T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF RESTORATIVE DENTISTRY

EVALUATION THE EFFECT OF HYDROGEN PEROXIDE WHITENING AGENTS WITH DIFFERENT CHEMICAL CONTENTS ON SHEAR BOND STRENGTH TO ENAMEL

MASTER THESIS

ESIN GOKCEK, DDS, MSc

ISTANBUL – 2024

T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF RESTORATIVE DENTISTRY

EVALUATION THE EFFECT OF HYDROGEN PEROXIDE WHITENING AGENTS WITH DIFFERENT CHEMICAL CONTENTS ON SHEAR BOND STRENGTH TO ENAMEL

MASTER THESIS

ESIN GOKCEK, DDS, MSc

SUPERVISOR

Assist. Prof. Dr. MUSTAFA ERSOY

ISTANBUL – 2024

THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Programme : Department of Restorative Dentistry, Esthetic Dentistry Master

Title of the Thesis : Evaluation the Effect of Hydrogen Peroxide Whitening Agents with Different Chemical Contents on Shear Bond Strength to Enamel

Owner of the Thesis : Esin Gökçek

Examination Date : 31.01.2024

This study has approved as a Master Thesis in regard to content and quality by the Jury.

	Title, Name-Surname (Institution)
Chair of the Jury:	Assoc. Prof. Dr. Ali Kağan GÖKÇE Yeditepe University, Restorative Dentistry
Supervisor:	Assist. Prof. Dr. Mustafa ERSOY Yeditepe University, Restorative Dentistry
Member/Examiner:	Assoc. Prof. Dr. Oktay YAZICIOĞLU İstanbul University, Restorative Dentistry

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated .... and numbered ....

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

31.01.2024

Esin Gökçek

ACKNOWLEDGEMENTS

I would like to thank all the participants here for their interest. Head of the Restorative Dentistry Department, I would like to thank **Prof. Dr. Esra CAN** for making me love restorative dentistry and helping me to choose this department for my master's degree, for always shedding light on the path of all her students, and for sharing her knowledge with all her interest. I would like to thank **Prof. Dr. Asım Mübin SOYMAN** for his teachings in the profession, his great interest, endless love and kindness.

Furthermore, I would like to express my gratitude to my advisor, **Assist. Prof. Dr. Mustafa ERSOY**, for providing me with his expertise, invaluable advice and guidance in the science of Restorative Dentistry throughout my undergraduate and graduate master's education, always answering my questions with great patience and constructively criticizing my mistakes. I also thank him for evaluating this research.

I would like to express my sincere gratitude to **Prof. Dr. Haktan YURDAGÜVEN** for all his efforts and valuable contributions throughout my education life. I would like to thank **Assoc. Prof. Dr. Ali Kağan GÖKÇE** for his clinical and academic support, motivation and contributions throughout my education.

Throughout my undergraduate and master's education, I extend my infinite thanks and gratitude to my dear professors, **Dr. Elif Türkeş BAŞARAN**, **Dr. Merve HALACOGLU**, **Dr. Merve YILDIRIM**, **Dr. Nazlı SIRINSUKAN**, **Dr. Burcu DIKICI**, and **Dr. Zeynep BATU**, for their valuable contributions to both my professional skills development and academic education, as well as for their guidance and advice.

I would like to express my endless gratitude to my beloved father, **Levent GOKCEK**, and my dear mother, **Zühal GOKCEK**, for supporting me throughout my education life, believing in me, and encouraging me to follow my dreams. I would like to thank my dear sister, **MSc. Dt. Seda Ceren GOKCEK**, my companion in my life, for guiding me and shedding light on my path and for always being by my side.

I am deeply thankful to my beloved darling **Sarp Kaya YETKIN**, who has always been by my side, providing endless support in all the decisions of my life.

TABLE of CONTENTS

LIST of TABLES

LIST of FIGURES

LIST OF SYMBOLS and ABBREVIATIONS

ACP	Amorphous Calcium Phosphate
a-HP	Alkaline Hydrogen Peroxide
ANUG	Acute Necrotizing Ulcerative Gingivitis
APF	Acidulated Phosphate Fluoride
°C	Degrees Celcius
Ca	Calcium
CP	Carbamide Peroxide
CPP	Casein Phosphopeptide
DSS	Aspartate Serine Serine
DPP	Dentin phosphoprotein
EAER	Electrically Accelerated and Enhanced Remineralization
F	Fluoride
FAP	Fluorapatite
HAP	Hydroxyapatite
HP	Hydrogen Peroxide
ISO	International Organization for Standardization
LED	Light Emitting Diode
µm	Micrometer
Mm	Millimeter
Mpa	Megapascal
N	Newton
n-HAp	Nanohidroksiapatit
Nm	Nanometer
P	Phosphate
PAMAM	Polyamidoamine
pH	Power of Hydrogen
SBS	Shear Bond Strength
SEM	Scanning Electron Microscope
STMP	Sodium Trimetaphosphate
TCP	Tricalcium Phosphate
W	Watt

ABSTRACT

GOKCEK E. Evaluation the Effect of Hydrogen Peroxide Whitening Agents with Different Chemical Contents on Shear Bond Strength to Enamel. Yeditepe University Institute of Health Sciences, Master Thesis, Istanbul.

The Purpose: The aim of this study is to investigate the effect of whitening agents containing n-HAp and the post-whitening waiting period on enamel bond strength.

Material & Methods: A total of 75 bovine teeth were used for this study. These samples were divided into two main groups based on the type of whitening agent used (n=30). Biowhiten Power HP with 40% HP containing n-HAp (Turkey) and Whiteness HP with 35% HP (FGM, Brazil) were selected as the whitening agents. Subsequently, the samples were further divided into two subgroups (n=15) to evaluate the waiting period of 1 or 7 days in artificial saliva at 37℃. A control group with the same sample size as the subgroups was also established. No whitening was performed on the control group, and the samples were kept in artificial saliva for 7 days after preparation. After completing the waiting period, the samples underwent the bonding procedure, and after 24 hours of storage in distilled water at 37℃, they were subjected to the shear bond strength (SBS) test.

Results: The shear bond strength of the control group was found to be higher than that of all other groups ($p< 0.005$). The shear bond strength of the samples subjected to a 7-day waiting period is higher than that of the samples left for one day ($p< 0.005$). Among the samples subjected to a 7-day waiting period, the shear bond strength of the samples whitened with Biowhiten 40% HP whitening agent containing n-HAp is higher than the shear bond strength of the samples whitened with Whiteness 35% HP ($p< 0.005$). Among the samples subjected to a 1-day waiting period, the shear bond strength of the samples whitened with Biowhiten 40% HP (n-HAp) is higher than the shear bond strength of the samples whitened with Whiteness 35% HP ($p< 0.005$).

Conclusion: It was concluded that in-office whitening reduces enamel bond strength. One of the recommended methods to increase bond strength is the waiting period. It has been determined that waiting periods of 1 day and 7 days after whitening are not sufficient to achieve ideal bond strength. Our study suggests that whitening agents containing n-HAp have a less negative impact on enamel bonding. While our study sheds light on the effect of n-HAp-containing whitening agents on enamel bond strength, further research on this topic is needed.

Key Words: In- Office bleaching, Waiting period, Shear bond strength, Nanohydroxyapatite.

ÖZET

GOKCEK E. Farklı Kimyasal İçeriklere Sahip Hidrojen Peroksit Beyazlatma Ajanlarının Minedeki Bağlanma Dayanımına Etkisi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Master Tezi, Istanbul.

Amaç: Bu çalışmanın amacı n-HAP içeren beyazlatma ajanlarının ve beyazlatma sonrası bekleme süresinin bağlanma dayanımına etkisini araştırmaktır

Gereç ve Yöntemler: Bu çalışma için toplam 75 adet sığır dişi kullanılmıştır. Bu örnekler kullanılan beyazlatma ajanının türüne göre 2 ana gruba ayrılmıştır (n=30). Beyazlatma ajanı olarak içerisinde n-HAp bulunduran Biowhiten Power HP 40% HP (Turkey) ile Whiteness HP 35% HP (FGM, Brazil) seçilmiştir. Daha sonra örnekle 37 ℃ yapay tükürükte 1 veya 7 gün bekleme süresini değerlendirmek için iki alt gruba ayrılmıştır (n=15). Alt gruplarla aynı örneklem sayısına sahip bir kontrol grubu oluşturulmuştur. Kontrol grubuna beyazlatma yapılmamıştır ve örnekler hazırlandıktan sonra 7 gün yapay tükürükte bekletilmiştir. Bekleme süresini tamamlayan örnekler bonding prosedürüne tabii tutulup 24 saat 37 ℃ distile suda bekletildikten sonra SBS (shear bond strength) testine tabii tutulmuştur.

Bulgular: Kontrol grubunun bağlanma dayanımı tüm gruplarınkinden yüksek bulunmuştur ($p<0.005$). Yedi gün bekleme süresine tabii tutulan örneklerin bağlanma dayanımı bir gün bekletilen örneklerinkinden yüksektir ($p<0.005$). 7 gün bekleme süresine tabii tutulan örnekler arasında; içerisinde n-HAp bulunduran Biowhiten 40% HP beyazlatma ajanıyla beyazlatılan örneklerin bağlanma dayanımı, Whiteness 35% HP ile beyazlatılan örneklerin bağlanma dayanımından yüksektir ($p<0.005$). 1 gün bekleme süresine tabii tutulan örnekler arasında; içerisinde Biowhiten 40% HP (n-HAp) beyazlatma ajanıyla beyazlatılan örneklerin bağlanma dayanımı, Whiteness 35% HP ile beyazlatılan örneklerin bağlanma dayanımından yüksektir.

Sonuç: Ofis tipi beyazlatmanın mine bağlanma dayanımını azalttığı ortaya çıkmıştır. Bağlanma dayanımını arttırmak için önerilen yöntemlerden biri bekleme süresidir. Beyazlatma sonrası 1 gün ve 7 gün bekleme süresinin ideal bağlanmayı sağlamak amacıyla yeterli olmadığı ortaya çıkmıştır. Çalışmamızda içerisinde n-HAp bulunduran beyazlatma ajanlarının mineye bağlanmaktaki negatif etkisinin daha az olduğu ortaya atılmıştır. Çalışmamız n-HAP içeren beyazlatma ajanlarının minedeki bağlanma dayanımına etkisine ışık tutsa da bu konuyla alakalı daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Ofis tipi beyazlatma, Bekleme süresi, Makaslama bağlanma dayanımı, Nanohidroksiapatit.

1 INTRODUCTION and PURPOSE

Aesthetic dentistry has envolved considerably in recent times. Discolored, damaged or misaligned teeth are no longer tolerated by patients, who increasingly desire to have the perfect smile. The smile has become a symbol of beauty, health and indicator of one's self esteem (1).

Previous studies have shown that whitening agents adversely decrease the shear bond strength of composite to enamel when bonding is performed immediately after the bleaching process. It is reported that the decrease of the bond strength is related to the presence of residual oxygen in the interprismatic space, wich prevents the adequate infiltration of the adhesive and its polymerization. At the same time, mineral dissolution from the enamel surface after bleaching, increase in enamel roughness and decrease in enamel hardness also reduce the bond strength to the enamel surface (2,3). The general approach to solve this situation is to delay the restorative procedures from 24 h to 3 weeks. A waiting period may not always be possible for many reasons such as lack of time, traveling commitments, personal occasion or aesthetics reasons due to the difference in color between the old restoration and tooth(4,5).

In recent years it has been concluded that the effect of whitening agents on enamel surface adversely affects bond strength. Many agents, such as antioxidants and nanaohydroxyapatite pastes, have emerged to restore the enamel surface (6). Further studies are needed to reveal the effect of Biowhiten, which is a new whitening agent and contains nanohydroxyapatite crystals unlike others, on the enamel bond strength. Unlike other studies, a new Turkish brand whitening agent used in this study, which has been studied in this way

The aim of the present study is to determine the effect of the whitening agent containing nanohydroxyapatite crystals on the enamel surface and the change in shear bond strength; these effects will be compared with the bleaching agent with other content in this study. Another purpose is to determine the ideal waiting time after bleaching.

2 LITERATURE REVIEW

2.1 History of Bleaching

In 1877, Chapple performed the first whitening process using oxalic acid. In 1879, Taft and Atkinson suggested chlorine as a whitening agent, moreover; Rossental proposed that ultraviolet waves could aid in the whitening process in 1911 (7). During the First World War, the effects of carbamide peroxide (CP), used in the treatment of acute necrotizing ulcerative gingivitis (ANUG), on tooth structure were discovered (8). Abbot pioneered the combined use of superoxol with heat and light in 1918. In 1970, Cohen suggested that 35% hydrogen peroxide could be used to bleach teeth with tetracycline discoloration. Zaragoza performed whitening with light activation using 70% hydrogen peroxide in 1984 (7). Towards the end of the 1980s, as people's desire to have perfect and white teeth increased, many more office and over-the-counter whitening products were introduced into the market (9). Klusmier discovered that an inflamed periodontium occurs after orthodontic treatment and introduced the concept of using carbamide peroxide gel to treat periodontal tissues. Thanks to this concept, it was accidentally discovered that peroxide had a lightening effect on enamel (9). Furthermore in 1989, Haywood and Heymann introduced the vital teeth whitening technique with night guard. In this technique, they introduced a system where the patient can perform the whitening process at home using a device similar to a night guard using 10% carbamide peroxide gel (10,11).

Whitening is the process of lightening the color of the tooth by oxidation of the pigments in the organic part of the enamel and dentin tissues using a chemical agent. Organic compounds called chromophores create the color of the tooth. These compounds form long-chain organic compounds by containing single and double bonds, heterotoms, carbonyl and phenyl rings in their structures. During bleaching process, the double bonds in the structure of the chromophore are opened. At this time, long chains are broken or some parts of the chain are oxidized. By opening the double bonds, smaller molecules are obtained. Since these molecules cannot reflect light, the teeth begin to appear in a lighter tone. (12)

2.2 Reasons of Bleaching to be Preferred in Aesthetic Dentistry

Due to humanity's intense interest in aesthetics in the 21st century, the demand for aesthetic dentistry has also increased. This high rated demand has contributed to the development of aesthetic dentistry. Patients increasingly desire a perfect smile instead of discolored, damaged or misaligned teeth (13). Nowadays, smile is a symbol of beauty and health. Moreover, it has also become an indicator of one's self-respect. For this reason, while restorating teeth, scientists have begun to develope minimally invasive approachs; rather than solutions that can cause more damage to the tooth, such as crown restorations (14).

2.3 Contents of Whitening Agents

Whitening products contain active ingredients, carriers, thickening agents, urea, surface moisturizers, preservatives and sweetening agents. Hydrogen peroxide as an active ingredient, glycerin as a carrier, and carbopol as a thickener are among the substances that can be used (15). In addition, by adding nano additives and alternative carrier substances to some new whitening agents, promising results have been achieved in terms of both accelerating whitening and minimizing reversible or irreversible damage to enamel.

Urea is found in saliva and gingival crevicular fluid and is produced naturally in the human body and has an anticariogenic effect. It is also included in whitening agents considering its effects on wound healing and saliva stimulation. It also stabilizes hydrogen peroxide and increases pH (16).

Surface moisturizers and carbapol enable hydrogen peroxide to penetrate the tooth surface more easily (16).

All whitening agents contain preservatives. Sodium stannate, citric acid, phosphoric acid are some of these preservatives. Thanks to these preservatives, metals such as magnesium, iron and copper cannot break down hydrogen peroxide. Thus, stabilization of the whitening gel is ensured (16).

2.3.1 Carbamide Peroxide

Carbamide peroxide is a stable structural complex. It reacts with water and breaks down into its active components. Since it is structurally stable, it deteriorates slowly. This creates a long-term active whitening process (17).

Moreover it is found in many home whitening products at a rate of 10% (18). In terms of whitening efficiency 10% carbamide peroxide is equivalent to 3.4% hydrogen peroxide. The main basis of the whitening reaction of carbamide peroxide is its reduction to hydrogen peroxide. In this reaction, urea, ammonia, carbonic acid and carbon dioxide are released as additional products to hydrogen peroxide. As a result, hydrogen peroxide is ionized and decomposed into water and oxygen. Thanks to the oxygen released, the color of the tooth becomes lighter (19). Whitening products containing carbamide peroxide generally contain carbopol and glycerin. Since they slow down the release of hydrogen peroxide, a long-term whitening process is created (20).

2.3.2 Hydrogen Peroxide

According to the chemical theory explaining the whitening reaction of hydrogen peroxide, active hydrogen peroxide breaks down into water (H_2O) and reactive oxygen (O2), and in a short period, free hydroxyl radicals (OH^-) are formed (21). It has high solubility (22). As a result of the breakdown of hydrogen peroxide, the generated free radicals diffuse into the interprismatic spaces of the enamel and carry small molecules detached from large organic molecules to the surface due to their foaming property. These free radicals react with organic molecules causing discoloration among inorganic salts in the enamel. As a result, simpler molecules that reflect light less are formed (23). Oxygen is released as a result of the breakdown of hydrogen peroxide. This released oxygen penetrates the tooth and breaks down the colored pigments. Thus, the color of the tooth lightens. When hydrogen peroxide is ionized, a higher amount of oxygen free radicals is released, while a lower amount of HO_2 free radicals is formed (Figure 1) (24,16).

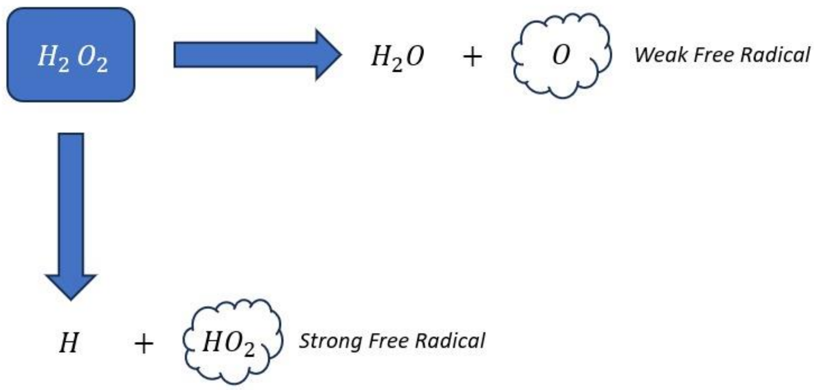

Figure 1. Ionisaiton of hydrogen peroxide

Hydrogen peroxide is an unstable compound, unlike carbamide peroxide. It breaks down into water and reactive oxygen radicals during the reaction. It is a colorless liquid with a bitter taste (24,16). It forms a solution with an acidic pH. The pH of the solution varies with concentration (22). HP is present in various concentrations in many whitening products, with the most commonly used concentrations being 30-35% (24,16).

Hydrogen peroxide ionizes only in the presence of catalysis and enzymes. However, certain enzymes in the oral cavity act as defense mechanisms against oxygen toxicity. Therefore, before whitening, teeth should be dry and cleaned (25,26).

The whitening process must be carried out using retractors and gum protectors as hydrogen peroxide can cause irritation to soft tissues. Additionally, due to its caustic and volatile effects, it should be stored in a cool environment +4 °C (26).

2.3.3 Other Agents

Other agents that do not contain hydrogen peroxide as an active ingredient often use sodium perborate. These products directly generate free radicals during the whitening reaction without converting to hydrogen peroxide. This allows the whitening process to take place (16).

In order to prevent potential issues such as wear, demineralization, reduction in hardness levels, and disruption in hydroxyapatite crystal structure that may occur in the enamel after whitening, next-generation biological whitening products have been introduced to the market. These products contain alkaline or neutral pH. For instance, alkaline-hydrogen peroxide (a-HP) agents aim to minimize demineralization and wear on the enamel surface. It has been revealed that nanohydroxyapatite (n-HAp) added to biological whitening products contributes to the remineralization of tooth enamel and also eliminates the sensitivity problem by filling microcracks and dentinal tubules (27).

2.4 Effect Mechanism of Whitening

Tooth whitening is a process where the color of the teeth is lightened through the oxidation of pigments in the organic part of enamel and dentin tissues using a chemical agent (12,28). The primary cause of color change in teeth is chromogens. The whitening process is based on the chemical breakdown of the chromogen's structure. Chromogens can be found in two forms: the first one is large organic compounds with double bonds, and the second one is compounds containing metallic elements (29).

These compounds form long-chain organic molecules with single and double bonds, heteroatoms, carbonyl, and phenyl rings in their structures. Whitening involves the opening of double bonds in the chromophore's structure. During this process, long chains break or some parts of the chain oxidize. The opening of double bonds results in smaller molecules. These molecules, as they do not reflect light, cause the teeth to appear in a lighter shade (12,28).

Whitening of chromophores containing metals is more challenging. Conversely, oxygen radicals released by hydrogen peroxide react with chromophores composed of organic compounds, causing oxidation. As a result, a lightening of the tooth color is observed (29).

2.5 Features of Enamel

The enamel is the outermost and visible layer of the tooth, providing an aesthetic appearance to the tooth. Originating from the ectodermal layer, the enamel tissue, created by ameloblast cells, is known as the body's hardest layer (30). Ameloblast cells lose their functional abilities after the completion of the formation of the tooth crown, preventing enamel tissue from repairing itself (31). Its high degree of mineralization and a regular crystal structure chemically make enamel the hardest tissue in the human body (30). However, despite the high resistance of enamel to wear, it is known to have a brittle structure. This is due to its low tensile strength (32). Enamel is composed of 95-98% inorganic material by weight, 1-2% organic material, and 4% water. The physical and chemical properties of enamel are closely linked to changes in its ionic structure. For example, the presence of carbonate in enamel increases crystal dissolution, while the inclusion of fluoride ions reduces dissolution (33). Tooth enamel consists of interlocking enamel prisms. Apatite crystals provide durability to enamel prisms and contribute to their structural identity. The fundamental component of the inorganic structure of enamel is these hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$]. Enamel prisms are formed by the assembly of approximately 1000 HAP crystals together (34). The chemical structure of hydroxyapatite is composed of ions arranged along the long axis of crystals around the C-axis of the hydroxyl column. The hydroxyl ion is surrounded by calcium ions (Ca II) forming an equilateral triangle. Phosphate ions are also included in this structure by forming another equilateral triangle at a 60-degree angle. Finally, this structure is enveloped by calcium ions (Ca I) forming a hexagon. The crystalline structure arises from the stacking of these hexagonal structures at a 60-degree angle with their neighbors (Figure 2). The spaces between enamel prisms are wide, filled with a matrix consisting of organic matter and water (35). Each crystal prism is surrounded by its sheath and has interprismatic areas rich in an organic matrix. The cross-sectional profile of the prisms can vary, appearing either circular or keyhole-shaped (Figure 3) (36).

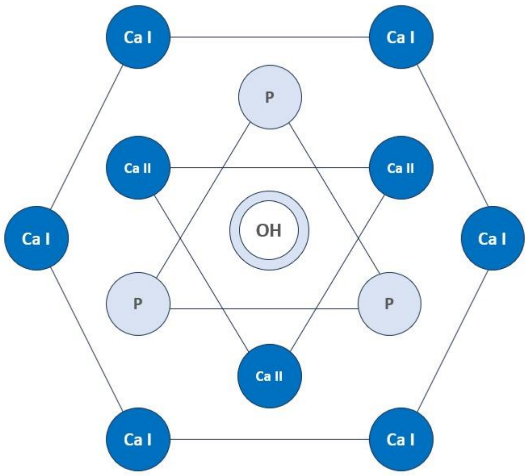

Figure 2. Crystal structure of hydroxyapatite

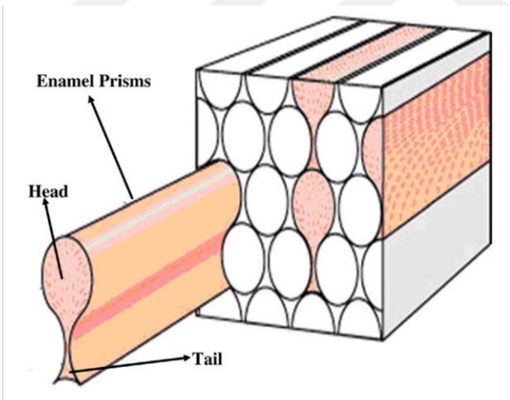

Figure 3. Keyhole shaped appearance of enamel prisms (37)

The structure of enamel prisms is responsible for the durability of enamel. In the central region of the head of enamel prisms, the long axes of apatite crystals are nearly parallel to the long axes of the enamel prisms. However, in the tail region, the crystals are inclined at increasing angles (up to 65 degrees) to the prism axis (30). The arrangement of crystal structures within prisms can make enamel tissue more sensitive or resistant to acid attacks (38). During acid attacks, the dissolution process occurs in the head or central part of the prism. While the tail region shows more resistance to acid attacks, interprismatic areas and peripheral regions of the prisms can experience up to 50% mineral loss (39).

The gaps between enamel prisms are referred to as pores. These pores give permeability feature to enamel while also influencing its density and hardness characteristics. When hydroxyapatite crystals start to dissolve due to acid attacks, the crystal sizes decrease, and the sizes of micropores increase. This leads to an increase in the porosity of enamel (40).

The organic structure of enamel consists of protein complexes, free amino acids, and lipids. The organic structure of enamel contains 16 different amino acids, including methionine and histidine. Enamelin and amelogenin in the protein structure are responsible for the organization of the organic structure (41,42).

2.6 Features of Bovine Enamel

Bovine teeth have been reported to be a reliable alternative to human teeth in experimental caries studies due to their morphological and histological similarity to human teeth. They are easily obtainable, larger in size, have a much flatter and thicker enamel surface compared to human tooth enamel, and exhibit a lower risk of decay compared to the human oral cavity. Additionally, they do not undergo any trauma during extraction, making them safely usable in experimental caries studies (43). When evaluating research conducted on hard dental tissues, especially limited tissue surfaces and thicknesses like enamel, similar results can be obtained through scientific studies using both human and bovine teeth. It is crucial to consider the histological differences between the two tissues, as they may play a role in the study outcomes. In a study conducted by Lippert and colleagues in 2013, comparing bovine teeth and human teeth enamel, they reported that the enamel of bovine teeth is more porous and has lower surface hardness compared to human teeth enamel. When the crystal structure of enamel is evaluated, there are some differences in the physical arrangement of prisms between bovine enamel and human enamel. Histological sections obtained from bovine enamel show larger crystallites, smaller prism diameters, and larger interprismatic areas compared to human enamel, which, similar to human enamel, contains "fibril-like" structures. In terms of elemental analysis, it is reported that bovine enamel has a higher carbonate content and lower fluoride concentration compared to human enamel. Despite the similarity in many histological structures with human enamel, the lower fluoride concentration in bovine enamel is suggested to be one of the factors affecting the rate of artificially induced demineralization lesions. The presence of F ions in polished bovine enamel at concentrations of less than 30 ppm has been scientifically proven to accelerate the formation of artificially induced demineralization lesions in bovine enamel by 1.4 times faster compared to healthy human enamel. In another study by the same researchers, in line with the mentioned reasons, it was reported that the surface microhardness of bovine enamel is less than that of human enamel (43,44).

2.7 Effects of Whitening Agents on Enamel

Whitening agents have been found to have various effects on enamel. One of these effects is the reduction in hardness of enamel, making it more sensitive to deformation and fractures. It has been observed that the Knoop hardness of enamel exposed to whitening agents significantly decreases (45). During the whitening process, the inorganic and organic components of enamel undergo oxidation reactions. This reaction leads to changes in the morphology of the enamel. The development of surface porosities and microcracks results in a decrease in hardness (46).

Potpcnick et al. reported in their study that the application of 10% carbamide peroxide did not cause a change in enamel surface hardness, but the use of 30% hydrogen peroxide resulted in a decrease in the surface hardness of both enamel and dentin (47).

McCracken and Haywood examined the impact of 10% carbamide peroxide on enamel surfaces and demonstrated that it affected the outermost 25 µm layer of the enamel (48).

Results from Scanning Electron Microscope (SEM) studies on the impact of whitening on enamel surfaces vary significantly. Rotstein et al. in their studies where they conducted histochemical analysis of enamel after whitening, reported a significant decrease in the Ca/P ratio in enamel following hydrogen peroxide application and an increase in Ca levels after carbamide peroxide application (49).

Another factor affecting the hardness of enamel after whitening is the pH of the whitening agent used. Whitening agents with neutral or alkaline pH create less change in enamel hardness compared to agents with acidic pH (50). In whitening performed using a whitening agent containing 25% hydrogen peroxide with a pH of 3.2, it has been observed that the decrease in enamel hardness is much greater than the reduction in enamel hardness observed in whitening performed using a whitening agent containing 38% hydrogen peroxide with a pH of 6.7 (51). In a similar study, it was found that the decreases in enamel hardness after treatments with two different whitening agents containing 10% carbamide peroxide, with pH values of 6.79 and 6.23, were not significantly pronounced (52). In an experiment conducted by S. Mundra and colleagues in 2015 using bovine teeth, hydroxyapatite whitening agents with pH in the range of 2.7 to 3.9 showed 2-3 times more enamel surface loss and reduction in hardness compared to hydroxyapatite agents with a neutral pH of 7.1 (53).

Araujo et al.'s research suggests that the reduction in enamel hardness after whitening is independent of the activation method by light. Regardless of the type of light used (LED, halogen, argon laser) or whether the agent is activated by light, a decrease of 5.81% in enamel hardness has been claimed. From this, it has been concluded that the changes occurring in the enamel are based on oxidation reactions, and this is related to the concentration and pH of the whitening agent (54). However, in another study, whitening with 30% hydrogen peroxide using a diode laser observed more damage in the enamel compared to whitening with 40% hydrogen peroxide without light activation. Based on this observation, it is believed that light activation has a direct impact on mineral loss in the enamel and damage to the interprismatic enamel (55).

In addition to these, the duration of application of the whitening agent also has an impact on the microhardness changes in the enamel. As the application time increases, the pH and concentration of the applied agent become more influential (56).

In another study, it was observed that some thickening agents used in whitening agents have an impact on enamel microhardness. For example, carbopol, an acidic component used as a thickening agent in whitening agents, is known to demineralize the enamel surface and prevent hydroxyapatite formation due to its high calcium-binding capacity (57).

While some in vitro studies indicate the formation of cracks, grooves, and porosities on the enamel surface following the whitening process, most in vivo studies suggest that effects that could increase external discoloration on the enamel surface after whitening are not significantly present (51). The pH and concentration of the agent, like their effect on enamel hardness, also lead to surface roughness in the enamel. As the concentration of the agent increases and the pH decreases, the surface roughness on the enamel increases.

Bleaching agents used in teeth whitening can penetrate the enamel surface, but the depth of penetration is dependent on the concentration of the applied agent and the application duration. However, most teeth whitening agents have an acidic pH, leading to the dissolution of inorganic material and erosion on the enamel surface (58). However, it should be noted that in many laboratory studies, for the purpose of achieving standardization, the more mineralized aprismatic enamel layer is shaved, requiring research to be conducted on a less mineralized layer. This situation can impact tissue loss on the enamel surface (58).

To reduce tissue loss and induce remineralization in enamel tissue after whitening, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is added to whitening agents (59).

2.8 Activation of Bleachig Agents

Whitening agents can activate on their own without needing any activation, or they can be activated with light, chemically mixed, or combination of both. Some chemical activators include manganese gluconate, manganese chloride, and iron sulfate. These chemical activators accelerate the chemical reaction of hydrogen peroxide on the enamel surface, enhancing the effectiveness of whitening. As a result, the duration of the whitening process is reduced, and sensitivity is minimized (60). Another perspective suggests that adding a photo activator to the whitening gel and activating the agent with ultraviolet light for 45 minutes provides a more effective whitening compared to a chemically activated system (61). Some studies claim that applying short-term light activation in in-office whitening may lead to damage in the pulp due to the absorbed energy converting to heat and simultaneously create a whitening illusion through enamel dehydration (62).

In a study conducted by Silva et al. on bovine teeth, they applied 35% HP whitening agent to the enamel surface of the teeth and then activated the agents with different light sources. When the SEM images were examined under 5000 magnification, no significant difference was found in terms of porosity in the enamel between the samples without any bleaching (A), without whitening agent activation (B), activated with quartz tungsten halogen (C), activated with light emitted halogen (D) (Figure 4) (63).

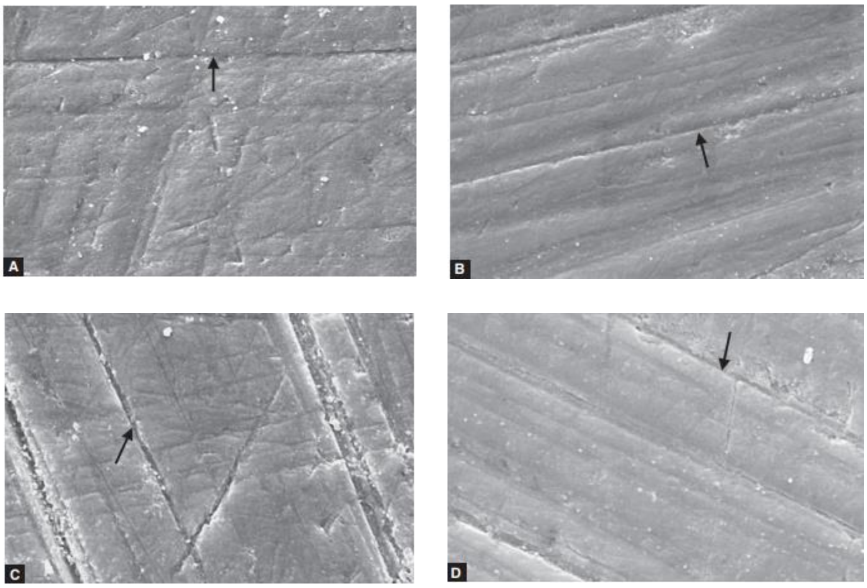

Figure 4. SEM images of enamel surface under 5000 magnification

2.9 Demineralisation

Tooth enamel is a calcified tissue that forms the outer protective structure of the anatomical crown of a tooth. Once enamel is formed, it cannot be biologically repaired or replaced. Therefore, throughout the lifespan of a tooth, there are cycles of enamel demineralization and remineralization that determine the degree of mineral balance and tissue integrity (64).

Demineralization occurring through biofilm (bacterial) or chemically (e.g. erosion) due to exogenous or endogenous acid sources is defined as the loss of calcified material from the tooth structure (65). As the pH falls below the critical value (pH = 5.5), the demineralization process begins in the enamel, accompanied by the dissolution of hydroxyapatite. Demineralization leads to the removal of calcium and phosphate ions from the tooth structure (66). The intercrystalline regions in the enamel structure serve as channels that allow acids to diffuse into the enamel, leading to the influence of crystallites. Over time, the crystal diameters decrease, prism sheaths dissolve, and the enamel gradually transforms into a more porous structure (67). Mineral loss leads to increased porosity, expansion of gaps between enamel crystals, and softening of the surface. This allows acids to penetrate into the depths of the tooth, causing mineral loss beneath the surface (68,69). It has been noted that there can be several changes in the enamel surface after whitening treatments, with demineralization being one of the most significant ones (70,71).

2.10 Remineralisation

Remineralization is the process of restoring minerals to the tooth structure that were previously lost through demineralization (65). Saliva contains $(Ca)^{+2}$ and $(PO)_4^{-3}$ ions throughout life to support the development of hard tissues (72). At physiological pH, saliva is supersaturated with $(Ca)^{+2}$ and $(PO)_4^{-3}$ ions, stabilized by phosphoproteins. This biological condition allows the ions to be biologically available to spread into lesions with mineral deficiency (73). It is known that fluoride-mediated saliva remineralization is limited to the outer 30 µm of the tooth (74). Surface remineralization that occurs is unable to improve the aesthetics or structural characteristics of the lesion immediately beneath the surface (73). Remineralization systems can be examined under two main categories: biomimetic systems and systems that enhance the effectiveness of fluoride.

Remineralized areas, due to the precipitation of calcium and phosphate from saliva, have a structure with less micro-porosity and a higher fluoride content compared to the original enamel (75). When remineralization occurs in the presence of fluoride, OH- ions within HAP crystals dissolve, and as a result, fluoride ions (F^-) replace them, transforming the compound into a stronger compound called fluorapatite (FAP) [$Ca_{10}(PO_4)_6F_2$] (76). FAP (fluorapatite) forms a more acid-resistant enamel surface compared to the original enamel structure containing HAP (hydroxyapatite) since FAP is less soluble in acid (77). The critical pH value is around 5.5 for HAP, whereas it is 4.5 for FAP (78).

Different forms of fluoride preparations are frequently used to prevent demineralization and promote remineralization on the tooth surface. It is known that fluoride forms fluorohydroxyapatite, accelerates the deposition of calcium and phosphate ions on the tooth surface, and has caries-preventive and remineralization effects (79,80). Fluoride acts as a remineralization agent by forming a calcium fluoride layer on the tooth surface. This accumulation later dissolves, diffuses into the enamel, supports remineralization, and leads to an increase in microhardness values (81,82).

One of the topical fluoride gels used is 1.23% acidulated phosphate fluoride (APF). APF contains 1.23% fluoride ions and has a pH between 3-4. The low pH is thought to increase fluoride uptake by the enamel, especially within the first 4 minutes (83). When the fluoride content in the enamel becomes sufficient, fluoride adheres to the crystal surface of the enamel, forming a remineralized structure resistant to acid known as fluorohydroxyapatite (84).

2.10.1 Biomimetic Systems of Remineralisation

Fluoride-containing oral care products are effective in remineralizing the enamel, but they do not have the potential to promote the formation of organized apatite crystals (85). Nowadays, the focus is on regeneration rather than repair of demineralized tissue, aiming to treat lost or damaged tissue with an exact replica of the previously healthy tissue (86). Moreover, enamel regeneration is quite difficult because mature enamel is acellular and cannot remodel, unlike bone or dentin. As a result, significant difficulties persist in the efforts to synthesize artificial enamel, particularly in the production of the complex hierarchical prism and interprismatic structures of enamel. At this point, biomimetic systems offer promising development (87).

2.10.1.1 8DSS Peptides Derived from Dentin Phosphoprotein

Dentin phosphoprotein (DPP) is the most abundant non-collagenous extracellular matrix component found in dentin and is known to play a critical role in tooth mineralization (88). Human DPP contains numerous repeating aspartate-serine-serine (DSS) nucleotide sequences, and studies have shown that DPP can form hydroxyapatite crystals in calcium phosphate solutions (89). Studies have emphasized the electrostatic force between negatively charged amino acids and positively charged calcium and phosphate ions, finding that this force plays a role in the tight binding of DSS peptides with cationic calcium and phosphate ions. In other words, 8DSS peptides prevent the leakage of calcium and phosphate ions from the enamel and also encourage the attachment of calcium and phosphate ions from the surrounding environment to the mineral environment formed on the enamel surface (90). An in vitro study conducted in 2016 provided strong evidence that the biomimetic 8DSS peptide, in addition to inhibiting enamel demineralization on its own, can significantly enhance fluoride from doing the same (91).

2.10.1.2 Self-Assembly P11-4 Peptides

An ideal enamel regeneration involves supporting deep remineralization of the lesion and replacing the compromised enamel matrix with a biomimetic matrix. The self-assembling peptide P11-4 can create such a three-dimensional matrix within the subsurface body of an initial enamel caries lesion and mimic enamel matrix proteins (92). These peptides spontaneously assemble into three-dimensional fibril scaffolds (93). The matrix of 5 P11-4 fibrils has a high affinity for $(Ca)^{+2}$ ions and serves as a nucleus for de novo hydroxyapatite (HA) formation, leading to the remineralization of the lesion body

(94). Studies have shown that microhardness increases after remineralization, and faster hydroxyapatite formation is observed (95). Since P11-4 relies on saliva-directed natural remineralization, its effectiveness will depend on the individual's saliva quality, especially its mineral content, pH, and flow rate (96).

2.10.1.3 Amelogenin

The enamel organic matrix which has a rich compound of amelogenin plays a critical role in the growth, shape formation, and organization of hydroxyapatite (HA) crystals during enamel mineralization. However, mature enamel lacks matrix proteins and cannot regenerate the mineral loss caused by tooth decay or erosion (85). Recently, attempts have been made to replicate the complex enamel microstructure using synthetic amelogenin systems. Recombinant pig amelogenin (rP172) has been reported to stabilize calcium phosphate clusters and promote the growth of enamel crystals in acid-roughened lesions. As a result, changes in the hardness and elastic modulus of the enamel have been achieved. The biomimetic redevelopment of hydroxyapatite (HA) crystals also creates a strong interface between the newly formed layer and the existing enamel, ensuring the effectiveness and durability of restorations (97). However, a disadvantage of amelogenin-mediated enamel regeneration is that the growth of the repaired enamel layer takes a long time. This situation complicates the clinical use of amelogenin (85).

2.10.1.4 Poly (Amido Amine) Dendrimers (PAMAM)

Poli (amido amine) (PAMAM) dendrimers are highly branched polymers. These amelogenin-like dendrimers, referred to as "artificial proteins," can mimic the functions of organic matrices while promoting the biomineralization of tooth enamel. Crystals formed by PAMAM organic structures exhibit similarities to the original prisms (98). However, similar to amelogenin, PAMAM-mediated enamel remineralization is a time-consuming process, and without enhancement, its practical applications in clinical settings may be challenging (99).

2.10.1.5 Accelerated and Enhanced Remineralization with Electricity

Electrically accelerated and enhanced remineralization (EAER), targeting initial and intermediate enamel lesions; It is a recently developed remineralization technology that aims to preserve healthy tissue, restore the carious lesion to its full depth and improve the mechanical properties of the treated enamel. iontopheresis uses to accelerate the flow of remineralizing ions into the deepest part of the subsurface carious lesion. This creates an environment that supports the remineralization of the lesion and allows it to mature by giving optimal hardness and mineral density to the repaired lesion. Lesions treated with EAER have an appearance very similar to healthy enamel, without the broken rods or distorted prisms visible under electron microscopy (100).

2.10.1.6 Nanohydroxyapatite (nHA)

Hydroxyapatite has a molecular formula of $Ca_{10}(PO_4)_6(OH)_2$ and is a calcium phosphate compound with a calcium-to-phosphorus ratio of 1:67. Hydroxyapatite can be synthesized or naturally extracted from bovine teeth or bones (101). Synthetic calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is chemically and biologically similar to the mineral component of human bone and teeth. It is classified as bioactive, belonging to a small group of substances, and is highly biocompatible (102). Other forms of calcium phosphate exist in nature, but hydroxyapatite is the most stable and least soluble of these (103). Unbonded surface atoms in a nanoparticle are more numerous than at micro or macro atomic scales. Thanks to this feature, nanoparticles have the ability to bond more with the surrounding structures. In this way, it can form stronger compounds (104).

A certain level of effectiveness is achieved when the particles are between 1 and 100 nm in size. The large reaction surface and small size increase the hydration of the material, thus gaining better physical and chemical properties (103). Synthetic hydroxyapatite particles at the nano scale show similarity to the apatite crystal structure of enamel, both morphologically and structurally. When used in orthopedic, dental, and maxillofacial applications, it serves as a material that supports bone development and osseointegration without disrupting or dissolving (105).

In the prevention of decay, nano-HA has been incorporated into toothpaste to provide ions that reduce demineralization and enhance remineralization. Nano particles can penetrate dental pores and create a protective layer on the tooth surface (106). In a study evaluating the remineralization effect of toothpaste containing nanohydroxyapatite on artificial cavities, SEM analysis showed a reduction in defects on the enamel surface, and the precipitation of numerous mineral salts. Researchers have suggested that nanohydroxyapatite can support remineralization for demineralized enamel (107). It has been determined that toothpaste containing nanohydroxyapatite particles supports remineralization, reduces the absorption of Streptococcus mutans virulence, and eliminates extrinsic discolorations due to its abrasive nature (108).

There are two views regarding the remineralization mechanism of nanohydroxyapatite. The first view suggests that remineralization occurs by the deposition of nano-particles into the voids on the demineralized enamel surface. The second view proposes that nanohydroxyapatite acts as a calcium source in the oral environment, contributing to remineralization (105). In other words, some propose that nanohydroxyapatite (nHA) acts as a calcium phosphate reservoir, maintaining a state of super-saturation with respect to enamel minerals. This is believed to inhibit demineralization and enhance remineralization (109).

Koçyiğit (2015) conducted an in vitro thesis study with deciduous teeth, evaluating the effectiveness of fluoride-containing and fluoride-free nanohydroxyapatite toothpaste in the remineralization of initial enamel lesions. According to the Micro-BT analysis, it was reported that the increase in mineral content in the initial enamel lesions in the nanohydroxyapatite toothpaste group was numerically higher than the fluoride group. Additionally, according to the SEM analysis, it was noted that surfaces treated with fluoride toothpaste were covered with CaF_2 (Calcium fluoride) crystals and did not exhibit a smooth surface. On the other hand, surfaces treated with nanohydroxyapatite toothpaste showed a homogeneous appearance across the entire surface, and the porous structure was completely lost (110).

97% of enamel consists of hydroxyapatite crystals with a size of 20-40 nm. It makes sense to use nano-sized HA particles after an erosive or abrasive attack on enamel. It has been proven that these particles spontaneously assemble in aqueous solutions and form enamel-like structures (104).

Another reported advantage of nHA is its ability to shift the oral flora to a more favorable condition. Saliva samples were taken before and after a 5-minute application of NHA paste to determine bacterial levels. It was revealed that the particles adhere to the tooth and plaque surfaces, selectively absorbing harmful bacteria such as Streptococcus mutans and Porphyromonas gingivalis. This capability might potentially help prevent the onset of plaque-related periodontal diseases by smoothing out rough enamel surfaces prone to plaque accumulation (111).

2.10.2 Fluoride Reinforced Remineralisation Systems

2.10.2.1 Calcium Phosphate Systems

Biomimetic enamel regeneration could be the future of fluoride-free remineralization; however, widespread clinical use requires time. Currently, calcium phosphate systems are predominantly used to enhance the remineralizing efficacy of fluoride in patients with a high risk of decay. One important criterion is solubility, with calcium phosphate systems having lower solubility negatively impacting remineralization. Calcium phosphate systems are categorized into three groups: stabilized amorphous calcium phosphate systems, crystalline calcium phosphate systems, and unstabilized amorphous calcium phosphate systems (112).

2.10.2.1.1 Stabilized Calcium Phosphates

2.10.2.1.1.1Casein Phosphopeptide Amorphous Calcium Phosphate

Various products have been developed to promote remineralization in the enamel (113). One of these products is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), a stabilized calcium phosphate. CPP-ACP acts as an agent that prevents enamel demineralization, initiates remineralization of the enamel surface, and increases the microhardness of softened enamel surfaces (114). Casein phosphopeptide (CPP) has the ability to stably retain amorphous calcium phosphate (ACP) in a nanocomplex structure in a solution of calcium and phosphate. Casein phosphopeptide molecules contain phosphoserine, which stabilizes amorphous calcium phosphate, increasing the solubility of calcium phosphate under neutral and alkaline conditions (115). As a result, it ensures the continued mineral density of the tooth. Additionally, exposure of CPP-ACP to acid leads to the release of ACP into the environment, and the released calcium and phosphate ions buffer the acidic environment, balancing the pH. Therefore, it helps prevent demineralization (116). In addition, CPP-ACP saturates the tooth surface by binding the free calcium and phosphate in the plaque localized on the tooth surface, and at the same time, it binds to the surfaces of bacterial cells in the dental plaque and prevents them from colonizing the tooth. In this way, CPP-ACP serves as a reservoir for tooth enamel to regain the minerals it loses during the demineralization process (117).

In a whitening study using bovine teeth, the enamel surface was whitened with 35% hydrogen peroxide for 14 days, and CPP-ACP was applied before, after or both before and after the procedure. It was concluded that in all of these protocols, changes in hardness and roughness on the enamel surface were significantly prevented (118). In a study conducted using human tooth enamel samples, it was revealed that the application of CPP-ACP paste after whitening significantly improved remineralization in the enamel (119). In another clinical study, the surface roughness of whitened enamel was evaluated after the use of remineralization agents. CPP-ACP, nanohydroxyapatite, or Novamin was applied 5 minutes after each whitening cycle. It was observed that the enamel roughness decreased by 50% after these applications (120).

2.10.2.1.2 Crystallized Calcium Phosphate Systems

2.10.2.1.2.1 Functionalized β-Tricalcium Phosphate(β-TCP)

TCP (Tricalcium Phosphate), primarily used for bone regeneration, is an absorbable material. Although there are many polymorphic structures, two polymorphic phases (α and β) are mainly employed as biomaterials. In dentistry, both α-TCP and β-TCP are utilized to remineralize subsurface lesions in toothpaste, reduce enamel demineralization in varnishes, and enhance bonding properties in adhesives (121). It is primarily designed to enhance the remineralization guided by saliva $(Ca)^{+2}$ and $(PO)_4^{-3}$ ions and increase the F^- ion activity on the tooth surface. Although currently available as a commercial product, data regarding its remineralizing effect is insufficient (121).

2.10.2.1.3 Unstable Calcium Phosphate Systems

2.10.2.1.3.1 Amorphous Calcium Phosphate

Amorphous calcium phosphate was the first product used as synthetic hydroxyapatite. Its unstable and reactive structure in aqueous environments leads to the release of calcium and phosphate ions, causing them to transform into crystalline phases due to microcrystal growth (122). One of the main concerns of using an unstabilized calcium phosphate system is its potential to support the accumulation of dental calculus on teeth. Additionally, ACP rapidly captures free F^- ions in the oral environment, reducing their availability for the remineralization of lesions (123).

2.10.2.2 Polyphosphate Systems

2.10.2.2.1 Sodium Trimetaphosphate

One way to maintain the anticariogenic efficacy of traditional toothpaste while reducing the potential risk of fluorosis is to partially replace fluoride with polyphosphate salts such as sodium trimetaphosphate (STMP), calcium glycerophosphate, or hexametaphosphate (124). Among polyphosphates, STMP is considered the most effective anticariogenic agent, not only preventing demineralization but also enhancing remineralization capabilities (125).

2.10.2.3 Natural Products

A fascinating approach to remineralization involves natural products of plant origin that show the ability to beneficially alter the demineralization/remineralization balance. Among the most promising is Galla chinensis, produced by parasitic leafhoppers, which has been found to inhibit demineralization and enhance remineralization and the effectiveness of fluoride (126). In addition, Hesperidin, a citrus flavonoid, and Arabic gum, an exudate from Acacia, are other natural products known to suppress acid-related demineralization and enhance remineralization, even under fluoride-free conditions (127).

2.11 Effect of Whitening on the Bonding of Adhesive Fillings

Tooth and dentin bonding may vary following whitening due to the presence of hydrogen peroxide (128). Several authors have found that the bonding strength of composite resin applied to whitened enamel is significantly lower compared to non-whitened enamel (129).

There are studies that give different results on the effect of the concentration of the applied agent on the bond strength. For example, in a study conducted by M. Turkun and colleagues in 2004 on bovine teeth, they concluded that the bonding strength on enamel surfaces treated with high-concentration carbamide peroxide was significantly lower compared to surfaces treated with low-concentration agents (130). On the other hand, Cavalli and colleagues found that carbamide peroxide concentration had no effect on bonding to enamel surfaces (131).

In whitened enamel, resin tags are fewer in number, less pronounced, and shorter compared to unbleached enamel (128). Research on the effects of peroxide-based materials on the bonding durability and structure of enamel has found a decrease in bonding strength (132). Many researchers have found that with the loss of prismatic form, the enamel gives an over-etched appearance and porosity increases, therefore the bonding on the enamel surface decreases (133). At the same time, calcium loss and decrease in microhardness negatively affect the bonding to the enamel (134).

Titley and colleagues, in their study, observed under SEM that the interface between bleached enamel and resin was significantly different from the interface between unbleached enamel and resin. In this study, many areas were found where the resin did not bond to the bleached enamel surfaces. The resin tags were weaker and fragmented, and it was concluded that they penetrated less deeply than in the unbleached samples (135).

In another study where Titley and colleagues examined the interface between bleached enamel and composite resin using SEM, they found an interface with a bubbly and porous appearance. It was suggested that these could be attributed to gas bubbles formed as a result of oxidation reactions. Additionally, it was revealed that the bonding of composite resin to enamel was lower immediately after bleaching (136). To eliminate residual oxygen from bleaching materials, in vitro samples are immersed in distilled water or artificial saliva. The goal here is to mimic the oral environment post-bleaching (137,138).

Zhao et al. demonstrated that under specific conditions, peroxide ions replace hydroxyl radicals in the apatite cage, forming peroxide-apatite. Additionally, when peroxide ions decompose, hydroxyl radicals re-enter the apatite cage, eliminating the structural damage caused by the combination of peroxide ions (139). Lai et al. proposed the hypothesis that the process of peroxide ion combination could be reversed by an antioxidant. They also suggested that sodium ascorbate alters the redox potential of the oxidized bonding substrate, allowing the free radical polymerization of adhesive resin to progress without premature termination, thus reversing impaired bonding (140,141).

This decrease in binding values is clinically important. Some authors reported that the negative effects of hydrogen peroxide on bonding were due to residual oxygen and emphasized that this situation prevented the polymerization of the resin (142).

To avoid clinical problems related to reduced bond strength after whitening treatment, various methods are recommended. The most widely recommended method is to delay the fabrication of restorations for a period ranging from 24 hours to 3 weeks after whitening treatment (143). In a study conducted by Lai and colleagues, it was reported that hydrogen peroxide reduced the bond strength of the composite, and they suggested that antioxidants or sodium ascorbate could be used to correct this condition (141). Such applications allow reducing the waiting time for restorative treatments after whitening (136, 141).

2.12 Bonding Strength Test Methods in Dentistry

In vivo tests have an important role in the evaluation of materials used in dentistry. However, these tests may not accurately evaluate the effects of different stresses occurring in the mouth on the restoration. Due to the rapid development of current binding agents and the search for the ideal product, in vitro bonding strength tests are needed (144).

The interfacial bond strength can be tested using various methods. Based on the dimensions of the bonded area, bond strength methods can be classified as macro (4-28 mm^2) or micro (approximately 1 mm^2) (144). Tensile or shear forces can be applied to the interface (145).

These tests include Macro Shear, Macro Tensile, Push-out, Micro Shear, and Micro Tensile (146).

2.12.1 Macro Shear Bond Strength Test

Force is applied to the test piece using a single-angle nail-shaped tip, a flat-edged chisel, or a wire loop attached to the force applicator arm of the machine to test the bond strength (147,148).

In the shear bond strength test method, a test is applied using an apparatus shaped like a blade edge to separate the connection with the tooth surface. In the ISO standard, it is specified that the working speed of the cutting edge of the test apparatus should be between 0.45 and 1.05 mm/min (149,150). In this test, which is based on the principle of applying force at a constant rate until rupture occurs on the surface where two materials are bonded to each other with a bonding agent, the value of the bond strength test result is measured by dividing the maximum force obtained by the surface area where bonding occurs. The value of the bond strength is expressed in pounds/$inch^2$, kg/cm^2, MN/m^2 or N/mm^2 (Mega pascals, MPa) (151,152). The typical bond strength in dentin is found to be approximately 10 MPa (153,154).

2.12.2 Macro Tensile Bond Strength Test

In tensile tests, the force distribution is more homogeneous compared to shear tests. This leads to much more stable results for bond strength testing. In macro tensile bond strength tests, it is crucial for the bonded interface to be aligned perpendicular to the loading axis. If not adjusted, the force may cause bending in the samples. Additionally, the testing apparatus must maintain the correct position between the tooth surface and the adhesive material. For these reasons, tensile tests require more technical precision compared to shear tests (155,156).

2.12.3 Push Out Test

This method is one of the tests used to measure bonding strength. Firstly, the surface of the tooth is prepared in a round slice shape. Then, an appropriately sized hole is drilled into the tooth surface for the material to be tested. The adhesive system is applied to the hole, and the material is placed inside. Subsequently, force is applied to the material with a pointed tip whose diameter-to-material ratio is less than 0.85 (157). The force at which separation occurs is measured. This method better simulates the clinical environment compared to shear and tensile tests because in this method, the material being tested is bonded to the tooth surface with an adhesive system (158).

2.12.4 Micro Shear Bond Strength Test

The micro-shear bond strength test was introduced to researchers in the year 2002 (159,160). The micro-shear bond strength test method is a testing technique in which samples, placed on dental tissue or a material with the help of a wire or a different apparatus, are used to measure the bond strengths. The surface area of the materials for which bond strength is measured is less than 1 mm^2. As an example of the micro-shear bond strength tests, the material to be tested is positioned on top of a composite cylinder using silicone tubes with a diameter of 0.5 mm and a height of 0.7 mm. Typically, 6 tube pieces are attached to the surface and filled with composite. The test procedures are similar to macro shear tests, and the negative features in stress distribution are also similarly comparable (161,162).

The advantages include the ability to calculate the average value for a single tooth, obtain multiple test samples from one tooth, conduct tests in the presence of irregular surfaces, and facilitate easier examination of samples under an electron microscope after experiments (163,164).

2.12.5 Micro Tensile Bond Strength Test

Various test setups are available for microtensile testing. The sample can be bonded using cyanoacrylate adhesive or attached to the test setup actively or passively with a handle. The gripping method prevents stress distribution in the specimen. The values for dentin bond strength range from 30 to 50 MPa. The MPa values obtained from the microtensile bond strength test method are higher than those found in macro tensile tests because the critical crack size is smaller in the microinterface (165,166). Evaluating force requires technical precision, as microcracks that may occur when samples are cut with a diamond bur can concentrate in the area where force is applied, leading to a lower bond strength value in the results (167,168).

3 MATERIAL and METHODS

3.1 Research Plan and Method

In this study, it is aimed to examine the effects of nanohydroxyapatite containing whitening agents and waiting period after whitening process onto enamel bond strength. As a result of power anaysis using the G power program, effect size d for shear bond strength: 1.078, standard deviation 4.4, Power 0.90 and the number of samples determined for α:0.05 is minimum n=12 samples for each group detected.

3.2 Selection and Preparation of Teeth Included in the Study

Among 90 incisor teeth obtained from bovines, those with visible cracks and fractures were eliminated and 75 teeth were selected and used in this study. After the extraction, any remaining tissue residues on the teeth were cleaned, and the teeth were washed under running tap water. Later, the teeth were stored in distilled water for 1 month with the addition of thymol at +4 °C.

3.3 Preparation of Sample Models

The roots of the teeth were separated from their crowns at the cemento-enamel junction using an ISOmet 1000 cutting machine (Buehler, Illinois USA) at a speed of 375 rpm.

The metal rings were filled with self-curing pink acrylic (Micryl, USA) (Figure 5), and tooth sections were mounted inside the acrylic with the buccal surfaces facing upward. The buccal surfaces of the teeth were embedded with acrylic to be at the same level, ensuring the formation of a single flat surface (Figure 6).

Figure 5. Self curing pink acrylic, Micryl, USA

Figure 6. Teeth sample embedded in acrylic

The central part of the teeth in the obtained molds was sequentially polished with 600, 800, and 1200 grit silicon carbide paper on a grinding machine (Buehler, Illinois, USA) at a speed of 600 rpm, with water cooling, at a 90-degree angle, to ensure uniform polishing of all areas (Figure 7). This process was carried out to standardize the samples among different groups.

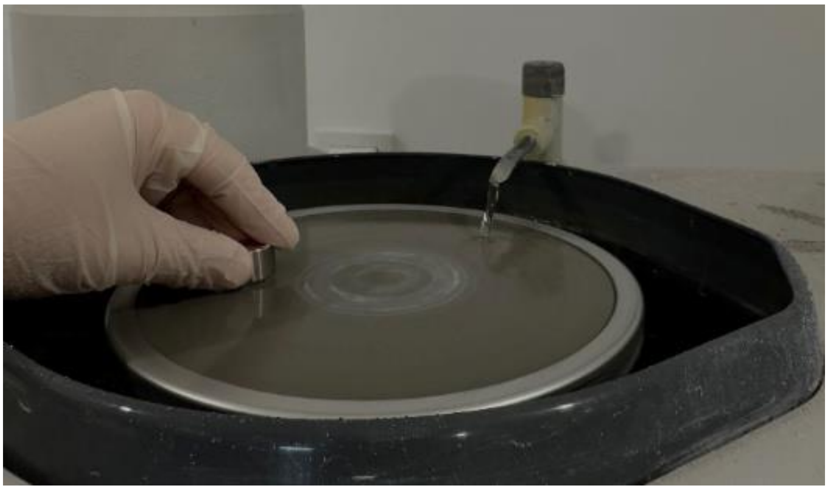

Figure 7. Enamel surface flatting of sample on grinding machine with water cooling

Out of 75 teeth, 30 were whitened with Biowhiten agent (40% HP Office Bleaching, Turkey), 30 were whitened with Whiteness agent (35% HP Office Bleaching, FGM, Brasil) and 15 teeth underwent no whitening treatment.

Table 1. List of all materials used in this study

Material Name	Brand and place of production	Code
Self-curing pink acrylic	Micryl, USA	23A768
Power HP 40%	Biowhiten, Turkey	K40H2306
Whiteness 35% HP	FGM, Brasil	080323
Proetch Jumbo 37% phosphoric acid	Promida, Turkey	210219
G-premio bond	GC, Japan	2212171
Gaenial Anterior, A2	GC, Japan	2105273

3.4 Determination of Groups and Waiting Times Before Bonding

After separating 15 teeth as the control group (Group A) out of a total of 75 teeth, the remaining 60 samples were divided into two main groups, each consisting of 30 samples: Group B with 40% HP Biowhiten, and Group C with 35% HP Whiteness. Subsequently, each group was further divided into two subgroups, each containing 15 samples. The samples in groups number 1 (Group B1, Group C1) were kept in artificial saliva at 37 ℃ for 1 day after bleaching. After whitening, groups 2 (Group B2, Group

C2) were kept in artificial saliva at 37 °C for 1 week (130). No bleaching was applied to the samples in the control group (Group A). It was kept in artificial saliva for 1 week just before the bonding procedure (130) (Table 2).

Table 2. List of groups in this study

Groups	Treatment regime
A (Control)	1-week immersion in artificial saliva
B1	Bleaching with Biowhiten, then 1-day immersion in artificial saliva
B2	Bleaching with Biowhiten, then 1-week immersion in artificial saliva
C1	Bleaching with Whiteness, then 1-day immersion in artificial saliva
C2	Bleaching with Whiteness, then 1-week immersion in artificial saliva

Artificial saliva's electrolyte composition has been prepared to resemble human saliva. It consists of 4.3 grams of xylitol, 0.1 grams of potassium chloride, 0.1 grams of sodium chloride, 5 milligrams of magnesium chloride, 1 gram of sodium carboxymethyl cellulose, 5 milligrams of calcium chloride, 1 milligram of potassium thiocyanate, 40 milligrams of potassium phosphate, and 100 grams of distilled ionized water (169).

3.5 Bleaching Treatment

For group B, after 30 teeth were removed from the artificial saliva, their labial surfaces were washed with water and dried, and biowhiten 40% nHP agent was applied to a thickness of approximately 2 mm (Figure 9). The whitening agent was applied in accordance with the manufacturer's recommendations, with three periods of 15 minutes each, totaling 45 minutes.

Figure 8. Biowhiten 40% nHP bleaching agent

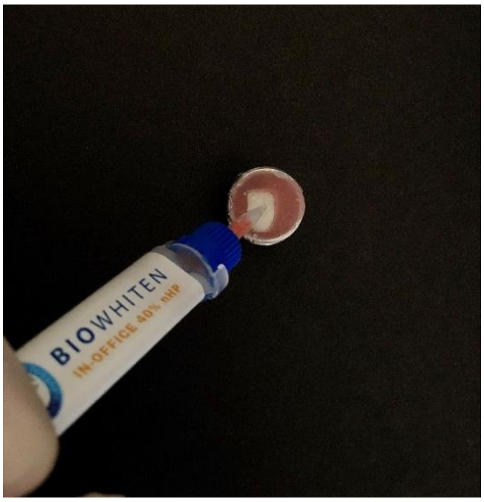

Figure 9. Application of Biowhiten

Figure 10. Biowhiten 40% nHP application tube

The whitening agent was activated throughout the procedure using an LED light device (High Strength 8 Lamp Bead, China) (Figure 11).

Figure 11. High Strength 8 Lamp Bead, China

At the completion of each 15-minute period, the agent on the tooth surface was removed using suction and a sterile pellet, and a new whitening agent was applied to the surface.

At the end of the 45-minute period, the samples were rinsed with air/water spray for 30 seconds. Subsequently, all samples were individually placed in distilled water and placed in an incubator (Memmert, Germany) under laboratory conditions at 37°C. The aim was to mimic the oral environment.

Figure 12. Incubator, Memmert, Germany

For group C, after removing the teeth from artificial saliva, the labial surface was rinsed with water and dried. Whiteness HP (35% HP, FGM,Brasil) was applied to the samples (Figure 13).

Figure 13. Whiteness HP 35% bleaching agent, FGM, Brasil

In a mixing bowl, following the manufacturer's instructions, 3 drops of hydrogen peroxide (phase 1) and 1 drop of thickening agent (phase 2) were added (Figure 15). The mixture was stirred in circular motions 25-30 times to activate the agent (Figure 14).

Figure 14. Bottles of Whiteness HP phase 1 and phase 2

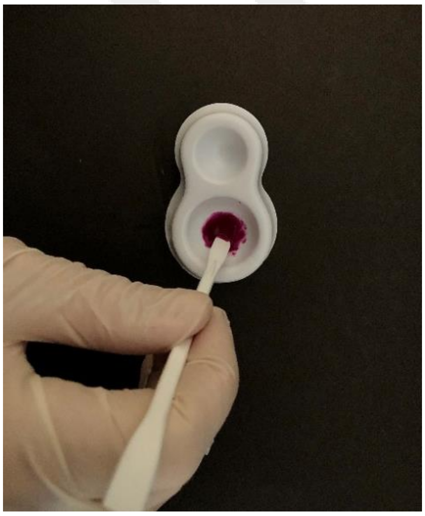

Figure 15. Mixing of agents with circular movements

Subsequently, the agent was applied to the enamel surface of the samples with an approximate thickness of 2 mm (Figure 16).

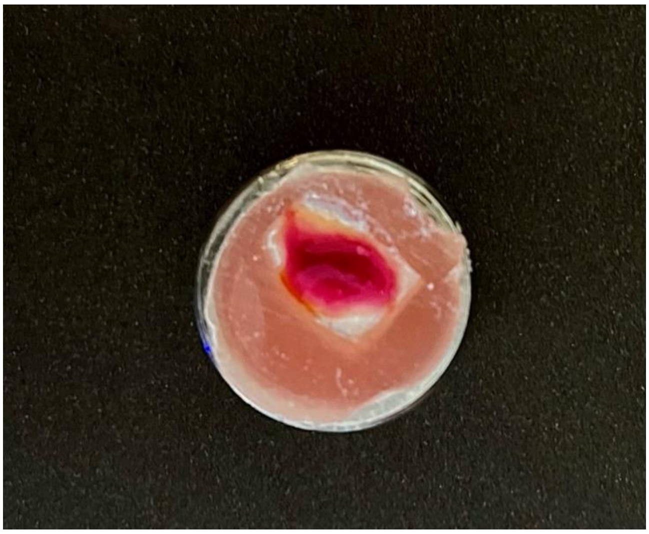

Figure 16. Application of Whiteness HP on enamel surface

After being activated with LED light for 15 minutes, the agent was removed from the surface with suction and sterile cotton and the whitening agent was applied to the surface again. This application was repeated 3 times, as recommended by the manufacturer, and the 45-minute whitening process was completed. The samples were rinsed with air/water spray for 30 seconds. Subsequently, all samples were individually placed in distilled water and placed in an incubator under laboratory conditions at 37°C.

3.6 Bonding Procedure

The teeth were removed from artificial saliva. After washing with air/water spray for 30 seconds, the surface was thoroughly dried with compressed air. Subsequently, 37% phosphoric acid (Promida, Proetch, Turkey) (Figure 17) was applied to the dried enamel surface for 15 seconds (Figure 18). After 15 seconds, the acid was removed from the surface using suction, and then the surface was washed for 30 seconds.

Figure 17. Promida 37% phosphoric acid, Proetch, Turkey

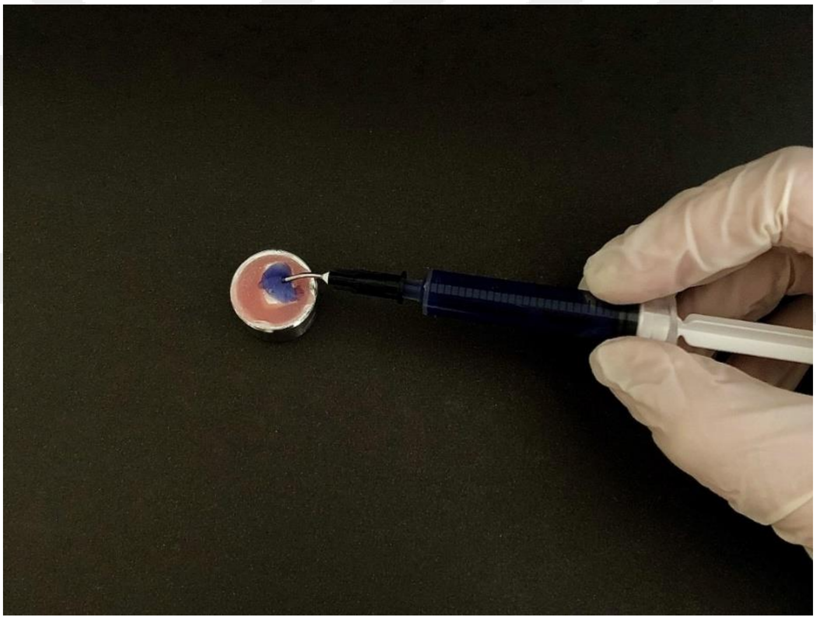

Figure 18. Application of 37% phosphoric acid on enamel surface for 15 seconds

After 15 seconds, the acid was removed from the surface using suction, and then the surface was washed for 30 seconds (Figure 19).

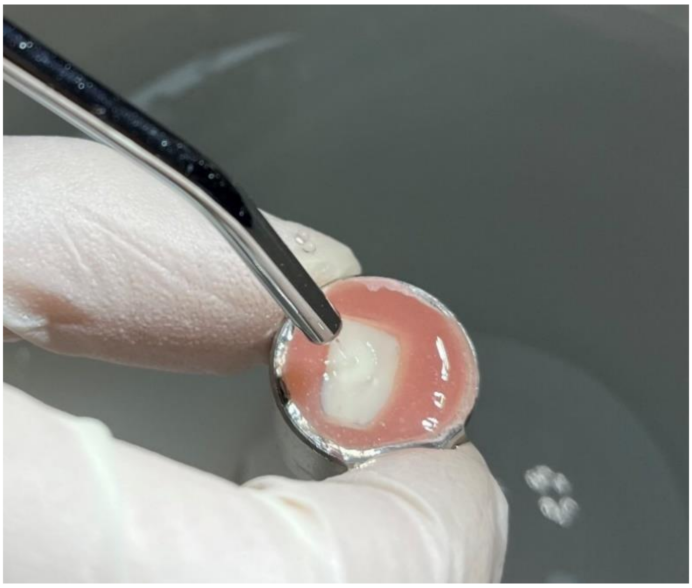

Figure 19. Cleaning of enamel surface with air and water

After drying the enamel surface, bonding resin (G-premio bond, GC, Japan) (Figure 21) was applied to the enamel surface using a microbrush for 10 seconds (Figure 20).

Figure 20. G-premio bond, GC, Japan

Figure 21. Application of bonding resin

After ensuring the even spreading of the bond on the surface with gentle air, it was polymerized for 20 seconds using a light curing unit (Ivoclar Vıvadent, Bluephase N G4, Liechtenstein) (Figure 22,23).

Figure 22. Light curing unit, Ivoclar Vivadent, Bluephase N G4, Liechtenstein

Figure 23. Polymerisation of bonding resin

To ensure the standardization of the application of composite resins to the enamel surface, a white sticker with a 4 mm diameter hole in the center was affixed to the sample in a way that it would align with the prepared surface and a transparent silicone guide was prepared with a central hole of 4 mm in diameter and 6 mm in height (Figure 25). The reason for making the guide transparent was to allow the passage of light completely. After placing the guide in the exact center of the prepared tooth surfaces, following the manufacturer's instructions, 2 mm of composite resin (Gaenial Anterior, A2,GC, Japan) was dispensed into the guide and polymerized from a distance of 10 mm for 20 seconds. This process was repeated three times gradually, creating vertically cylindrical posts with a height of 6 mm on the enamel surface (Figure 26). After removing the samples from the silicone mold, they were placed in distilled water and incubated at 37°C for 1 day.

Figure 24. Fixation of white sticker on the enamel surface

Figure 25. Application of composite restoration into silicon guide

Figure 26. Samples obtained by removing the silicone mold

3.7 Evaluation of Shear Bond Strength

After removal from the incubator, the samples were subjected to shear bond strength tests at a rate of 0.5 mm/min using an Instron Universal Testing Machine (Model 3345, USA) (Figure 27).

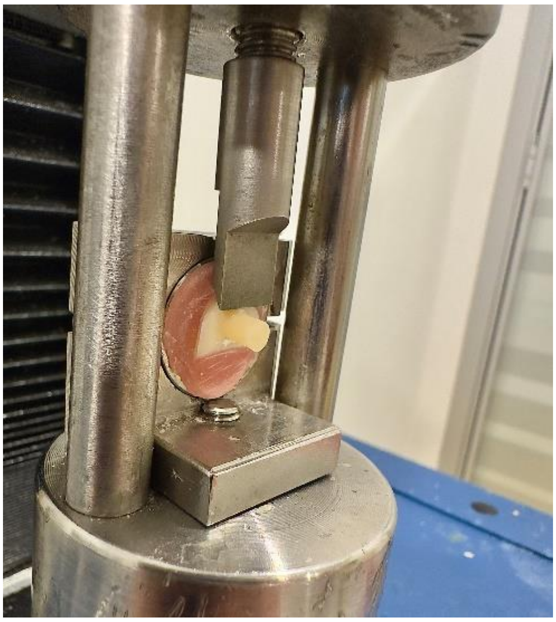

Figure 27. Shear bond strength test on Instron Universal Testing Machine

The load at the moment of failure was recorded using Bluehill 3 software version 3.62 (Figure 28). The shear bond strengths of the samples were calculated by folllowing equation below and expressed in units of MPa.

$$S = T/A$$

Here, S, T, and A represent shear bond strength, applied tension and bonded area respectively (170).

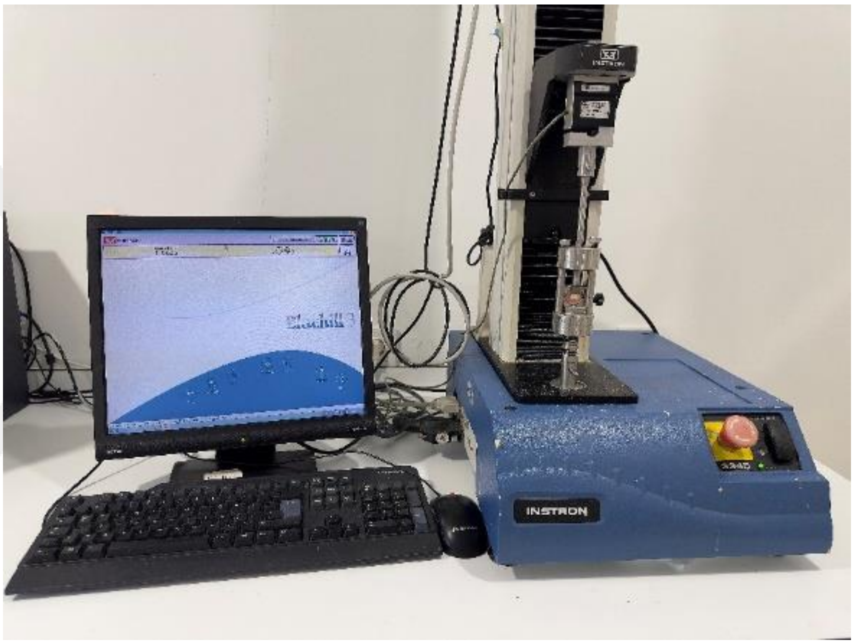

Figure 28. Bluehill 3 software version 3.62 and Instron Universal Testing Machine

3.8 Methods Of Statistical Analysis

In evaluating the findings of the study, IBM SPSS Statistics 22 software was utilized for statistical analyses. The normal distribution of parameters was assessed through Kolmogorov-Smirnov and Shapiro-Wilk tests, and it was determined that the parameters were normally distributed. Two-way ANOVA test was employed for assessing the joint effect of whitening agent and different waiting days within artificial saliva on shear bond strength when comparing quantitative data. Student t-test was used for pairwise comparisons between parameters. Significance was evaluated at the $p<0.05$ leve

4 RESULTS

Table 3. Maximum load in compressive strength and maximum load values of the B1 group (Bleaching with Biowhiten, then 1-day immersion in artificial saliva)

	Maximum Load in Compressive Strength (Mpa)	Maximum Load (N)
Sample 1	7.38745	92.83344
Sample 2	7.54044	94.75591
Sample 3	7.19319	90.39233
Sample 4	9.13755	114.82578
Sample 5	7.71901	96.99999
Sample 6	6.65703	83.65469
Sample 7	7.34627	92.31599
Sample 8	7.66605	96.3344
Sample 9	7.34963	92.3522
Sample 10	9.00423	113.15049
Sample 11	7.32411	92.03746
Sample 12	9.24928	116.22985
Sample 13	8.17704	102.75574
Sample 14	6.8037	85.49778
Sample 15	8.29335	104.21729

Table 4. Maximum load in compressive strength and maximum load values of the B2 group (Bleaching with Biowhiten, then 7-day immersion in artificial saliva)

	Maximum Load in Compressive Strength (Mpa)	Maximum Load (N)
Sample 1	12,0206	151,05534
Sample 2	12,96793	162,95975
Sample 3	12,04281	151,33437
Sample 4	12,35132	155,21123
Sample 5	12,46479	156,63715
Sample 6	11,70796	147,12662
Sample 7	10,60577	133,276
Sample 8	10,84655	136,3017
Sample 9	12,61438	158,51695
Sample 10	12,55164	157,72852
Sample 11	12,86269	161,63736
Sample 12	10,35314	130,10135
Sample 13	12,31562	154,76268
Sample 14	11,30723	142,09084
Sample 15	11,8003	148,28693

Table 5. Maximum load in compressive strength and maximum load values of the C1 group (Bleaching with Whiteness, then 1-day immersion in artificial saliva)

	Maximum Load in Compressive Strength (Mpa)	Maximum Load (N)
Sample 1	5,25524	66,03925
Sample 2	4,9467	62,16201
Sample 3	7,10893	89,33345
Sample 4	5,3626	67,38843
Sample 5	6,55982	82,43311
Sample 6	6,73301	84,60948
Sample 7	6,57234	82,59052
Sample 8	6,33643	79,62587
Sample 9	6,02028	75,65304
Sample 10	5,32004	66,85361
Sample 11	6,72221	84,47375
Sample 12	5,91598	74,34243
Sample 13	6,48907	81,54401
Sample 14	9,21249	115,76749
Sample 15	7,22559	90,79948

Table 6. Maximum load in compressive strength and maximum load values of the C2 group (Bleaching with Whiteness, then 7-day immersion in artificial saliva)

	Maximum Load in Compressive Strength (Mpa)	Maximum Load (N)
Sample 1	9,24717	116,20336
Sample 2	9,16791	115,20731
Sample 3	9,51105	119,51939
Sample 4	11,41957	143,50259
Sample 5	10,39476	130,62436
Sample 6	9,89366	124,32736
Sample 7	10,77956	135,45998
Sample 8	10,59948	133,19698
Sample 9	10,35494	130,12402
Sample 10	9,87108	124,04366
Sample 11	8,45083	106,19623
Sample 12	9,59335	120,5536
Sample 13	11,62911	146,13573
Sample 14	9,6939	121,81707
Sample 15	8,29891	104,28719

Table 7. Maximum load in compressive strength and maximum load values of the A group (1-week immersion in artificial saliva)

	Maximum Load in Compressive Strength (Mpa)	Maximum Load (N)
Sample 1	16,57483	208,28542
Sample 2	16,82387	211,41502
Sample 3	18,18537	228,52409
Sample 4	16,26562	204,39983
Sample 5	17,34702	217,98911
Sample 6	15,17315	190,67137
Sample 7	16,73567	210,30663
Sample 8	15,46682	194,36179
Sample 9	15,52103	195,04295
Sample 10	14,73362	135,14818
Sample 11	15,26944	191,88138
Sample 12	18,96163	238,27884
Sample 13	16,91128	212,51343
Sample 14	15,87056	199,43532
Sample 15	15,11488	189,93912

Table 8. Evaluating the effect of bleaching agents and waiting period on shear bond strength

Shear Bond Strength	**Type III Sum of Squares**	**Df**	**Mean Square**	**F**	**P**
Bleaching agents	43,308	1	43,308	51,685	0,001*
Waiting period	220,758	1	220,758	263,46	0,001*
Bleaching agents * Waiting period	1,302	1	1,302	1,554	0,218

Two-way ANOVA test **p<0.05*

There is a statistically significant difference between whitening agents in terms of shear bond strength averages (p:0.001; p<0.05) (Table 8).

There is a statistically significant difference in terms of shear bond strength averages between waiting periods (1 and 7 days) (p:0.001; p<0.05) (Table 8).

The common effect of the whitening agent and waiting times on shear bond strength is not statistically significant (p:0.218; p>0.05) (Table 8).

Table 9. Evaluating the effect of bleaching agent types and waiting time on shear bond strength

	Biowhiten	Whiteness
Waiting period	**Ort±SS**	**Ort±SS**
1. day	7,79±0,81 ^{Aa}	6,39±1,05 ^{Ba}
7. day	11,92±0,82 ^{Ab}	9,93±0,96 ^{Bb}

Two-way ANOVA test

Different capital letters (A-B) in rows indicate the difference between whitening agents, different lowercase letters (a-b) in columns indicate the difference between waiting periods.

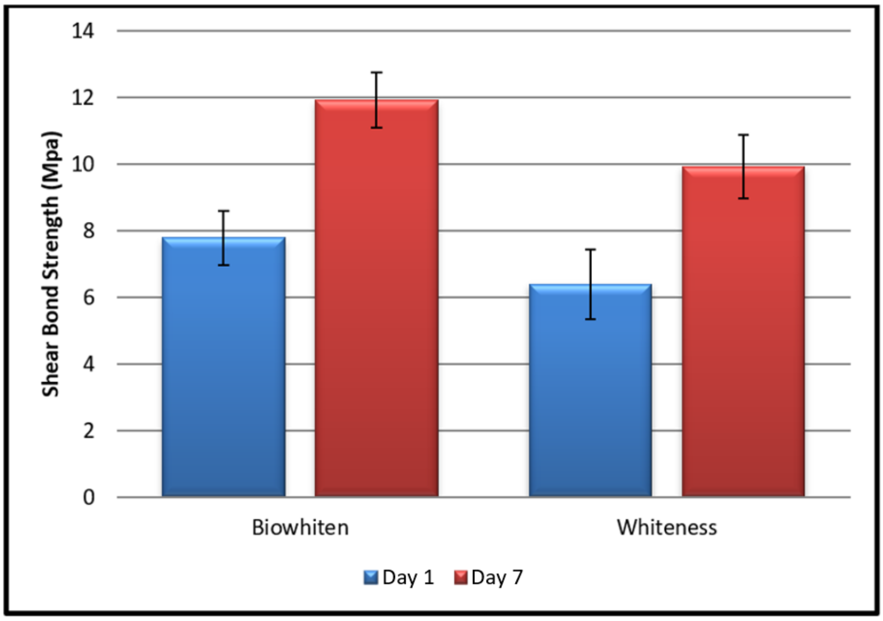

Figure 29. Shear bond strength of B1, B2, C1, C2 groups according to waiting periods

When Biowhiten is used as whitening agent; The shear bond strength average at the end of the 7th day (11.92±0.82) is statistically significantly higher than that at the end of the 1st day (7.79±0.81) (p:0.001; $p<0.05$) (Figure 29).

When Whiteness is used as whitening agent; The shear bond strength average at the end of the 7th day (9.93±0.96) is statistically significantly higher than the shear bond strength average at the end of the 1st day (6.39±1.05) (p:0.001; $p<0.05$) (Figure 29).

The average shear bond strength of the Biowhiten agent (7.79±0.81) at the end of the 1st day is statistically significantly higher than the Whiteness agent (6.39±1.05) (p:0.001; $p<0.05$) (Figure 29).

The average shear bond strength of the Biowhiten agent (11.92±0.82) at the end of the 7th day is statistically significantly higher than the Whiteness agent (9.93±0.96) (p:0.001; $p<0.05$) (Figure 29).

Table 10. Comparisons of whitening groups with the Control group

	Shear Bond Strength Ort±SS	P
Control Group	16,33±1,2	0,001*
Biowhiten 1.day	7,79±0,81	0,001*
Biowhiten 7.day	11,92±0,82	0,001*
Whiteness 1.day	6,39±1,05	0,001*
Whiteness 7.day	9,93±0,96	0,001*

Student t test *p<0.05*

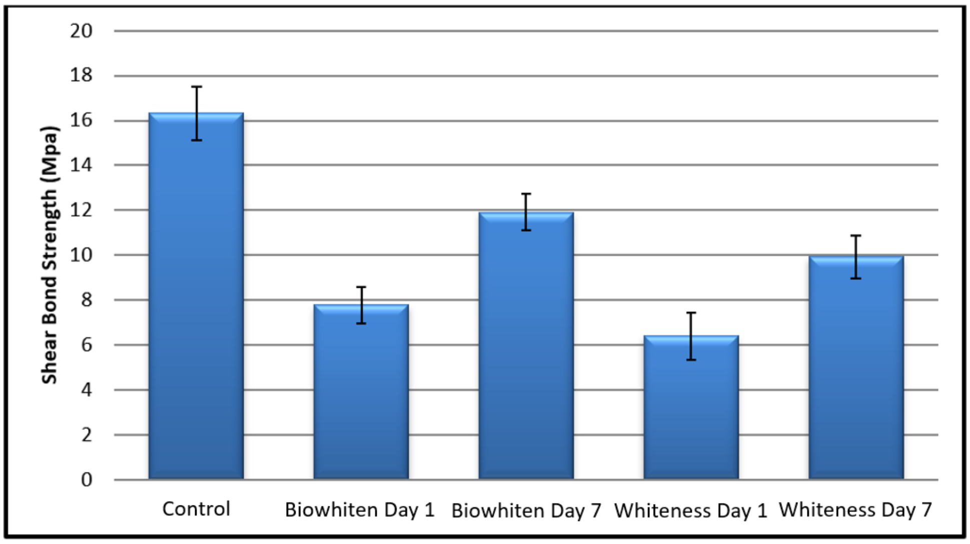

Figure 30. Graphic of shear bond strength of all groups (A, B1, B2, C1, C2)

The shear bond strength average of the control group (16.33±1.2) is statistically significantly higher than the average of the Biowhiten agent on the 1st day (7.79±0.81) (p:0.001; $p<0.05$) (Table 10, Figure 30).

The shear bond strength average of the control group (16.33±1.2) is statistically significantly higher than the average of the Biowhiten agent on the 7th day (11.92±0.82) (p:0.001; $p<0.05$) (Table 10, Figure 30).

The shear bond strength average of the control group (16.33±1.2) is statistically significantly higher than the average of the Whiteness agent on the 1st day (6.39±1.05) (p:0.001; $p<0.05$) (Table 10, Figure 30).

The shear bond strength average of the control group (16.33±1.2) is statistically significantly higher than the average of the Whiteness agent on the 7th day (9.93±0.96) (p:0.001; $p<0.05$) (Table 10, Figure 30).

5 DISCUSSION

In the 21st century, along with humanity's intense interest in aesthetics, there has been an increased demand for aesthetic dentistry as well. With people increasingly desiring a perfect smile, interest in non-invasive whitening has also grown (13).

Whitening agents contain active ingredients, carriers, thickening agents, urea, surface moisturizers, preservatives, and sweetening agents (15). Compounds such as hydrogen peroxide, carbamide peroxide, and sodium perborate serve as active ingredients (16). Hydrogen peroxide, with high solubility, is a preferred active component in whitening agents and is commonly found in various products on the market (24). Among the different concentrations of hydrogen peroxide in whitening products, the most preferred concentrations are typically in the range of 35-38% (16,24).

Peroxides used in whitening penetrate the enamel surface. The concentration of the applied agent affects the penetration depth (58). Additionally, the pH of the used agent has a significant impact on deformations, hardness reduction, and roughness on the enamel surface (51). As the concentration of the agent increases and the pH decreases, the deformations on the enamel surface also increase (51).

Some new whitening agents in the market aim to minimize reversible or irreversible damage occurring on the enamel by incorporating nano additives. The addition of nanohydroxyapatite (n-HAp) to whitening agents aims to minimize demineralization and wear on the enamel surface (27).

Post-whitening bonding to enamel and dentin can be affected by the presence of hydrogen peroxide (128). It has been observed that the bonding of composite resin to whitened enamel is lower compared to non-whitened enamel (129). The resin tags in whitened enamel are fewer in number and shorter (128). An increase in porosity and a decrease in microhardness in whitened enamel after whitening negatively affect bonding (134). Due to the residual oxygen released during the oxidation reaction during whitening, it has been found that there are areas of non-bonding at the enamel-composite interface when immediate composite bonding is performed after whitening, and polymerization does not occur effectively (136).

To avoid potential clinical problems arising from reduced bonding strength after whitening, it is recommended to delay post-whitening restoration for a period ranging from 24 hours to 3 weeks (142). However, assessing the effectiveness of bonding to enamel after whitening is not only crucial in in vitro studies but also highly significant in in vivo studies. In in vivo studies, bonding strength cannot be directly measured, and for evaluating bonding, long-term clinical follow-up is required to detect microleakage and restoration color changes (171). However, there are researchers who argue for the presence of changes on the enamel surface that affect post-whitening bonding (172-174), while some researchers contend that there are no significant differences on the enamel surface after whitening (175-178). In conclusion, although there is no definitive consensus, the continuous introduction of new whitening products to the market has led to a rapid increase in studies examining changes that may occur on the enamel surface after whitening. Research in this area includes investigations into the bonding of composite and porcelain restorations to enamel after whitening, as well as studies on the marginal leakage of restorations (179,180).

After tooth whitening, a decrease in bond strength has been reported, and many studies are being conducted to improve this condition. To address this issue, the use of phosphoric acid in the composite resin bonding procedure after whitening has been experimented with by many researchers. In our study, we utilized the one-bottle universal bonding system, GC Premiobond. Following the manufacturer's recommendations, the enamel was etched with 37% phosphoric acid for 15 seconds, then the universal bond was applied to the surface for 10 seconds and polymerized for 20 seconds. However, in our study, a statistically significant difference was found between the enamel bond strength of the samples stored in artificial saliva at 37 °C for 24 hours after whitening and the samples in the non-whitened control group. M. Miyazaki and colleagues conducted a study on bovine teeth, evaluating the enamel bond strength using two-step self-etching primer systems without phosphoric acid application after in-office whitening (Hi-Lite). After surface smoothing with 600-grit SiC paper for standardization, the Hi-Lite whitening agent was applied to the enamel surface. After the whitening procedure, the samples were stored in distilled water at 37 °C for 24 hours, and then the bonding procedure was completed using three different self-etching primer systems (Imperva Fluora Bond, Mac Bond II, Clearfil SE Bond) and the control material, the one-bottle adhesive system Single Bond. After another 24-hour immersion in distilled water at 37 °C, a slight difference in enamel bond strength was observed between the non-whitened control group and the samples immersed in distilled water for 24 hours at 37 °C after whitening. However, this difference was not statistically significant. The result of this study suggests that, after 24 hours of post-whitening waiting period, using two-step self-etching primer systems can result in less impact on enamel bond strength (181). Considering that the same bonding agent was used for both the control and other groups, and phosphoric acid application was applied to all samples, the differences found in our results could be attributed to the effectiveness of the whitening agent used in the study conducted by Miyazaki and colleagues.

Nowadays, manufacturers have developed universal adhesive systems with the aim of creating an adhesive system suitable for use on every surface. Universal adhesive systems can be used with or without prior acid etching, and selective etching, which involves acid etching only the enamel surface, is also among the recommended methods. It is claimed that universal adhesives can bond to dental tissues as well as other dental materials (182). However, issues related to the bonding lifespan of these systems have been reported(183). In our study, all specimens, including the control group, were acid-etched for 15 seconds with 37% phosphoric acid before the bonding procedure, then rinsed for 30 seconds and dried. Subsequently, universal GC Premio-bond was applied to the enamel surface for 10 seconds, ensuring spreading with air spray, and polymerized for 20 seconds. The high bond strength observed in the control group without tooth whitening in our study supports the idea that combining universal adhesive systems with phosphoric acid application can achieve high bond strength results. Additionally, the presence of residual oxygen on the surface after tooth whitening may contribute to increased bonding by being removed with the effect of acid application in etch & rinse systems. Uçar and colleagues conducted a study in 2019 using 60 mandibular premolar teeth where vital whitening was performed. Subsequently, a whitening agent containing 40% hydrogen peroxide (Opalescence Boost; Eltradent, USA) was applied every three days for 40 minutes, and all teeth were immersed in artificial saliva for two weeks. After the waiting period, the teeth were divided into 4 groups of 15. Two of these groups were treated with universal adhesive in the etch and rinse system, one received universal adhesive in the self-etch system, and another had universal adhesives applied without acid etching. The aim was to determine the effect of universal adhesives on the bond strength of whitened teeth. After the application of the adhesive system and composite resin, the specimens were stored in artificial saliva at 37 °C for 24 hours. According to the results of the one-way ANOVA test, it was determined that the bond strength of the Single Bond

Universal group used in the etch-and-rinse system was statistically significantly higher than the other groups, followed by the One Step Universal group without acid etching. No statistical difference was observed between the other two groups. In this context, it is thought that the shear bond strength values of universal adhesives will increase when used in the etch & rinse system(184).

Halabi and colleagues investigated the effect of two different self-etch adhesive systems and two different waiting periods on bond strength after teeth whitening on bovine teeth. For the adhesive system, they used Clearfil SE Bond 2 (Kuraray, Japan), a two-step self-etch adhesive, and G Premio Bond (GC, Japan), a universal adhesive, both applied in a one-step self-etch mode. The whitening agent used was 35% HP Ti ON In-Office (GC). 144 teeth divided into three main groups, each containing 48 samples: the group subjected to bonding immediately after whitening, the group subjected to one week of waiting after whitening, and the control group with no whitening. Later, each of these three main groups was further divided into four subgroups: those are treated with Clearfil SE Bond, treated with Clearfil SE Bond after the application of 40% phosphoric acid, treated with G Premio Bond, and treated with G Premio Bond after the application of 40% phosphoric acid. The samples subjected to waiting periods were kept in distilled water at 37 °C for seven days. According to the results of the shear bond strength (SBS) test, the bond strength of the control groups with no whitening was statistically significantly higher than the other groups. However, in the SE Bond applied control groups, the bond strength of samples with acid etching (40.7 +/- 6.1 MPa) was statistically significantly higher than the bond strength in the self-etch mode (23.1 +/- 6). In the G Premio Bond applied control group, although there was no significant difference between the bond strength in the self-etch mode (18.3 +/- 5.4) and samples with acid etching (16.8 +/- 4.1), the self-etching group had a higher bond strength. In both bonding applications, it is observed that acid etching, except for the GP Bond control group, increases the bond strength. When evaluated in terms of waiting period, it was statistically significantly higher that the bond strength of samples with a one-week waiting period compared to those with a one-day waiting period. Regarding the bonding agent used, it is noted that the bond strength of all samples using SE Bond is statistically significantly higher than G Premio Bond samples. The highest bond strength was found in the control group treated with SE Bond after acid etching (185). In our study, G Premio Bond was combined with acid etching in all samples. However, in our study, the bond strength of the control group with no whitening was statistically significantly higher than the samples subjected to one day and one week of waiting, while in this study, no statistically significant difference was found between the bond strength of the control group treated with acid etching and G Premio Bond (16.8 +/- 4.1) and the group treated with acid etching and G Premio Bond after one week of waiting (16.0 +/- 5.6). On the other hand, the difference between the control group treated with SE Bond after acid etching (40.7 +/- 6.1) and the samples waiting for one week (23.3 +/- 5.3) in the same study suggests that this result may be due to technical sensitivity in the study.

Numerous studies have been conducted on the waiting period after teeth whitening. Many authors present findings supporting an increase in bond strength with the extended waiting period following whitening. Our study also concluded that an increase in waiting time corresponds to an increase in bond strength. Considering studies conducted under laboratory conditions, it can be suggested that the waiting solution is the factor contributing to this effect. When an artificial saliva is used as the waiting solution, the remineralization process positively influences the bonding. In a study conducted by Titley and colleagues using bovine teeth, in the first group, after applying a 35% HP whitening agent (Drug Trading Co Ltd., Toronto) for 60 minutes, 37% phosphoric acid was applied to the enamel surface. The teeth in the second group were stored in saline solution for 60 minutes and then etched with 37% phosphoric acid for 60 seconds. The teeth in the third group were etched for 60 seconds, followed by the application of a 35% HP whitening agent for 60 minutes. The fourth group, after etching for 60 seconds, was stored in saline solution for 60 minutes. All groups were stored in distilled water at 37 degrees Celsius for half of the specimens for 1 day and the other half for 7 days. After the waiting period bonding procedure was done. After the bonding procedure, the specimens were stored in distilled water at 37 degrees Celsius for 24 hours and then subjected to shear bond strength testing. According to the statistical analyses, the bonding strength of the control group without whitening application (Group 2 & 4) was significantly higher compared to the other groups. However, no significant difference was found among the waiting periods (186).

The reduction in bond strength after bleaching can lead to several clinical problems. Waiting for a certain period for intraoral demineralization is suggested as one of the methods to avoid these issues (7). In our study, the samples subjected to a one-day waiting period showed considerably lower bonding strengths compared to those subjected to a one-week waiting period. Vyver and colleagues evaluated the effect of waiting time on bond values to the enamel after bleaching. For this, after bleaching with hydrogen peroxide, the enamel was etched with 37% phosphoric acid, followed by a shear bond strength (SBS) test. All samples were randomly divided into four groups. No bleaching was done in the first group. In the second group, composite was immediately bonded to the samples after bleaching. The third group was subjected to a one-week waiting period after bleaching for the bonding procedure, and the fourth group had a two-week waiting period. As a result of the SBS test, the samples in the control group, Group 1, showed the highest bond strength (25.1 +/- 3.7). The subsequent highest values were observed in Group 4 (23.1 +/- 3.7), Group 3 (19.2 +/- 3.2), and Group 2 (14.2 +/- 4.9), respectively. From this, it can be inferred that the immediate bonding values after bleaching were found to be quite low, while a one-week waiting period significantly approached the bonding values to an optimum level (187).

Bittencourt and colleagues used unerupted third molars to examine the effect of post-bleaching waiting time on shear bond strength. The teeth were divided into 200 fragments, with 100 each for transversal and longitudinal sections, comprising 100 enamel and 100 dentin fragments. The samples were divided into five groups. The first group served as the control, with no bleaching applied. The second group received the application of 35% Whiteness HP (FGM, Brazil) immediately followed by the application of composite resin on the tooth surface. The third group underwent a waiting period of 7 days for bonding procedures after bleaching, the fourth group waited for 14 days, and the fifth group waited for 21 days. The shear bond strength (SBS) test results revealed that the control group's bond strength was statistically significantly higher compared to the group undergoing immediate restorative treatment (Group 2). However, among all enamel and dentin segment samples, no statistically significant difference was found in bond strengths among the control group and those with 1-week, 2-week, and 3-week waiting periods (188). In contrast, our study found that, after a 1-week waiting period, all samples treated with both Biowhiten and Whiteness bleaching agents had statistically significantly lower bond strength compared to the control group with no bleaching. The reason for this might be that all samples in our study relied on bonding to the enamel surface. Therefore, we believe that a waiting period longer than one week could provide more suitable bonding in ideal conditions.

The changes occurring on the enamel surface after bleaching are known to have a direct impact on adhesion. It is believed that the concentration of the bleaching agent affects post-bleaching adhesion (81). As the concentration increases, enamel hardness decreases, and roughness increases (85). In our study, Power HP (Biowhiten, Turkey) with 40% hydrogen peroxide and Whiteness (FGM, Brazil) with 35% hydrogen peroxide were used. The results indicated that the bond strength of samples treated with 40% HP Biowhiten was significantly higher than those treated with 35% HP Whiteness. We believe that this may be attributed to the higher concentration of hydrogen peroxide in Biowhiten and the presence of n-HAp particles, which could result in fewer defects on the enamel surface. However, Sadeghion and colleagues conducted a study in 2021, examining the shear bond strengths of orthodontic brackets without using adhesive resin on 60 maxillary and mandibular premolar teeth that were bleached using different bleaching protocols. Subsequently, the samples were randomly divided into a total of 4 groups, each consisting of 15 teeth. The first group (Group C) was designated as the control group and immersed in artificial saliva. The second group (Group HB) underwent at-home bleaching with 20% carbamide peroxide (Opalescence; Ultradent Product, USA) for 4 hours each day for 7 days. The third group (Group OB) underwent in-office bleaching with 45% carbamide peroxide (Opalescence; Ultradent Product, USA) for 30 minutes. The fourth group (Group L-OB) underwent in-office bleaching with 40% hydrogen peroxide (Opalescence; Ultradent Product, USA) for 60 seconds, activated by diode laser (810 nm wavelength, 2.5 W) from a distance of 1 mm. As a result, the bond strength of the control group (Group C) was found to be higher (12.04 MPa) compared to the other groups, while the bond strength of Group HB (7.45 MPa) was higher than Group L-OB (7.39 MPa) and Group OB (6.62 MPa). However, the difference among these three groups is not statistically significant. Consequently, it is concluded that bleaching with 20% or 40% carbamide peroxide affects bond strength, but there is no significant difference between them (189).

In our study, despite the higher HP concentration in the Biowhiten agent, the bond strength was higher. From this, it can be considered that when additives supporting remineralization, such as nanohydroxyapatite, are incorporated into the bleaching agent, the increase in concentration may result in less difference in bond strength. However, when studies conducted without the addition of nanoparticles to whitening agents are evaluated, El-Din and colleagues evaluated the bond strength in bovine teeth using different concentrations of bleaching agents. They divided 72 bovine teeth into three main groups, with each group containing 24 teeth. The first group was kept in distilled water at 37 °C without bleaching (control group). The second group was subjected to in-office bleaching with 38% hydrogen peroxide (Opalescence Xtra Boost, Ultradent, USA) for 30 minutes. The third group was bleached with 10% carbamide peroxide (CP) (Opalescence, Ultradent, USA) every day for 6 hours for 5 days. All groups were further divided into two subgroups, with half of the samples using ethanol-based Single Bond (3M ESPE, USA), and the other half using acetone-based One Step (BISCO, USA) adhesive systems immediately after bleaching. When evaluating the SBS test results, it was observed that the bond strength of the non-bleached control groups was the highest, followed by the groups treated with 10% CP. The samples treated with 38% HP exhibited the lowest bond strength. Regarding the applied adhesive systems, although the bond strength in samples where Single Bond adhesive was used was higher than in those using One Step adhesive, there was no statistically significant difference between the two. From this, it can be inferred that increasing the concentration of the bleaching agent independently may lead to a decrease in bond strength (190).

One of the methods tried to increase bonding strength after whitening is to perform reduction on the enamel surface after whitening. In 2019, Cheng and colleagues investigated the effect of post-whitening surface reduction on the enamel's bond strength. In this context, 48 bovine teeth, each group containing 24 teeth, were randomly divided into 2 groups. In group B (bleaching group), samples were subjected to in-office whitening with 40% HP gel (Opalescence, Ultradent, USA) in two sessions, one week apart. The whitening agent was applied to the enamel surface three times during each session, with 15 minutes each time. During the waiting period, all teeth were stored in artificial saliva at 37 degrees Celsius. In group C (control group), samples were not subjected to whitening. After the whitening process, enamel surface reduction was performed by using diamond burs and discs under water cooling, reducing 0.5 mm of the enamel surface. Following this procedure, bonding procedure was done. According to the results of the shear bond strength (SBS) test, the control group without whitening showed the highest bond strength (25.21 +/- 5.53), while the bleaching group exhibited the lowest bond strength (21.41 +/- 7.22). However, it was concluded that the 0.5 mm reduction from the enamel surface after whitening did not alter the bond strength. Cheng and colleagues did not observe statistically significant differences in the impact of reduction from the enamel surface on enamel bonding in teeth subjected to whitening (191).

After whitening, it is known that the increase in porosity at the microscopic level, the decrease in microhardness, and surface irregularities of the enamel surface reduce the bond strength of the enamel. In the oral cavity, the presence of saliva allows numerous demineralization and remineralization cycles, and the buffer effect of saliva helps balance the decreased calcium and phosphate ratio after whitening over time. To mitigate these negative effects on the enamel surface after whitening, many studies have been conducted. One approach is the application of nanohydroxyapatite pastes to the tooth surface before or after whitening, while another involves incorporating nanohydroxyapatite particles into whitening agents (192). Nanohydroxyapatite is a biomaterial that undertakes the task of biologically replacing lost structure. In this context, it is believed that the application of nanohydroxyapatite paste increases Knoop hardness. In our study, the higher bond strength observed in samples treated with a teeth whitening agent containing nanohydroxyapatite supports this notion. Although our study did not assess factors such as microhardness and mineral content, considering the direct impact of these factors on bond strength, it is observed that Whiteness HP 35% agent without n-HAp particles significantly reduced bond strength compared to Biowhiten HP 40% agent containing n-HAp particles. However, there are also studies that present different results in this regard. In a study conducted by Gomes and colleagues in 2017, the effect of applying nanohydroxyapatite paste to the enamel surface before whitening on enamel hardness was investigated. The nanohydroxyapatite paste for this study was prepared under laboratory conditions and stored at 37 °C. Patients were instructed to use fluoride-free toothpaste, a soft toothbrush, and dental floss prophylactically at least 2 weeks before the study. Third molars were preferred for sample teeth. A total of 60 samples (3x3x3mm) were prepared. The study aimed to utilize saliva in the oral environment, and therefore, samples were cemented on the buccal surface of the first molar teeth using Rely X Automix (3M ESPE, Brazil) composite resin. The samples were divided into three groups: the group without whitening, the group whitened with 35% Whiteness HP (FGM, Brazil), and the group whitened with 35% Whiteness HP after the application of nano-HAp paste. The nano-hydroxyapatite paste was obtained by mixing 1g of nano-HAp powder with 1ml of distilled water and applied to the surface for 10 minutes before whitening. After treatment, the composite pieces were removed from the tooth surface using orthodontic pliers. Knoop microhardness was measured before and after whitening, and the final average was calculated. The statistical results of the study suggested that the use of nano-hydroxyapatite paste before whitening was significant in maintaining Knoop hardness and encouraging less mineral loss (193). There is a need for studies comparing the effect of nano-hydroxyapatite paste and whitening agents containing nano-hydroxyapatite on the tooth surface in this regard.

Monterubbianesi and colleagues conducted an in vitro study in 2021 to evaluate the color, hardness, and microstructure of a home-use hydrogen peroxide (HP) whitening agent enriched with nanohydroxyapatite. In the whitening procedure, half of the samples were subjected to whitening with 6% HP (Optident, UK) for at least 50 minutes daily for 7 days, while the other samples underwent whitening with 6% HP (6Hp-nHA) (Biowhiten, Turkey) for the same duration. During the whitening procedure, the teeth were kept in artificial saliva at 37 degrees Celsius. The control group did not undergo any whitening. SEM images revealed irregularities on the enamel surface in samples whitened with Optident, with no discernible enamel rods or interrods. In contrast, samples whitened with Biowhiten exhibited a more organized enamel surface with visible enamel rods and interrods. At the end of the treatment, the microhardness of the control group and the group treated with Biowhiten were similar, while the microhardness of the group treated with Optident was found to be significantly lower than the other two groups, although not statistically significant. The difference in color change between the two groups was not statistically significant (194). From this, although SEM image examinations were not conducted in our study, we believe that minimal changes on the enamel surface could potentially enhance bond strength. The lack of a significant difference in enamel hardness between the two experimental groups in this study may be attributed to the very low concentrations of the agents. Considering the impact of microhardness on bond strength, we believe that further scientific research is needed.

After teeth whitening, some factors affecting bond strength include the decrease in the Ca/P ratio and microhardness value of the enamel surface. In 2018, Kütük and colleagues investigated the effects of desensitizing agents on the enamel surface in in-office whitening. Additionally, in this study, one group had nanohydroxyapatite paste applied before teeth whitening. Extracted human incisors were used in the study. All samples were stored in artificial saliva for 14 days after treatment. The examinations revealed that desensitizing agents applied before or after whitening did not affect color change. However, when combined with these agents, whitening treatments increased the microhardness of enamel after a 14-day waiting period, with no significant change in Ca and P ratios (195). In this context, it is possible to conclude that adding nanohydroxyapatite paste to the whitening agent increases microhardness, indirectly enhancing bond strength.

In our study, the n-HAp-containing Biowhiten whitening agent, as claimed by its manufacturer, increases alkalinity when added to the whitening agent. The alkaline nature of the whitening agent accelerates the oxidation reaction, allowing free radicals to form more quickly and easily (196). On the other hand, the Whiteness HP, another agent used in our study, has a neutral pH value. The higher bond strength in samples whitened with the n-HAp-containing agent suggests that, considering the pH value, the alkalinity of the Biowhiten groups resulted in less surface alteration on the enamel. This, in turn, implies a higher bond strength.

Nanohydroxyapatite is known as a biomaterial. The working principle of a biomaterial is to replace deficient tissue in a biologically suitable manner. In this context, nanohydroxyapatites, having the same particle size as enamel hydroxyapatites, can easily react with them. Dabanoğlu et al. have indicated that hydroxyapatite can accumulate on the tissue, forming a layer without chemically affecting the tooth(196).

Asaki et al. claim that when n-HAp is used with a teeth-whitening agent, minerals are stored on the enamel surface, increasing surface roughness. This, in turn, enhances surface adhesion, reducing adhesive failure (197). A limitation of our study is the lack of surface roughness and SEM evaluations, so the impact of n-HAp particles on teeth whitening in our study should be supported by additional research.

Ferraz and colleagues conducted a study in 2018 to evaluate the effects of adding different concentrations of n-HAp to a 35% hydrogen peroxide (HP) whitening agent on the surface enamel and deep dentin of bovine teeth, as well as the bond strength in enamel. The treatment was applied in five groups as follows: The first group did not undergo whitening (negative control), the second group underwent whitening with 35% HP (Whiteness HP, FGM, Brazil) (3 x 15 minutes) (positive control), the third group underwent whitening with 35% HP + 5% n-HAp agent, the fourth group underwent whitening with 35% HP + 10% n-HAp agent, the fifth group underwent whitening with 35% HP + 15% n-HAp agent. Whitening treatments were performed in three sessions with a one-week interval between each. Throughout the procedure, the samples were stored in artificial saliva at 37℃. After whitening, 24 hours later, the enamel surfaces were treated with 35% phosphoric acid for 30 seconds, followed by the application of Single Bond (3M ESPE, USA) bonding agent for 20 seconds and polymerization. Filtek Z350 XT (3M ESPE, USA) composite resin was used, and after 24 hours, the samples were subjected to the shear bond strength (SBS) test. The examinations revealed that adding n-HAp to the whitening agent did not make a significant difference in terms of whitening effectiveness and did not have a significant impact on bond strength. However, samples whitened with 35% HP + 10% n-HAp showed a lower adhesive failure rate compared to the 35% HP group, similar to the samples without whitening (6). In our study, when the whitening agent containing n-HAp was compared with the Whiteness HP whitening agent used by Ferraz and colleagues, the samples whitened with the n-HAp-containing agent showed a statistically significantly higher bond strength.

6 CONCLUSION

Within the limitations of this in-vitro study, the following conclusions may be drawn

1.The waiting period after teeth whitening affects bond strength, and a one-week waiting period is not sufficient to achieve optimal bonding.

2.The addition of n-HAp biomaterials to teeth whitening agents increases the bonding strength to enamel.

3. One week waiting period is not enough to establish ideal bond strength after whitening with nanohydroxyapatite containing agents.

It was concluded that there was a statistically significant difference in bonding strength between the whitening agent containing n-HAp (Biowhiten) and the other whitening agent (Whiteness HP).

7 REFERENCES

1. Chan MYS, Mehta SB, Banerji S. An evaluation of the influence of teeth and the labial soft tissues on the perceived aesthetics of a smile. Br Dent J. 2017;223(4):272-278.

2. Oliveira MTD, Andrade MACD, Michels M. Oxygen Release, Microleakage, and Shear Bond Strength of Composite Restorations After Home Dental Bleaching. Rev Odonto Cienc. 2011;26:45-49

3. Kum KY, Lim KR, Lee CY, Park KH, Safavi KE, Fouad AF, Spångberg LS. Effects of removing residual peroxide and other oxygen radicals on the shear bond strength and failure modes at resin-tooth interface after tooth bleaching. Am J Dent. 2004;17(4):267-270.

4. Oz FD, Kutuk ZB. Effect of various bleaching treatments on shear bond strength of different universal adhesives and application modes. Restor Dent Endod. 2018;43(2).

5. Xu Y, Zhou J, Tan J. Use of grape seed extract for improving the shear bond strength of total-etching adhesive to bleached enamel. Dent Mater J. 2018;37(2):325-331.

6. Ferraz LN, Júnior WFV, Ambrosano GMB, Giorgi MCC, Aguiar FHB, Lima DANL. Effect of different concentrations of nanohydroxyapatite on tooth bleaching effectiveness and enamel bond strength. Braz Dent Sci. 2018;21(1):17-25.

7. Zaragoza VMT. Bleaching of vital teeth: Technique. Esto Modeo. 1984;9:7-30.

8. Banerjee A, Millar BJ. Minimally Invasive Esthetics. Elsevier; 2015.

9. Haywood VB. Nightguard Vital Bleaching. Quintessence International (Berlin, Germany: 1985). 1989;20:173-176.

10. Matis BA, Gaião U, Blackman D, Schultz FA, Eckert GJ. In vivo degradation of bleaching gel used in whitening teeth. Journal of the American Dental Association. 1999;130(2):227-235.

11. Haywood VB, Heymann HO. Night guard vital bleaching. Quintessence International. 1989;20(3):173-176.

12. Bayırlı G. Diş renkleşmeleri ve Beyazlatma. In: Endodontik Tedavi 1. İstanbul Üniversitesi Basımevi ve Film Merkezi. 1998; 585-62, İstanbul.

13. Chan MYS, Mehta SB, Banerji S. An evaluation of the influence of teeth and the labial soft tissues on the perceived aesthetics of a smile. Br Dent J. 2017;223:272-278.

14. Bezerra-Júnior DM, Silva LM, Martins LM, Cohen-Carneiro F, Pontes DG. Esthetic Rehabilitation with Tooth Bleaching, Enamel Microabrasion, and Direct Adhesive Restorations. Gen Dent. 2016;64(2):60-64.

15. E. Thickett, M.T. Cobourne. New Developments in Tooth Whitening: The Current Status of External Bleaching in Orthodontics. J Orthod. 2009;36(3):194-201.
16. Greenwall L. Bleaching Techniques in Restorative Dentistry: An Illustrated Guide. Martin Dunitz Ltd, London, 2001.
17. Price RB, Sedarous M, Hiltz GS. The pH of Tooth-Whitening Products. J Can Dent Assoc. 2000;66(8):421-426.
18. Fasanaro TS. Bleaching teeth: History, chemicals, and methods used for common discoloration. J Esthet Dent. 1992;4(3):71-78.
19. Gökay O, Müjdeci A. Ağartma ajanları uygulanmış ve uygulanmamış dişlerde restorative materyaller ve mine dokusu arayüz ilişkisinin SEM ile değerlendirilmesi. Ankara Üniversitesi Diş Hekimliği Fakültesi Dergisi. 1998;25(3):229-239.
20. Alaçam T, Uzel İ, Alaçam A, Aydın M. Endodonti. Barış Yayınları. Ankara; 2000; 583-604.
21. Fasanaro TS. Bleaching teeth: history, chemicals, and methods used for common tooth discolorations. J Esthet Restor Dent. 1992; 4(3): 71-8.
22. Walsh LJ. Safety Issues Relating to the Use of Hydrogen Peroxide in Dentistry. Aust Dent J. 2000;45(4):257-269.
23. Karadaş M, Seven N. Vital Dişlerde Beyazlatma. Atatürk Üniversitesi Diş Hekimliği Fakültesi Dergisi. 2014; 9(9): 126-35
24. Feinman RA, Goldstein RE, Garber DA. Bleaching Teeth. Quintessence Publishing, Chicago, 1987.
25. Frysh H, Bowles W, Baker F, Rivera-Hidalgo G, Guillen G. "Effect of pH on bleaching efficiency." J Dent Res. 1993; 72:384.
26. Carlsson J. "Salivary peroxidase: An important part of our defence against oxygen toxicity." J Oral Pathol. 1987; 16:412-416.
27. Ferraz LN, Júnior WFV, Ambrosano GMB, Giorgi MCC, Aguiar FHB, Lima DANL. Effect of different concentrations of nanohydroxyapatite on tooth bleaching effectiveness and enamel bond strength. Braz Dent Sci. 2018;21(1):17-25.
28. Joiner A. Tooth colour: a review of the literature. J Dent. 2004;32(1):3-12.
29. Carey CM. Tooth Whitening: What We Now Know. J Evid Based Dent Pract. 2014;14:70-76.
30. Roberson TM, Lundeen TF. Sturdevant's Art And Science Of Operative Dentistry Cariology (5 Bs.). St. Louis, Missouri: Mosby Elsevier; 2006.

31. Fincham AG, Moradian-Oldak J, Simmer JP. The structural biology of the developing dental enamel matrix. J Struct Biol. 1999;126:270-299.
32. Berkovitz BKB, Holland GR, Moxham BJ. Oral anatomy, embryology and histology. Mosby Incorporated; 2002.
33. Elliott JC, Wilson RM, Dowker SEP. Apatite Structures. International Centre for Diffraction Data, Advances in X-Ray Analysis. 2002;45.
34. Boyde A. Enamel. In: Oksche A, Vollrath L, editors. Handbook of Microscopic Anatomy. Berlin: Springer Verlag; 1989. p. 309-473.
35. Robinson C, Shore RC, Brookes SJ, Strafford S, Wood SR, Kirkham J. The chemistry of enamel caries. Crit Rev Oral Biol Med. 2000;11(4):481-495.
36. Roberson TM, Heymann HO, Swift EJ. Sturdevant's Art and Science of Operative Dentistry Cariology. 5th ed. Orlando; 2002. p. 476-483.
37. Roy S, Basu B. Mechanical and tribological characterization of human tooth. Materials Characterization. 2008;59(6):747-756.
38. Piesco NP, Simmelink J. Histology of enamel. In: Avery JK, editor. Oral Development and Histology. 3rd ed. New York: Thieme; 2002. p. 153-171.
39. Bishara SE, Ostby AW. White spot lesions: Formation, prevention, and treatment. Semin Orthod. 2008;14:174-182.
40. Rozzi RF. Enamel structure and development and its application in hominid evolution and taxonomy. J Hum Evol. 1998;35:327-330.
41. Veis A. A Window On Biomineralization. Science. 2005;307:1419-1420.
42. Zachrisson BU, Brobakken BO. Clinical Comparison Of Direct Versus Indirect Bonding With Different Bracket Types And Adhesives. Am J Orthod Dentofacial Orthop. 1978;74:62-78.
43. Lippert F, Butler A, Lynch RJM. Characteristics Of Methylcellulose Acid Gel Lesions Created In Human And Bovine Enamel. Caries Res. 2013;47(1):50-5.
44. Lippert F, Hara AT. Fluoride Dose-Response Of Human And Bovine Enamel Caries Lesions Under Remineralizing Conditions. Am J Dent. 2012;25(4):205-9.
45. Mondelli RF, Garrido Gabriel TRC, Piola Rizzante FA, Magalhães AC, Soares Bombonatti JF, Ishikiriama SK. Do Different Bleaching Protocols Affect the Enamel Microhardness? Eur J Dent. 2015;9(1):25-30.
46. Markovic L, Jordan RA, Lakota N, Gaengler P. Micromorphology of Enamel Surface after Vital Tooth Bleaching. J Endod. 2007;33(5):607-10.

47. Potocnik I, Koseck L, Gaspersic D. Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content. J Endod. 2000;26:203-206.
48. McCracken MS, Haywood VB. Effects of 10% carbamide peroxide on the subsurface hardness of enamel. Quint Int. 1995;26:21.
49. Rotstein I, Dankner E, Goldman A, Heiling I, Stabholz A. Histochemical analysis of dental tissues following bleaching. J Endod. 1996;22:23.
50. Furlan IS, Bridi EC, Amaral F, França F, Turssi CP, Basting RT. Effect of High- or Low-Concentration Bleaching Agents Containing Calcium and/or Fluoride on Enamel Microhardness. Gen Dent. 2017;65(3):66-70.
51. Eva K, Marijan M, Mira R, Ivan S, Katica P, Zrinka T. Surface Changes of Enamel and Dentin after Two Different Bleaching Procedures. Acta Clin Croat. 2013;52(4):413-429.
52. Rodrigues JA, Basting RT, Serra MC, Rodrigues AL Jr. Effects of 10% Carbamide Peroxide Bleaching Materials on Enamel Microhardness. Am J Dent. 2001;14(2):67-71.
53. Mundra S, Mohan V, Gwyer J, Young N, Franklin SE, Gerhardt LC. Hardness, Friction, and Wear Studies on Hydrogen Peroxide Treated Bovine Teeth. Tribol Int. 2015;89:109-118.
54. Araujo FO, Baratieri L, Araújo E. In Situ Study of In-Office Bleaching Procedures Using Light Sources on Human Enamel Microhardness. Oper Dent. 2010;35(2):139-146.
55. Coceska E, Gjorgievska E, Coleman NJ, Gabric D, Slipper IJ, Stevanovic M, Nicholson JW. Enamel Alteration Following Tooth Bleaching and Remineralization. J Microsc. 2015;232:44
56. Jurema ALB, de Souza MY, Torres CRG, Borges AB, Caneppele TMF. Effect of pH on Whitening Efficacy of 35% Hydrogen Peroxide and Enamel Microhardness. J Esthet Restor Dent. 2018;30(2):E39-E44
57. Basting RT, Rodrigues AL Jr, Serra MC. The Effect of 10% Carbamide Peroxide, Carbopol and/or Glycerin on Enamel and Dentin Microhardness. Oper Dent. 2005;30(5):608-616.
58. Demarco FF, Meireles SS, Sarmento HR, Dantas RVF, Botero T, Tarquinio SBC. Erosion and Abrasion on Dental Structures Undergoing At-Home Bleaching. Clin Cosmet Investig Dent. 2011;3:45-52

59. George L, Baby A, Dhanapal TP, Charlie KM, Joseph A, Varghese AA. Evaluation and Comparison of the Microhardness of Enamel after Bleaching with Fluoride-Free and Fluoride-Containing Carbamide Peroxide Bleaching Agents and Post-Bleaching Anticay Application: An In Vitro Study. Contemp Clin Dent. 2015;6(Suppl 1):S163-S166
60. Batista G, Barcellos D, Torres C, Goto E, Pucci C, Borges AB. The Influence of Chemical Activation on Tooth Bleaching Using 10% Carbamide Peroxide. Oper Dent. 2011;36(2):162-168
61. Ziemba S, Felix H, MacDonald J, Ward M. Clinical Evaluation of a Novel Dental Whitening Lamp and Light-Catalyzed Peroxide Gel. J Clin Dent. 2004;16(4):123-127
62. Polydorou O, Hellwig E, Hahn P. The Efficacy of Three Different In-Office Bleaching Systems and Their Effect on Enamel Microhardness. Oper Dent. 2008;33(5):579-586
63. Silva AF, Demarco FF, Meereis CT, Cenci MS, Piva E. Light-Activated Bleaching: Effects on Surface Mineral Change on Enamel. J Contemp Dent Pract. 2014;15(5):567-572.
64. Angelova Volponi A, Zaugg LK, Neves V, et al. Tooth Repair and Regeneration. Curr Oral Health Rep. 2018;5(4):295-303.
65. Fontana M, Young DA, Wolff MS, Pitts NB, Longbottom C. Defining Dental Caries for 2010 and Beyond. Dent Clin North Am. 2010;54:423-440.
66. Featherstone JD. The Science and Practice of Caries Prevention. J Am Dent Assoc. 2000;131:887-899
67. Hara AT, Zero DT. The Caries Environment: Saliva, Pellicle, Diet, And Hard Tissue Ultrastructure. Dent Clin North Am. 2010;54:455-467.
68. Pamir T. Başlangıç Çürüklerinin Durdurulması ve Ozon ve Sealantların Kullanımı. Turkiye Klinikleri J Dent Sci - Spec Top. 2014;5(3):43-60.
69. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A. Dental Caries. Nat Rev Dis Primers. 2017;3:1-16.
70. Cervantes A, Bolanho A, Valera MC, Araújo MA. Microhardness Study of Bovine Enamel to Bleaching Treatment Activated for Different Sources of Light. Braz Dent Sci. 2006; 9:78-86..
71. Giannini M, Silva AP, Cavalli V, Paes Leme AF. Effect of Carbamide Peroxide-Based Bleaching Agents Containing Fluoride or Calcium on Tensile Strength of Human Enamel. J Appl Oral Sci. 2006;14:82-87.

72. Farooq I, Bugshan A. The Role of Salivary Contents and Modern Technologies in the Remineralization of Dental Enamel: A Narrative Review. F1000Res. 2020;9:171.
73. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New Approaches to Enhanced Remineralization of Tooth Enamel. J Dent Res. 2010;89(11):1187-1197.
74. Schmidlin P, Zobrist K, Attin T, Wegehaupt F. In vitro Re-hardening of Artificial Enamel Caries Lesions Using Enamel Matrix Proteins or Self-assembling Peptides. J Appl Oral Sci. 2016;24(1):31-36.
75. Selwitz RH, Ismail AI, Pitts NB. Dental Caries. Lancet. 2007;369:51-59.
76. Kim HW, Kim HE, Knowles JC. Fluor-hydroxyapatite Sol-gel Coating on Titanium Substrate for Hard Tissue Implants. Biomaterials. 2004;25(14):3351-3358.
77. Featherstone JD. Dental Caries: A Dynamic Disease Process. Aust Dent J. 2008;53:286-291.
78. Buzalaf MA, Pessan JP, Honorio HM, ten Cate JM. Mechanisms of Action of Fluoride for Caries Control. Monogr Oral Sci. 2011;22:97-114.
79. Moi GP, Tenuta LM, Cury JA. Anticaries Potential of a Fluoride Mouthrinse Evaluated In Vitro by Validated Protocols. Braz Dent J. 2008;19:91-68.
80. Groeneveld A, Van Eck AA, Backer Dirks O. Fluoride in Caries Prevention: Is the Effect Pre- or Post-Eruptive? J Dent Res. 1990;69(Spec No):751-5; discussion 820-3
81. Bayrak S, Tunc ES, Sonmez IS, Egilmez T, Ozmen B. Effects of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) Application on Enamel Microhardness After Bleaching. Am J Dent. 2009;22:393-396.
82. White DJ, Featherstone JD. A Longitudinal Microhardness Analysis of Fluoride Dentifrice Effects on Lesion Progression In Vitro. Caries Res. 1987;21:502-512.
83. Marinho VC, Higgins JP, Logan S, Sheiham A. Topical Fluoride (Toothpastes, Mouthrinses, Gels, or Varnishes) for Preventing Dental Caries in Children and Adolescents. Cochrane Database Syst Rev. 2003;4:CD002782.
84. Jiang H, Bian Z, Tai BJ, Du MQ, Peng B. The Effect of a Bi-Annual Professional Application of APF Foam on Dental Caries Increment in Primary Teeth: 24-Month Clinical Trial. J Dent Res. 2005;84:265-268.
85. Alkilzy M, Tarabaih A, Santamaria RM, Splieth CH. Self-assembling Peptide P11-4 and Fluoride for Regenerating Enamel. J Dent Res. 2017;97(2):148-154.
86. Moradian-Oldak J. Protein-Mediated Enamel Mineralization. Front Biosci (Landmark Ed). 2012;17:1996-2023.

87. Yang Y, Lv XP, Shi W, Li JY, Li DX, Zhou XD, Zhang LL. 8DSS-Promoted Remineralization of Initial Enamel Caries In Vitro. J Dent Res. 2014;93(5):520-524.
88. Liang K, Xiao S, Shi W, Li J, Yang X, Gao Y, Li J. 8DSS-Promoted Remineralization of Demineralized Dentin In Vitro. J Mater Chem B. 2015;3(33):6763-6772.
89. Zheng W, Ding L, Wang Y, Han S, Zheng S, Guo Q, Li W, Zhou ZX, Zhou X, Zhang L. The Effects of 8DSS Peptide on Remineralization in a Rat Model of Enamel Caries Evaluated by Two Nondestructive Techniques. J Appl Biomater Funct Mater. 2019;17(1).
90. Yang Y, Lv X, Shi W, Zhou X, Li J, Zhang L. Synergistic Inhibition of Enamel Demineralization by Peptide 8DSS and Fluoride. Caries Res. 2016;50(1):32-39.
91. Chen M, Yang J, Li J, Liang K, He L, Lin Z, Li J. Modulated Regeneration of Acid-Etched Human Tooth Enamel by a Functionalized Dendrimer That Is an Analog of Amelogenin. Acta Biomater. 2014;10(10):4437-4446.
92. Dawasaz AA, Togoo RA, Mahmood Z, Azlina A, Ponnuraj KT. Effectiveness of Self-Assembling Peptide (P11-4) in Dental Hard Tissue Conditions: A Comprehensive Review. Polymers (Basel). 2022;14(4):792
93. Alkilzy M, Santamaria RM, Schmoeckel J, Splieth CH. Treatment of Carious Lesions Using Self-Assembling Peptides. Adv Dent Res. 2018;29(1):42-47.
94. Schlee M, Schad T, Koch JH, Cattin PC, Rathe F. Clinical Performance of Self-Assembling Peptide P11-4 in the Treatment of Initial Proximal Carious Lesions: A Practice-Based Case Series. J Investig Clin Dent. 2018;9(1)
95. Schmidlin P, Zobrist K, Attin T, Wegehaupt F. In Vitro Re-hardening of Artificial Enamel Caries Lesions Using Enamel Matrix Proteins or Self-Assembling Peptides. J Appl Oral Sci. 2016;24(1):31-36.
96. Mukherjee K, Ruan Q, Liberman D, White SN, Moradian-Oldak J. Repairing Human Tooth Enamel with Leucine-Rich Amelogenin Peptide–Chitosan Hydrogel. J Mater Res. 2016;31(05):556-563.
97. Prajapati S, Ruan Q, Mukherjee K, Nutt S, Moradian-Oldak J. The Presence of MMP-20 Reinforces Biomimetic Enamel Regrowth. J Dent Res. 2018;97:84-90.
98. Sun M, Wu N, Chen H. Laser-assisted Rapid Mineralization of Human Tooth Enamel. Sci Rep. 2017;7(1).
99. Bordea IR, Candrea S, Alexescu GT, Bran S, Băciuț M, Băciuț G, Todea DA. Nano-hydroxyapatite Use in Dentistry: A Systematic Review. Drug Metab Rev. 2020:1-14.

100. Pitts NB, Wright JP. Reminova and EAER: Keeping Enamel Whole through Caries Remineralization. Adv Dent Res. 2018;29(1):48-54.
101. Barakat NAM, Khil MS, Omran AM, Sheikh FA, Kim HY. Extraction of Pure Natural Hydroxyapatite from the Bovine Bones Bio Waste by Three Different Methods. J Mater Process Technol. 2009;209(7):3408-3415.
102.Besinis A, van Noort R, Martin N. Infiltration of Demineralized Dentin with Silica and Hydroxyapatite Nanoparticles. Dent Mater. 2012;28(9):1012-1023.
103. Souza BM, Comar LP, Vertuan M, Fernandes Neto C, Buzalaf MAR, Magalh~aes AC. Effect of an Experimental Paste with Hydroxyapatite Nanoparticles and Fluoride on Dental Demineralisation and Remineralisation In Situ. Caries Res. 2015;49(5):499-507.
104. Tao J, Pan H, Zeng Y, Xu X, Tang R. Roles of Amorphous Calcium Phosphate and Biological Additives in the Assembly of Hydroxyapatite Nanoparticles. J Phys Chem B. 2007;111(47):13410-8.
105. Görken FN, Erdem AP, İkikarakayalı G, Sepet E. Nano-hidroksiapatitli (nHap) Diş Macunlarının Mine Remineralizasyonu Üzerine Etkileri. Istanbul Univ Dis Hekim Fak Derg. 2013;47(2):81-88.
106. Huang S, Gao S, Cheng L, Yu H. Remineralization Potential of Nano-Hydroxyapatite on Initial Enamel Lesions: An In Vitro Study. Caries Res. 2011;45(5):460-468.
107. Lv KL, Zhang JX, Meng XC, Li XY. Remineralization Effect of the Nano-HA Toothpaste on Artificial Caries. Key Eng Mater. 2007;330-332:267-270.
108. Kani T, Kani M, Isozaki A, Shintani H, Ohashi T, Tokumoto T. Effect of Apatite-Containing Dentifrices on Dental Caries in School Children. J Dent Health. 1989;39(1):104-109.
109. Ekambaram M, Mohd Said SNB, Yiu CKY. A Review of Enamel Remineralisation Potential of Calcium- and Phosphate-based Remineralisation Systems. Oral Health Prev Dent. 2017;15(5):415-420.
110. Koçyiğit C. Başlangıç Mine Lezyonlarının Remineralizasyonunda Florid İçeren ve İçermeyen Nanohidroksiapatitli Diş Macununun Etkinliğinin Değerlendirilmesi. Yayınlanmamış Doktora Tezi, Ankara: Ankara Üniversitesi Sağlık Bilimleri Enstitüsü; 2015.
111. Fujimaru T, Ishizaki T, Hayman R. Benign Shift in the Oral Flora Induced by Nano-Hydroxyapatite. J Dent Res. 2015;94

112. Fernando JR, Shen P, Sim CPC, Chen Y, Walker GD, Yuan Y, Reynolds C, Stanton DP, MacRae CM, Reynolds EC. Self-assembly of dental surface nanofilaments and remineralisation by SnF2 and CPP-ACP nanocomplexes. Sci Rep. 2019;9:1285.
113. Reynolds EC. Remineralization of Enamel Subsurface Lesions by Casein Phosphopeptide-Stabilized Calcium Phosphate Solutions. J Dent Res. 1997;76:1587-1595.
114. Kamath U, Sheth H, Mullur D, Soubhagya M. The Effect of Remin Pro on Bleached Enamel Hardness: An In-Vitro Study. Indian J Dent Res. 2013;24:690-693.
115. Rose RK. Effects of an anticariogenic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques. Arch Oral Biol. 2000;45:569-575.
116. Jiang H, Bian Z, Tai BJ, Du MQ, Peng B. The Effect of a Bi-Annual Professional Application of APF Foam on Dental Caries Increment in Primary Teeth: 24-Month Clinical Trial. J Dent Res. 2005;84:265-268.
117. Reynolds EC. Anticariogenic Complexes of Amorphous Calcium Phosphate Stabilized by Casein Phosphopeptides: A Review. Spec Care Dentist. 1998;18:8-16.
118. Gama Cunha AG, De Vasconcelos M, Alcantara A, Dutra Borges BC, De Oliveira Vitoriano J, Alves-Junior C, Machado CT, Dos Santos AJS. Efficacy of In-Office Bleaching Techniques Combined with the Application of a Casein Phosphopeptide-Amorphous Calcium Phosphate Paste at Different Moments and Its Influence on Enamel Surface Properties. Microsc Res Tech. 2012;75(8):1019-1025
119. Penumatsa NV, Kaminedi RR, Baroudi K, Barakath O. Evaluation of Remineralization Capacity of Casein Phosphopeptide-Amorphous Calcium Phosphate on the Carbamide Peroxide Treated Enamel. J Pharm Bioallied Sci. 2015;7(Suppl 2):S583-S586.
120. da Rosa NT, Alexandrino L, de Lima GYS, de Melo AC, Alves E, Silva C. An In Situ Evaluation of Bioactives on the Morphology of Bleached Enamel. J Contemp Dent Pract. 2015;17(3):192-197.
121. Karlinsey RL, Pfarrer AM. Fluoride Plus Functionalized βTCP. Adv Dent Res. 2012;24(2):48-52.
122. Takeshita EM, Danelon M, Castro LP, Cunha RF, Delbem ACB. Remineralizing Potential of a Low Fluoride Toothpaste with Sodium Trimetaphosphate: An In Situ Study. Caries Res. 2016;50(6):571-578.

123. Fernando JR, Shen P, Sim CPC, Chen Y, Walker GD, Yuan Y, Reynolds C, Stanton DP, MacRae CM, Reynolds EC. Self-assembly of dental surface nanofilaments and remineralisation by SnF2 and CPP-ACP nanocomplexes. Sci Rep. 2019;9:1285.
124. Freire IR, Pessan JP, Amaral JG, Martinhon CCR, Cunha RF, Delbem ACB. Anticaries Effect of Low-Fluoride Dentifrices with Phosphates in Children: A Randomized, Controlled Trial. J Dent. 2016;50:37-42.
125. Huang X, Deng M, Liu M, Cheng L, Exterkate RAM, Li J, Zhou X, Ten Cate JM. Comparison of Composition and Anticaries Effect of Galla Chinensis Extracts with Different Isolation Methods. Open Dent J. 2017;11:447-459.
126. Islam SM, Hiraishi N, Nassar M, Sono R, Otsuki M, Takatsura T, Yiu C, Tagami J. In vitro Effect of Hesperidin on Root Dentin Collagen and De/Remineralization. Dent Mater J. 2012;31:362-367.
127. Farooq I, Ali S, Siddiqui IA, et al. Influence of Thymoquinone Exposure on the Micro-Hardness of Dental Enamel: An In Vitro Study. Eur J Dent. 2019;13(3):318–322.
128. Nathoo S, Chmielewski M, Kirkup R. Effects of Colgate Platinum Professional Toothwhitening System on Microhardness of Enamel, Dentin, and Composite Resins. Compend Suppl. 1993;17:S627-S630.
129. Sung EC, Chan M, Mito R, Caputo AA. Effect of Carbamide Peroxide Bleaching on the Shear Bond Strength of Composite to Dental Bonding Agent Enhanced Enamel. J Prosthet Dent. 1999;82:595–598.
130. Türkün M, Kaya AD. Effect of 10% Sodium Ascorbate on the Shear Bond Strength of Composite Resin to Bleached Bovine Enamel. J Oral Rehabil. 2004;31(12):1184-1191. https://doi.org/10.1111/j.1365-2842.2004.01369.x
131. Cavalli V, Reis AF, Giannini M, Ambrosano GM. The Effect of Elapsed Time Following Bleaching on Enamel Bond Strength of Resin Composite. Oper Dent. 2001;26:597–602.
132. itley K, Torneck C, Ruse N. The Effect of Carbamide-Peroxide Gel on the Shear Bond Strength of a Microfil Resin to Bovine Enamel. J Dent Res. 1992;71(1):20-24.
133. Josey AL, Meyers IA, Romaniuk K, Symons AL. The Effect of a Vital Bleaching Technique on Enamel Surface Morphology and the Bonding of Composite Resin to Enamel. J Oral Rehabil. 1996;23:244–250.

134. Hegedüs C, Bistey T, Flóra-Nagy E, Keszthelyi G, Jenei A. An Atomic Force Microscopy Study on the Effect of Bleaching Agents on Enamel Surface. J Dent. 1999;27:509–515.
135. Titley KC, Torneck CD, Smith DC, Chernecky R, Adibfar A. Scanning Electron Microscopy Observations on the Penetration and Structure of Resin Tags in Bleached and Unbleached Bovine Enamel. J Endodont. 1991;17:72–75.
136. Titley KC, Torneck CD, Ruse ND. The Effect of Carbamide-Peroxide Gel on the Shear Bond Strength of a Microfil Resin to Bovine Enamel. J Dent Res. 1992;71:20–24.
137. Torneck CD, Titley KC, Smith DC, Adibfar A. Effect of Water Leaching on the Adhesion of Composite Resin to Bleached and Unbleached Bovine Enamel. J Endodont. 1991;17:156–160.
138. Miles PG, Pontier J, Bahiraei D, Close J. The Effect of Carbamide Peroxide Bleach on the Tensile Bond Strength of Ceramic Brackets: An In vitro Study. Am J Orthodont Dentofac Orthop. 1994;106:371–375.
139. Zhao H, Li X, Wang J, Qu S, Weng J, Zhang X. Characterization of Peroxide Ions in Hydroxyapatite Lattice. J Biomed Mater Res. 2000;52:157–163.
140. Lai SCN, Tay FR, Cheung GSP, Mak YF, Carvalho RM, Wei SHY, Toledano M, Osorio R, Pashley DH. Reversal of Compromised Bonding in Bleached Enamel. J Dent Res. 2002;81:477–481.
141. Lai SCN, Mak YF, Cheung GSP, Osorio R, Toledano M, Carvalho RM, Tay FR, Pashley DH. Reversal of Compromised Bonding to Oxidized Etched Dentin. J Dent Res. 2001;80:1919–1924.
142. Titley K, Torneck C, Smith D, Chernecky R, Adibfar A. Scanning electron microscopy observations on the penetration and structure of resin tags in bleached and unbleached bovine enamel. J Endod. 1991;17(2):72-5.
143. Garcia-Godoy F, Dodge W, Donohue M, O'quinn J. Composite resin bond strength after enamel bleaching. Oper Dent. 1993;18:144-
144. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Sinono Y, Fernandes CA, Tay F. The microtensile bond test: a review. J Adhesive Dent. 1999;1:299-309.
145. Choudhary K, Nandlal B. Comparative evaluation of shear bond strength of nano-hydroxyapatite incorporated glass ionomer cement and conventional glass ionomer

cement on dense synthetic hydroxyapatite disk: An in vitro study. Indian J Dent Res. 2015;26:170-5.

146. Sirisha K, Rambabu T, Shankar YR, Ravikumar P. Validity of bond strength tests: A critical review: Part I. J Conserv Dent. 2014;17:305-11.

147. Devatha AB, Lakshmi MN, Kumar NB, Erukala S, Valluri R, Ealla KKR. A comparative study of shear bond strength of direct bonding system with and without a liquid primer: an in vitro study. J Pharm Bioallied Sci. 2019;11:515-22.

148. Kansal R, Rani S, Kumar M, Kumar S, Issar G. Comparative evaluation of shear bond strength of newer resin cement (RelyX Ultimate and RelyX U200) to lithium disilicate and zirconia ceramics as influenced by thermocycling. Contemp Clin Dent. 2018;9:601-6.

149. Technical specification ISO/TS 11405. Dental materials – testing of adhesion to tooth structure. Switzerland; 2003.

150. Inokoshi M, De Munck J, Minakuchi S, Van Meerbeek B. Meta-analysis of bonding effectiveness to zirconia ceramics. J Dent Res. 2014;93:329-34.

151. Craigs GV. Restorative dental materials. Br Dent J. 2012;213:96-8

152. Salz U, Bock T. Testing adhesion of direct restoratives to dental hard tissue - a review. J Adhes Dent. 2010;12:343-71

153. Drummond JL, Toepke TR, King TJ. Thermal and cyclic loading of endodontic posts. Eur J Oral Sci. 1999;107:220–4.

154. Otani A, Amaral M, May LG, Cesar PF, Valandro LF. A critical evaluation of bond strength tests for the assessment of bonding to Y-TZP. Dent Mater. 2015;31:648-656.

155. Van Noort R, Noroozi S, Howard IC, Cardew G. A critique of bond strength measurements. J Dent. 1989;17:61-67.

156. Kelly JR, Benetti P, Rungruanganunt P, Bona AD. The slippery slope: critical perspectives on in vitro research methodologies. Dent Mater. 2012;28:41-51.

157. Chen WP, Chen YY, Huang SH, Lin CP. Limitations of push-out test in bond strength measurement. J Endod. 2013;39:283-287.

158. Ungor M, Onay EO, Orucoglu H. Push-out bond strengths: The Epiphany-Resilon endodontic obturation system compared with different pairings of Epiphany, Resilon, AH Plus and gutta-percha. Int Endod J. 2006;39:643–647.

159. Shimada Y, Senawongse P, Harnirattisai C, Burrow MF, Nakaoki Y, Tagami J. Bond strength of two adhesive systems to primary and permanent enamel. J Oper Dent. 2002;27:403-409
160. Pamato S, do Valle AL, de Andrade GH, Vidotti HA, Só MV, Pereira JR. Does hybridized dentin affect bond strength of self-adhesive resin cement? J Clin Exp Dent. 2016;8:409-414.
161. Sadr A, Ghasemi A, Shimada Y, Tagami J. Effects of storage time and temperature on the properties of two self-etching systems. J Dent. 2007;35:218-225.
162. Flury S, Peutzfeldt A, Lussi A. Two pre-treatments for bonding to non-carious cervical root dentin. Am J Dent. 2015;28:362-366.
163. Armstrong S, Geraldeli S, Maia R, Raposo LH, Soares CJ, Yamagawa J. Adhesion to tooth structure: a critical review of "micro" bond strength test methods. Dent Mater. 2010;26:50-62.
164. Ezoji F, Tabari K, Jaberi Ansari Z, Torabzadeh H, Kharrazi Fard MJ. Shear bond strength of a resin cement to different alloys subjected to various surface treatments. J Dent (Tehran). 2016;13:29-39.
165. Samsfi S, Van Noort R. Do dentin bond strength tests serve a useful purpose? J Adhes Dent. 1999;1:57-67.
166. Thammajaruk P, Buranadham S, Thanatvarakorn O, Ferrari M, Guazzato M. Influence of glass ceramic coating on composite zirconia bonding and its characterization. Dent Mater. 2019;35:105-113.
167. Versiuis A, Tantbirojn D, Dougias WiH. Why do shear bond tests pull out dentin? J Dent Res. 1997;76:1298-1307.
168. Wang R, Shi Y, Li T, Pan Y, Cui Y, Xia W. Adhesive interfacial characteristics and the related bonding performance of four self-etching adhesives with different functional monomers applied to dentin. J Dent. 2017;62:72-80
169. Han Y, Mo S, Jiang L, Zhu Y. Effects of antioxidants on the microleakage of composite resin restorations after external tooth bleaching. Eur J Dent. 2014 Apr;8(2):147-153.
170. Konno ANK, Sinhoreti MAC, Consani S, Sobrinho LC, Consani RLXC. Storage effect on the shear bond strength of adhesive systems. Braz Dent J. 2003;14(1):June.

171. Oktay EK. Clinical Comparison of the Effects of Different Vital Tooth Whitening Systems on Tooth Color. Hacettepe University Institute of Health Sciences, Doctoral Dissertation, Ankara; 2006.
172. Hegedüs C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. J Dent. 1999;27(7):509-515.
173. Buchalla W, Attin T. External bleaching therapy with activation by heat, light, or laser. Dent Mater. 2007;23:586-596.
174. Haywood VB. Historical development of whiteners: clinical safety and efficacy. Dent Update. 1997;24(3):98-104.
175. Ernst CP, Marraquin BB, Zönnchen BW. Effects of hydrogen peroxide-containing agents on the morphology of human enamel. Quintessence Int. 1996;27:53-56.
176. Gultz J, Kaim J, Scherer W, Gupta H. Two in-office bleaching systems: a scanning electron microscope study. Compend Contin Educ Dent. 1999;20:965-968.
177. Kwon YH, Huo MS, Kim SK, Kim YJ. Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. J Oral Rehabil. 2002;29:473-477.
178. Spalding M, Taveira LA, de Assis GF. Scanning electron microscopy study of dental enamel surface exposed to 35% hydrogen peroxide: alone, with saliva, and with 10% carbamide peroxide. J Esthet Restor Dent. 2003;15:154-164.
179. Lewinstein I, Hirschfeld Z, Stabholz A, Rotstein I. Effect of hydrogen peroxide and sodium perborate on the microhardness of human enamel and dentin. J Endod. 1994;20:61-63.
180. Bağış YH, Ertaş E. Kompozit restorasyonların yapımından önce ve sonra uygulanan vital ağartma işlemlerinin mikrosızıntı üzerine etkiler. AÜ Dişhek Fak Derg. 2000;27:137-142.
181. Miyazaki M, Sato H, Sato T, Moore BK, Platt JA. Effect of a whitening agent application on enamel bond strength of self-etching primer systems. Am J Dent. 2004;17(3):151–155.
182. Thanaratikul B, Santiwong B, Harnirattisai C. Self-etch or etch-and-rinse mode did not affect the microshear bond strength of a universal adhesive to primary dentin. Dent Mater J. 2016;35(2):174-179.
183. Sauro S, Mannocci F, Toledano M, Osorio R, Thompson I, Watson TF. Influence of the hydrostatic pulpal pressure on droplets formation in current etch-and-rinse and self-

etch adhesives: a video rate/TSM microscopy and fluid filtration study. Dent Mater. 2009;25(11):1392-1402.
184. Uçar EN, Arslan S, Demirbuğa S, Çayabatmaz M. Üniversal adezivlerin vital beyazlatma yapılmış dişlerde bağlanma dayanımına etkisi. Selçuk Dent J. 2019;6(1):10-17.
185. Halabi S, Matsui N, Nikaido T, Burrow MF, Tagami J. Effect of Office Bleaching on Enamel Bonding Performance. J Adhes Dent. 2019;21(2):167–177.
186. Titley KC, Torneck CD, Smith DC, Adibfar A. Adhesion of composite resin to bleached and unbleached bovine enamel. J Dent Res. 1988;67(12):1523–1528.
187. van der Vyver PJ, Lewis SB, Marais JT. The effect of bleaching agent on composite/enamel bonding. J Dent Assoc S Afr. 1997;52(10):601–603.
188. Bittencourt ME, Trentin MS, Linden MS, de Oliveira Lima Arsati YB, França FM, Flório FM, Basting RT. Influence of in situ postbleaching times on shear bond strength of resin-based composite restorations. J Am Dent Assoc. 2010;141(3):300–306.
189. Sadeghian S, Garavand S, Davoudi A. Effect of different bleaching treatment protocols on shear bond strength of bonded orthodontic brackets with no-primer adhesive resin. J Orthod Sci. 2021;10:11.
190. Nour El-din AK, Miller BH, Griggs JA, Wakefield C. Immediate bonding to bleached enamel. Oper Dent. 2006;31(1):106–114.
191. Cheng YL, Musonda J, Cheng H, Attin T, Zheng M, Yu H. Effect of surface removal following bleaching on the bond strength of enamel. BMC Oral Health. 2019;19(1):50.
192. Sasaki RT, Catelan A, Bertoldo Edos S, Venâncio PC, Groppo FC, Ambrosano GM, Marchi GM, Lima DA, Aguiar FH. Effect of 7.5% hydrogen peroxide containing remineralizing agents on hardness, color change, roughness and micromorphology of human enamel. Am J Dent. 2015 Oct;28(5):261-267.
193. Gomes YSL, Alexandrino LD, Alencar CM, Alves EB, Faial KC, Silva CM. In situ Effect of Nanohydroxyapatite Paste in Enamel Teeth Bleaching. J Contemp Dent Pract. 2017 Nov;18(11):996-1003.
194. Monterubbianesi R, Tosco V, Bellezze T, Giuliani G, Özcan M, Putignano A, Orsini G. A Comparative Evaluation of Nanohydroxyapatite-Enriched Hydrogen Peroxide Home Bleaching System on Color, Hardness and Microstructure of Dental Enamel. Materials (Basel). 2021;14(11):3072.

195. Kutuk ZB, Ergin E, Cakir FY, Gurgan S. Effects of in-office bleaching agent combined with different desensitizing agents on enamel. J Appl Oral Sci. 2018;27:e20180233.
196. Dabanoglu A, Wood C, García-Godoy F, Kunzelmann KH. Whitening effect and morphological evaluation of hydroxyapatite materials. Am J Dent. 2009 Feb;22(1):23-9.
197. Sasaki RT, Catelan A, Bertoldo Edos S, Venâncio PC, Groppo FC, Ambrosano GM, et al. Effect of 7.5% hydrogen peroxide containing remineralizing agents on hardness, color change, roughness and micromorphology of human enamel. Am J Dent. 2015 Oct;28(5):261-7.

8 APPENDICES

8.1 Appendix 1. Curriculum Vitae

Personal Informations

Name	Esin	**Surname**	Gökçek

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Master	Restorative Dentistry, Master	Yeditepe University	2024
University	Dentistry	Yeditepe University	2020
High school	Science	Ramazan Yaman Science High Schcool	2014

Languages	**Grades (#)**
English (Tıpdil)	67

Work Experience

Position	**Institute**	**Duration (Year - Year)**
Dentist	Dr. Neslihan Aktaş Clinic	2022-2023
Dentist	Dentataşehir Ağız ve Diş Sağlığı Polikliniği	2021-2022
Dentist	Istanbul Diş Akademisi Polikliniği	2020-2021
Dentist	Dentama Ağız ve Diş Sağlığı Polikliniği	2020-2021

Others (Projects / Certificates / Rewards)

10.CONSEURO Kongresi (European Fderation of Conservative Dentistry)
İstanbul Diş Hekimleri Odası 20 Mart Oral Sağlık Sempozyumu
4. Genç EDAD (Estetik Diş hekimliği Akademisi Derneği) Samsun
Diş Hekimliği Lazer Akademisi Derneği 2. Sempozyumu
Dentbizz Kariyer ve Kişisel Gelişim Eğitim Programı
YUDSA 6. Uluslararası 8. Ulusal Öğrenci Bilimsel Araştırma Günleri
25. Uluslararası Türk Diş Hekimleri Birliği Kongresi ve Expodental
61. Avrupa Diş Hekimliği Öğrencileri Birliği Toplantısı Amsterdam
23. Uluslararası Türk Diş Hekimleri Birliği Kongresi ve Expodental