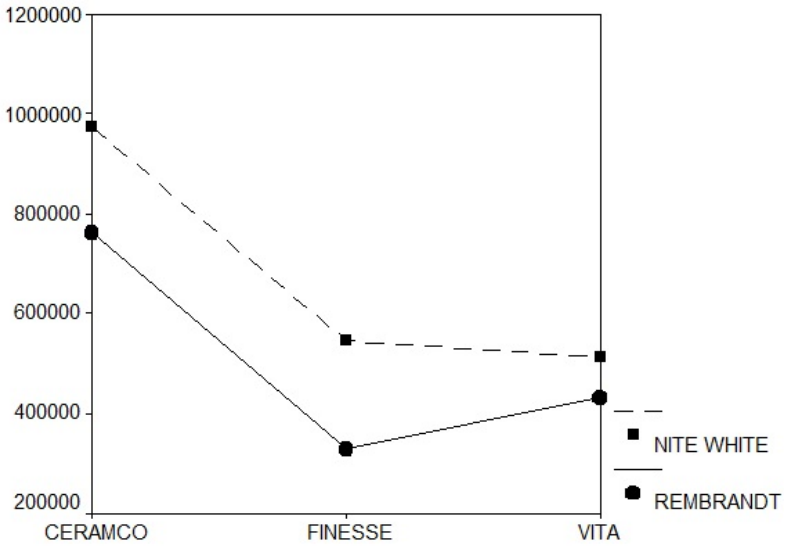
Table 1. Mean, median and standard deviation values of bacterial adhesion colony count before (Sm1) and after (Sm2) porcelain bleaching agent application without pellicle layer.

	Sm1			**Sm2**		
	Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
CN	76000	90000	28362.73	1050000	1000000	497214.5
CR	68000	60000	33598.94	830000	900000	391010.1
FN	64000	60000	33065.59	610000	500000	275681.0
FR	78000	60000	40496.91	405000	275000	238572.1
VN	46000	45000	21187.0	560000	500000	218327.0
VR	75000	75000	22236.4	505000	500000	235053.2

The Kruskal-Wallis test was used to determine whether there was a statistically significant difference between the FN and FR, CN and CR, and VN and VR groups. Only between the FN and FR groups, a significant difference was found ($P < 0.05$). There was no significant difference between CN and CR, and between VN and FR groups ($P = 0.09$, $P = 0.08$). The highest bacterial adhesion was seen in the CN group (974.000), and the lowest in the FR group (327.000). It was determined that Nite-White bleaching agent caused more bacterial retention than Rembrandt ($P = 0.007$).

The graphic of bacterial adhesion change averages as a result of porcelain bleaching agent interaction is shown in Figure 2.

Figure 2. Change in mean difference in S. mutans bacterial adhesion as a result of porcelain-bleaching agent interaction.



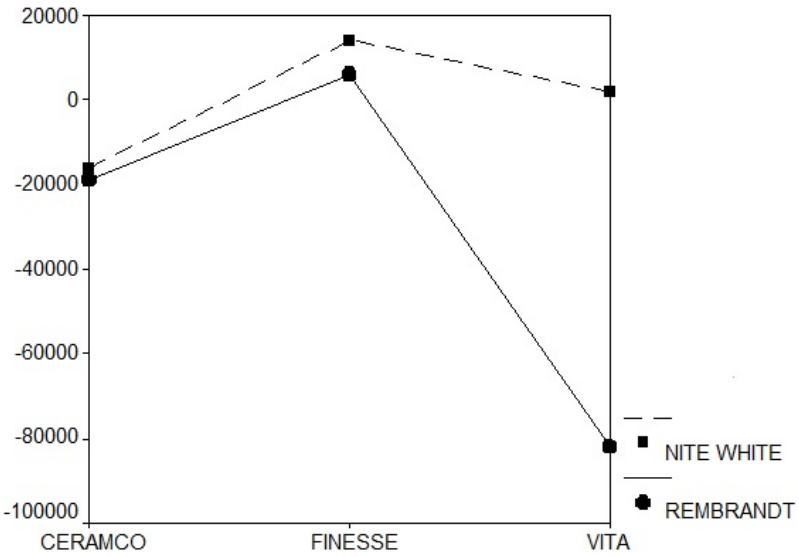
The mean, median and standard deviation values of S. mutans bacteria colony counts before and after bleaching of samples with pellicle layer are given in Table 2.

Table 2. Descriptive analysis of bacterial adhesion colony numbers before (Smp1) and after (Smp2) bleaching on porcelain surfaces with pellicle layer.

Porcelain bleaching	**Smp1**			**Smp2**		
	Mean	**Median**	**Standard deviation**	**Mean**	**Median**	**Standard deviation**
CN	111000	100000	33813.21	95000	100000	43779.75
CR	91000	100000	33730.47	72000	65000	26161.89
FN	60000	50000	42947.0	74000	70000	24129.28
FR	73000	70000	43217.79	790000	80000	19692.07
VN	64000	65000	17126.98	66000	55000	20655.91
VR	156000	150000	37475.92	74000	80000	28362.73

The values of S. mutans bacteria colony counts before and after bleaching in porcelain samples with pellicle layer were compared in pairs with Kuruskal-Wallis test. Statistically significant difference was found only between the VN and VR groups ($P < 0.05$). There was no significant difference between FR and FN and VN and VR groups ($P > 0.05$). The graph of the average of bacterial adhesion change as a result of the interaction of the bleaching agent on the porcelain groups with a pellicle layer is shown in Figure 3.

Figure 3. Difference mean change in S. mutans bacterial adhesion on porcelain with pellicle layer formed as a result of porcelain-bleaching agent interaction.



The descriptive statistical results of the surface roughness values measured before bleaching (SRa1) and the values measured after bleaching (SRa2) are given in Table 3.

Table 3. Mean, median and standard deviation of SRa1 and SRa2 values.

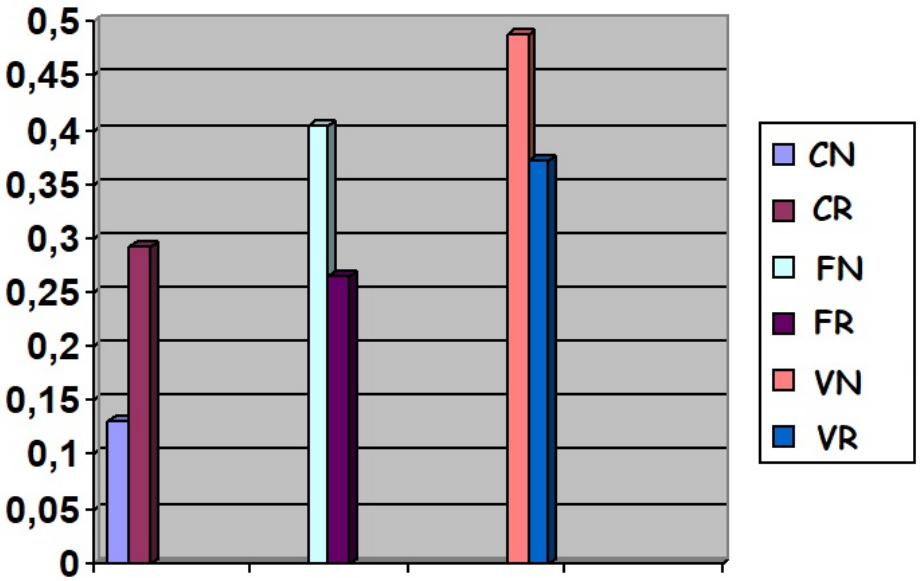
	SRa1			**SRa2**		
	Mean	Median	S.deviation	Mean	Median	S.deviation
CN	1.0270	1.0500	0.1253	1.1580	1.200	0.1421
CR	0.6900	0.6600	0.3226	0.9830	0.9900	0.4564
FN	0.7370	0.7350	0.1710	1.1400	1.200	0.2171
FR	0.8220	0.8500	0.1830	1.8680	1.1500	2.3398
VN	1.7900	1.7750	0.5254	2.2790	2.100	0.6380
VR	2.0420	2.0450	0.3954	2.3140	2.300	0.4186

Whether the difference between the surface roughness values measured before and after the bleaching process was statistically significant was investigated using the Kruskal-Wallis test. Statistically significant difference was found between CN and CR groups ($P < 0.05$). Alt-

hough bleaching increased the surface roughness values of the samples, there was no statistically significant difference between VN and VR groups ($P > 0.05$) and FN and FR groups ($P > 0.05$).

The graphic of the average surface roughness variation as a result of the porcelain bleaching agent interaction is shown in Figure 4.

Figure 4. Average values of the surface roughness change.



Correlation between the surface roughness and bacterial adhesion (without and with pellicle formation) resulting from vital bleaching was investigated with Spearmen's correlation coefficient. Correlation coefficients are given in Table 4.

Table 4. Spearman's correlation coefficients between bacterial adhesion and surface roughnesses.

Correlation (rs)	CN	CR	FN	FR	VN	VR
Without pellicle	-.166	0.131	0.167	0.389	0.537	0.571
With pellicle	0.209	-.178	0.154	0.674	0.467	0.387

The relationship between the surface roughness after bleaching and the adhesion of "S. mutans" bacteria on porcelain surfaces without a pellicle layer was not found statistically significant both without pellicle and with pellicle ($P > 0.05$).

Discussion

Aesthetic concerns are problems that have an important place in modern life and can be solved with many alternative methods. The use of bleaching agents at home or under the supervision of a physician in dental aesthetics has the potential to cause detrimental effects on the tooth surface along with its positive results.[12] One of these effects is the increase in bacterial adhesion. The first step in plaque and biofilm formation is the adhesion of bacteria called primary colonizer to the pellicle surface. Among these bacteria, streptococci come first, and S. mutans is reported as the most effective bacterial species in plaque formation.[13] Adhesion of S. mutans is an important marker in dental plaque accumulation and caries formation due to its primary colonizer feature. Studies reporting that S. mutans can change the local environment by creating an exopolysaccharide (EPS) rich and low pH environment, thus creating a suitable niche for other acidogenics; proves that this bacterium is the leading bacterium in the development of caries.[14] For these reasons, NTCC 10919 (ATCC 33402) strain of S. mutans was used to detect bacterial adhesion in our study.

There are many studies reporting a significant increase in the adherence of Streptococcus mutans, especially after the application of bleaching agents.[15]

In our study, when the bacterial adhesion before and after bleaching was examined, it was determined that both bleaching agents increased the adhesion of S. mutans bacteria on porcelain surfaces after bleaching. We attribute this to the fact that the bleaching process increases the surface roughness of the porcelain surfaces and the amount of bacteria attached accordingly. Bacterial retention was higher in Finesse porcelain after bleaching. However, when the difference between the groups was examined, only the difference between FN and FR groups was statistically significant.

In many studies in the literature, it was stated that there was a positive correlation between surface roughness and bacterial adhesion, and that rough surfaces increased plaque formation and maturation. In the study of Go et al.[16] it was reported that as the surface roughness increases, bacterial adhesion also increases and rough surfaces pave the way for plaque formation. The accumulation of dental plaque increases on rough surfaces. Therefore, the risk of both caries formation and periodontal disease increases. In the present study, by measuring the surface roughness before and after bleaching, it was investigated whether the bleaching process would increase the amount of roughness, whether there was a correlation between the amount of roughness and bacterial adhesion, and the effect of the acquired pellicle layer on bacterial

adhesion. In the study of Jeon et al.[17], a positive correlation was found between the surface roughness and adhesions of S. mutans and Porphyromonas gingivalis; as the incubation times increased, the adhesion of S. mutans increased, while the adhesion of P. gingivalis decreased.

Kawai et al. [18] investigated bacterial adhesion (Streptococcus sobrinus) and glucan synthesis of bacteria on porcelain discs with different surface roughness. They found a positive correlation between surface roughness and bacterial adherence**.**[18]

Gürgan et al.[19], on the other hand, found that there was no change in surface roughness in the enamel samples in which 10% KP was applied for 30 days, compared to the enamel samples that did not apply KP, but there were significant differences in the bacterial adhesion before and after bleaching, and the bacterial adhesion increased after bleaching.

In another study, there was a significant increase in both surface roughness and S. mutans adhesion after the application of vital bleaching agent, but there was no linear relationship between S. mutans colony count and roughness.[20]

In the present study, no statistically significant relationship was found between the surface roughness and bacterial adhesion in the sample groups, in which a pellicle layer was formed or not after bleaching. However, the correlation coefficients between surface roughness and bacterial adhesion were found to be higher in groups without pellicle layer.

In a study by McConnell et al.[4], the effects of surface roughness and surface composition on bacterial plaque formation in hydroxyapatite (HAP), enamel and polished enamel were investigated; it was reported that the roughness of the surfaces with a pellicle layer decreased and accordingly, the number of adhered bacteria decreased.[4]

It was also reported that high-energy surfaces (hydrophilic) accumulated more plaque than low-energy surfaces (hydrophobic), and lipophilic components modulated the bioadhesion process of the pellicle to oral hard tissues with oral biofilm formation**.**[21]

In addition to the effect of the pellicle layer in bacterial adhesion, the properties of the surface where the adhesion takes place are also important. Restorative and prosthetic materials after polishing affect bacterial adhesion. Critical surface energy, zeta potential, surface roughness, and saliva flow rate also have an effect on adhesion.[13]

Steinberg et al.[22] investigated the effectiveness of the salivary biofilm layer in the adhesion of bacteria to bleached and untreated restorative materials. No difference was found between the amounts of saliva protein adsorption in restorative material samples with or without bleaching. However, the total salivary protein content decreased adhesion of S. mutans, Streptococcus sobrinus and Actinomyces viscosus after bleaching.[22]

In the literature, it was stated that dental materials covered with pellicle layer homogenize the total free surface energies. Sipahi et al. [23] stated that the salivary biofilm layer reduced the total free surface energy in dental materials.

When the bacterial adhesion was examined after the formation of the acquired glycoprotein layer, bacterial adhesion decreased in the CN, CR, and VR groups, and increased in the VN, FR and FN White groups. The authors think that the formation of a pellicle layer covers the roughened surfaces, reducing the surface roughness, changing the material hydrophobicity, zeta potential, and contact angle.

Examining the bacterial adherence in porcelains with and without pellicle layer formed after bleaching, decreases were observed in bacterial adherence in all pellicle formed groups compared to the groups without pellicle layer. We attribute this to the change in surface roughness. The rough surface turned into a flatter surface with the formation of the pellicle layer. For this reason, non-pellicle surfaces are rougher and bacterial adhesion is higher. In other words, the formation of a pellicle layer has reduced the importance of surface roughness and has made surface roughness a less effective factor in bacterial adhesion.

In our study, we examined bacterial adhesion in different porcelains with and without pellicle layer before and after bleaching. The relationship between surface roughness and bacterial adhesion was also tried to be explained. There are two different views in the literature review regarding the adhesion of bacteria on the upper surface of different materials. Surface roughness is effective in bacterial plaque adhesion. The second opinion is that the material type is also important. Although we used different porcelain types in our study, we only examined the roughness of the surface conditions of the porcelains. We think that the bleaching process changes the free surface energy, zeta potential and wettability, contact angle of the porcelain besides the surface roughness, and accordingly, bacterial adhesion is also affected by other factors. More detailed studies are needed on how bleaching affects the surface properties of restorations. By taking these factors into consideration, it will be possible to explain the bacterial adhesion more comprehensively.

Conclusion

Different vital bleaching agents could produce different degrees of surface roughness on the surface of different dental porcelains.

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