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VAN YÜZÜNCÜ YIL ÜNİVERSİTESİ  
Sağlık Bilimleri Enstitüsü



**EVALUATION OF THE EFFICACY OF FLUORIDATED AND  
NON-FLUORIDATED REMINERALIZATION AGENTS APPLIED  
BY DIFFERENT METHODS ON REMINERALIZATION OF  
EARLY ENAMEL LESIONS**

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**VAN-2024**

## ETİK BEYAN

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SAĞLIK BİLİMLERİ ENSTİTÜSÜ MÜDÜRLÜĞÜ'NE

Yüksek Lisans/Doktora tezi olarak hazırlayıp sunduğum “*Farklı Yöntemler ile Uygulanan Florürlü ve Florürsüz Remineralizasyon Ajanlarının Erken Mine Lezyonlarının Remineralizasyonu üzerine Etkilerinin Değerlendirilmesi*” başlıklı tezim; bilimsel ahlak ve değerlere uygun olarak tarafımdan yazılmıştır. Tezimin fikir/hipotezi tümüyle tez danışmanım ve bana aittir. Tezde yer alan deneysel çalışma/araştırma tarafımdan yapılmış olup, tüm cümleler, yorumlar bana aittir. Bu tezdeki bütün bilgiler akademik kurallara ve etik ilkelere uygun olarak hazırlanıp, bu kural ve ilkeler gereği, çalışmada bana ait olmayan tüm veri, düşünce ve sonuçlara atıf yapılmış ve kaynak gösterilmiştir.

Yukarıda belirtilen hususların doğruluğunu beyan ederim.

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Tarih: 10/06/2024

İmza:

## **ETHICAL DECLARATION**

T.C

To The,

VAN YUZUNCU YIL UNIVERSITY

HEALTH SCIENCE INSTITUTE DIRECTORATE,

My thesis titled “Evaluation of The Efficacy of Fluoridated and Non-Fluoridated Remineralization Agents Applied by Different Methods on Remineralization of Early Enamel Lesions” I prepared and presented as a PhD thesis; written by me in accordance with scientific ethics and values. The idea/hypothesis of my thesis belongs to me and my thesis advisor completely. The experimental study/research in the thesis was made by me and all sentences and comments belong to me. All the information has been prepared in accordance with academic rules and ethical principles in this thesis, and according to these rules and principles in the study; all the data, thoughts and results that doesn't belong to me have been cited and referenced.

I declare that the above points are true.

The student's name:

Mays Marwan Abedalaziz ALREFAI

Date: 10/06/2024

Signature:

## ACKNOWLEDGEMENTS

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## ÖZET

**ALREFAI M, Farklı Yöntemler ile Uygulanan Florürlü ve Florürsüz Remineralizasyon Ajanlarının Erken Mine Lezyonlarının Remineralizasyonu üzerine Etkilerinin Değerlendirilmesi, Van Yüzüncü Yıl Üniversitesi, Sağlık Bilimleri Enstitüsü, Restoratif Diş Tedavisi Anabilim Dalı, Doktora Tezi, Van, 2024.** Çalışmada parmak ve diş fırçası ile uygulanan amorf kalsiyum fosfat (ACP) ve hidroksiapatit (HA) içeren florürlü ve florürsüz macunların mine demineralizasyonu üzerindeki etkisi değerlendirildi. İnsan dişleri kullanıldı. Yeni başlayan çürük benzeri lezyonlar oluştuktan sonra, bunlar her grupta 13 örnek içeren 9 gruba ayrıldı; bunların 10'u mikrosertlik testi ve 3'ü SEM analizi içindi. Örnekler ph siklus modeli ile 8 saat aralıklarla demineralize edildi, demineralizasyon sonrası tüm gruplara 3 dakika boyunca remineralizasyon ajanları uygulandı ve daha sonra yapay tükürük ile temizlendi. Örnekler 28 gün boyunca 16 saat boyunca yapay tükürük içerisinde saklandı. Örnekler başlangıçta, demineralizasyondan sonra ve 28 günlük remineralizasyondan sonra analiz edildi. Numuneler yüzey mikrosertliği (SMH) ve SEM ile analiz edildi. T-testi ve Mann-Whitney U testi, One-way ANOVA, Tukey HSD ve Tamhane's T2 ve Bonferroni testi ( $p<0,050$ ). Remineralizasyon sonrasında parmakla ve diş fırçası uygulama yöntemleri arasında anlamlı bir fark görülmez. HA bazlı ACP bazlı malzemelerden daha yüksek mikrosertlik değerlerine sahiptir. Florürlenmiş olanlar flor gruplara göre daha yüksek etkinliğe sahiptir. Florürsüz ACP bazlı gruplar ile diğer Florürlü (HA ve ACP) ve Florürsüz HA bazlı gruplar arasında önemli bir fark vardır. Kontrol grubu ile Florürsüz ACP bazlı gruplar arasında anlamlı bir fark yoktur. SEM analizinin remineralizasyonundan sonra, kontrol grubu, Florürsüz ACP bazlı gruplarla aynı şekilde düzensiz pürüzlü yüzeye sahip çoklu gözenekliliğe sahiptir; florürlü ACP bazlı gruplar, düzensiz pürüzlü yüzey ile tam kaplamaya sahipken, HA bazlı gruplar, pürüzsüz ve düzenli yüzeylerle tam kaplamaya sahiptir.

**Anahtar Kelimeler:** Erken mine lezyonları, Florürlü, Florürsüz, Remineralizasyon, Taramalı Elektron Mikroskobu, Hidroksiapatit.

## ABSTRACT

**ALREFAI M, Evaluation of the Efficacy of Fluoridated and Non-Fluoridated Remineralization Agents Applied by Different Methods on Remineralization of Early Enamel Lesions, Van Yüzüncü Yıl University, Institute of Health Sciences, Department of Restorative Dentistry, PhD Thesis, Van, 2024.** This study evaluated the effect of pastes containing amorphous calcium phosphate (ACP) and hydroxyapatite (HA) with and without fluoride applied by finger and toothbrush on enamel demineralization. Human teeth were used. After artificial caries lesions were formed, they were addressed to 9 groups each group contained 13 specimens, 10 for microhardness test and 3 for SEM analysis. The specimens were demineralized by the pH cycling model for 8-hour intervals, after demineralization the remineralization agents were applied to all groups for 3 minutes and then cleaned with artificial saliva. Specimens were stored in artificial saliva for 16 hours for 28 days. Specimens were analyzed at baseline, after demineralization, and after 28 days of remineralization. Specimens were analyzed by surface microhardness (SMH) and SEM. T-test and the Mann-Whitney U test, One-way ANOVA, Tukey HSD and Tamhane's T2 and Bonferroni test ( $p<0.050$ ). After remineralization, there is no significant difference between finger and toothbrush application methods. The HA-based has a higher microhardness mean value than ACP-based materials. The fluoridated has higher efficacy than the non-fluoridated groups. There is a significant difference between non-fluoridated ACP-based groups and the other fluoridated (HA and ACP) and non-fluoridated HA-based groups. There is no significant difference between the control group and non-fluoridated ACP-based groups. After remineralization of the SEM analysis, the control group has multi porosity with irregular rough surface same as non-fluoridated ACP-based groups, fluoridated ACP-based groups have complete coverage with irregular rough surface, while HA-based groups have complete coverage with smooth regular surface.

**Keywords:** Early enamel lesions, Fluoridated, Non-fluoridated, Remineralization, Scanning Electron Microscopy, Hydroxyapatite.

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## ACRONYMS AND ABBREVIATIONS

|                                                   |                                                     |
|---------------------------------------------------|-----------------------------------------------------|
| <b>Ca<sup>2+</sup></b>                            | : Calcium ion                                       |
| <b>Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub></b> | : Calcium Phosphate                                 |
| <b>CaCl<sub>2</sub></b>                           | : Calcium Chloride                                  |
| <b>CaF<sub>2</sub></b>                            | : Calcium Fluoride                                  |
| <b>CPP-ACFP</b>                                   | : Casein Phosphopeptide Amorphous Calcium Fluoride  |
| Phosphate                                         |                                                     |
| <b>CPP-ACP</b>                                    | : Casein phosphopeptide Amorphous Calcium Phosphate |
| <b>DCPD</b>                                       | : Dicalcium Phosphate Dihydrate                     |
| <b>EDX</b>                                        | : Energy Dispersive X-ray                           |
| <b>F-</b>                                         | : Fluoride                                          |
| <b>FA</b>                                         | : Fluoroapatite                                     |
| <b>H</b>                                          | : Hydrogen                                          |
| <b>HA</b>                                         | : Hydroxyapatite                                    |
| <b>HCA</b>                                        | : Hydroxycarbonate apatite                          |
| <b>HPO<sub>4</sub><sup>2-</sup></b>               | : Phosphate ion                                     |
| <b>KCl</b>                                        | : Potassium Chloride                                |
| <b>KHN</b>                                        | : Knoop Hardness Value                              |
| <b>KOH</b>                                        | : Potassium Hydroxide                               |
| <b>n-HAP</b>                                      | : Nanohydroxyapatite                                |
| <b>NaF</b>                                        | : Sodium Fluoride                                   |
| <b>O<sub>3</sub></b>                              | : Ozone                                             |
| <b>P</b>                                          | : Phosphorus                                        |
| <b>pH</b>                                         | : Power Of Hydrogen                                 |
| <b>ppm</b>                                        | : Parts Per Million                                 |
| <b>SEM</b>                                        | : Scanning Electron Microscope                      |
| <b>SMFP</b>                                       | : Sodium Monofluorophosphate                        |
| <b>SnF<sub>2</sub></b>                            | : Stannous Fluoride                                 |
| <b>TCP</b>                                        | : Tricalcium phosphate                              |
| <b>β-TCP</b>                                      | : β-tricalcium phosphate                            |

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## 1. INTRODUCTION

Dental caries is a chronic, preventable disease mediated by biofilms that are affected by diet. With time, the accumulation of fermentable carbohydrates on the tooth surface leads to an imbalance of the oral biofilm, which is the main reason for this multifactorial oral disease. Not everyone with teeth, biofilm, and carbohydrate consumption will eventually develop caries lesions. Several protective and risk-modifying variables affect the development of dental caries. Some bacteria metabolize refined carbohydrates to produce organic acid byproducts and energy. If these organic acids remain in the biofilm for a long enough time, they can reduce the pH below a crucial value (Ritter et al., 2019). Therefore, hydroxyapatite (HA) dissolves when  $\text{pH} < 5.5$  due to acid released by bacteria through metabolic products or by eating acidic foods, initiating the demineralization process (Khijmatgar et al., 2020).

The tooth experiences demineralization, or a net loss of minerals, because of the low pH drives calcium and phosphate out of the tooth and into the biofilm to reach equilibrium (Ritter et al., 2019). Early enamel lesions are the stage of dental caries development that occurs before cavitation. When the dynamic equilibrium between demineralization and remineralization shifts toward demineralization over time, enamel caries will take place. These events occur several times a day during the life of a tooth. They are caused by interaction between several variables including the type and the amount of microbiological flora in the biofilm, dietary habits, oral hygiene, genetics, tooth anatomy, dentin and enamel constitution, use of therapeutic agents like fluorides, composition, flow, and buffering ability of saliva. Renewal of partially demineralized enamel is possible when the pH in the biofilm reaches a neutral level, and when the concentration of soluble calcium and phosphate is supersaturated in comparison with that in the tooth (Nazari et al., 2012; Pitts et al., 2017; Ritter, et al., 2019).

Because there were no feasible alternatives, the surgical method—which is considered invasive—was used to remove caries many years ago. Recently, caries management has shifted toward the development of methods for early detection of early carious lesions and non-invasive treatment methods. Treatment of early caries lesions by remineralization is considered a valuable non-invasive treatment approach. It may be feasible to remineralize carious and white-spot lesions using a range of already accessible

treatments (Naveena et al., 2014). These agents could be fluoridated and non-fluoridated agents.

The aim of this in vitro study was to evaluate the effects of two remineralizing materials (each substance has a fluorinated and non-fluorinated form), which are applied in two different ways (with finger and brush) based on the manufacturer's instructions, on remineralization of early enamel lesions.

This study is expected to provide information about the effectiveness of the materials in terms of composition and the optimal application methods to ensure better results for remineralizing early enamel lesions.

The null hypotheses in this study are:

1. There is no significant statistical difference between the fluoride and non-fluoridated groups regarding microhardness values.
2. Between the groups using toothbrushes and finger applications, there is no significant statistical difference.
3. There is no significant statistical difference between different material groups
4. There is no statistically significant difference between all groups.
5. There is no statistically significant difference between the baseline, demineralization, and remineralization groups.

## **2. BACKGROUND**

### **2.1. Enamel Structure**

Enamel is the hardest tissue in the body that covers the anatomical crown of a tooth. Enamel is derived from epithelial tissue that originates from a cell called ameloblast, which is ectodermal in origin (Lacruz et al., 2017; Ritter et al., 2019). Dental enamel is a highly mineralized, outermost, translucent, avascular, unrepairable covering that protects the human clinical crown and can tolerate stresses and physio-chemical action (Hu et al., 2007; Qamar et al., 2017).

It is considered, a wear-resistant coating, by forming an insulating barrier, it shields the tooth from external forces like heat, pressure, and chemicals that could harm the underlying dental pulp's tissue (Lacruz et al., 2017). It is semipermeable, so it allows entry for some ions such as fluoride (Ritter et al., 2019). Comparing the surface and subsurface enamel layers, the surface layer is less porous, radio-opaque, and harder (Qamar et al., 2017). Since ameloblasts cease to function after the complete formation of the tooth's crown, the enamel tissue cannot repair itself after formation; its structure only permits mineral loss or gain; and saliva's remineralization potential and protective effect are the only physiological repair tools available (Hu et al., 2007). The thickness of enamel varies depending on the area of the tooth. It is thicker in the incisal and occlusal areas of the crown and reduces progressively towards the cemento-enamel junction (CEJ) (Ritter et al., 2019).

Tooth enamel's chemical composition varies greatly with 80–95% of its content being inorganic and the remaining 1–2% organic by volume, where this ratio varies in terms of weight and the water content is 3–4% water, and 92–96% inorganic. Tissue fluid and/or organic matter comprise 5 to 20% of the tissue volume. The inorganic component is most often hydroxyapatite crystals (HA) (Robinson et al., 1995; Qamar et al., 2017). HA crystals are tightly packed, highly organized crystallites, which make up approximately 95% of enamel's weight and 87% of its volume. Mature enamel contains less than 1% organic substance, compared to other mineralized tissues that contain over 20% organic substance. The volume of crystals in bone, dentin, and

cementum is a thousand times less than that of enamel crystallites (Simmer and Hu, 2001).

Proteins like tuftelin, amelogenin, and ameloblastin make up most of the organic component; trace levels of proteoglycans and lipoids usually fade as enamel matures. Teeth enamel is inert, brittle, and unrepairable, due to its distinctive composition. Thus, for the tooth to function properly, underlying dentin support is necessary (Qamar et al., 2017).

A simple formula that corresponds to the representation of enamel, dentin, and bone mineral is  $\{Ca_{10-x}(Na)_x(PO_4)_6-y(CO_3)_z(OH)_2-u(F)_u\}$ . The stoichiometric formula of HA is  $Ca_{10}(PO_4)_6(OH)_2$ , which essentially makes up the mineral component of enamel. Enamel may have many elemental replacements; for example, calcium may be substituted with sodium, magnesium, zinc, etc.; phosphate may be replaced with carbonate; hydroxyl may be substituted with fluoride. The behavior of HA, particularly concerning its solubility at low pH, can be greatly influenced by these defects and replacements. Specifically, areas in the crystal that are rich in carbonate and lack calcium are more vulnerable to acid demineralization, but resistance to acid demineralization can be increased by replacing hydroxyl with fluoride. In Figure 1, the solubility variations are schematically presented (Featherstone, 1999; West and Joiner, 2014).

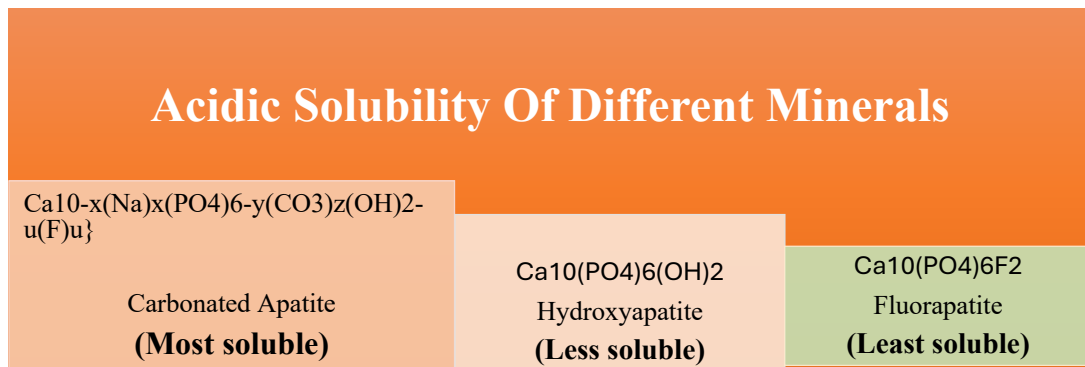


**Figure 1.** The acidic solubility review of minerals is illustrated schematically.

The enamel rod, interrod, and rod sheath are the three primary structural elements that make up the enamel. The building unit of enamel is the enamel rod, the cross-sectional profile of the prisms resembles a circular keyhole shape. Enamel rods is made up of millions of tiny, elongated apatite crystallites that differ in terms of size and form.

The mineral composition of enamel is determined by the density of the crystals or prisms, which varies. As we move from the enamel surface to the dentin, porosity, liquid, and organic material tend to increase while crystal density decreases. What gives the enamel rod its strength and structural character is the tightly packed crystallites arranged in a unique orientation pattern.

The apatite crystallites' long axes in the head's center region (body) are almost parallel to the rod's long axis, but in the tail region, the crystallites incline at progressively greater angles (65 degrees) to the rod's axis. The crystals shift from this orientation near the borders of each prism, creating an interface where there is typically more intercrystalline space between the prisms. Organic compounds and water fill the spaces between the crystals, creating 15% by volume of diffusible spaces that permits the flow of ions and tiny molecules like calcium and hydrogen peroxide as well as lactic acid.

The average length of crystallite is about 160 nm and its average width is 20 to 40 nm, while its thickness is about 25 nm. Each crystal is enclosed by an organic matrix. Rod sheath is a sheath of organic matrix that envelops each rod. The crystals within the rod are arranged around each other with a patchy organic meshwork separating them. It has been proposed that the crystallites' orientation affects their vulnerability to acidic environments, either as a result of etching or the caries process. The head region of each rod experiences a greater amount of acid-induced mineral loss or demineralization. Relatively resistant to acidic demineralization are the head area's periphery and the tail region according to Ritter et al. The interrod regions, which are found in between the enamel rods, are recognized for having a higher organic content and distinct crystallite orientations from the intrarod portions of the enamel rods (Robinson et al., 1971; Robinson et al., 2000; West and Joiner, 2014; Beniash et al., 2019; Ritter et al., 2019).

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porosity, protein, and crystal distribution might be extremely complicated in some areas. What gives the enamel rod its strength and structural character is the tightly packed crystallites arranged in a unique orientation pattern. The apatite crystallites' long axes in the head's center region (body) are almost parallel to the rod's long axis, but in the tail region, the crystallites incline at progressively greater angles (65 degrees) to the rod's axis. The crystals shift from this orientation near the borders of each prism, creating an interface where there is typically more intercrystalline space between the prisms. Organic compounds and water fill the spaces between the crystals, creating 15% by volume of diffusible spaces that permits the flow of ions and tiny molecules like calcium and hydrogen peroxide as well as lactic acid. The average length of crystallite is about 160 nm and its average width is 20 to 40 nm, while its thickness is about 25 nm. Each crystal is enclosed by an organic matrix. Rod sheath is a sheath of organic matrix that envelops each rod. The crystals within the rod are arranged around each other with a patchy organic meshwork separating them.

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The different structural arrangement of the HAp crystals results in different etching patterns at the rod enamel scale. According to Silverstone et al. (Silverstone et al., 1975), three distinct etching types can be distinguished when lactic acid is applied to enamel. Type 1 consists of a preferential loss in the enamel center of the rod; Type 2 is characterized by a mostly intact enamel rod compared to mineral loss at the rod's boundaries; and Type 3 is characterized by a random pattern that is not consistent with the shape of the enamel rods. The identification of etching of Type 1, 2, or 3 for a particular agent is believed to be reliant on the variation in HAp crystal orientation

concerning acid attack direction; however, no systematic evidence or interpretation has been demonstrated at this time. The type of etching is largely determined by the composition of the acidic chemical species, as it is evident that different etchant solutions react differently on different crystal faces (Salvati et al., 2021).

Previous nanoindentation studies of the enamel rod and interrod regions have found that the hardness and elastic modulus of the enamel rod region are higher than those of the interrod regions. The different crystallite directions at the two locations could be the reason for the variances observed by Habelitz et al (2001). According to Ge et al., the enamel rod was 50% tougher and had a higher elastic modulus than the interrod region (Habeltiz et al., 2001; Ge et al., 2005; Ang et al., 2012).

Enamel is more resistant to acidic attack than dentine due to its chemical composition and structure. This can be explained by the fact that enamel has 4% fewer organic components than dentin, which has 25%. Moreover, enamel has larger crystals than dentin, which results in less surface area available and makes it more resistant to acid attack. As a result, caries progression and enamel demineralization are slower in enamel than in dentine. Furthermore, enamel remineralizes more quickly than dentin does in similar circumstances (Amaechi and Loveren, 2013).

### **2.1.1. The effect of each elemental substitution on the hydroxyapatite**

#### **Carbonate**

Acid phosphate (type B) or hydroxyl (type A) can both be substituted with carbonate. The substitutions during crystal formation are dependent upon the local  $p\text{CO}_2$ . At higher  $p\text{CO}_2$  carbonate can replace hydroxyl group. The exchange of sodium for calcium is also considered to occur with the substitution of carbonate for phosphate. In such a situation, sodium would represent carbonate concentrations. The apatite phase becomes less stable and more acid soluble because of these replacements due to a poorer fit of carbonate in the lattice. When considering the solubility product of enamel in comparison to stoichiometric apatite, carbonate substitution, in conjunction with ion gaps, is the significant factor. Carbonate incorporation appears to occur only during tissue development, in contrast to fluoride, possibly due to the presence of  $p\text{CO}_2$  in the crystals'

immediate surroundings. Concentrations increase from the 2% level on the enamel surface to 4-6% levels in the dentin. Fissure enamel in molar teeth is one example of a region where the gradient is frequently broken up by pockets of quite high concentrations (Robinson et al., 2000).

### Magnesium

Magnesium can partially replace calcium, but this substitution is regarded to be limited—possibly as much as 0.33%. Magnesium is assumed to be present in distinct, more acid-soluble phases or on crystal surfaces. Due to its charge density, magnesium would destabilize the apatite lattice in a manner close to that of carbonate, increasing the solubility product necessary for precipitating phases in the process. It's also important to keep in mind that magnesium and carbonate work well together to enhance the hydroxyapatite lattice's integration of the minerals as well as the apatite mineral's acid solubility. Magnesium is distributed similarly to carbonate and is also likely incorporated during enamel development. The concentration of magnesium increases from around 0.2% on the outside of the tissue to approximately 0.5% on the inside. Compared to carbonate, gradients are typically less uniform, with discrete pockets of high concentration commonly appearing in the area around the dentin, where protein concentrations are typically highest (Robinson et al., 2000).

### Fluoride

According to traditional theories, fluoride ions fill hydroxyl vacancies in c-axis columns or displace hydroxyl ions to cause fluoride incorporation. A significantly closer fit for fluoride within the Ca II triangles results from the high charge density and symmetry of the fluoride ion (Robinson et al., 2000). This lowers lattice energy and stabilizes the crystal structure. This consequently decreases the solubility product for fluoride minerals making it resistant to dissolve fluoridated crystals and simpler to redeposit fluoridated crystals, for example, at lower solution concentrations. This process is essential for fluoride's function of preventing and controlling tooth caries.

Fluoride, or fluoridated hydroxyapatite, is not distributed uniformly. The concentration of fluoride is highest on the surface of the enamel and decreases

significantly as it moves toward the inner portion of the tissue. The high concentration at the tissue's surface most likely results from unerupted enamel collecting fluoride ions from tissue fluids when fluoride accumulates during development. Any fluoride that enters the tissue would be successfully picked up by the development of a more stable fluoridated mineral at the enamel surface, preventing fluoride ions from passing down to the tissue's deeper layers. Wear causes the loss of the outer surface's fluoride. This is not to claim that the fluoride present at the surface of the enamel is limited to fluoridated apatite. The components comprising fluoride that have been found at the surface of enamel vary from indistinct fluoride phosphate complexes to extended calcium fluoride hydrogen-bonded complexes. All of them, however, concentrate large amounts of fluoride ions at the tooth's surface, the site of the carious attack (Robinson et al., 2000).

#### Other minor inorganic constituents

There may also be trace quantities of sodium, chlorine, and even lead. A more thorough description of them has been neglected because there haven't been many studies of their impact on caries at endogenous concentrations, except sodium, which has been implicated in apatite instability (Robinson et al., 2000).

#### Non-apatitic mineral phases

Although substituted hydroxyapatite, as previously mentioned, is thought to make up most of the enamel mineral phase, there have been indications of the existence of additional distinct mineral phases, especially magnesium and carbonate. These could be localized at the contact between prisms or at the crystal surfaces, possibly as a result of recrystallization that occurred during development. There have been proposals for the  $\text{Ca Mg}(\text{CO}_3)_2$  and  $\text{Ca}_9\text{Mg}(\text{PO}_4)_6(\text{HPO}_4)$  phases (Robinson et al., 2000).

#### Organic components

Organic substances are also present in dental enamel, but they are typically found in small amounts. This presents as tiny peptides and amino acids that spread throughout the developed tissue. This is most likely the remaining pieces of the initial developing matrix, which were maybe held in place by adhering to the crystals of hydroxyapatite. Nonetheless, there is also an insoluble protein substance that is mostly associated with

the enamel tufts and is found in higher quantities close to the dentin as well as in places with less tight crystal packing, such as the cusps and fissure regions. The presence of amelin (ameloblastin/sheathelin) in the tuft protein has been identified, but its composition is unknown (Robinson et al., 2000).

## **2.2. Dental Caries Definition And Pathogenesis**

Caries of the teeth is a chronic, preventable disease mediated by biofilms that is affected by diet (Ritter et al., 2019). Tooth caries can affect primary and permanent dentition, crown, and root surfaces. All dental tissues could be affected including enamel, dentin, and cementum. In addition to smooth, pitted, and fissured surfaces. It starts with molecular alterations that are sub-clinical and progresses to infect the dentin tissue, which can have either an intact surface or clear cavitation. When acidogenic bacteria interact with substrate in addition to different host components, such as saliva and teeth, for long time, this will result in dental decay. The oral biofilm becomes unbalanced over time when fermentable carbohydrates build up on the surface of teeth. Changes in the microbiology of the complex biofilm are the cause of the condition called caries. It also depends on the flow and content of saliva, the amount of carbohydrates consumed in the diet, exposure to fluoride, and preventive behaviors like brushing teeth (Selwitz et al., 2007; Ritter et al., 2019).

An unequilibrium in the oral environment, specifically a physiological imbalance between dental minerals and oral bacterial biofilms, is the cause of tooth decay. On the teeth, the bacteria live in tiny colonies and are encased in an organic matrix composed of cell-secreted proteins, carbohydrates, and DNA. In addition to strengthening the bacteria's resistance to antimicrobial agents, this matrix shields them from desiccation, host defenses, and assault. In both health and disease, an abundance of bacteria and their byproducts accumulate in a biofilm on the enamel of the tooth, and teeth offer non-shedding surfaces for the colonization of microorganisms. Weak organic acids are produced by endogenous bacteria in the biofilm, primarily mutant streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) and *Lactobacillus* species, as a consequence of the metabolism of carbohydrate fermentation. This acid's capacity to bring local pH levels below the critical threshold demineralizes the tooth tissues. If the

migration of calcium, phosphate, and carbonate out of the tooth is allowed to continue, cavitation will ultimately take place. (Selwitz et al., 2007; Ritter et al., 2019).

### **2.2.1. Demineralization**

Mineral breakdown from the enamel theoretically starts with every drop in plaque pH, although this dissolution does not always happen. Despite this, the pH value of the plaque is far more significant than the pH value of saliva. The acid concentration in the plaque fluid needs to be high enough to permeate into the enamel for dissolving to take place. Plaque Mineral dissolution does not occur when the pH is neutral or nearly neutral, indicating that the plaque fluid is saturated with calcium and phosphate. But pH decreases below a certain point. The pH level at which the mineral dissolution process starts is known as "Critical pH". As it falls, the  $\text{Ca}_3(\text{PO}_4)_2$  saturation in the dental plaque falls, and dissolving happens from the tooth surface (Zero, 1999).

A drop in the pH of the biofilm causes demineralization, or an overall reduction of minerals, in the tooth, this will lead to minerals (calcium and phosphate) moving from the tooth toward the biofilm to reach equilibrium initiating the demineralization process (Ritter et al., 2019; Khijmatgar et al., 2020).

While 5.5 is generally regarded as the critical pH for mineral dissolution from enamel tissue, this value is not constant. It can change based on the kind of acid present in the oral environment, the concentration of fluoride, the concentration of calcium and phosphate ions, or the characteristics of mineral dissolution in specific tooth regions. Drops in the pH of the plaque; are directly linked to the intricate interactions that occur between bacteria, saliva, and food (28). When the dynamic equilibrium between demineralization and remineralization shifts toward demineralization over time, enamel caries will take place. Therefore, HA dissolves when the level of the pH is below a crucial level ( $\text{pH} < 5.5$ ) (Nazari et al., 2012).

### **2.2.2. Remineralization**

The restoration of a demineralized tooth area completely has been referred to as "remineralization". Which was clinically observed by the elimination of white spot lesions and the chalky white appearance of eroded enamel. Instead, the process of mineral

deposition can be referred to as "remineralization". This definition states that remineralization happens both during the formation of caries and during neutral pH periods when minerals precipitate from saliva in enamel defects (Cate, 1990).

According to Cochrane et al. define remineralization as: "The process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel, to produce net mineral gain" (Carey, 2023). Enamel remineralization can be achieved by increasing the concentration of these ions in dental plaque and the oral cavity. The concentration of these ions in dental plaque has increased with the development of various materials (Soltanimehr et al., 2019).

The basis of the remineralization concept is the replacement of minerals lost from tooth enamel by improving the ability of saliva to remineralize enamel surfaces (Abdelmegid et al., 2019). Therefore, when the pH in the biofilm reaches a neutral level and the concentration of soluble calcium and phosphate is supersaturated relative to that in the tooth, regeneration of partial demineralized enamel is conceivable (Ritter et al., 2019).

Usually, especially when fluoride is present, remineralization is a self-limiting surface phenomenon that stops ions from entering the lesion deeply. The fast accumulation of fluorapatite leads to superficial remineralization, which forms a surface layer that is more resistant to demineralization. But this tougher outer layer also keeps the calcium phosphate ions out of the lesion's interior, which is necessary for deep remineralization (Mount, 2005).

Demineralization and remineralization occur on a regular basis during the day for most persons. Remineralization occurs frequently, especially in cases where saliva functions as a buffer to regulate the pH of the biofilm. Compared to the original tooth structure, remineralized areas have less microporous structure and a higher concentration of fluoride because of salivary calcium and phosphate uptake (Selwitz et al., 2007). Dental caries can advance or reverse depending on the "caries balance," or the equilibrium between pathogenic factors that encourage demineralization and protective ones that shift the scales in favor of remineralization. Figure 4. provides an overview of the caries balance (Featherstone and Chaffee, 2018).

### **2.3. Methods Used In Caries Detection**

Initial carious lesions can be identified early, treated with remineralization products, and both function and appearance preserved (Pretty, 2006). Several techniques are employed in clinics to detect caries. Caries were discovered in dental clinics using various techniques. These techniques: Optical Coherence Tomography, Electrical Conductivity Measurement, Ultrasonic Method, Traditional Methods include [Visual-Tactile Methods, Radiographic Methods, Digital Radiographs], Caries Detection Dyes, Improved Visual Techniques including [Fiber Optic Transillumination (FOTI), Digital Fiber Optic Transillumination (DIFOTI)], Fluorescent Techniques include [Laser Fluorescence Method-DIAGNOdent, DIAGNOdent Pen, FACE], Camera and Fluorescent Technical Combinations include [Quantitative Light Effect Fluorescent-QLF, Canary, Soprolife, and Soprocare, Lifedt, Saproimaging, VistaCam] (Selwitz et al., 2007; Vinod et al., 2020).

### **2.4. Caries Prevention And Risk Factors**

Several protective and risk-modifying variables affect how dental caries develop (Ritter et al., 2019). Since most risk factors are alterable; an individual's risk of dental caries changes over time. Risk factors include the following: Physical and biological factors that contribute for enamel or root caries include recession of the gums, insufficient levels of saliva and its constituents, high concentrations of cariogenic bacteria, insufficient fluoride exposure, immune system variables, and individuals with particular medical conditions. A person's controllable lifestyle and behavioral characteristics influence their risk of dental caries. such as inadequate dental hygiene, improper eating practices—such as consuming refined carbs frequently—frequent use of sugar-containing medications, and improper baby-feeding techniques. Additionally, an individual's socioeconomic status, level of education, dental insurance coverage, under orthodontic treatment, use of teeth sealants, use of defective or poorly-fitting partial dentures, and poverty are all responsible for dental caries susceptibility. Children who have a history of caries, exhibit signs of the disease, or whose parents or siblings have intense caries should also be at a higher risk of developing the disease. The primary caregiver, typically the mother, is the main source from which infants get mutans streptococci, a cariogenic bacterial species that has been extensively investigated. Nonetheless, there is evidence

that taking into account risk factors such as the existence of lactobacilli or mutans streptococci, low socioeconomic status, a history of caries, salivary flow, and fluoride history, and the dentist's assessment can have a positive effect (Selwitz et al., 2007). An overview of the elements that contribute to the caries process are shown in Figure 2. (Ritter et al.,2019).

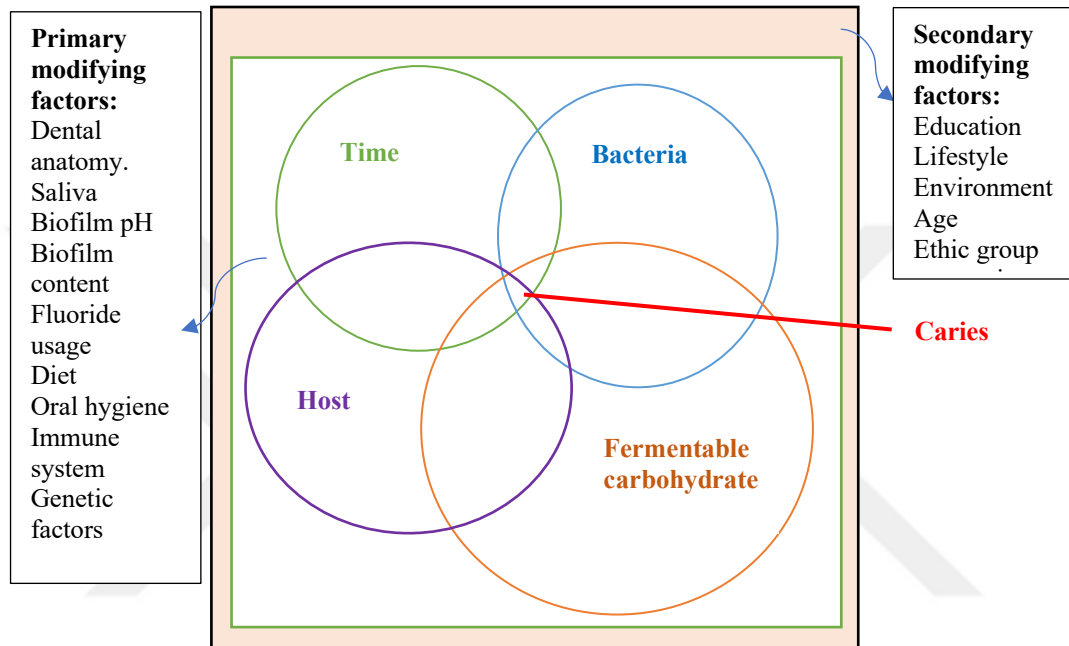


**Figure 2.** Keyes-Jordan diagram with modifications (Ritter et al.,2019).

Simply, tooth decay is due to by the gradual interaction of fermentable carbohydrates on the tooth (host) with cariogenic oral flora (biofilm). In reality, not everyone with teeth, biofilm, and carbohydrate consumption will eventually develop caries, making dental caries acquisition and activity significantly more complex. Dental caries is influenced by several protective and modifiable risk factors (Ritter et al.,2019).

Preventive dental care approaches aim to decrease the number of acidogenic bacteria that induce tooth demineralization, stop the evolution of early caries, and prevent the progression of early lesions into cavitated lesions. The preventive dental care approaches include increasing the resistance of the tooth surface to demineralization, modifying the metabolism of the biofilm, and raising the pH of the biofilm. A caries prevention program's main objectives are to: change the biofilm into a non-pathogenic biofilm and establish an environment that encourages remineralization. The first step in

prevention should be to consider the possibility of keeping the patient in a noncariogenic biofilm. The patient may have non-controllable factors such as the fluoride exposure history, immune system function, salivary gland function, or overall health, all of which have a substantial influence on the patient's caries risk. On the other hand, there are controllable factors including nutrition, dental treatment (which may involve sealants and restorations), oral hygiene practices, and the use of medications to modify the biofilm are typically within the patient's control (Ritter et al., 2019).

### **2.5. Early Enamel Lesion**

Early enamel lesion is the stage of dental caries development that occurs before cavitation, it is characterized by subsurface demineralization under a non-cavitated enamel surface (Sadikoglu, 2020). The typical whitish appearance of these lesions is due to increased porosity inside the lesion body. Consequently, the term "white" is typically applied to these lesions. (Torres et al., 2011). This is the first type of dental caries that can be seen clinically and is probably reversible. Non-cavitated enamel caries lesions and early lesions are other names for initial enamel lesions (Paula et al., 2017; Ritter et al., 2019).

When the dynamic equilibrium between demineralization and remineralization shifts toward demineralization over time, enamel caries will take place (Nazari et al., 2012). These events happen many times a day during the life of a tooth. They are caused by interaction between several variables including the type and the amount of microbiological flora in the biofilm, dietary habits, oral hygiene, genetics, tooth anatomy, the constitution of dentin and enamel, use of therapeutic agents like fluorides, composition, flow, and buffering ability of saliva (Pitts et al., 2017; Ritter et al., 2019).

White-colored staining in enamel can arise from sources other than early enamel lesions. Trauma and other factors before or after tooth eruption could be implicated in tooth discoloration. It's crucial to make an accurate diagnosis to choose the best treatment for each type of tooth discoloration, which may include microabrasion, whitening, resin infiltration, and, in severe circumstances, restorations. Molar incisor hypomineralization (MIH), traumatic hypocalcification, and fluorosis are disorders brought on by defects in the enamel's development. White spot lesions are color changes brought on by caries that

are noticed after tooth eruption. All these circumstances are associated with a decrease in the mineral phase of enamel, a change in its chemical composition, and consequently, a change in its optical characteristics (Torres et al., 2008; Denis et al., 2013).

### **2.5.1. Clinical features of early enamel lesion**

Initial enamel lesions may become partially and completely invisible when the surface is hydrated, while in enamel hypocalcification situations, they are less affected by the hydration or dryness of the tooth. Differentiating the origin of these lesions is crucial since they may be inherited or the result of dental caries. Hypocalcified enamel does not an issue other than aesthetic concern (Iijima, 2008; Ritter et al., 2019).

When Utilizing an explorer for tactile examination, the initial lesion is undetectable, due to the unchanged surface texture of an initial lesion. In comparison to normal, healthy enamel, a more advanced lesion has an uneven, rough surface. Active caries can be detected by softened, chalky enamel that can easily chipped away with an explorer. But if an explorer tip is used incorrectly, it might produce cavitation in non-cavitated areas, necessitating restorative intervention most of the time. Although visual inspection is less reliable in recognizing them, similar lesions can form on proximal flat surfaces. Occasionally, early enamel lesions appear as thin radiolucency limited to the enamel's surface on radiographs. Likely, histologic modification of the underlying dentin has already taken place when a proximal lesion is clearly apparent radiographically, regardless of whether the lesion is cavitated or not (Ritter et al., 2019). The clinical characteristics of normal and altered enamel are listed in Table 1.

The damaged area's color appears opaque and whiter due to a decrease in the mineral concentration, which also impairs the enamel's translucent quality. As a result, white spot lesions are another name for early enamel lesions. They are recognized as early lesions by dentists since they are the first apparent signs in the early stages of caries. It is important to keep in mind that, nevertheless, demineralization cannot be seen until it is at least 300–500 µm in depth at the surface. The enamel tissue appears translucent due to the micropores that are present on the enamel bulk, which has a refractive index of 1.62. Nonetheless, this refractive index decreases because of the ongoing demineralization that increases the subsurface micro-porosity. These pores are filled with water that has a

refractive index of 1.33 when the enamel surface becomes moist. Since water's refractive index is so similar to that for normal enamel, the lesion cannot be detected and its opacity on the enamel surface will not be evident. However, when the tooth is allowed to air dry, air with a refractive index of 1.0 will fill the pores in the lesion, making the opaque enamel lesions apparent and easily separated from the healthy enamel surface. This method has several benefits, including ease of use, low cost, and clinical validity; nevertheless, the main drawback is the difficulty of standardizing the procedure (Iijima, 2008; Sadikoglu, 2020).

**Table 1.** The clinical traits of both normal and modified enamel (Ritter et al.,2019).

| Wetted                                    | Dried       | Surface Texture | Surface Hardness |
|-------------------------------------------|-------------|-----------------|------------------|
| Translucent                               | Translucent | Smooth          | Hard             |
| Opaque (white)                            | Opaque      | Smooth          | Hard             |
| • Translucent (early lesion)              | Opaque      | Rough           | Softened         |
| • Opaque (more persistent initial lesion) | Opaque      | Rough           | Softened         |
| Shiny/ or dark                            | Shiny/ dark | Smooth          | Hard             |

Clinical characteristics of initial enamel lesion include:

1. Chalky white appearance when dried with loss of normal translucency due to change of light characteristics.
2. A fragile surface layer of pits and fissures that is easily damaged by probing.
3. Discoloration potential has been increased, as a consequence of increased porosity under the surface.
4. Reduction of subsurface density can be detected radiographically, by transillumination, or by modern laser devices.
5. The potential for remineralization especially with the use of advanced remineralization treatments, thus; increasing resistance to further acid attacks (Mount, 2005 ).

## **2.6. Early Enamel Carious Lesion Layers**

During the first stage of enamel demineralization, interprismatic minerals loss takes place. In the following phase, a well-defined surface layer developed, that represents early enamel lesions. These investigations have shown that an enamel lesion has a porous, mineral-rich surface layer with morphology slightly different from sound enamel, whereas the subsurface area, or the body of the lesion has a low mineral concentration (10–70 vol%) (Kudiyirickal and Ivančáková, 2008). In the caries lesion, four zones related to the porosity were identified. These Translucent zone followed by dark zone, then the body of the lesion followed by intact surface of the tooth (Robinson et al., 2000).

There are two demineralization zones:

1. The 1% pore volume translucent zone that runs along the lesion's advancing front.
2. The body of the lesion which is more than 5 up to 25% pore volume and is located 15-30  $\mu\text{m}$  below the intact enamel surface above it.

Additionally, there are two remineralization zones:

1. Dark zone (2-4% pore volume) that is located superficially to the translucent zone, close to the advancing front.
2. The intact surface layer that covers the lesion, ranging from 1 to less than 5% of the total pore volume (Hicks et al., 2005; Koopa et al., 2015).

### **2.6.1. The early development of subsurface enamel lesions**

Early enamel lesions appear due to the HA prism's dissolution that makes up the enamel surface. Early enamel lesion resembles a translucent zone where a small amount of minerals is lost. A surface zone with a porosity similar to that of the surrounding healthy enamel will emerge if demineralization continues without remineralization, and this zone will remain intact until cavitation. The lesion's body (water imbibition) forms as a result of continued mineral removal from the underlying enamel, separating the

translucent zone at the advancing front from the overlying surface zone. Mineral phases from the translucent zone will reprecipitate in the black zone, which forms when lesion development takes a long time. Lesion establishment will result soon in the absence of a dark zone, rapid frontal advancement, a big, severely demineralized lesion body, and a thin surface zone. After the lesion has reached a particular level of demineralization, it will appear as a white spot and be clinically noticeable (Hicks et al., 2005; Kudiyirickal and Ivančáková, 2008; Roopa et al., 2015).

## **2.7. History Of Early Enamel Lesion Treatment**

In the past, dental professionals treated dental caries as an infectious condition brought on by bacteria that are harmful and contagious. Therefore, eliminating possible breeding areas, killing pathogens, thoroughly disinfecting infectious locations, and preventing infection recurrence were the goals of treatment. Following the discovery of caries lesions, full caries removal and cavity restoration were frequently the main goals of caries treatment. It was also advised to remove healthy dental hard tissue in order to prevent cavities. This dental caries treatment approach is essentially an operative one that is carried out surgically. Caries prevention or control by this method is impossible since it ignores the etiological elements that cause the disease. The effectiveness of dental restorations, which have a finite lifespan, basically affects how well the procedure succeeds. The rate of dental restoration failure was considerably higher in patients with a high caries risk than in those with a low caries risk. According to these investigations, there was insufficient control over dental caries with the surgical management of caries (Yu et al., 2021).

A new idea that places greater emphasis on managing etiological factors has replaced the prior surgical method of managing caries. The composition of the oral microbiota changes in response to environmental changes, such as higher sugar intake. The micro-community's equilibrium is disrupted by the enrichment of acidogenic and aciduric bacteria, which leads to tooth caries. By decreasing the targeting of pathogenic microbes, enhancing oral hygiene, and altering the environmental conditions that favor the cariogenic bacterium, caries can be prevented, according to the ecological plaque theory. The minimal intervention dentistry approach, which seeks to maintain dental structure and pulpal vitality in order to prolong dental life, is consistent with the modern

cariology philosophy. The primary objective is to enhance oral health by allowing the disease to heal. Throughout the whole caries development process, it illustrates the use of nonrestorative treatment to prevent the loss of minerals from caries. For effective caries control, nonrestorative management must be considered first.

By handling the many etiological features of dental caries, nonrestorative management strategies can be divided into four basic categories. These elements include keeping dental plaque under control, reducing the risk of caries, protecting tooth hard tissue, and regular follow-up for long period of time (Yu et al., 2021).

There are three possible levels of treatment: primary, secondary, and tertiary. Patients at the primary level are motivated or informed about maintaining good oral hygiene and taking preventative steps against dental caries. Lesions that still in the early stages of development are kept from developing into more severe phases at the secondary preventive level. In tertiary prevention, function is restored and disease-related consequences are minimized in an effort to lessen or completely eliminate the effects of an existing disease. Enhancing the live's quality of individuals afflicted with illnesses is another goal of tertiary prevention (John et al., 2015).

## **2.8. Early Enamel Lesion Treatment Approaches**

The plaque biofilm, diet, vulnerable tooth hard tissue, and time are variable etiologies that involved in the the development dental decay. Caries etiology and risk factors should be considered during nonrestorative dental caries treatment. Consequently, dental plaque reduction, lowering risk of caries, preserving the hard tissue of teeth, and regular maintenance for long time are essential for the nonrestorative management of tooth decay (Yu et al., 2021).

### **2.8.1. Plaque control**

#### Oral Hygiene Education

The traditional oral hygiene education was widely used in dentistry, its impact on improving oral hygiene and reducing caries is regrettably questionable. It was suggested that motivational interviewing could increase the efficacy of dental hygiene education.

This is a patient-focused counseling technique meant to enhance a patient's internal motivation and modify their behavior related to oral health. According to a systematic evaluation, motivational interviewing outperformed traditional oral hygiene education in terms of enhancing oral hygiene. In patients with high caries risk, interviews could successfully inhibit caries; however, in those with low caries risk, its impact was negligible (Yu et al., 2021).

### Brushing Teeth

Brushing teeth is the most frequently used oral hygiene method for reducing plaque mechanically. Both electrical and manual toothbrushes are efficient at eliminating plaque, according to the American Dental Association. According to a review, powered toothbrushes are better than manual toothbrushes at controlling plaque over the long and short term. The frequency of brushing your teeth affects caries development. Compared to people with regular toothbrushing practices, those with irregular brushing habits exhibited a greater prevalence of caries. When Comparing between groups that brush twice a day, with those who brush less frequently had a significant prevalence of dental decay. Remarkably, when tooth brushing was done at least three times a day, the incidence of dental caries did not change. Consequently, brushing twice a day is recommended to prevent caries (Yu et al., 2021).

### Interdental Cleansing

Dental plaque is removed from the interdental spaces that are inaccessible to toothbrushing using interdental cleaning instruments. Interdental brushes, floss, mouth irrigators, and tooth-cleaning sticks are popular self-practice tools for interdental cleaning. A review found that flossing of the teeth or the usage of interdental brushing in addition to brushing of the teeth was more effective at reducing dental plaque than just brushing alone. The use of interdental cleaning to inhibit the formation of caries is not well-supported by research (Yu et al., 2021).

### Antimicrobial Disinfectant

To prevent caries and control plaque, chlorhexidine is a broad-spectrum, biocompatible antimicrobial disinfectant. Chlorhexidine comes in a variety of forms, such

as chewing gum, mouthwash, varnish, gel, and spray. 0.05% chlorhexidine mouthwash and 1% chlorhexidine gel are typical formulations. Studies conducted in laboratories have shown how effective it is at controlling plaque. In general, clinical research did not support the use of chlorhexidine to reduce dental caries (43). There is little evidence to support the use of chlorhexidine gels or varnishes to stop *Streptococcus mutans* or prevent dental caries. Moreover, chlorhexidine can change salivary acidity and raise the oral microbiome's capacity for caries formation.

Povidone-iodine is additional wide-spectrum antibacterial disinfectant used in dentistry. Prior to dental procedure, some dentist recommend their patients to rinse their mouths with 1% povidone-iodine. Dental practitioners also utilized povidone-iodine with fluoride varnish to stop and remineralize white spot lesion. Clinical research has shown that administering povidone-iodine and fluoride together reduces the occurrence of caries in kids who are at high risk for tooth decay (Yu et al., 2021).

In toothpaste and chewing gum, xylitol, a sugar alcohol, can be added. According to a clinical investigation, using fluoridated toothpaste containing 10% xylitol decreased the incidence of dental cavities by 13% more than using regular fluoride toothpaste. Nonetheless, laboratories studies indicate that xylitol diminishes the cariogenic potential of dental plaque and prevents the growth of cariogenic bacteria. There is not enough clinical evidence to support the use of xylitol in the prevention of dental cavities, nor is there adequate data to support the caries prevention abilities of other sugar alcohols (Yu et al., 2021).

Bacteria resistant to acid growth are inhibited by the amino acid arginine. The content of arginine varies throughout toothpastes and mouthwashes. A review found that arginine-fluoride toothpaste with 1.5% was better than fluoride toothpaste and reduced caries incidence by 12% to 21%. Dental substances have also introduced additional antibacterial components as hexetidine, chitosan, and prebiotics to manage plaque and prevent caries. However, little evidence exists to substantiate the application of these medications in the treatment of dental caries (Yu et al., 2021).

### **2.8.2. Caries risk reduction**

The risks of dental caries can be minimized by determining risk factors and protective factors for dental caries and developing a customized treatment strategy. Caries arise when the protective factors are outweighed by the risk factors. Caries can be avoided if the risk factors for the condition are outweighed by the preventative factors.

There are two types of caries risk factors: related to the oral environment and related to the patient. Patient-level risk factors, such as inadequate diet, low recall compliance, and bad oral hygiene habits, can be found through a patient's medical and dental history. Intraoral caries risk indicators, With a thorough clinical examination, conditions including obvious plaque accumulation and teeth with deep pits and fissure can be discovered. Protective variables include sealants, resin infiltrations, dental hygiene practices, enough salivary flow and salivary composition, and other elements. Moreover, the lack of protective elements is regarded as a risk factor, indicating a higher chance of dental caries (Yu et al., 2021).

Treatment processes pertaining to diagnosis, prevention, and restorative care are part of the minimal intervention caries treatment approach. A computer program called Cariogram is intended to visually represent a patient's risk of caries. Once the patient's caries risk has been established, a customized plan for dental care must be established for the patient's caries management. Brushing twice a day with fluoride toothpaste, encouraging involvement, and following dental instructions are advised for those who are at low risk. Individuals who are a moderately to highly susceptible to tooth decay may want to think about controlling their diet, increasing the concentration of fluoride products, applying fluoride with greater frequency, sealant or resin infiltration, or enhancing hyposalivation (Yu et al., 2021).

Dental cavities are linked to the use of free sugar, which is any mono- and disaccharides that are added to food by food producers, chefs, or consumers, as well as the natural sugars present in honey, syrups, and fruit juices. Reducing the quantity and frequency of meals and beverages that contain free sugar should be the main focus of dietary recommendations meant to minimize the incidence of dental caries. Patients should be encouraged to consume more almonds, milk, and various fresh, sugar-free fruits and vegetables. Research must back up this personalized nutritional advice. Fissure sealing is advised for patients with deep pit and fissure sites or pit and fissure decay.

The risk of caries is moderate to high for these people. Resin infiltration is advised for non-cavitated enamel lesions at the interproximal surface that reach the outer third of the dentine. (Yu et al., 2021).

### **2.8.3. Maintaining of dental hard tissue**

Restoration intervention should be used for most cavitated caries lesions that cannot be cleaned up or sealed. Treatment should be non-invasive for cleanable active cavitated lesions or active non-cavitated lesions. When a carious lesion is limited to an area outside the outer third of the dentine, it is recommended to intervene non-invasively. Whenever caries extend to the middle third of the dentine, restorative therapy becomes necessary. The main objective of nonrestorative treatment is to remineralize dentine and carious enamel. During tooth formation, the mineral that makes up teeth may absorb contaminants, which could alter the mineral's solubility. Crystals that contain impurities like  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $(\text{CO}_3)^{2-}$ , or  $(\text{HPO}_4)^{2-}$  may dissolve selectively and make the substance more soluble. During the demineralization and remineralization processes, contaminants will be eliminated. When the pH rises above 5.5, remineralization occurs, but the impurities that make the mineral more soluble won't be absorbed. Higher-quality minerals will be produced via cycles of demineralization and remineralization as long as remineralization can maintain up with demineralization (Amaechi and Loveren, 2013).

### **2.8.4. Long-term follow up**

Maintaining dental health and managing dental cavities require routine recall visits and long-term follow-ups. Even after getting treatment, patients at a high risk of caries are more likely to see an increase in pre-existing cavities or to develop new ones. To reassess the therapeutic efficacy of the implemented caries management measures, routine recalls are required. The patient's risk of dental caries determines the recall frequency. For people who are at a high risk of dental cavities, the recall period may last three months, and for people who are at a low risk, it may last two years. Notably, due to the lack of readily available research at this time, the recall interval is frequently established by the clinical judgments of the most experienced practitioners (Yu et al., 2021).

## **2.9. Remineralization Therapy**

It's usual practice to apply topical remineralization agents such as fluoride or calcium-based treatments to prevent demineralization and/or encourage remineralization. Tri-calcium phosphate, casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and nano-hydroxyapatite are common calcium-based products used to remineralize carious lesions. An ideal remineralization material should meet the following criteria:

- Subsurface diffusibility.
- No excess calcium delivery.
- Don't form calculus.
- Can work at low pH.
- Effective in people with xerostomia.
- Enhances the salivary remineralization potential (Arifa et al., 2019).

## **2.10. Fluoridated Remineralization System**

Soi et al. discovered four fluoride modes of action (2013). First, fluoride stops demineralization by combining with enamel apatite crystals to generate fluorapatite crystals, which are more resistant to acid attack than HA crystals. Second, fluoride promotes remineralization by encouraging the aggregated growth of calcium and phosphate ions, which stimulates the production of new fluorapatite crystals. Third, it inhibits the production of phosphoenol pyruvate, an essential bacterial intermediate of the glycolytic pathway that deters acid-producing carious bacteria. Moreover, fluoride ions are held in the tooth hard tissue, oral mucosa, and dental plaque in order to lessen demineralization and encourage remineralization (Yu et al., 2021).

Furthermore, there are other chemical forms of fluoride, such as amine fluoride ( $C_{27}H_{60}F_2N_2O_3$ ), stannous fluoride ( $SnF_2$ ), sodium fluoride (NaF), sodium monofluorophosphate ( $Na_2FPO_3$ ), and mixtures of these (Amaechi and Loveren, 2013).

### **2.10.1. Fluoride in the teeth**

The total amount of fluoride present in the tooth during its formation is expressed by fluoride concentration. The total amount of fluoride available during tooth production is reflected in the concentration of fluoride found in teeth. The enamel's surface has the highest quantities of fluoride, which can range from 2000 mg/kg in non-fluoridated areas to 3000 mg/kg in fluoridated areas. These concentrations, which vary depending on whether the area is fluoridated or not, drop abruptly after the outermost 10–20 µm of enamel to values ranging from hundreds of mg/kg to about 50 mg/kg. Up to the enamel-dentine junction, the fluoride level stays rather stable after this depth. Dentine, as opposed to enamel, keeps absorbing fluoride throughout life. Dentine receives all of its fluoride through the systemic route. The concentration of fluoride in human enamel can only be permanently changed in subsurface areas following full development, such as when there is damage from caries, erosion, or abrasion. Fluoride diffusion from the oral cavity, which varies based on exposure to dietary fluoride sources, medical substances, saliva, and plaque in the teeth, can, nevertheless, cause fluoride concentrations in the outer surface to rise. De- and remineralization processes have little effect on these concentrations. (O'Mullane et al., 2016). It was found that the usual enamel fluoride concentrations were insufficient to provide effective caries prevention (Buzalaf et al., 2011).

### **2.10.2. Systematic fluoride**

#### Fluoridated drinking water

The gold standard for effectively monitoring fluoride amount in drinking water is widely acknowledged to be the use of an ion-specific electrode (O'Mullane et al., 2016). The initial method of using fluoride to prevent cavities was to add regulated amounts of fluoride to public water systems. Pregnant women are advised to take fluoride supplements to prevent dental cavities in their future babies as a result of the positive outcomes of this measure. Before the 1970s, when fluoride was first found to provide cariostatic benefits when consumed from "systemic" sources, it was thought that fluoride's cariostatic mechanism mostly depended on its uptake in forming enamel. Formation of Fluorhydroxy-apatite, a mineral phase that is more resistant to dissolution in the future. For this reason, it was believed that consuming fluoride was obligatory and

that developing dental fluorosis was a required risk to take advantage of the cariostatic benefits of fluoride (Buzalaf et al., 2011).

Dean found that 1 mg/L is the ideal water fluoride concentration based on his preliminary studies on fluoride levels in the water. This 1.0 mg/l recommendation has been changed to a range (0.7-1.2 mg/l); the lower the recommended fluoride level in drinking water, the higher the community's temperature. This is because individuals in hotter regions consume more water than people in colder climates. This range was established as the guideline for fluoride content in drinking water by the US Public Health Service in 1962, and it has been widely applied ever since. But by the 1990s, it was evident that not all places in the world could use these criteria. The introduction of air conditioning, rising consumption of processed foods and soft drinks, and the availability of alternative fluoride sources—particularly the use of fluoride-containing toothpaste beginning in the 1970s—all rendered this range outdated, even in the United States, where it was first used. It was likely not appropriate to follow the recommended range due to the wide diversity of dietary behaviors observed in many various races and cultures in other parts of the world, especially in the tropical and subtropical regions of Africa and Asia. Following awareness of this circumstance, nations started controlling the amount of fluoride in drinking water (O'Mullane et al., 2016). The standard value for fluoride in drinking water according to the World Health Organization (WHO) is 1.5 mg/l (Bergmann and Bergmann, 1995).

#### Fluoridated salt

Using salt as a carrier for other minor components is not an unusual thought, since it is the primary source of iodine supplementation in numerous countries and has been demonstrated to successfully treat goiter caused by iodine deficiency. Following its introduction in Switzerland over 20 years ago, fluoridated salt has gained widespread acceptability worldwide (Bergmann and Bergmann, 1995).

Fluoridated salt is available to consumers through various methods, such as bread, large kitchens, school lunches, and home salt. It has both surface and systemic effects. Since 1986, there has been a steady growth in the community's use of fluoridated salt as a preventive measure against tooth caries (O'Mullane et al., 2016).

Studies on salt intake and total fluoride exposures must be taken into account when determining the ideal fluoride concentration; a minimum value of 200–250 mg/kg of salt is commonly accepted (World Health Organization, 2019). The following are some benefits of using salt to provide fluoride: By itself, salt is a supplement. When compared to fluoridating drinking water, salt gives the customer greater freedom of choice. In contrast to the daily consumption of fluoride tablets or drops, lifetime daily compliance requires no special attention. When taking the supplement with its highest concentrations of fluoride in plasma, small doses are given throughout the day rather than as a single bolus dosage once a day. It is extremely inexpensive. The primary drawback is that it is widely acknowledged that a high sodium intake increases the risk of hypertension (Bergmann and Bergmann, 1995).

#### Fluoridated milk

Around the same period, Switzerland (1953), the United States of America (USA) (1955), and Japan (1952) all proposed adding fluoride to the milk they produced (Bánóczy et al., 2013). There is evidence that fluoridating milk can effectively reduce dental cavities, especially in children. Various nations have implemented programs integrating milk fluoridation with school health and nutrition programs (Toumba et al., 2019).

Early studies revealed that fluoride added to milk is well absorbed, although more slowly than from fluoridated water, and has no effect on the flavor or other qualities of the milk. It was deemed beneficial because fluoridated milk is only required for those who most need it and accept to receive it, and fluoride is added to a diet that is crucial for newborns and early children. Because milk has high levels of protein, fat, and minerals that have cariostatic qualities, the caries-prevention action of fluoride can even be strengthened by the milk carrier. Based on investigations, the current understanding states that the majority of the fluoride added to milk forms a soluble complex with the milk's protein component, from which the fluoride can be released in an ionic form to become bioavailable. Fluoride absorption is slower when it is consumed with food, in contrast when it is consumed alone. Furthermore, the amount of proportion absorbed is influenced by the diet's calcium intake (Bánóczy et al., 2013). Breast milk is generally low in fluoride and is therefore best for newborn health. Therefore, encourage moms to breastfeed even in places where fluoride exposure is high (World Health Organization, 2019).

The age and history of fluoride exposure—which is ascertained by a questionnaire and measurement of urine fluoride excretion—will decide the dosage. The milk has different forms that vary between the countries but it does not affect fluoride availability and effectiveness. Furthermore, the price of milk that has been fluoridated and that has not is the same (O'Mullane et al., 2016).

#### Fluoride supplements (Drops and Tablets)

In places where water fluoridation was not available, fluoride tablets, lozenges, and drops were initially used to supply systemic fluoride. The efficacy of fluoride toothpaste had not been conclusively proven at the time of its introduction. Effective fluoride toothpaste started to become widely accessible in the middle of the 1970s. It was also acknowledged around this time that, when applied correctly, fluoride's post-eruptive effects were strong enough to maintain dental health. According to these results, fluoride drops and tablets are less effective caries-prevention tools. Furthermore, while applying them, caution should be used to ensure that the products have enough substantivity in the mouth cavity to have a topical effect as well (Toumba et al., 2019).

Supplements should only be given to children who are extremely vulnerable to dental cavities and whose primary source of drinking water is deficient in fluoride. Recommendations for fluoride supplementation should consider the possibility of fluorosis in addition to the substance's ability to prevent dental caries. The objective should be to strike the best possible balance between both the benefits and drawbacks of using fluoride. The likelihood of developing enamel fluorosis and the caries-preventive efficacy of dietary fluoride supplementation can be influenced by several factors. Age, the amount of fluoride in the child's main water sources, and the child's caries risk status are some of these variables. Table 2. displays the recommended dosages of fluoride supplements based on the fluoride level in drinking water (Rozier et al., 2010).

**Table 2.** Fluoride supplement doses.

| Age               | <0.3 ppm | 0.3 ppm-0.6 ppm | >0.6 ppm |
|-------------------|----------|-----------------|----------|
| 0-6 month         | 0        | 0               | 0        |
| 6 month -3 years  | 0.25 mg  | 0               | 0        |
| 6 years- 16 years | 1 mg     | 0.50 mg         | 0        |

### 2.10.3. Topical fluoride

Topical fluoride works as a cariostatic agent by promoting remineralization and preventing demineralization. It might also have inhibitory effects on pathogens that cause caries. By definition, treatment with fluoride that applies fluoride to tooth enamel in high quantities is referred to as "topically applied fluoride." Currently, the most often utilized vehicles are toothpaste, mouth rinses, gels, and varnishes that contain fluoride, either separately or in combination. Topical fluoride mechanism of action include:

Boost remineralization because studies have shown that remineralization happens spontaneously in if there is of low fluoride concentrations once an acidic stimulus is generated. Through surface adhesion to the partially dissolved sub-surface crystal in the carious lesion and attraction of  $\text{Ca}^{2+}$  and  $\text{HPO}_4^{2-}$ , fluoride facilitates remineralization. Consequently, a layer that is significantly less soluble than the initial material precipitates as a result of a chemical interaction that affects carbonate removal and fluoride inclusion. Thus, after every cycle of breakdown and reprecipitation, the enamel becomes more resistant to subsequent acidic challenges (Miller et al., 2012).

Prevent demineralization because low fluoride levels in saliva and plaque fluid (sub-ppm range: 0.03 ppm or higher) significantly lower the degree of enamel demineralization throughout the process of dental caries. Fluoride in the plaque biofilm will follow the cariogenic acid challenge into the tooth's subsurface, where it will cling loosely to the surface of the crystal to keep it from dissolving. Because the crystal has a layer of loosely linked fluoride, its characteristics are similar to FA, which significantly resist the acidic dissolution by carious bacteria (Miller et al., 2012).

Impact on bacterial caries ; one possible aspect of fluoride's cariostatic activity is its potential to suppress cariogenic organisms. Direct suppression of crucial cellular enzymes, such as regulatory phosphatases and heme-containing or other metalloenzyme enzymes, by fluoride (either alone or in conjunction with metals) might influence bacterial metabolism. Additionally, it might function as Hydrofluoric acid (HF), a transmembrane proton conductor, increasing the permeability of the bacterial membranes to protons. Although fluoride in its ionized state cannot pass through bacterial cell walls, it can enter the cells as HF (Miller et al., 2012).

Acidic environments facilitate the easier formation of this compound. Consequently, the fluoride present in the plaque reacts with hydrogen ion when bacteria breakdown carbohydrates in food to make acids, and the resulting HF that diffuses into the cell quickly. HF dissociates in  $H^+$  and  $F^-$  once it enters the cytoplasm, which is more alkaline than the plaque fluid. This latter can block glycolytic enzymes, especially enolase, and acidify the surrounding environment. Both of these actions cause the bacteria's glycolytic activity to diminish, which in turn reduces the formation of acid. Only high fluoride concentrations (such as toothpaste having 5,000 ppm of fluoride) or fluoride formulations containing other antibacterial ingredients (such as amine and stannous fluoride) have been shown to have significant antibacterial benefits in clinical trials (Miller et al., 2012).

#### Toothpaste with fluoride:

In the 1950s, fluoride toothpaste began to become widely available worldwide. Over 95% of toothpaste sales currently consist of fluoridated toothpaste. The primary means of maintaining dental health and contributing to the decline in dental caries that developed nations have seen since the 1960s is the usage of fluoridated toothpaste. In addition, toothpaste is the most effective way to provide fluoride (Miller et al., 2012).

Since ancient times, toothpaste has been commonly used as a cleaning product. But only in the past century have efficient treatments—most notably fluoride—been added to their recipe, greatly enhancing the oral health of people all over the world. Given their significant influence on toothpaste's mode of action, two of the formulation's constituents need more discussion:

- Abrasive substances
- Therapeutic compounds.

For a toothpaste to work as an effective dental stain and plaque remover, it must contain abrasive compounds. Because a toothbrush alone cannot effectively remove pellicles, toothbrushing compliance is decreased when using an abrasive-free recipe. This leads to raise in discoloration susceptibility and quick dental biofilm reform. Different toothpastes contain different abrasives, like calcium and sodium fluoride. Because

sodium fluoride is highly soluble, it releases fluoride ions, which then react with calcium to generate insoluble calcium fluoride precipitate, which is useless for remineralization. In order to prevent interactions with calcium from calcium-based abrasive systems and to promote remineralization, the fluoride ion needs to be in a more stable formula. Since fluoride is covalently bonded to the phosphate group in sodium monofluorophosphate, which is the more stable form, it cannot react with calcium ions released from the abrasive system. Instead, it releases monofluorophosphate when ionized. With regard to the activity of non-specific oral phosphatases, the monofluorophosphate ion will only release ionic fluoride in the mouth. However, because some fluoride is released from the monofluorophosphate ion during toothpaste storage and reacts with calcium to generate insoluble fluoride, the durability of the activity of these toothpaste formulations needs to be evaluated over time (Cury and Tenuta, 2014).

Fluoride is the primary element in toothpaste, which is frequently sold as a paste or gel and is used in conjunction with a toothbrush to preserve and improve oral health. The bioavailability of fluoride is crucial for its caries-prevention action. Fluoride's solubility and surface adherence are essential factors that determine its bioavailability. Different fluoride compounds have been employed in toothpaste formulations, including sodium fluoride, stannous fluoride, amine fluoride, and sodium monofluorophosphate (Miller et al., 2012).

The typical toothpaste has 1000–1100 ppm of fluoride, however, several countries provide toothpaste with greater fluoride concentrations (1500 ppm) and lower (around 500 ppm) than those found in normal toothpaste. While lower-fluoride toothpaste is advised for younger children in various European nations, there is no scientific evidence that toothpaste with a lower fluoride content can prevent tooth decay (Ercan et al., 2010). In contrast, toothpastes with higher fluoride concentrations showed better caries prevention. Furthermore, it is abundantly evident that using fluoride toothpaste and brushing twice a day or more reduces dental caries more than brushing just once a day or less monofluorophosphate (Miller et al., 2012).

#### Mouthrinse with fluoride

Mouthrinse is a dental hygiene product that, when used with fluoride, may protect teeth from cavities by acting as a bactericidal and bacteriostatic agent against oral bacteria. The development of mouthrinses into a commercial product began only in the late 1960s when a conclusive experiment revealed that chlorhexidine was capable of eliminating dental plaque bacteria. Since then, several investigations have been conducted to determine whether fluoride mouthrinses are an effective caries prevention measure. Only a small number of the research that is currently available in the literature mention the use of amine fluoride mouthrinses; for most of the studies, the fluoride salt used in these studies is sodium fluoride (NaF). Fluoride levels range from 230 parts per million to 900 parts per million. After brushing, mouthwash (approximately 20 ml, or 2/3 fl oz) is often used twice a day to rinse the mouth. Usually, the wash is gargled or swished for thirty seconds or less, and then it is spat out. Fluoride bio-availability is related to more than just the mouthrinse's fluoride salt content when it comes to the ability of the fluoride to react with dental enamel. A low pH may have a beneficial effect on fluoride's interaction with dental enamel.

Furthermore, studies have demonstrated that frequent mouthwashing with a minimal level of fluoride (0.025% NaF) causes a considerable increase in fluoride absorption that is comparable to that of mouthwashing with a high concentration (0.05% NaF). In most community-based oral health prevention programs, mouthrinses were tried. There is widespread agreement that this fluoride delivery technology has shown to be a successful means of preventing dental caries (Miller et al., 2012).

#### Fluoridated varnishes

Under the brand name Durahat, fluorinated varnishes were initially made available in Europe in 1964. Fluorinated varnishes have been demonstrated to be both safe and highly effective in preventing caries in clinical investigations that have lasted over 25 years. Studies conducted on patients have indicated that using fluorinated varnishes can reduce dental cavities by 25% to 75% (Ercan et al., 2010).

To extend the fluoride-dental enamel contact duration, the first fluoride varnishes were created and commercialized in the 1960s under the brand name Duraphat, 15% sodium fluoride varnish, which is equal to 22,600 ppm fluoride ion. Enamel is a good

surface for fluoride varnishes to stick to, and the calcium fluoride (CaF<sub>2</sub>) that forms after application serves as a long-term fluoride reservoir.

In non-fluoridated regions, varnishes were applied as a means of prevention. The discovery of methods for permanently fixing fluoride in the form of fluorapatite in enamel was prompted by observations of increased fluoride concentration in enamel following topical fluoride applications. Saliva interacts with fluoride varnishes, forming CaF<sub>2</sub> compounds on enamel. CaF<sub>2</sub> was supposed to dissolve easily from the enamel shortly after application, leading to the long-standing assumption that it was an undesirable side reaction. However, pellicle proteins and secondary phosphates maintain CaF<sub>2</sub> at neutral pH, as demonstrated by multiple experiments carried out in the 1980s, CaF<sub>2</sub> acts as a sustained source of fluoride following treatment when the pH of the plaque lowers, causing it to disintegrate and release fluoride ions. Fluoride varnishes are a viable solution to prevent preschoolers from consuming too much fluoride because they are accepted and simple to apply. After applying the varnish, the child can immediately close his mouth since it hardens when it comes into touch with the saliva and forms a layer that adheres to tooth surfaces. Nonetheless, it is advised that patients avoid eating for two hours after applying the varnish and skip brushing that day. This gives the varnish several hours to stay in touch with the dental enamel. The American Dental Association (ADA) recently declared that caries in children's and adolescents' primary and permanent dentition can be effectively prevented by using fluoride varnish every six months (Miller et al., 2012).

### Fluoride gel

In the past, fluoride gels have been a standard part of dental procedures and caries prevention measures around the world. In recent years, concerns have been made over their affordability and the potential for excessive fluoride exposure, particularly in light of the high fluoride level in certain gels. It is possible to expertly or independently apply fluoride gels once or multiple times a year while under supervision. Expertly gels application often use trays, while self-applied gels might use a toothbrush or a tray. Biting is used to hold the tray in the mouth. Patients should wait at least 30 minutes after application before rinsing, eating, smoking, or drinking. Application takes around four minutes. It's important to distinguish fluoride gels from various fluoride toothpastes, which can also be found in gel form. The "classic" fluoride gels are used very

infrequently, don't include abrasives, and often have a fluoride concentration that is far higher than that of fluoride toothpaste. Typically, professional applications occur twice a year for four minutes each of acidulated phosphate fluoride APF gels (12,300 ppm fluoride, or 1.23%), and NaF gel (9,050 ppm fluoride, or 0.90%) (Miller et al., 2012).

The agents that are most frequently utilized include 1.23% acidified phosphate fluoride, 2% sodium fluoride, and 8–10% stannous fluoride. Traditional fluoride gels are typically made of phosphate fluoride that has been acidified and is non-abrasive. Gels containing amine fluorides, SnF<sub>2</sub>, and NaF are also utilized. Cellulose is added to the fluoride mixture during gel manufacture, increasing its viscosity. Since 1968, APF preparations have been approved; their pH ranges from 3 to 4 and they have 1.23% fluoride ions (12300 ppm). The theory for their acidic preparation is that low pH promotes enamel's absorption of fluoride (Ercan et al., 2010).

### **2.11. Non-fluoridated Remineralization Systems**

Calcium phosphate is the primary form of calcium found in the blood and milk of cows. Since calcium and phosphate are the main components of HA crystals, their concentrations in saliva and plaque have a significant impact on the processes of tooth demineralization and remineralization. A higher calcium diet may encourage the remineralization of enamel (Arifa et al., 2019).

Concerning hard tissue maintenance and development, saliva is considered the source of bioavailable ions (calcium and phosphate) throughout life. Thus, ensuring the diffusion of these ions to mineral-deficient lesions at normal pH. The remineralization of saliva is insufficient to remineralize white spot lesions as several studies showed that the remineralization of white spot lesions is unaffected with salivary remineralization. In addition to that; mineral gain is only a slow process and superficial due to the low concentration of ions from saliva toward the the lesion (Philip, 2019). Efficient Non-fluoridated remineralization systems with low fluoride concentrations are needed to reduce the risks of high fluoride (Philip, 2019).

#### **2.11.1. Biomimetic remineralization**

Biomimetic techniques developed from advances in tissue engineering have shown great promise in the regeneration of the hierarchical enamel microstructure. Fluoride-containing dental care products are good at remineralizing enamel, but they can't encourage the growth of ordered HA crystals. Currently, there is an effort to switch from reparative to regenerative biomineralization therapies, which entail the replacement of diseased dental tissues with tissues that are similar to the original one. Enamel regeneration is extremely difficult since mature enamel is acellular and does not resorb or reconstruct itself as bone or dentine do (Philip, 2019).

#### Dentine Phosphoprotein-Derived 8DSS Peptides

The most prevalent non-collagenous extracellular matrix component in dentine, dentine phosphoprotein (DPP), is recognized to be essential for tooth mineralization. Numerous repeated aspartate-serine-serine (DSS) nucleotide sequences have been found in human DPP, and studies have demonstrated that DPP can produce hydroxyapatite (HA) crystals in calcium phosphate solutions, which is thought to encourage the development of HA. The aspartate-serine-serine (8DSS) octuplet repeats are the most effective in inducing biomineralization among the DPP-derived peptides. 8DSS peptides have two mineral-binding sites; they may attach firmly to the HA surface as well as free  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions. Two mechanisms mediate biologically guided remineralization. In demineralized dentine, they first prevent  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions from dissolving, and then they encourage the ions to be captured and form newly deposited minerals on demineralized enamel.

Small apatite crystals were uniformly deposited in the newly formed mineral, and its properties—such as its hardness, elastic modulus, and decreased surface roughness—were noticeably better. Clinical application of 8DSS peptides is probably going to pose certain difficulties because the proof of concept has only been demonstrated in vitro systems currently. For instance, it's unclear if these peptides can withstand oral cavity enzymatic action. Another limitation of 8DSS is that, if left unchecked, its high level of calcium binding could result in calculus development (Philip, 2019).

#### Self-Assembling P11- 4 Peptides

Replacing the damaged enamel matrix with a biomimetic matrix that promotes thoroughly remineralization of enamel lesions would be the optimal method for enamel regeneration. P11-4, a monomeric peptide of 11 amino acids, is an impressive discovery in this discipline. When faced with local conditions like high ionic strength and acidic pH in the lesion body, this well-designed peptide self-assembles into hierarchical 3-dimensional fibrillar scaffolds. With its strong attraction for  $\text{Ca}^{2+}$  ions, the P11-4 fibrillar matrix serves as a nucleator for the de novo HA production that causes the lesion body to remineralize. P11-4 fibers in the lesion body accelerated the synthesis of HA and enhanced the microhardness of the remineralized subsurface lesion, according to an analysis of in vitro data. The isotropic P11-4 easily diffuses into the initial lesion's body due to low viscosity.

In the presence of positively charged ions (cation) and pH less than 7.4, the isotropic P11-4 turns into an elastomeric nematic gel, which facilitates the assembly of the three-dimensional fiber matrix and the lesion's subsequent biomineralization (Philip, 2019).

#### Nanohydroxyapatite (n-HA)

Since synthetic nanohydroxyapatite (n-HA) resembles the apatite crystal found in enamel in terms of form, structure, and crystallinity, it is regarded as one of the most biocompatible and bioactive materials. The nanoparticles can form a strong bond with bacteria, plaque fragments, and enamel surfaces. Because of the tiny particles that make up nHA, it has a much larger surface area for binding and can be used as a filler to fix tiny cracks and holes in the enamel surface. Dynamic pH-cycling tests conducted in the laboratory have demonstrated that n-HA can remineralize early enamel lesions as effectively as fluoride, if not more so. Significant potential for enamel remineralization near restoration margins was observed in another in vitro study using n-HA gel. The exact process by which nHA performs its biomimetic function is unknown, however, some studies propose that it does so by either depositing apatite nanoparticles in enamel lesions or defects or by forming a new layer of synthetic enamel around the tooth.

Because n-HA preserves the excess minerals in enamel and serves as a calcium phosphate reservoir, it inhibits demineralization and encourages remineralization.

Moreover, it is demonstrated that, under neutral conditions, n-HA preferentially facilitates the remineralization of the outer enamel caries lesion; complete remineralization of the lesion is not seen. Further research is required before clinicians recommend nHA oral products as a substitute for fluoride mouthwashes or dentifrices (Philip, 2019).

### **2.11.2. Calcium phosphate-based systems**

The three types of innovative calcium phosphate remineralization systems that are now on commerce were classified by Cochrane et al. as follows: crystalline calcium phosphate systems, unstabilized amorphous calcium phosphate systems, and stabilized amorphous calcium phosphate systems (Cochrane et al., 2010).

#### Unstabilized calcium phosphates

##### Amorphous calcium phosphate ACP

Amorphous calcium phosphate ACP is the first solid phase to precipitate from a high overabundance calcium phosphate solution. Because of its disorganized structure, ACP reacts strongly with body fluids, which causes quick apatite reprecipitation. It is readily converted into apatitic compounds or stable crystalline phases such as octacalcium phosphate. It serves as a bridge between biomineralization and the bioapatite precursor (Zhao et al., 2011; Arifa et al., 2019).

Unstabilized ACP is the foundation of ACP technology, in which the phosphate salt and calcium salts are administered intraorally in separate forms or combined in a product with low water activity. The salts break down when they come into contact with saliva, releasing phosphate and calcium ions. As a result of mixing calcium and phosphate ions leads to the immediate precipitation of amorphous calcium phosphate (ACP) or, in the presence of fluoride, as amorphous calcium fluoride phosphate (ACFP). These phases (ACP and ACFP) have the potential to be extremely unstable in the intraoral environment, and they may quickly change into a more crystalline, thermodynamically stable phase (such as hydroxyapatite and fluorhydroxyapatite). To encourage enamel subsurface lesion remineralization, calcium and phosphate ions must be momentarily accessible before phase change with time (Cochrane et al., 2010).

ACP and ACFP are applied by two systems; the first system is a system of a single phase in which ACP is inhibited from early reaction and generation of apatite before use by stabilizing it or manufactured without water. Dental composites and chewing gum with ACP are two examples of single-phase systems that are available. The second system is a two-phase system in which all components ( calcium, phosphate, and fluoride) are physically separated until applied, thus, calcium phosphate can not form before use. The calcium component of toothpaste is separated from the phosphate and fluoride-containing components; these two components are merged during dispensing and brushing, demonstrating a two-phase system (Tung, 2004).

In the mouth, the unstabilized ACP/ACFP may change into phases that are less soluble, which could contribute to the development of dental calculus. The ability of accessible fluoride ions to assist the remineralization of subsurface enamel lesions would be reduced if fluoride-containing apatite were to develop intraorally. But some of the intra-oral ACP/ACFP phases may be stabilized by phosphoproteins in saliva, pellicle, and plaque that aren't functioning to their maximum potential. This could account for both the favorable in situ model results and the bioavailability of these technologies in the presence of saliva. To prove the unstabilized ACP/ACFP technologies' effectiveness in avoiding coronal caries and their safety — which is demonstrated by the absence of dental calculus promotion —long-term randomized controlled caries clinical trials must be carried out (Cochrane et al., 2010).

#### Stabilized calcium phosphates

##### Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP)

Approximately 80% of the total protein in bovine milk is made up of casein, the main phosphoprotein that stabilizes calcium and phosphate, mostly as calcium phosphate micellular complexes (Gurunathan et al., 2012).

Trypsin partial enzymatic digestion results in the formation of casein phosphopeptide (CPP), which is then purified by ultrafiltration into stable complexes that function as calcium and phosphate carriers (Cross et al., 2007; Cochrane and Reynolds, 2009). It has been demonstrated that casein phosphopeptides (CPP) stabilizes calcium

and phosphate, keeping them in a soluble or amorphous form known as amorphous calcium phosphate (ACP) (63). CPP stabilizes ACP clusters through an active series Ser(P)-Ser(P)-Ser(P)-Glu-Glu, that have phosphoseryl residues, exhibiting a remarkable ability to stabilize calcium and phosphate as nanoclusters of ions in a metastable solution and preventing ACP clusters from growing to a critical size that facilitates nucleation, precipitation and phase transformation maintaining it in soluble bioavailable status (Cross et al., 2005; Zhao et al, 2011; Gurunathan et al., 2012).

It can accomplish this at both basic and acidic pH environmental situations, and if fluoride ions are present. CPP stabilized ACP; when creating casein phosphopeptide stabilized amorphous calcium phosphate CPP-ACP, if there is a fluoride ion in the environment; casein phosphopeptide stabilized amorphous calcium fluoride phosphate CPP-ACFP forms nanocomplexes (Amaechi and Loveren, 2013). Because they dissolve easily in saliva, casein phosphopeptide-amorphous calcium phosphate nanocomplexes can localize in dental plaque through the development of a diffusion gradient. Thus, CPP-ACP can serve as biological calcium phosphate carriers, increasing bioavailable calcium and phosphate ion levels in plaque fluid and saliva considerably without precipitating calcium salts randomly (Philip and Walsh, 2018).

The CPP-ACP is thought to have its anticariogenic properties through localizing ACP in dental plaque, which buffers free calcium and phosphate ions activities. This helps to keep tooth enamel in a supersaturated state, which reduces demineralization and promotes remineralization (Reynolds, 1998). The natural pH of 7 is significantly lowered below the critical value of 5.5 due to the acid generated by acidogenic bacteria's digestion of dietary sucrose in dental biofilm. This allows acids to penetrate inside enamel and dissolve calcium phosphate minerals. In these acidic conditions, the CPP-ACP breaks down and separates, releasing calcium and phosphate ions. Demineralization is prevented in this way (Chhabra and Chhabra, 2018).

Moreover, the elevated calcium phosphate in the plaque enhances the remineralization of the enamel by buffering free calcium and phosphate ion activities and preserving a condition of overabundance of the ions near the tooth. Different in vitro investigations conducted recently have demonstrated the ability of CPP ACP to enter enamel subsurface lesions, where it can release weakly bonded calcium and phosphate

ions where they settle into crystal gaps. In this way, CPP-ACP plays a role in controlling of crystal formation and preventing crystal demineralization (Chhabra and Chhabra, 2018).

According to Schüpbach et al., the CPP-ACP prevents cariogenic streptococci from adhering to the tooth surface, which prevents the development of cariogenic plaque (Schüpbach et al., 1996). Through its capacity to bind to (pellicles, plaque, and bacteria), via binding to the surface of bacterial cells, intercellular plaque matrix components, and adsorbed macromolecules on the tooth surface. CPP-ACP competes with free calcium for plaque calcium binding sites, thus; reducing the bridging of calcium between pellicle and attached cells (plaque) and between cells themselves (Rose, 2000). Also, the bacteria is inhibited from binding to the tooth by the ability of CPP-ACP to mask salivary molecule's binding sites for streptococci (Chhabra and Chhabra, 2018). CPP-ACP has bactericidal or bacteriostatic effects through increasing extracellular free calcium concentration. (Rose, 2000). Moreover, it has been shown that the enzymatic degradation of the CPP results in ammonia, which inhibits demineralization and encourages remineralization, raising the pH of the plaque (Amaechi and Loveren, 2013).

With its ability to remineralize, CPP-ACP integrates into the pellicle and plaque, causing an ecological change in the bacterial population. This alteration, combined with the plaque's altered cariogenic capability, results in the production of a less cariogenic plaque (Chhabra and Chhabra, 2018).

#### Crystalline calcium phosphates

Calcium phosphate has a variety of crystalline forms and thus; different solubilities of each of these crystalline phases, and some of them have been investigated as possible means for remineralization by supplying calcium and phosphate ions to subsurface enamel lesions. The low solubility of the calcium phosphate phases, which prevents the calcium and phosphate ions from being available for remineralization, is the issue with introducing crystalline material to the oral cavity to encourage enamel remineralization. To release ions that can diffuse into the enamel subsurface lesion, these crystalline calcium phosphate phases need to be separated from the product before coming into contact with saliva and then dissolved. Saliva must be undersaturated

concerning that crystalline phase for the calcium phosphate phase to dissolve. At the typical pH range of saliva, these crystalline calcium phosphate phases will not dissolve. Moreover, it presents difficulties for substantial amounts of solid calcium phosphate phases to be localized at the tooth surface (Cochrane et al., 2010).

#### Calcium sodium phosphosilicate (Bioactive Glass)

Relatively recently, bioactive glass has been shown to promote bone regeneration and treat illnesses or trauma-related bone abnormalities. A bioactive glass containing sodium, calcium, and phosphorus was the first bioactive glass to be introduced in 1969. There are various kinds of bioactive glass available today, including phosphate- and silicate-based bioactive glass (Dai et al., 2019).

The manufactured mineral called sodium calcium phospho silicate contains the elements naturally present in the body: sodium, calcium, phosphorous, and silica. Particles quickly release sodium, calcium, and phosphorous ions into saliva or water upon contact, allowing saliva to remineralize the tooth surface. In contrast to alternative calcium phosphate technologies, bioactive glass releases ions that directly form hydroxycarbonate apatite (HCA) without the need for an intermediary amorphous calcium phosphate phase. In vitro research has demonstrated that these particles can release ions and convert them into HCA for up to two weeks (Madan et al., 2011). Acting as reservoirs, they can release calcium and phosphate ions over days. The calcium-phosphate complexes can crystallize into hydroxycarbonate apatite at the end (Khijmatgar et al., 2020).

Because carbonated apatite makes up the surface of the bioactive glass, it adheres strongly to the tooth mineral, naturally increasing the RM capacity (Tulumbacı and Oba, 2019). Adjuvant surface treatments, such as calcium sodium phosphosilicate bioactive glass, are used in conjunction with various superficial treatments to improve remineralization. There are techniques like adding polyacrylic acid to the surface and pre-treating the lesion surface with air abrasion. The polyacrylic acid (COOH) functional group can bind to calcium and phosphate ions to form nano precursors (Alafifi and Hassanein, 2020). Polyacrylic acid (PAA) is thought to expose the subsurface lesion available for remineralization (Salomão et al., 2016). Better remineralization is achieved

when chemical and mechanical surface treatments are used in combination rather than just one of the two techniques (Pliska et al., 2012).

#### Functionalized $\beta$ -tricalcium phosphate (fTCP)

Functionalized  $\beta$ -tricalcium phosphate (fTCP) was created by modifying  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) through the addition of carboxylic acids and surfactants (Karlinse et al., 2009). Functionalized  $\beta$ -TCP serves as a targeted low-dose delivery system when applied to teeth via dentifrices or mouthwashes. In addition, providing a barrier to avoid early fluoride-calcium interactions (Karlinsey1 and Pfarrer, 2012). Its main goal was to increase fluoride ion activity on the surface of teeth, where remineralization is primarily encouraged by salivary  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions. Data on the remineralizing efficacy of this commercial product are confined to in vitro research, despite it being accessible already. fTCP appears to offer just a tiny number of unbound ions during the brief brushing period before being expectorated from the mouth, in contrast to the pH-responsive CPP-ACP complexes that can deliver stable  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions for a longer duration of time (Philip, 2019).

#### Dicalcium Phosphate Dihydrate (DCPD)

DCPD is a precursor for apatite that can be added to dental products to increase the remineralization effectiveness of fluoride. DCPC facilitates the conversion of apatite to fluorapatite in the presence of fluoride (Amaechi and Loveren, 2013). The application of fluoride at high concentrations (such as topical fluoride) the main agent formed is calcium fluoride. During the acidogenic caries condition, the DCPD facilitates the conversion of calcium fluoride present to fluorapatite (Wefel and Harless, 1987).

To strengthen the remineralizing effects of the fluoride component, this substance has been added to various fluoride toothpaste. When compared to traditional silica toothpaste, the incorporation of DCPD in a toothpaste raises the amounts of free calcium ions in plaque fluid, and these levels stay elevated for up to 12 hours after the toothbrush (Walsh, 2009).

Several investigations were carried out elucidate the function of DCPD in promoting fluoride activity in the oral cavity. The first was

to determine whether using DCPD dentifrices raises the amounts of free  $\text{Ca}^{+2}$  ions and the level of enamel saturation in plaque fluid, they researched plaque fluid. According to the findings of these investigations, DCPD would dissolve in the mouth since the plaque fluid was undersaturated in this regard. Comparing DCPD dentifrice to silica dentifrices and NaF/silica dentifrice, the calcium activity in plaque fluid was found to be considerably higher 12 hours after treatment. The second one was performed by Ca labeling investigation to find out if Ca from DCPD could be found in demineralized enamel and entire plaque following in in vivo applications of a DCPD/fluoride dentifrice.

The Ca levels in enamel were measured using Secondary Ion Mass Spectrometry (SIMS) both before and after a six-day in vivo treatment with a DCPD/fluoride dentifrice.

The findings demonstrated that 18 hours after treatment, Ca from DCPD was found in plaque and integrated into enamel. The results of these research demonstrate that, when combined with fluoride, brushing with a DCPD dentifrice adds more exogenous calcium to the oral environment and promotes better tooth remineralization (Sullivan et al., 1997).

### **2.11.3. Xylitol**

Recent research on eroded teeth in vitro has demonstrated the potential for remineralization of xylitol, a natural herbal product with antimicrobial properties that helps transport and access calcium into the lesion pores. Xylitol is not a source of calcium or phosphate (Gargouri et al., 2020). Studies have shown that the nonfermentable sugar alcohol xylitol exhibits both cariostatic and noncariogenic characteristics. Due to *S. mutans*' inactivation and plaque's decreased capacity to produce acids and polysaccharides, it possesses anticarcinogenic effects (Arifa et al., 2019).

Cariious microorganisms do not metabolize xylitol and xylitol does not reduce the plaque pH. Since plaque pH does not decrease, enamel demineralization is prevented and plaque bacteria cannot multiply. When gum sweetened with xylitol is chewed, remineralization increases because plaque pH does not decrease. It causes the palate's tiny salivary glands to produce more saliva that is mineral-rich and alkaline when taken as

mints or gum. Regular use of xylitol-sweetened chewing gum has specific inhibitory effects on the growth of mutans streptococci in the mouth. Additionally, chewing xylitol gum stimulates saliva flow, which increases the buffering effect on plaque (Makinen et al., 1995; Burt, 2006; Arifa et al., 2019) . Regarding xylitol's increased efficacy, particularly when paired with fluoride, there are conflicting reports from the studies (Arifa et al., 2019).

#### **2.11.4. Polyphosphate systems**

Traditional toothpaste's fluoride content can be partially replaced with inorganic polyphosphate salts to reduce the risk of fluorosis and maintain its preventative efficacy in high-risk patients and children. Polyphosphate salts can be found as calcium glycerophosphate, sodium trimetaphosphate (STMP), or hexametaphosphate (Zaze et al., 2013; Philip, 2019).

Given its dual properties of promoting remineralization and inhibiting demineralization, STMP is thought to be the most potent anti-ager among polyphosphates (Freire et al., 2016; Takeshita et al., 2016). Since phosphates are intensely attracted to hydroxyapatite, they can inhibit ion release and minimize the possibility of enamel dissolution. Adsorption of polyphosphonates happens fast and competes with ionic fluoride adsorption. For the product to function better, there must be an appropriate ratio of fluoride and phosphate (Zaze et al., 2013).

Compared to other phosphates, STMP is a condensed inorganic phosphate that has a stronger ability to bind to phosphate sites on the enamel surface and stay adsorbed for a longer time. As a result, during a cariogenic challenge, an enamel surface protective barrier is formed, limiting the amount of acid that diffuses into the enamel. Furthermore, the protective barrier against acidic dissolution does not hamper the diffusion of calcium and fluoride ions into the enamel. Danelon et al. and Takeshita et al., have demonstrated that adding STMP to a low-fluoride product had remineralization effects that were comparable to those of a 1,100-ppm fluoride formulation (Danelon et al., 2013; Takeshita et al., 2016). To determine whether STMP can affect the reversal of non-cavitated lesions, more clinical research is required (Philip, 2019).

#### **2.11.5. Ozon**

A molecule of natural gas called ozone (O<sub>3</sub>) is made up of three oxygen atoms. Living creatures are shielded from UV radiation by the overabundance of ozone found in the stratospheric layer of the atmosphere. The world naturally purifies itself through the ozone layer, which is heavier than air and mixes with various pollutants it encounters on its descent from high altitudes to the earth (Tiwari et al., 2017).

German chemist Christian Friedrich Schonbein, considered the founder of ozone therapy, made the first observation of ozone in 1840. The first dentist to employ ozone in his practice was Dr. Edward Fisch, and Dr. Erwin Payr, a German surgeon, reported his surgical outcomes utilizing ozone. Ozonized water was utilized during dental surgery to enhance the local oxygen supply, encourage hemostasis, and inhibit the growth of microorganisms (Azarpazhooh and Limeback, 2008).

Ozone therapy has been developed as an innovative method for treating caries, disinfecting the cavity, and lowering the amount of caries-related microorganisms in light of the increasing popularity of pharmaceutical techniques for treating dental decay.

Theoretically, by lowering the number of bacteria in active caries lesions, ozone can momentarily arrest the growth of caries and postpone or eliminate the need for restoration. This is ascribed to ozone's strong antibacterial qualities as well as the fact that it converts cariogenic bacteria's pyruvic acid to carbon dioxide and acetate (Almaz and Sönmez, 2015; Tiwari et al., 2017). There are several ozone therapy applications in practically every dental care field. Immunostimulant, analgesic, antihypnotic, detoxic, antibacterial, bioenergetic, and biosynthetic actions are just a few of its unique characteristics. Better-designed, randomized clinically controlled trials are required for caries management, even though the procedure's non-invasive, painless, and atraumatic characteristics boost patient acceptability and compliance and make it an excellent therapeutic choice for young patients in that particular (Tiwari et al., 2017).

#### **2.11.6. Chlorohexidine gluconate**

Chlorhexidine gluconate is a wide-spectrum antimicrobial agent that disrupts the cell membrane (Brookes et al., 2020). It is derived from guanidine and was first produced

in England at Imperial Chemical Industries in 1950 (Szymańska et al., 2017). The most effective antibacterial agent available right now for treating dental decay and mutant streptococci. It can be found in the form of gluconate, hydrochloride, and acetate (diacetate) salts (Pallavi, 2011). It is most stable in the form of its salts and it is an excellent base. Digluconate salt is the most often used preparation due to its high water solubility (Fardai and Turnbull, 1986). It has been shown that using chlorhexidine gluconate to prevent tooth decay is a non-invasive method of managing dental decay and represents a contemporary approach to treating caries. It has been described as a secure and efficient antiseptic for lowering the concentrations of mutant streptococci in saliva and plaque. Various application techniques have been suggested to prevent tooth decay. Mouthwashes (0.12% and 0.2%), gels (0.12% and 1%), and varnishes (up to 40%) are the most often used products (Pallavi, 2011).

Chlorhexidine has a bacteriostatic effect in low concentration by altering the cell membrane permeability allowing the leaching of ions such as potassium, and in high concentration. It has a bactericidal effect; by allowing all essential intracellular cell components to leak leading to cell lysis and death (Brookes et al., 2020). In addition to penetration through the cell membrane and precipitation of the cytoplasm by complex forming with phosphorylated compounds like adenosine triphosphate (ATP) and nucleic acids (Szymańska et al., 2017).

Dental caries is primarily caused by plaque formation, which includes bacteria like *Streptococcus mutans* and *lactobacilli* spp., which produce lactic acid in the presence of carbohydrates. According to Brookes et al.'s most recent 2020 review of the oral use of chlorhexidine, 0.2% chlorhexidine can be used every day because it lowers plaque concentrations. Nevertheless, other research indicates that while applying 0.2% chlorhexidine gel lowers plaque, it has no effect on dental caries and does not lower *Streptococcus mutans*. Furthermore, there isn't much proof that applying a chlorhexidine varnish to the tooth's surface lowers the incidence of tooth decay. It is currently advised to use a mouthwash containing 0.05% sodium fluoride every day to prevent tooth decay. In order to avoid diminishing the benefits of fluoride toothpastes, there should be a delay of at least 30 minutes to 2 hours between teeth brushing and using chlorhexidine mouthwash if mouthwashes are to be used for plaque reduction in addition to brushing

and cleaning interdental areas. This is due to the possibility that mouthwash could remove the fluoride from toothpaste as well as the possibility that CHX rinses could interact with the anionic ingredients in many kinds of toothpaste, like sodium monofluorophosphate and SLS, and lessen the benefits of fluoride on the remineralization of enamel lesions (Brookes et al., 2020).

#### **2.11.7. Arginin**

Arginine is a natural component of human saliva. Saliva peptides and proteins contain plenty of arginine (Cheng et al., 2015). Moreover, it can be found as naturally occurring amino acids in food proteins which is considered as an organic compound based on prebiotics. Prebiotics are described as a "substrate that provides a health benefit and is selectively utilized by host microorganisms." When oral bacteria that express the Arginine Deaminase System (ADS) metabolize arginine, raising pH levels and decreasing tooth demineralization, arginine is therefore categorized as a prebiotic. With notable anti-carries advantages, it was recently added as an addition to fluoride toothpaste and other oral care products (Bijle et al., 2018; Pørksen et al., 2023).

Arginine has been linked to changes in the pH and ecology of dental biofilms, according to several studies. To enhance the prevention of cariese lesion with further remineralization, toothpaste containing insoluble calcium and 1450 ppm fluoride (sodium monofluorophosphate) (with 1.5% arginine content) was issued. This might have the benefit of supporting mechanical plaque control, which would maintain the host's biofilm homeostasis. The formulation of arginine + fluoride + calcium consistently produced better results than non-arginine formulations, according to several randomized clinical trials. According to the results of a recent systematic review and meta-analysis, this formulation may have a better anti-carries effect. According to the results of another systematic review, there is not enough data at this time to demonstrate the superior anti-carrying efficacy of toothpaste containing arginine because of the high risk of commercial bias in the trials studied. In conclusion, clinical experiments have demonstrated that this formulation alone has the potential to greatly enhance fluoride toothpaste's performance; however, final recommendations need to be made following the approval of results from highly qualified, long-term, and possibly less biased investigations (González-Cabezas and Fernández, 2018).

### **2.11.8. Chitosan**

The most common naturally occurring biopolymer, chitin is a cationic amino polysaccharide that is formed from the cell walls of fungi and insects as well as the exoskeletons of crustaceans (shrimp and crab)( Tharanathan and Kittur, 2003).

Chitin is considered a precursor of chitosan when chitin is deacetylated. Since chitosan has bactericidal and/or bacteriostatic properties, it has been employed in research on caries prevention. Furthermore, chitosan has biocompatible and biodegradable properties and is thought to be harmless. Chitosan affected the de-remineralization process of tooth enamel and led to the inhibition of mineral loss in 55-81% percent of enamel depending on the duration of the exposure and concentration. Arnaud et al. assumed that chitosan may act as a mechanical barrier in the prevention of acid penetration in their in vitro pH cycle studies. These studies examined the effect and anticaryogenicity of chitosan in the de-remineralization processes. Chitosan has minimal effect on remineralization (Arnaud et al., 2010).

Furthermore, a promising biomaterial is the chitosan-amelogenin hydrogel, which contributes mineral content and forms an enamel-like structure that modifies the Ca-P organization. Chitosan hydrogel containing amelogenin-derived peptides was shown to have antibacterial and remineralization capability, and its clinical utility was validated (Fakhri et al., 2020).

### **2.11.9. Natural products**

Natural products are one alternate strategy for reducing and preventing deterioration. Natural products that have anticaries properties include food, beverages, flowers, and traditional herbs. It has been established that polyphenol compounds make up the majority of the active components that have a role in bacterial inhibition and antiplaque effect in addition to remineralization/demineralization regulation in the hard tissue of the teeth. Certain natural substances work well to alter the remineralization balance, even though the majority of them are researched as antibacterial agents. For the majority of natural compounds, the exact processes underlying antagonistic action are yet unknown. Future research must focus more on finding new and efficient natural products

through in situ, animal, and in vitro studies, and emerging technologies like nanotechnology must be used to boost the anticaries properties of these products (Cheng et al., 2015).

Recently, *Galla chinensis*, a type of Chinese herbal medicine, has gained a lot of interest and may be a useful tool in the research for new bioactive chemicals with anticaries properties (Huang et al., 2010).

Huang et al. (2010), investigated the synergistic effects of *Galla chinensis* and nano-hydroxyapatite on the remineralization of early enamel defects. After thorough research, it was discovered that nano-HA could not considerably lower the lesion depth and would only aid in mineral deposition, which predominates in the lesion's external layer. Reducing lesion depth and increased mineral deposition in the lesion body was seen in the *Galla chinensis*–nano-HA combination therapy group. In the meantime, compared to the *Galla chinensis* group, noticeably more mineral deposition was also seen in the lesion's outer area.

The study conducted by Cheng et al. (2008), intended to determine the impact of *Galla chinensis* on the in vitro remineralization of simulated early enamel caries. Additionally, the study investigated the combined effects of *Galla chinensis* and fluoride on enhancing dental enamel remineralization. They discovered that *Galla chinensis*' chemical components could improve the remineralization of dental enamel. They also enhanced remineralization when associated with fluoride.

Coffee is one of the most popular drinks in the world, primarily due to its potential health advantages (Daglia et al., 2007). Given its safety and well-liked flavor and aroma, this opens up a potentially useful field of use (Antonio et al., 2012). According to Antonio et al. (2010) and Cheng et al. (2015), coffee is not only the most consumed beverage globally but also contains a high concentration of polyphenols, which have antimicrobial properties. Antonio et al. (2010) and Antonio et al. (2012) have demonstrated in their papers that *Coffea canephora* extract has been shown in the literature to have antibacterial properties against *Streptococcus mutans*. It has also been shown to have inhibitory effects on the demineralization of tooth enamel in biofilm circumstances.

### **3. MATERIAL AND METHODS**

This study was carried out at Van Yüzüncü Yıl University Faculty of Dentistry, Department of Restorative Dental Treatment, under in vitro conditions, with the approval of the Van Yüzüncü Yıl University Clinical Research Ethics Committee (decision number: 06/15.02.2023 and 09/28.02.2024).

The microhardness test method was used to assess and compare the remineralization efficacy, and the scanning electron microscope (SEM) test method assessed the surface morphology and characteristics. A single researcher performed all procedures to avoid individual variations.

#### **3.1. Materials**

##### **3.1.1. Tools Used in the preparation of tooth samples**

Pumice, polishing brush, diamond chamfer cutting bur, silicon carbide paper sander (Mager Scientific, Dexter, MI, USA), self-cure acrylic repair powder (Integra, bg-dental, Ankara, Turkey), self-cure acrylic material liquid (SC, IMICRYL, Konya, Turkey) (Figure 3), paper stickers, and nail polish (Flormar No:68, Kosan Koz. San. Ne Tic. A.S. G.O.S.B., Kocaeli, Turkey).



**Figure 3.** Self-cure acryl used in the preparation of the samples.

### **3.1.2. The used solutions**

Two separate solutions were used in our study. These solutions were prepared, and their pH was adjusted in the faculty of Veterinary in the lab of the Department of Biochemistry. These are the demineralization solutions of pH: 2.3 to be used in the demineralization cycle with the remineralization solution with pH: 7 which is used during the remineralization period Figure 3.2. The content and pH values of remineralization and demineralization solutions in 5 liters of distilled water are shown in Table 3. and Table 4, respectively. The measurement of each component is measured using the Sensitive scale. The pH was measured using (HANNA, USA), Figure 4.



**Figure 4.** The sensitive scale used in the preparation of the solutions.

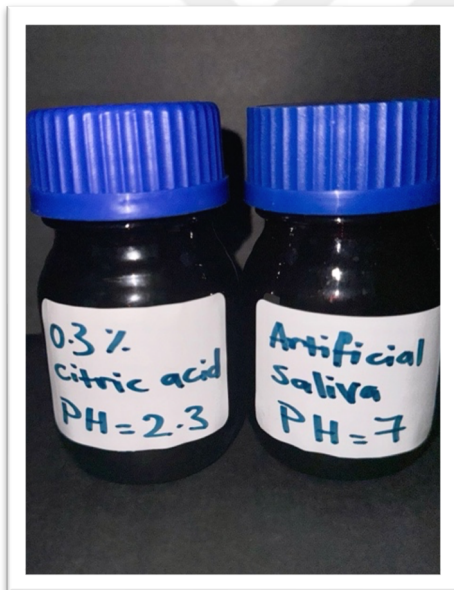


**Figure 5.** The remineralization and demineralization solutions.

**Table 3.** Remineralization solution content and pH value.

| Remineralization solution ingredients                           |                                               |
|-----------------------------------------------------------------|-----------------------------------------------|
| CaCl <sub>2</sub>                                               | 0.550 grams of calcium chloride               |
| KCl                                                             | 18.6 grams of potassium chloride              |
| KH <sub>2</sub> PO <sub>4</sub>                                 | 13.60 grams of potassium dihydrogen phosphate |
| NaNO <sub>3</sub>                                               | 0.05 grams of sodium nitrate                  |
| pH: 7 (The pH adjustment was done using potassium chloride KOH) |                                               |

**Table 4.** Demineralization solution contents and pH.

| Demineralization solution ingredients                            |                         |
|------------------------------------------------------------------|-------------------------|
| C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>                     | 15 grams of citric acid |
| pH: 2.3 (The pH adjustment was done using hydrochloric acid HCL) |                         |

### 3.1.3. The materials used in this study and their ingredients

In the current in vitro study, two materials are used one is hydroxyapatite-based (HA) and the other is amorphous calcium phosphate-based (ACP). Each of them has two forms one fluoridated and the other is non-fluoridated. All of the used materials presented in Figure 6. The ingredients of the materials listed in Table 5.



**Figure 6.** a) Enamelon, is fluoridated ACP-based material, b) KAREX gelée, is non-fluoridated HA-based material, c) Zen CP+, a non-fluoridated ACP-based material, d) Remin Pro, is a fluoridated HA-based material.

**Table 5.** The materials and their ingredients used in the study.

| Material  | Ingredients                                                                                                     | Manufacturer                       | LOT Number |
|-----------|-----------------------------------------------------------------------------------------------------------------|------------------------------------|------------|
| Remin Pro | Remin Pro is a water-based cream that contains hydroxyapatite, sodium fluoride (1450 ppm fluoride) and xylitol. | VOCO GmbH, Cuxhaven, Germany       | 101881     |
| Karex     | Karex contains hydroxyapatite, calcium, and xylitol.                                                            | Dr. Kurt Wolff, Bielefeld, Germany | 007512     |
| Zen CP+   | Calcium Magnesium Phosphate, Potassium Nitrate, Glycerin, Water,                                                | DentMat, USA                       | 2308100028 |

The continuation of Table 5 is on the next page

The continuation of Table 5. The materials and their ingredients used in the study.

| Material | Ingredients                                                                                                                                                                                                                                                                                                                                                                                                                               | Manufacturer        | LOT Number |
|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|------------|
| Zen CP+  | Carbopol 980, Xanthan gum, Calcium Chloride, Monosodium Phosphate, Potassium Hydroxide, Sodium Benzoate, N&A Raspberry flavor.                                                                                                                                                                                                                                                                                                            | DentMat, USA        | 2308100028 |
| Enamelon | Active ingredients include stannous fluoride 0.45% (0.15% w/v fluoride ion). Amorphous calcium phosphate technology. All aided in anticavity and sensitivity treatment. Inactive ingredients include glycerin, PEG-12, silica, calcium/sodium pvm/ma copolymer, calcium sulfate, cocamidopropyl betaine, lauroyl sarcosine, titanium dioxide, poloxamer 407, flavors, potassium acesulfame, monosodium phosphate, dimethicone, sucralose. | Premier Dental, USA | E322GW     |

#### 3.1.4. Toothbrush and its characteristics

The toothbrush used in this study is an electric toothbrush (Oral-B VITALITY PRO, BRAUN, Hungary) with three modes of action: daily, sensitive, and extra-sensitive. The extra-sensitive mode was used. Every group had its toothbrush head which was changed every week. Figure 7.



**Figure 7.** Electric toothbrush with special different heads for each group.

### **3.1.5. Toothbrush-holding machine**

A custom-made machine was prepared to hold the toothbrush in its place, it was made in the form of two pieces one holding the toothbrush and the other to fix the weight of 200 grams on the top of the toothbrush to simulate light brushing force. Figure 8

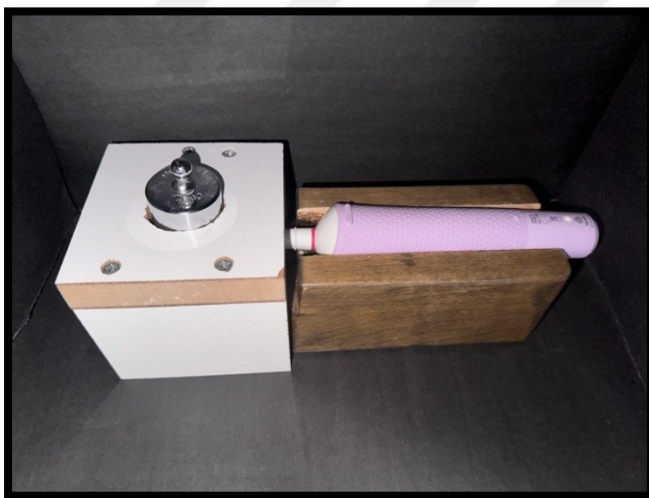


**Figure 8.** The custom-made toothbrush holding machine.

### **3.1.6. Microhardness testing machine**

The prepared samples were subjected to microhardness analysis at the baseline, after demineralization, and after 28 days of remineralization using a Vickers hardness measurement device (Shimadzu HMV-700 Shimadzu, Kyoto, Japan) Figure 9.



**Figure 9.** Microhardness testing machine.

### **3.1.7. Scanning Electron Microscope (SEM)**

Three samples from each group were used in SEM analysis, the specimens were examined at the Scientific Application and Research Center of Van Yüzüncü Yıl University. Using a Scanning Electron Microscope (FESEM) (Zeiss Sigma 300, Germany) Figure 10.

## **3.2. Methods**

### **3.2.1. Teeth selection criteria**

In our study conducted under in vitro conditions, 117 teeth were used in the study, which were premolars and molars extracted for periodontal and orthodontic reasons. 90 teeth were used for the microhardness test and 21 teeth were used for surface morphology evaluation by SEM test.

It was determined that there were no visible structural defects such as caries, fractures, cracks, demineralization, hypomineralization ,or discoloration on the tooth surfaces by examining them under a microscope.

### 3.2.2. Teeth preparation

After extraction, the teeth were cleaned with an ultrasonic scaler and then pumice and a polishing brush and stored in 0.5 % chloramine T solution for one week for disinfection and stored for a maximum of 2 months. The water was changed weekly for one month like the study of (Erkut et al., 2014; Shihabi et al., 2021).

First, the roots of the teeth were separated 2 mm away from the cemento-enamel junction with the help of diamond chamfer bur under water cooling. Each tooth was embedded in the self-cure acrylic by using 1.3 cm high cylindrical silicone molds with a diameter of 1.5 cm, which were impeded inside the cold cure acrylic to have the buccal surface parallel to the surface of the mold.

To protect the samples from the heat generated during the polymerization of acrylic, the samples were kept on a glass slab during polymerization. Following the polymerization of the acrylic the tooth surface was flattened by using silicon carbide paper (1000 grit followed by 1200 grit) under water to obtain a smooth surface for the microhardness test.

After that, a 4 mm × 4 mm square area was left open by placing a sticker on the buccal surfaces of the samples, and the other areas were covered with nail polish to ensure good isolation. The prepared specimen is shown in Figure 10.

For morphology characteristics evaluation by scanning electron microscopy SEM, the specimens were cut into smaller sizes to be easily vacuumed by the machine and were given color code and numbered to distinguish between the sample groups as shown in Figure 11.



**Figure 10.** the prepared tooth specimen.



**Figure 11.** the prepared specimen for SEM analysis.

### **3.2.3. Working groups preparation**

After the samples were prepared, they were divided into 9 groups giving them letter codes with numbering for each group for microhardness values, each group contains 10 specimens. For the SEM test, the specimens were color-coded, and numbers for each group, each group contains 3 specimens.

The groups for the microhardness test are prepared as follows:

KP: non-fluoridated HA-based material with finger application.

KF: non-fluoridated HA-based material with toothbrush application.

RP: fluoridated HA-based material with finger application.

RF: fluoridated HA-based material with toothbrush application.

EP: fluoridated ACP-based material with finger application.

EF: fluoridated ACP-based material with toothbrush application.

ZP: non-fluoridated ACP-based material with finger application.

ZF: non-fluoridated ACP-based material with toothbrush application.

CB: control group (demineralized without treatment) kept in artificial saliva.

The groups for the SEM test are prepared in accordance to color, as follows:

Black: EF, Brown: EP, Red: ZF, Pink: RF, Yellow: KF, Orange: ZP, Green: RP, Blue: KP, Transparent: CB.

#### **3.2.4. Baseline microhardness measurement**

The created samples were subjected to microhardness tests utilizing Vickers hardness measurement device (Shimadzu HMV-700 Shimadzu, Kyoto, Japan). The test was done for the specimens before any surface treatment, after lesion formation, and post-experiment.

Clear images were obtained by placing the prepared enamel samples under the test microscope, moving the microscope table in a 4x4 mm<sup>2</sup> region at X40 magnification, and avoiding the edge areas. The apparatus automatically determined the microhardness value of the enamel sample after 200 grams of force were applied to the samples for 10 seconds. The length of the diagonals of the shape formed by the diamond tip on the enamel was measured and the device automatically calculated the microhardness value of the enamel sample. Three separate measurements were made from various positions

within a  $4 \times 4 \text{ mm}^2$  region, and the results were recorded. The specimens were kept in distilled water at  $37^\circ\text{C}$  inside the incubator Figure 12.



**Figure 12.** The incubator used to keep the specimens at  $37^\circ\text{C}$ .

### **3.2.5. Generating the initial enamel lesions and microhardness measurement**

Initial enamel lesions were accomplished by subjecting the enamel specimens to 4 erosive cycles with an 8-h interval. The specimens were immersed in 0.3% citric acid solution (pH 2.3) for 2 min, followed by 2 h in artificial saliva as a remineralizing solution Figure 13. demonstrate the cycle.

The samples underwent a 10-second distilled water washing after every immersion, followed by a gentle paper towel drying. Every cycle saw a renewal of the solution. Once the samples were dried, opaque white lesions appeared on the surfaces. The specimens were kept in distilled water to prevent dehydration during the period until the hardness values were measured after the formation of the lesion. The hardness measurements of the samples after the formation of lesions were repeated in the same procedure using Vicker's hardness measurement device. The measurements were repeated and recorded 3 times each and the average of the results was recorded.

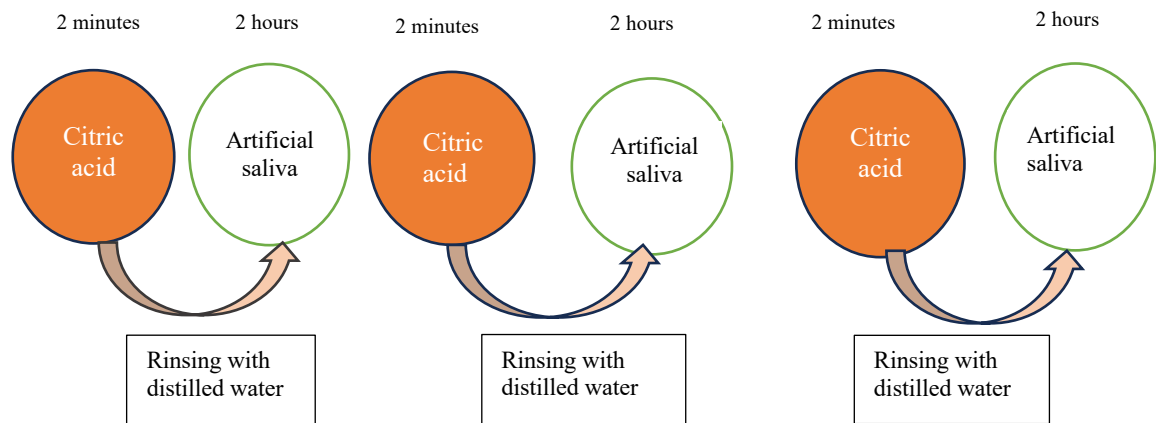


**Figure 13.** The Early enamel lesion formation, the cycle of 8 hours-interval.

### 3.2.6. Application of the materials

The materials were applied for each group for 30 seconds, by toothbrush or by finger, and left for 150 seconds according to manufacturer instructions. After the application of the material was finished, the specimen was cleaned with a syringe containing artificial saliva and kept for approximately 16 hours in refreshed artificial saliva at 37°C inside the incubator. This procedure repeated for 28 days. The procedure was done by one operator.

### 3.2.7. Surface microhardness measurement after remineralization

The specimens were kept in distilled water to prevent dehydration during the period of hardness measurement. The hardness measurements of the samples after the formation of lesions were repeated in the same procedure using a Vickers hardness measurement device. The measurements were repeated and recorded 3 times each and the average of the results was recorded.

### 3.2.8. Surface morphology test by Scanning Electron Microscope

The surface morphology and characteristics assessed used by scanning electron microscope testing machine SEM. The 21 specimens were prepared as previously mentioned before. The prepared specimens for SEM analysis were first coated with gold using the Quorum 100 Sputter Coater (Quorum Technologies, East Sussex, UK) to make the surfaces electrically conductive Figure 14. Then, the samples placed on aluminum

stabs to be examined by SEM at 10 kV with a secondary electron (SE2) detector using a Scanning Electron Microscope (FESEM) (Zeiss Sigma 300, Germany) Figure 15.

For surface morphology analysis. Images were taken from each sample at x500, x1000, and x5000 magnifications. The surface morphologies of the enamel surface of the samples were recorded by SEM at baseline, after demineralization, and after remineralization. The images for the specimens were taken at 3 periods of time, at baseline, after demineralization, and after remineralization.



**Figure 14.** Gold sputtering machine (Quorum Technologies, East Sussex, UK).



**Figure 15.** Scanning Electron Microscope.

### **3.3. Statistical Analysis**

For Independent samples, the T-test was used to compare normally distributed data according to paired groups, and the Mann-Whitney U test was used to compare non-normally distributed data. One-way ANOVA was used to compare normally distributed data according to three or more groups, and multiple comparisons were examined with Tukey HSD and Tamhane's T2 tests. Repeated analysis of variance was used to compare normally distributed microhardness values over time, and multiple comparisons were examined with the Bonferroni test. The significance level was taken as  $p < 0.050$ . Data were analyzed with IBM SPSS V23. Compliance with normal distribution was examined with Shapiro-Wilk tests.

## 4. RESULTS

### 4.1. Results Related to Microhardness

In this thesis study, The effectiveness of various toothpaste kinds in remineralization with and without fluoride applied in different application methods (finger and toothbrush) was evaluated using the Vickers hardness measurement method on human enamel samples.

Vicker's hardness values (VHN) were evaluated in various commercially available toothpaste groups with the control group (CB ). They are fluoridated HA-based with finger method (RP), fluoridated HA-based with toothbrush (RF), non-fluoridated HA-based with finger (KP), non-fluoridated HA-based with toothbrush (KF), non-fluoridated ACP-based with finger (ZP), non-fluoridated ACP-based with toothbrush (ZF), fluoridated ACP-based with finger (EP), and fluoridated ACP-based with toothbrush (EF). Hardness measurements were examined comparatively using a Vickers hardness device (Shimadzu HMV-700 Shimadzu, Kyoto, Japan).

There is no significant variance between the fluoridated and non-fluoridated groups at baseline ( $p = 0.804$ ) or following demineralization ( $p = 0.722$ ). However, The values of the fluoridated and non-fluoridated groups at remineralization varied statistically significantly ( $p = 0.002$ ) the mean microhardness values illustrated in (Table 6. and Figure 16.)

**Table 6.** Comparison of the results of surface microhardness values of two materials (fluoridated and non-fluoridated), at baseline, after-demineralization, and after-remineralization (mean, standard deviation, minimum, maximum, median). The Independent samples are normally distributed, T-test.

| Fluoridated                       |                      |                         | Non-Fluoridated      |                         |                    |              |
|-----------------------------------|----------------------|-------------------------|----------------------|-------------------------|--------------------|--------------|
|                                   | Mean±<br>s.deviation | Median<br>(min-<br>max) | Mean±<br>s.deviation | Median<br>(min-<br>max) | Test<br>Statistics | P*           |
| <b>Baseline</b>                   | 336.8 ± 50,0         | 339.2<br>(205- 469)     | 334.3 ± 37.5         | 339.8<br>(251-<br>404)  | 0.249              | 0.804        |
| <b>After<br/>demineralization</b> | 227 ± 44.5           | 227.5<br>(115-326)      | 223.9 ± 32.8         | 224<br>(155 -<br>279)   | 0.357              | 0.722        |
| <b>After<br/>remineralization</b> | 368.6 ± 39.6         | 365.7<br>(256-495)      | 337.4 ± 45,1         | 331<br>(252-454)        | 0.357              | <b>0.002</b> |

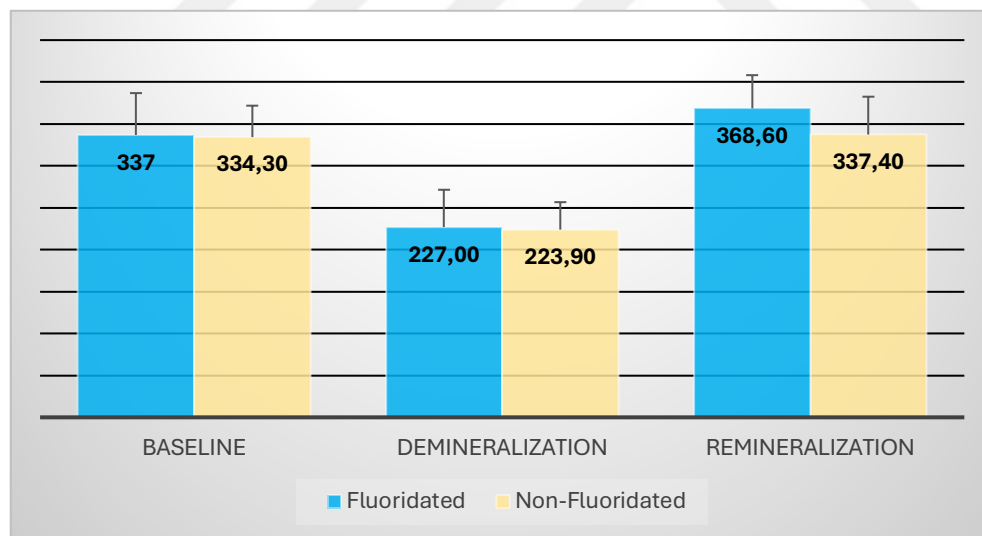


**Figure 16.** The values at baseline, after demineralization, and after remineralization for fluoridated and non-fluoridated groups. After remineralization the fluoridated group has higher value.

In comparison between the Hydroxyapatite-based materials (HA) groups and the Amorphous calcium phosphate-based groups (ACP), there is no statistically significantly variance between groups at baseline ( $p=0.206$ ) and after demineralization ( $p=0.678$ ).

There is a statistically significant difference at remineralization ( $p=0.027$ ). The mean values are demonstrated in (Table 7. and Figure 17.).

**Table 7.** Comparison of the results of surface microhardness values of two materials (HA-based and ACP-based), at baseline, after-demineralization, and after-remineralization (mean, standard deviation, minimum, maximum, median). Independent samples were normally distributed (T-test\*) and non-normally distributed (Mann-Whitney U test\*\*).

| Hydroxyapatite-based materials    |                      |                         | Amorphous calcium phosphate-based materials |                         |                    |                |
|-----------------------------------|----------------------|-------------------------|---------------------------------------------|-------------------------|--------------------|----------------|
|                                   | Mean±<br>s.deviation | Median<br>(min-<br>max) | Mean±<br>s.deviation                        | Median<br>(min-<br>max) | Test<br>Statistics | P*             |
| <b>Baseline</b>                   | 340 ± 45             | 341.7<br>(205-469)      | 331.1 ± 43                                  | 325<br>(254-430)        | 668.5              | 0.206*         |
| <b>After<br/>demineralization</b> | 227.3 ± 40.9         | 224.5<br>(115-318)      | 223.6 ± 37.2                                | 224.5<br>(166-326)      | 0.417              | 0.678**        |
| <b>After<br/>remineralization</b> | 364.1 ± 45           | 365.7<br>(256-495)      | 341.9 ± 42.8                                | 347.2<br>(252-437)      | 2.261              | <b>0.027**</b> |

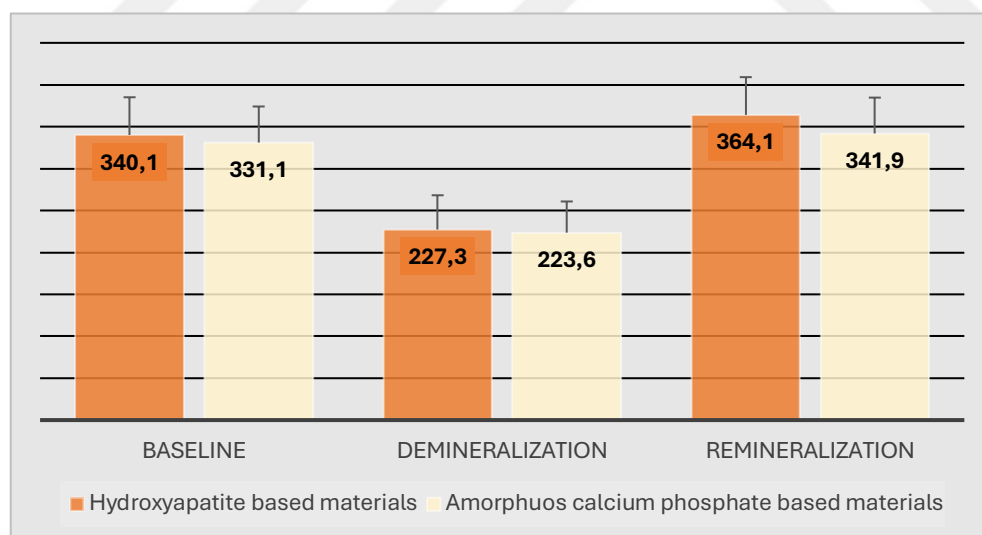


**Figure 17.** The values at baseline, after demineralization, and after remineralization for HA-based and ACP-based groups.

In comparison between the finger application method groups and the toothbrush application method groups, there is no significant variance among groups in baseline ( $p=0.058$ ). After demineralization, microhardness mean values show a significant

difference ( $p=0.006$ ). There is no statistically significant variance among the remineralization values of toothbrush and finger application method groups ( $p=0.118$ ). The average mean values are demonstrated in (Table 8. and Figure 18.)

**Table 8.** Comparison of the results of surface microhardness values of two application methods (toothbrush and finger), at baseline, after-demineralization, and after-remineralization (mean, standard deviation, minimum, maximum, median). The Independent samples are normally distributed, T-test.

| Finger application method         |                      |                         | Toothbrush application method |                         |                    |              |
|-----------------------------------|----------------------|-------------------------|-------------------------------|-------------------------|--------------------|--------------|
|                                   | Mean±<br>s.deviation | Median<br>(min-<br>max) | Mean±<br>s.deviation          | Median<br>(min-<br>max) | Test<br>Statistics | P*           |
| <b>Baseline</b>                   | 344.8 ± 48,5         | 344<br>(205-469)        | 326.2 ± 37,1                  | 325.5<br>(251-404)      | 1.926              | 0.058        |
| <b>After<br/>demineralization</b> | 237.2 ± 40,7         | 243<br>(115-326)        | 213.7 ± 33,5                  | 208.5<br>(155-279)      | 2.825              | <b>0.006</b> |
| <b>After<br/>remineralization</b> | 360.9 ± 49,3         | 359.7<br>(256 -495)     | 345.1 ± 39,4                  | 352.3<br>(252-406)      | 1.582              | 0.118        |

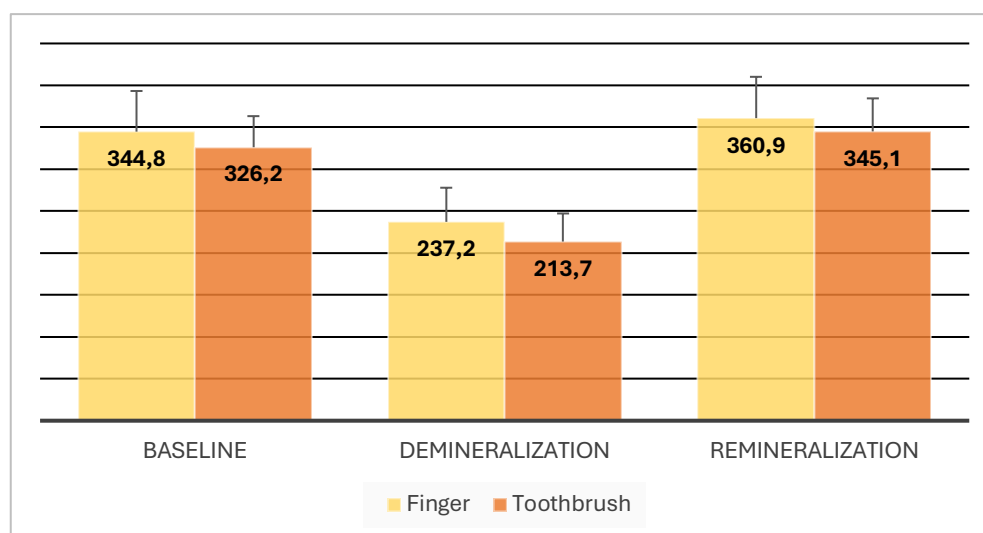


**Figure 18.** The values at baseline, after demineralization, and after remineralization for finger and toothbrush groups.

At baseline, a difference with statistical significance was discovered across all the groups ( $p = 0.044$ ). Consequently, the pairwise comparisons, there is no difference between them.

At demineralization, a difference with statistical significance was discovered across all the groups ( $p = 0.013$ ). Consequently, the pairwise comparisons, there is a difference between ZF and EP. There is no difference between other groups.

At remineralization, a difference with statistical significance was discovered across all the groups ( $p < 0.001$ ). As a result of the pairwise comparisons, there is a difference between the CB group and KF, KP, EP, EF, RF, and RP groups. As the control group has lowest mean microhardness value. There is no significant difference between the CB group and ZF and ZP groups where all the results has low mirohardness mean values. There is a significant difference between the ZF group and EP, RP, and KP groups. The ZF group has lowest mean microhardness value. The mean microhardness values of baseline, after demineralization, and after remineralization are demonstrated in (Table 9. and Figure 19.) the ZF group has lowest mean microhardness value.

**Table 9.** Comparison of the microhardness values of all groups, at baseline, after-demineralization, and after-remineralization. One-way analysis of variance; a-d; The groupings that share the same letter are identical; means.deviation; median(min-max).

| Group                  | Baseline             |                      | After demineralization        |                      | After remineralization         |                      |
|------------------------|----------------------|----------------------|-------------------------------|----------------------|--------------------------------|----------------------|
|                        | Mean±<br>s.deviation | Median<br>(min-max)  | Mean±<br>s.deviation          | Median<br>(min-max)  | Mean±<br>s.deviation           | Median<br>(min-max)  |
| <b>KF</b>              | 334.6 ±<br>37.4      | 332.8<br>(251 - 383) | 216.5 ±<br>35.4 <sup>ab</sup> | 213.2<br>(155- 279)  | 345.7 ±<br>40.7 <sup>cd</sup>  | 334.7<br>(304-406)   |
| <b>RF</b>              | 343.4 ±<br>19,7      | 345<br>(308 - 372)   | 229.7 ±<br>30.5 <sup>ab</sup> | 224.2<br>(189- 272)  | 366.7 ±<br>23.2 <sup>cd</sup>  | 375.5<br>(325-401)   |
| <b>EF</b>              | 313.5 ±<br>35.7      | 305.8<br>(266 - 383) | 207.7 ±<br>36.1 <sup>ab</sup> | 192<br>(168 - 278)   | 356.8 ±<br>23.2 <sup>cd</sup>  | 359.5<br>(308 - 393) |
| <b>ZF</b>              | 313.5 ±<br>46.3      | 309<br>(254 - 404)   | 201 ±<br>29.2 <sup>a</sup>    | 199<br>(166 - 259)   | 311,2 ±<br>45.1 <sup>ac</sup>  | 308.7<br>(252 - 376) |
| <b>KP</b>              | 355.4 ±<br>20,7      | 365<br>(320 - 381)   | 247.5 ±<br>21.1 <sup>ab</sup> | 254.5<br>(217 - 276) | 371.1 ±<br>41.7 <sup>bd</sup>  | 366.2<br>(324 - 454) |
| <b>RP</b>              | 326.5 ±<br>77.7      | 327.5<br>(205 - 469) | 215.5 ±<br>62.2 <sup>ab</sup> | 206<br>(115 - 318)   | 372.9 ±<br>65.7 <sup>bd</sup>  | 365.7<br>(256-495)   |
| <b>EP</b>              | 363.7 ±<br>39.5      | 355<br>(315 - 430)   | 255.2 ±<br>31.9 <sup>b</sup>  | 245.7<br>(224 - 326) | 378.1 ±<br>33.5 <sup>bd</sup>  | 378.2<br>(334-437)   |
| <b>ZP</b>              | 333.7 ±<br>33.8      | 343.5<br>(267 - 371) | 230.7 ±<br>28.4 <sup>ab</sup> | 235.2<br>(189 - 268) | 321.4 ±<br>31.9 <sup>abd</sup> | 320.3<br>(267-378)   |
| <b>CB</b>              | 326.3 ±<br>32.2      | 329<br>(252 - 364)   | 211.6 ±<br>30.6 <sup>ab</sup> | 214.8<br>(150 - 246) | 282.7 ±<br>18.8 <sup>a</sup>   | 284.3<br>(252 - 305) |
| <b>Test Statistics</b> | 2.295                |                      | 2.643                         |                      | 15.05                          |                      |
| <b>P*</b>              | <b>0.044</b>         |                      | <b>0.013</b>                  |                      | <b>&lt;0.001</b>               |                      |

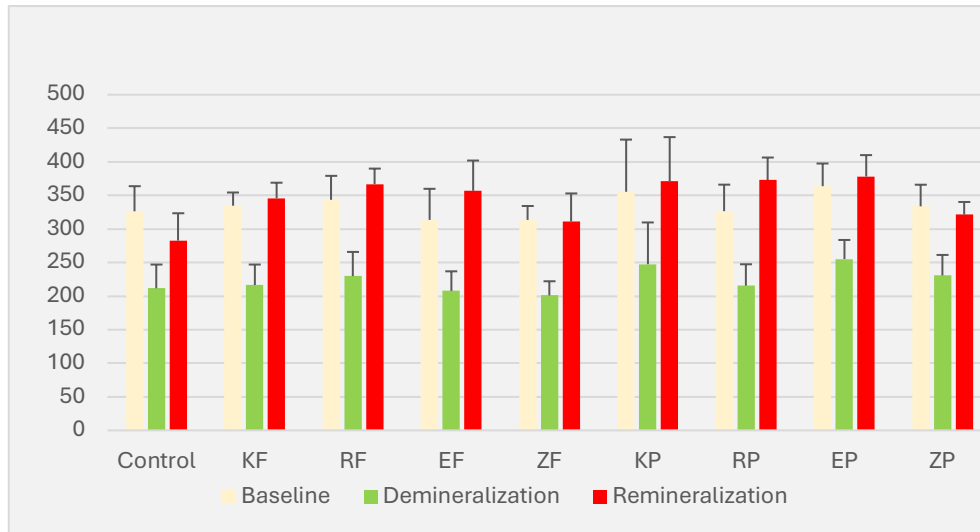


**Figure 19.** The values at baseline, after demineralization, and after remineralization for all groups.

There is a statistically significant difference between the baseline, demineralization and remineralization groups ( $p < 0.001$ ). The three groups are different from each other. Baseline mean value is 334.5, Demineralization mean value is 223.9 and Remineralization mean value is 345.2.

**Table 10.** Comparison of microhardness mean value between baseline, demineralization and remineralization groups. Analysis of Variance in Repeated Measurements; a-c: There is no difference between groups with the same letter.

|                                   | Mean±<br>s.deviation      | Median (min-<br>max) | Test<br>Statistics | P*               |
|-----------------------------------|---------------------------|----------------------|--------------------|------------------|
| <b>Baseline</b>                   | 334.5 <sup>c</sup> ± 42.8 | 338.8<br>(205 - 469) | 727.246            | <b>&lt;0.001</b> |
| <b>After<br/>demineralization</b> | 223.9 <sup>b</sup> ± 38.2 | 224<br>(115 -326)    |                    |                  |
| <b>After<br/>remineralization</b> | 345.2 <sup>a</sup> ± 48.3 | 349<br>(252 - 495)   |                    |                  |

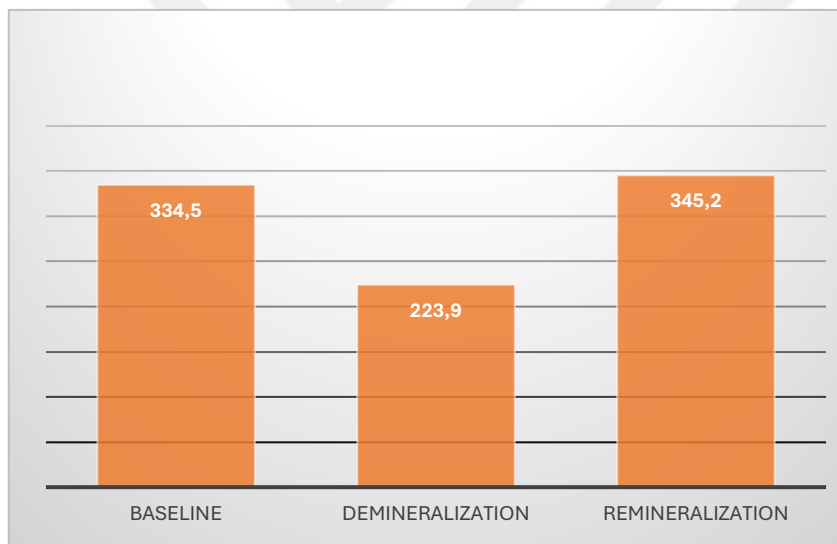


**Figure 20.** The microhardness mean values comparisons between Baseline, Demineralization, and Remineralization groups.

#### 4.2. Results Related To Scanning Electron Microscope SEM

As a result of the evaluation of the enamel samples that were prepared initially by SEM; It was observed that the healthy normal enamel surface represents the tooth's natural shape, that have a smooth, homogenous structure. Figure 20. describes the appearance of normal enamel morphology.

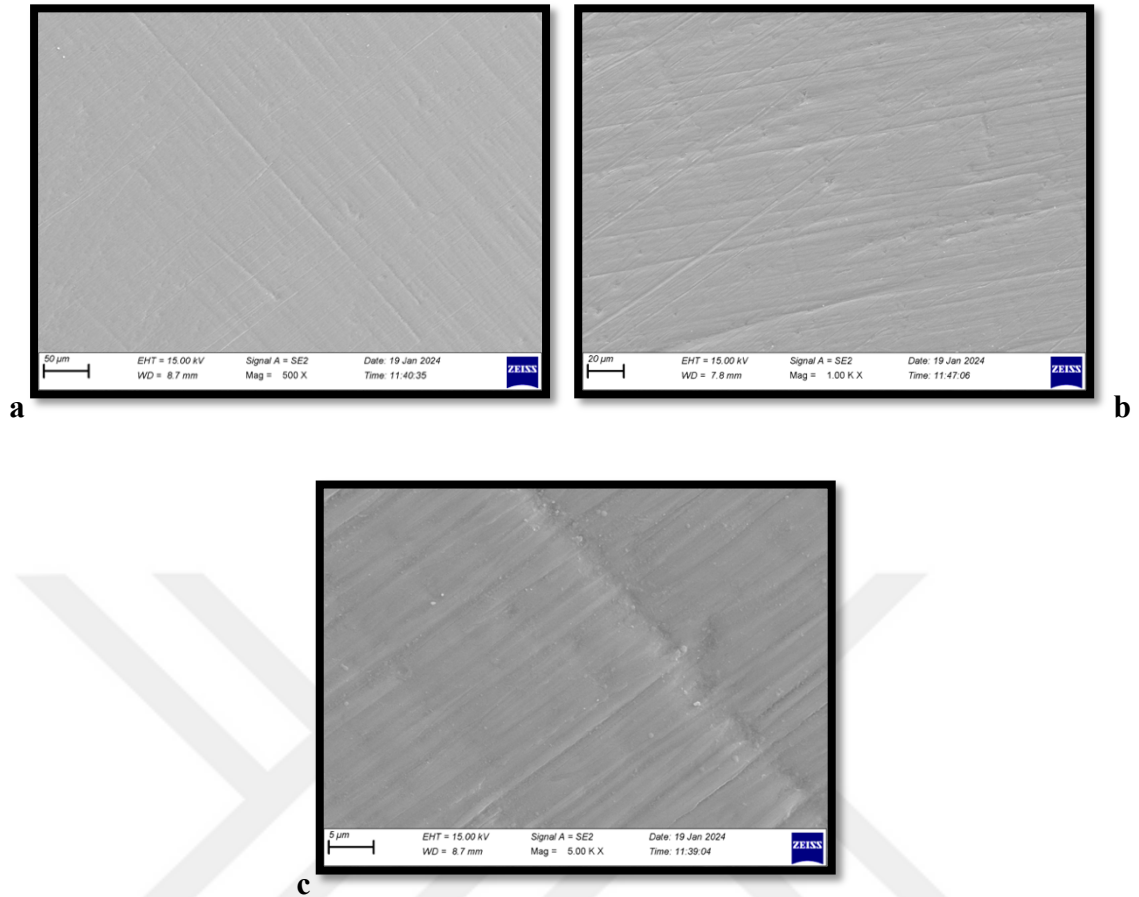


**Figure 21.** a) Image of the intact enamel surface at  $\times 500$  magnification, b) Image of the intact enamel surface at  $\times 1000$  magnification, c) The intact enamel surface at  $\times 5000$  magnification.

All of the samples' demineralized surfaces showed evidence of loss of integrity of the enamel surface layer and exposure of micropores which is in compatible with prism structure. After evaluation of the samples, the integrity of the enamel prisms was disrupted and there was more dissolution in the core part of the prisms rather than in the periphery, causing a honeycomb-like appearance Figure 21.

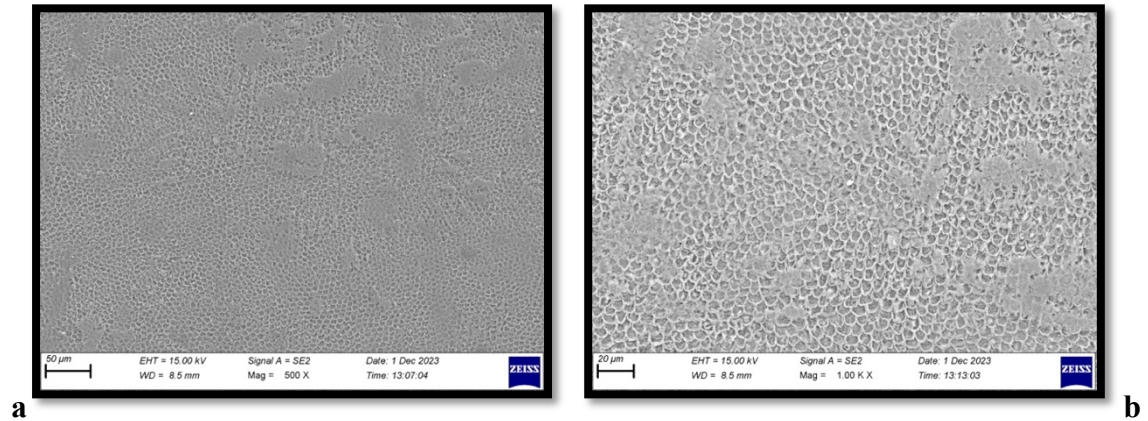


**Figure 22.** a) Image of the demineralized surface at  $\times 500$  magnification, b) Image of the demineralization surface at  $\times 1000$  magnification with honeycomb appearance.

After the treatment of initial enamel lesions with fluoridated ACP-based toothpaste, it was observed that the enamel surfaces were covered with Calcium fluoride crystals. The remineralization pattern is non-homogeneous and irregular. The surfaces were almost completely covered. There were small globules formed in some regions combined to create larger globules. The surface is more homogenous than the non-fluoridated ACP-based group Figure 22.

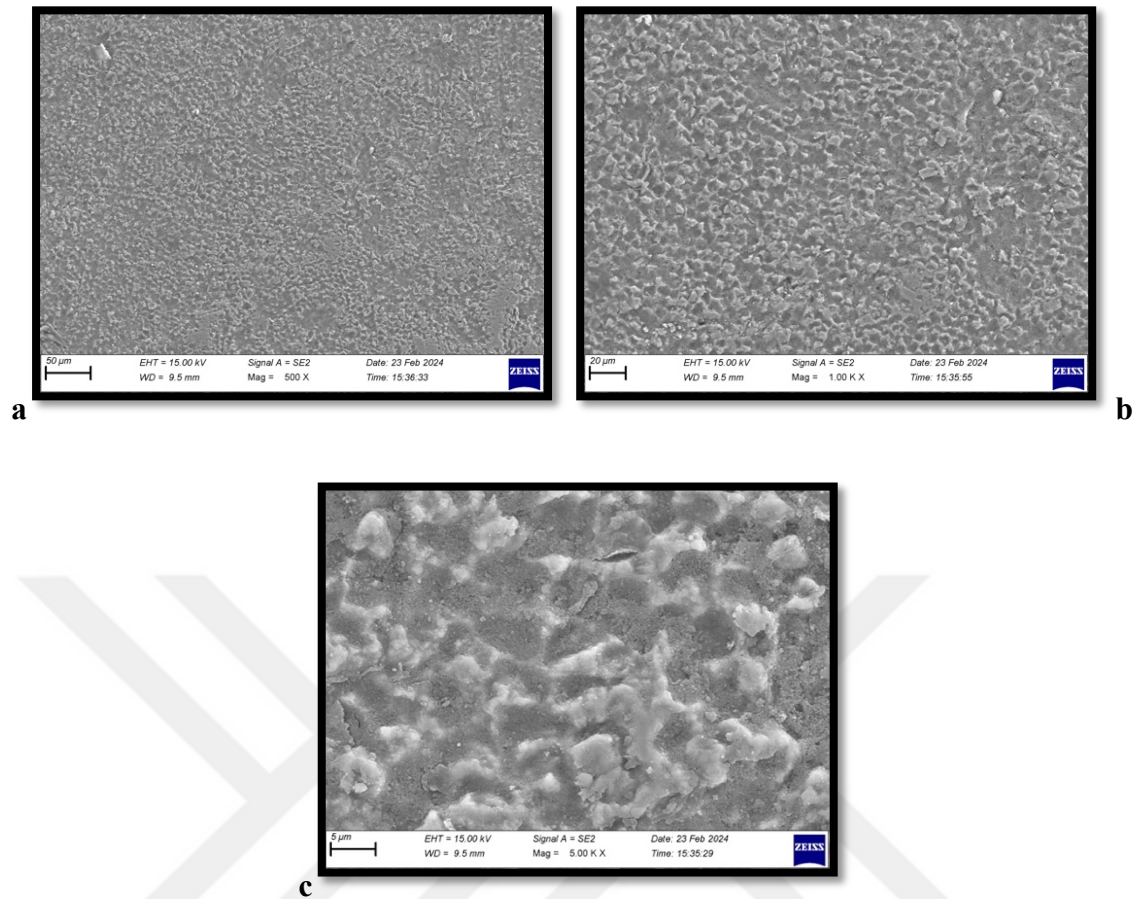


**Figure 23.** a) Image of fluoridated ACP-based material remineralization at  $\times 500$  magnification, b) Image of surface remineralization at  $\times 1000$  magnification c) Image of surface remineralization at  $\times 5000$  magnification.

After the treatment of initial enamel lesions with non-fluoridated ACP-based toothpaste, it was observed that the enamel surfaces were poorly covered with Calcium phosphate crystals and a lot of microporosity. The remineralization was a non-homogeneous irregular rough surface pattern Figure 23.

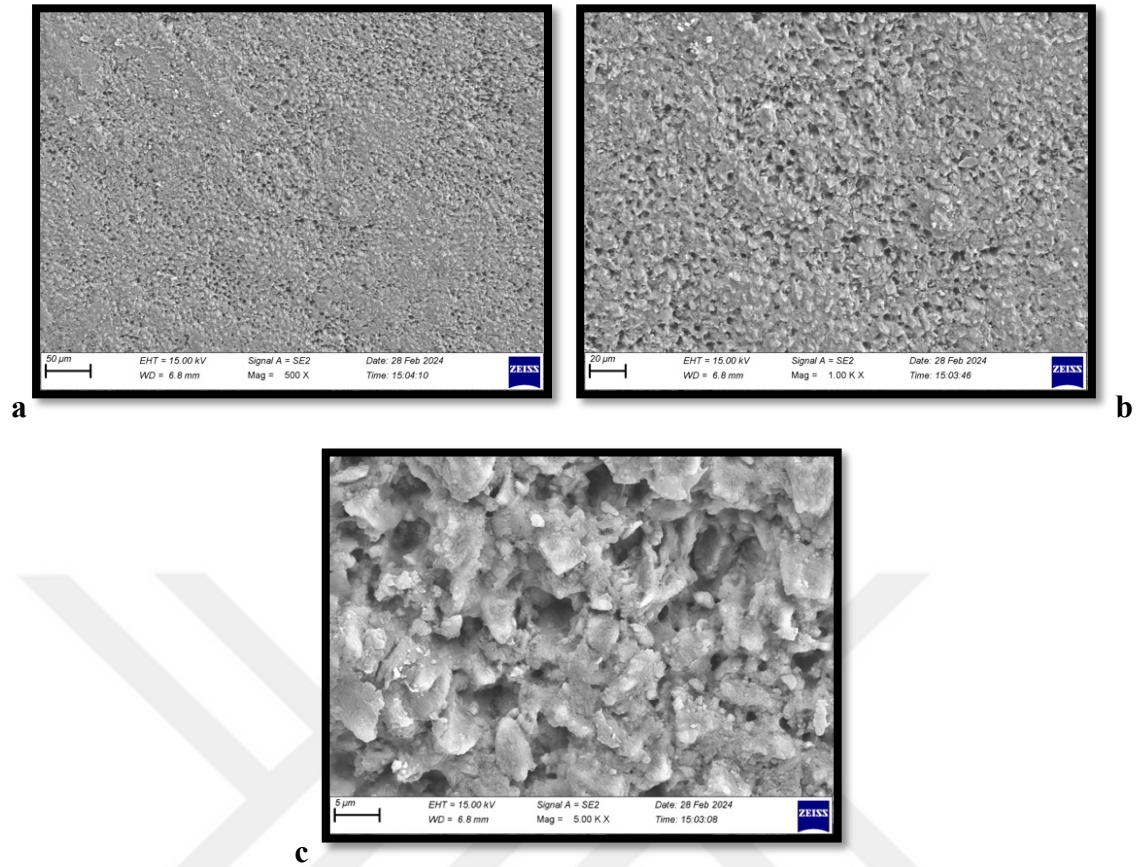


**Figure 24.** a) Image of non-fluoridated ACP-based material remineralization at  $\times 500$  magnification, b) Image of surface remineralization at  $\times 1000$  magnification c) Image of surface remineralization at  $\times 5000$  magnification with a lot of uncovered microporosity.

After the treatment of initial enamel lesions with fluoridated HA-based toothpaste, it was observed that the enamel surfaces were entirely covered with calcium fluoride crystals with successful surface regeneration. The remineralization did not exhibit a homogeneous smooth regular surface. Small globules formed in some regions combined to form larger globules. However, it was noticed that the small micropores were not covered. Figure 24.

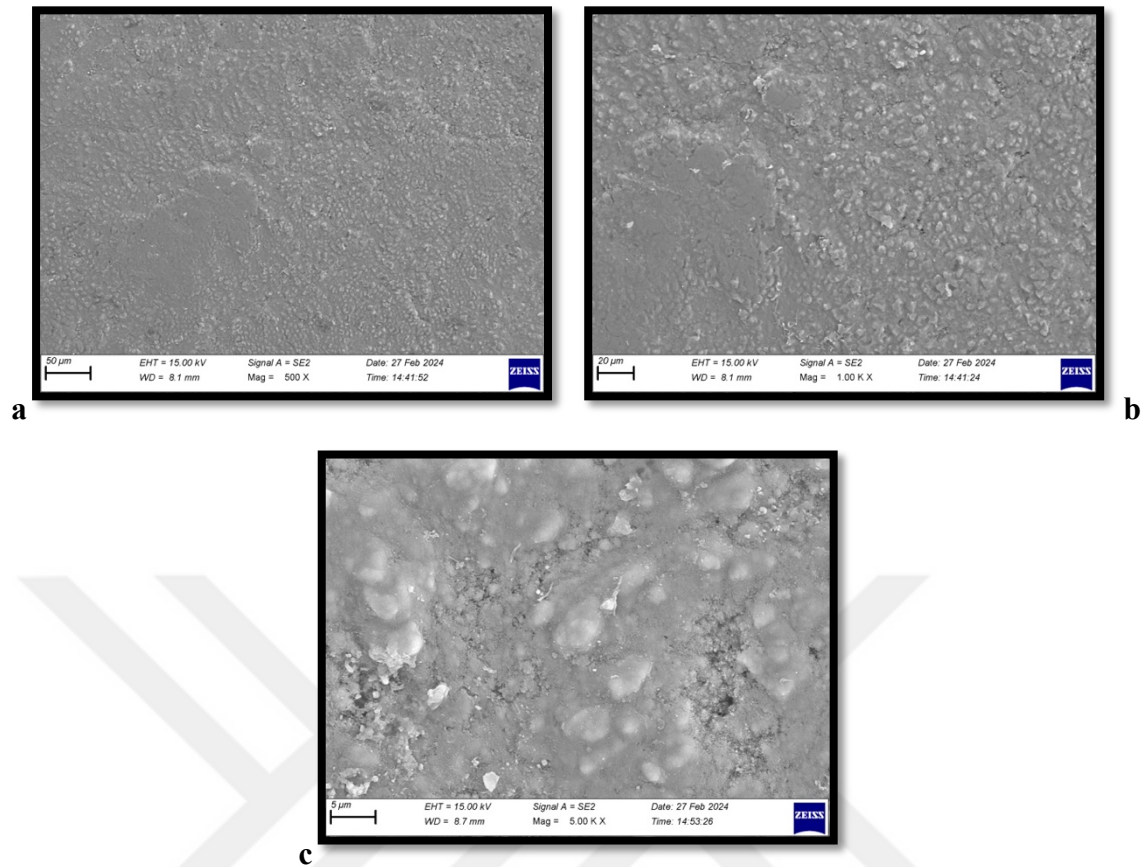


**Figure 25.** a) Image of fluoridated HA-based material remineralization at  $\times 500$  magnification, b) Image of surface remineralization at  $\times 1000$  magnification c) Image of surface remineralization at  $\times 5000$  magnification with complete surface reconstruction.

After the treatment of initial enamel lesions with non-fluoridated HA-based toothpaste, it was observed that the enamel surfaces were completely covered with hydroxyapatite. A homogeneous smooth surface with a complete covering of all microporosity and successful surface regeneration. There are small mineral precipitate of calcium phosphate above the apatite layer that acts as a reservoir to protect against acid attacks Figure 25.

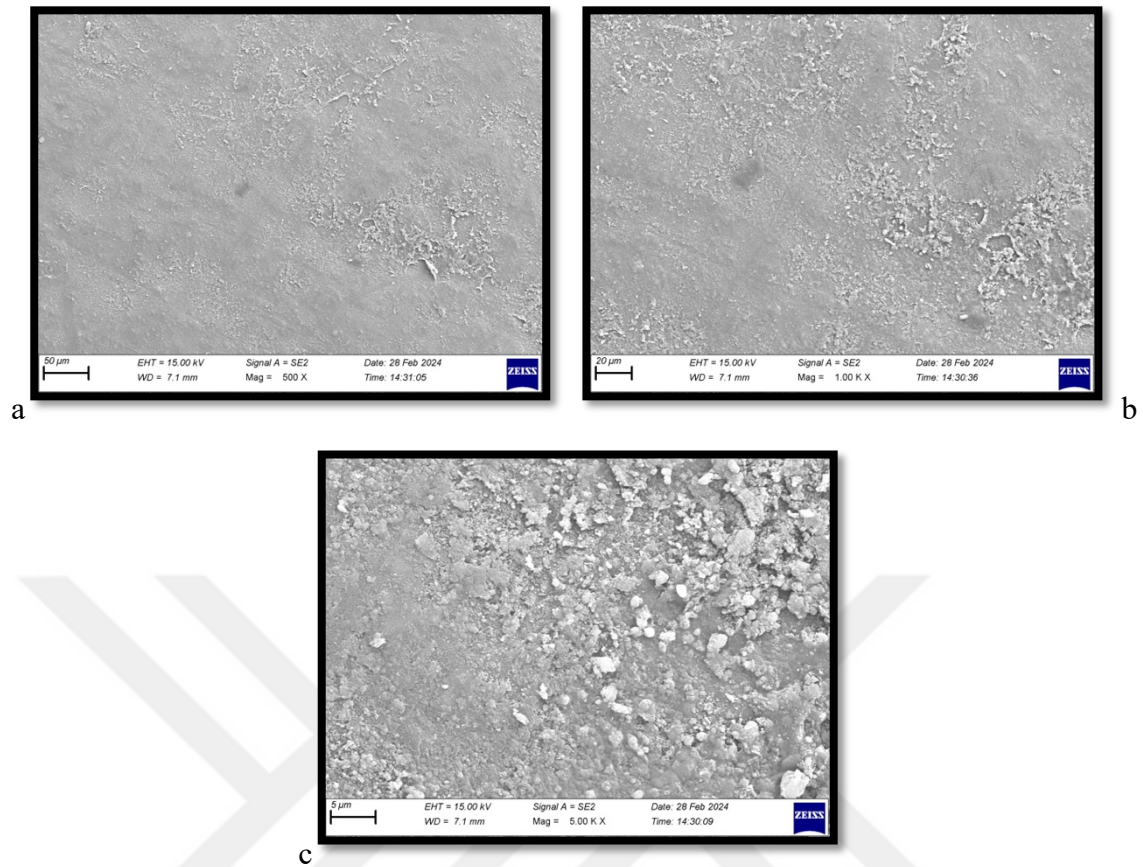


**Figure 26.** a) Image of non-fluoridated HA-based material remineralization at  $\times 500$  magnification, b) Image of surface remineralization at  $\times 1000$  magnification c) Image of surface remineralization at  $\times 5000$  magnification with complete surface reconstruction and formation of a highly mineralized layer. There is a small mineral precipitate of calcium phosphate above the apatite layer.

The control group shows many microporosity and loss of surface integrity, irregular rough, and non-homogeneous surfaces Figure 26.

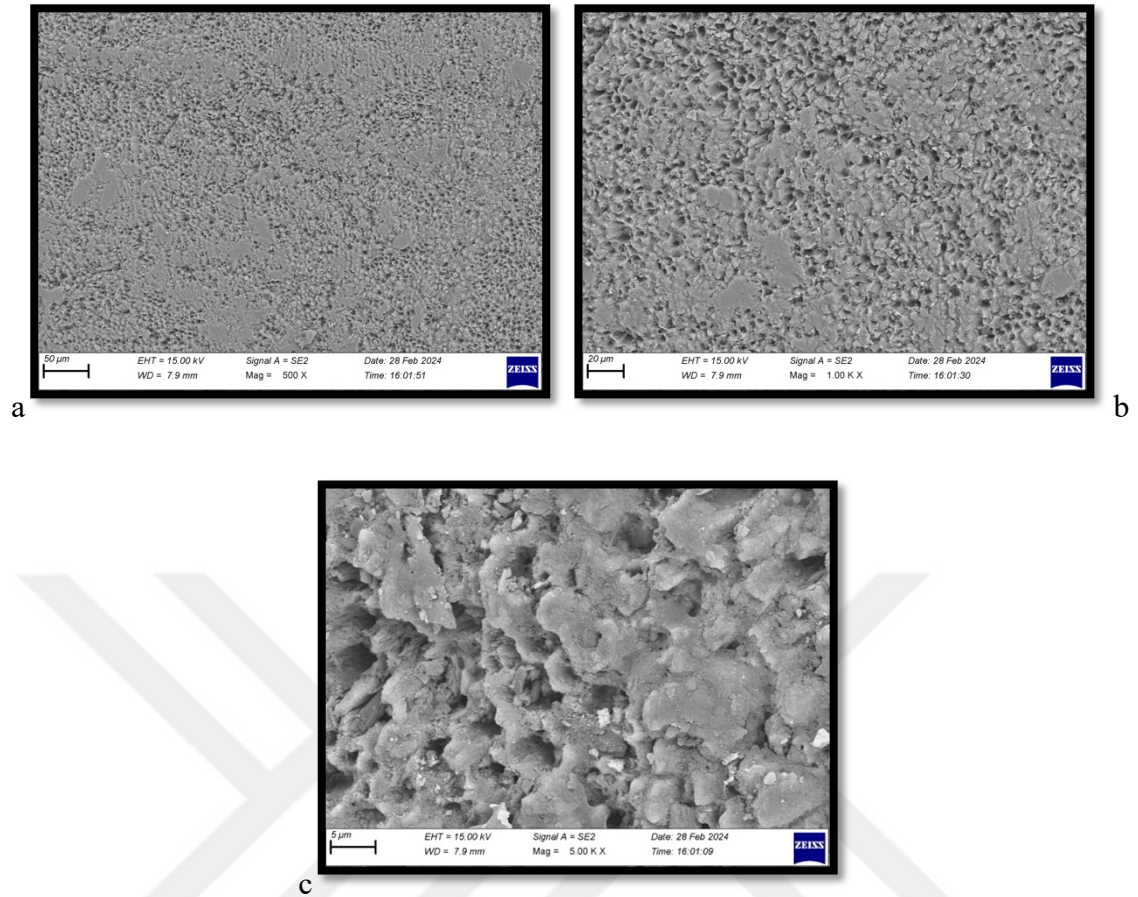


**Figure 27.** a) Image of control group remineralization at  $\times 500$  magnification, b) Image of surface remineralization at  $\times 1000$  magnification c) Image of surface remineralization at  $\times 5000$  magnification with loss of surface integrity, rough and multi porosities (honycomb demineralization pattern).

## 5. DISCUSSION

The 2017 Global Burden of Disease Study found that 2.3 billion people and over 530 million children worldwide suffer from dental caries of permanent teeth, respectively (Cubukcu et al., 2021).

Dental caries should be treated as conservatively as possible, and the invasive procedure should be the last choice. Removing the tooth's carious section and extending the cavity into areas considered susceptible to caries were key components of G. V. Black's classic restorative technique. Preventing extension has replaced G. V. Black's concept of extension for prevention. The prevention and early detection of caries are now the main priorities in dentistry. As a result, the idea of minimally invasive dentistry (MID) was developed (Mirsiaghi et al., 2018).

The principles of Minimum Invasive Dentistry (MID) are assessing caries risk, minimizing intervention when necessary, remineralizing early carious lesions, preventing and controlling the spread of disease by lowering cariogenic bacteria, and repairing rather than replacing damaged restorations (Mirsiaghi et al., 2018). Many treatments have been proposed to reduce early caries lesions. These include teaching patients about oral hygiene, applying remineralization agents topically, and adding remineralizing agents to restorative materials (Ghadirian et al., 2020).

The non-cavitated lesion can be remineralized. It is highly beneficial to employ this strategy to preserve dental structures and dentition for a much longer life, even though it is not easier than the interventional approach. Furthermore, it is unprofessional to sacrifice natural tooth structure with broadly created cavities in the hopes that it will somehow prevent disease (Dallı et al., 2012).

Generally, the topical fluoride application can reduce decay of the teeth by 30% to 70 %. The reduction of caries incidence and risk can be achieved by reducing the frequency of fermentable carbohydrate consumption such as (glucose, sucrose, and fructose), in addition to replacing them by sugar alternatives such as xylitol. Prevention of caries also can be achieved by fluoridated water, in addition to fluoride-releasing

preventive and restorative materials, using pit and fissure sealants, and practicing good dental hygiene and plaque removal (Kudiyirickal and Ivančáková, 2008).

Numerous systematic reviews have demonstrated the essential function that fluoride plays in preventing caries since it is the most important agent in preventing demineralization, the development and progression of caries lesions (Benson et al., 2013; Shahid, 2017). Fluoride can be delivered systemically or topically using products like toothpastes, mouthwashes, gels, varnishes, oral fluoride release equipment, and topical fluoride combination applications. These days, there are more concerns over the use of high-fluoridated materials because of the increasing hazard of probable fluorosis of the teeth resulting from children being exposed to fluoride from many sources and the recent classification of fluoride as a chemical and neurotoxic substance (Philip, 2019).

Based on studies conducted in both fluoridated and non-fluoridated areas, four main risk factors have been found for fluorosis: using fluoridated drinking water, toothpaste containing fluoride, fluoride supplements, and newborn formulae before the age of eight years (Mascarenhas, 2000).

Burt and Szpunar (1988). According to their research, the percentage of fluorosis markedly increased as the fluoride concentration in the community water raised from zero to 1.2 ppm. In fluoridated areas, the incidence of fluorosis ranges from 7.7% to 69%, while in non-fluoridated areas, it ranges from 2.9% to 42% (Mascarenhas, 2000).

As a preventative precaution against caries, children in fluoride-deficient areas are advised to take fluoride supplements. Several studies have demonstrated that, although inefficiently children in fluoridated areas are also provided supplements. In fluoridated areas, odds ratio for fluorosis from supplement use reached 24, but in non-fluoridated areas, where fluoride supplements are often advised, the odds ratios varied between 1.7 and 8 (Mascarenhas, 2000).

Before 1979, the producers of infant formula in North America voluntarily lowered and controlled the amount of fluoride in their products, which had previously been found in infant formulas at variable and high amounts. The risk of fluorosis in children born after 1979 has been assessed by Pendrys and Katz (1998). According to

their findings infant formula is considered a fluorosis risk factor in fluoridated regions. According to Mascarenhas and Burt's (1998) research, using fluoride toothpaste before the age of six is related to elevated probability of developing fluorosis in the subjects of the study. Furthermore, excessive fluoride intake leads to fluoride toxicity can lead to major health problems including toxicity in bone and teeth that lead to fluorosis in the teeth and joint pain, limited joint movement, and limb motor dysfunction. It can lead to toxicity in the lungs, liver, kidney, immune system, reproductive system, and nervous system (Zuo et al., 2018).

It is also observed that remineralization by salivary fluoride is limited to the tooth's outer 30  $\mu\text{m}$ . Neither the structural characteristics nor the appearance of the subsurface lesion are enhanced by only surface remineralization. Moreover, in some populations, the recent epidemiological information is worrying, as the incidence of caries is unaffected or increased despite fluoridated toothpaste usage. These findings are claimed to the fact that the food worldwide is changing to be more sugary and processed which limits the fluoride remineralization potential (Philip, 2019).

Under normal physiological conditions, salivary homeostatic processes and fluoride are often adequate to remineralize early lesions; but, in high-caries environments, these systems are insufficient. Boosters to increase the remineralizing and preventive efficaciousness of fluoride can also assist other population groups under risk are (xerostomia patients, and old people at risk of root decay). The growing worries that children today may be more susceptible to dental fluorosis due to many sources of fluoride exposure are more relevant. In all age groups, high doses of topical fluoride has ability to enhance the risk of caries, sometimes known as "fluoride syndrome." New remineralization technologies are needed to complement the fluoride and enhance the remineralization potential to initial enamel caries (Philip, 2019).

The purpose of the current in vitro experimental study is to evaluate the efficacy of newly developed non-fluoridated remineralization agents on the remineralization of initial enamel lesions compared to fluoridated agents—additionally, the possibility of replacing fluoridated agents with non-fluoridated ones.

Even with years of research and new anti-caries treatments in the market, tooth decay continues to be one of the chronic conditions with the greatest incidence affecting a significant number of people worldwide. Numerous in vitro and in vivo studies on cariology have been published during the past few decades. There are different types of research to study cariology include in vitro research comprised almost all of the research (84%), followed by in situ studies (15%), and the least in vivo studies (1%). (Yu et al., 2017).

For an equilibrium between the benefits and drawbacks of in vitro and in vivo research, in situ research is suggested. The fundamentals of the chemical and physical characteristics of the dental ecosystem are provided by this research. In situ models need to adequately imitate uncontrolled intraoral environments and combine highly controlled experimental factors to gather enough information about the methods and materials to be used before clinical application. Consequently, the cariogenic environment, dental biofilm, regular carbohydrate loading, and effective reaction time are the four fundamental components of in situ investigations and may be used for either human or animal tooth substrate. Study designs for in situ investigations are highly complex and have various limitations since they involve several combinations of natural and experimental variables. Natural caries-filled extracted teeth were employed as evaluation specimens in several in situ research. Because of this, it was challenging to compare the parameters from various caries using natural caries models to assess the effectiveness of careis preventive substances (Yu et al., 2017).

There are various benefits to evaluating the overall impact of an intervention on carious teeth in an oral environment using in vivo studies. These benefits include having the potential to more closely simulate the natural caries formation process through the induction of caries lesions in animals, which offers superior scientific control and easier standardization than in situ investigations. Furthermore, most of the variables that lead to the formation of caries may be replicated, including the effect of diet, microbiology, strcuture of the tooth, and de- and remineralization actions, as well as their interactions. Despite the above mentioned benefits, animal studies do not predict clinical outcomes when it comes to anti-caries effect assessments for therapies like fluoride therapy. In certain animal studies, the outcomes of in vitro and in situ tests were different.

Furthermore, the mechanism of demineralization-remineralization of dental hard tissue has been difficult to study because of the oral environment's unexpected oscillations and the ethical concerns associated with human in vivo research (Yu et al., 2017).

In vitro research is frequently used by researchers studying cariology to examine the demineralization-remineralization process. One of the several benefits of in vitro investigations is that they may mimic the oral environment from many angles and provide more controlled situations than an actual environment. Moreover, it has the least time and expense among all laboratory studies and is simple and controllable to meet the study requirement. Furthermore, in vitro study designs can range from simple to complicated depending on the goals of the research. The authors of in vitro research indicate that it is nearly hard to replicate the intricate process of natural caries formation, even with a complex design. As a result, the in vivo ecosystem's realism is compromised by the design. However, relevant and practical results can still be obtained by researchers (Yu et al., 2017).

In light of these benefits, the demineralization-remineralization efficacy of fluoridated and non-fluoridated remineralization agents was assessed in vitro. Human teeth are the most therapeutically suitable substrate, according to Mellberg (1992) and Ögaard and Rolla (1992), who established the essential criteria for dental substrate selection in in vitro and in situ research. When attempting to forecast a product's impact on caries inhibition, the selection of sound enamel will be essential. Based on their analysis, the extracted human premolars and molars were the best specimens for this study.

It should be remembered, though, that the composition of human dental tissue can differ depending on several factors, including age (dentin sclerosis and post-eruption maturation), environmental factors (diet, fluoride exposure, previous caries assault), and genetic influences. These variations can have a significant impact on how the body reacts to acid attacks (Buzalaf et al., 2010).

According to Aydın et al. (2015) found that the different storage mediums including de-ionized water, 0.2% glutaraldehyde, HBSS, 0.1% NaOCl, and 0.1% thymol, were acceptable for in vitro tests and have no influences on the microhardness

of the enamel when stored for 2 months. Thus, the extracted human molar and premolar teeth in this study were kept in deionized water that was renewed daily for a maximum of two months before the beginning of the experimental research. The teeth were kept in 0.5 % chloramine T solution for one week for disinfection after that it was kept in deionized water until use in the thesis experimental study (Shihabi et al., 2021).

The analysis of tooth demineralization/remineralization Involves a range of quantitatively and technically advanced methods, from direct measurements of mineral gain/loss (e.g., microradiography) to indirect measurements of changes in tooth mineral properties (e.g., polarized light photomicroscopy (PL), microhardness methods, and iodide permeability). Even though these approaches are indirect, they are nevertheless quantitative and may evaluate alterations to actual physical characteristics to varying degrees of accuracy (White et al., 1992).

Using reference point indentation and micro-Raman spectroscopy, Seyedmahmoud et al. (2017) investigated the correlation between dentin's hardness and microstructure. He found that as dentin minerals increased the hardness increased. Moreover, Akkus et al. (2017) studied the Relationship between the mineral concentration and micro-hardness of healthy enamel and found that a positive relationship between mineral content and hardness using Raman microscope and evaluated with a Brinell hardness measurement device. This explains that the surface microhardness (SMH) test is the best complement to direct approaches (White et al., 1992). Due to this reason, in this thesis study, the evaluation of the mineral/loss gain in the teeth was interpreted with surface microhardness values.

Knoop hardness (KHN), Vickers hardness (VHN), and Brinell hardness values can be used to identify the microhardness values of the specimens. The Vickers and Knoop microhardness testing techniques are commonly used in dentistry. In the Vickers hardness test it is simple to identify even the smallest variations in this square-shaped indentation that was acquired following the test, and inaccurate readings can be avoided. However, faults that may arise in the Knoop hardness test where it is more challenging to detect since it forms a rhombic structure on the sample surface with parallel opposing surfaces (Jyothi et al., 2014).

In the current study, the Vickers hardness measurement method was preferred to evaluate the surface hardness of the samples. It is easy and more precise to measure the square-shaped indentation obtained in Vicker's hardness device.

Different loads and dwell times are utilized to assess the tooth enamel's microhardness. Studies that use varying load values at varying times have been published in the literature by Ahrari et al. (2018) and Behrouzi et al. (2020).

Lee et al. (2010), Montasser et al. (2015), and Alagha and Alagha, (2021) have investigated the enamel's microhardness values while subjecting it to a 200 g load for 10 seconds. Similar to previously described studies, a 200 g load was applied for 10 seconds in this experimental study to assess the microhardness values of human enamel. Microhardness measurements were taken and recorded at baseline, following lesion formation, and following the completion of the 28-day remineralization procedure.

Obtaining the smooth surfaces of the specimens is needed for the microhardness test, as it influences the study's findings (De Abreu et al., 2011). The specimens' uneven surfaces lead the microhardness machine's diamond tip to make a bigger indentation, which will result in a small incorrect microhardness value (Jyothi et al., 2014). Furthermore, a "prism-free enamel layer" is consisting of parallel-arranged, 30-micrometer-thick apatite crystals free of prisms is present on the external enamel surface. High mineralization and acid resistance characterize this layer (Ritter et al., 2019).

To achieve smooth and flat enamel surfaces and remove this mineral-rich, acid-resistant layer, the enamel surfaces of each specimen in this thesis experimental study were polished using silicon carbide paper grits ranging from 1000 grit to 1200 grit as done by Shimojima et al. (2023).

Chemical and biofilm models are the two types of demineralization-remineralization models employed in in vitro research. Cariogenic bacteria are thought to be the primary factor influencing caries lesions in biofilm models. It can be created as combined bacteria or as a single strain. Microcosm biofilms generated from dental plaque or saliva can closely simulate original tooth plaque's physiological and microbiological characteristics. On the other hand, chemical models imitate the caries by simulating the

demineralization and remineralization processes with acid or acid buffer. Most in vitro research has created synthetic demineralized lesions or caries on enamel and/or dentin using chemical models. The ease of use, affordability, efficiency (saving time), repeatability, and stability of the experiment are among the benefits of chemical modeling (Yu et al.,2017).

Chemical models are classified into simple mineralization and pH cycle models. In simple mineralization models, mild organic acids and acid buffers such as lactic acid/acetic acid or acidic gels create demineralized lesions. These mild acids create demineralized lesions that are more like natural caries than inorganic acids. Acid erosion models can be created using inorganic acids (hydrochloric acid, phosphoric acid and citric acid). These inorganic acids are used for cariology research. It is more acidic than those used and pH values can be as low as 1.0 (Yu et al.,2017).

The pH-cycling approach is a type of modern chemical model based on an approach where acid challenges were introduced periodically to disrupt a pH-neutral environment. These experiments simulate the dynamics of the oral environment, including the loss and gain of minerals that occur during the caries-forming process when sugars are metabolized to produce caries lesions, this is a significant benefit of the pH-cycling approach. Additional benefits are the reduced sample size needed and the high degree of scientific control that leads to decreased intrinsic variability in in vitro models. The remineralization solution typically has an acidity of  $\text{pH} = 7$ , which is neutral. Depending on the different substrates and demineralized acid, the pH of the demineralization solution ranged from 4.4 to 5.5 (Yu et al.,2017).

In this thesis study, two different solutions were prepared: a Remineralization solution that is used to remineralize the enamel lesion and to be used in the artificial caries phase of the pH cycle. The other solution is a demineralization solution to be used in the demineralization part of the pH cycle.

In our study, similar to the study of Üstün and Güven study (2022), a pH cycling chemical model in demineralization was used by preparing a solution of 0.3% citric acid, which was prepared by diluting 15 grams of citric acid powder in 5 liter of distilled water; since it will promote the formation of subsurface lesion. A demineralization solution was

prepared by adjusting the pH with hydrochloric acid as in the study of Nikiforuk and Sreebny (1953). The cycle is demonstrated schematically in Figure 3.14. The solutions were renewed between each cycle as in study of Üstün and Güven study (2022), to inhibit the accumulation of the substances results from demineralization and the pH change, and the specimens were cleaned with a artificial saliva containing syringe between each cycle, then dried (Esfahani et al.,2015).

The remineralization solution formula was prepared as listed In Table 3. the remineralization pH was adjusted to 7 by potassium hydroxide (KOH) as in Üstün and Güven study (2022).The remineralization agents were applied to all of the specimens once daily according to the instructions of manufacturers. After each application specimens were cleaned by a syringe containing artificial saliva (Esfahani et al., 2015), then dried and stored in renewed artificial saliva solution and stored at 37°C as Üstün and Güven study (2022).

The manufacturers recommend different application methods for remineralization materials, including the toothbrush, finger, and tray application methods. Some studies used toothbrushes to apply remineralization agents directly by designing toothbrush holding machines with 200-gram load simulating brushing force of 2 newton and using soft bristles toothbrush as in the study of Maciel et al. (2023). Another study used a manual toothbrush to apply remineralization agents and a 200-gram load as in the study of Guntermann et al. (2022). A cotton applicator brush was also used to apply the materials as in the studies of Kaur et al. (2015), Kilic and Gurbuz (2020), a soft applicator brush in the study of Salinovic et al. (2021). In study of Keratibumrunpong et al. (2024), compares the two application methods for remineralization agents which are by paint-on (by brush) and tray application. They found that the paint-on application of APF gel or the tray application of APF had a same effecacy in remineralization of the enamel.

Singh et al. (2017), studied and compared the relative efficiency of n-HA (Aclaim), n-HA (Apagard), Clinpro Tooth Crème, and Colgate total in remineralization. In their study, they used two application methods finger and toothbrush methods.

In the current study, the efficacy of two application methods (finger and toothbrush), of four remineralization agents that are of two basic materials with and

without fluoride that include (fluoridated hydroxyapatite-based, non-fluoridated hydroxyapatite-based, fluoridated ACP-based, and non-fluoridated ACP-based materials) used in remineralization of early enamel lesions.

The electric toothbrush is fixed on a custom-made holding machine with a 200-gram axial load simulating brushing load as in the study of Hassan et al. (2021), Figure 8. In addition, the finger application method was done with a gloved finger. The materials were applied for 3 minutes according to manufacturer instructions. (application session was for 30 seconds), according to a study by Gavrilă et al. (2015) and left for 150 seconds on the tooth surface, the total application time is 3 minutes. After that, the teeth were cleaned with a syringe of artificial saliva as recommended by the manufacturer (spit out excess materials and not drink and eat for 45 minutes following application) and like the study of Nalawade et al. (2021). The specimens were returned to refreshed artificial saliva at 37°C and stayed for 16 hours. The procedure was repeated daily for 28 days as in Esfahani et al. (2015). Regarding remineralization time, as time increases the remineralization extent increases too (Salinovic et al., 2021).

At baseline ( $p = 0.804$ ) and demineralization ( $p = 0.722$ ), the fluoridated and non-fluoridated groups do not differ significantly from one another. This is explained by the fact that the application of acid causes a significant decrease in microhardness values due to mineral loss, bringing the values in each group down to similar values (Salinovic et al., 2021). At remineralization, the fluoridated and non-fluoridated groups differ significantly from one another, which supports the rejection of the null hypothesis that at remineralization, the fluoridated and non-fluoridated groups do not differ significantly from one another.

This corresponds with the results of a study by Rirattanapong et al. (2010), which showed that toothpaste containing fluoride at 250, 500, and 1,000 ppm produced noticeably more remineralization than toothpaste without fluoride, because the fluoride-containing toothpaste was better at delaying the advancement of dental caries. Furthermore, Dijkman et al.'s (1990) study demonstrated that whereas brushing samples treated with fluoride-free toothpaste did not alter lesion depth, using toothpaste containing 1250 ppm reduced lesion depth by roughly 35%.

Moreover, in the study of Thimmaiah et al. (2019) that supports our study result, they investigate the remineralization capability of (CPP-ACFP), (TCP), and (n-H) (which is non-fluoridated) toothpastes quantitatively using (EDX) and (SEM). All specimens were prepared and demineralized. Following 30 days of remineralization, the SEM-EDX was used to assess the specimens' calcium and phosphorus levels. After remineralization, the Ca/P mass percentage was significantly greater with CPP-ACFP and TCP, followed by n-HA.

They supported our study result where the fluoridated ACP-based materials significantly increased the remineralization efficacy. We can conclude that when comparing HA-based materials and ACP-based materials, Fluoride has a substantial effect on their effectiveness. Thus, the formation of fluorapatite instead of hydroxyapatite will enhance the enamel's microhardness.

The finding of the current study is contraindicated with Chaudhary et al.'s (2013) findings, where the fluoride free toothpastes increased the enamel hardness more than sodium monofluorophosphate. Moreover, the findings of Kasemkhun and Rirattanapongs' study (2021) have contraindicated to our study, indicating that toothpaste containing, two non-fluoridated agents, was just as effective as fluoridated toothpaste in remineralizing primary teeth. This may be because saliva contains calcium and phosphate, which fluorides need for remineralization process. As a result, the remineralizing impact of calcium phosphate-based agents may be just as effective as fluoride.

When comparing the materials we find that there is no statistically significant variance among the baseline mean values of the Hydroxyapatite-based material and Amorphous calcium phosphate-based material groups ( $p = 0.206$ ). Furthermore, there is no statistically significant variance among the demineralization values of the Hydroxyapatite-based material and Amorphous calcium phosphate-based material groups ( $p = 0.678$ ).

There is a significant variance among the remineralization values of the Hydroxyapatite-based material and Amorphous calcium phosphate-based material groups ( $p = 0.027$ ). The average value of the hydroxyapatite-based material group was

higher (364.1) than the average value of the amorphous calcium phosphate-based material group (341.9). This provides evidence against the null hypothesis, which contends that there is no statistically significant difference in the remineralization of early enamel lesions between materials based on HA and those based on ACP.

This is attributed to the mechanism of action of enamel biomimetic nanohydroxyapatite. There are two theories in Huang et al. (2011), One theory states that nano-HA acts as a “reservoir” for calcium and phosphate, thereby promoting continuous remineralization and minimizing demineralization. This theory is consistent with the traditional paradigm of “top-down” ion-mediated crystalline growth, which explains the complex biomineralization strategies found in nature. The other theory proposed that because of its distinctive features, such as its superior deposition abilities, which are consistent with the “bottom-up” theory of particle-mediated nano precursor assembly and mesocrystalline transformation in the biomineralization process, on enamel lesions, nano-HA shows significant remineralization effectiveness. Pepla et al.’s study (2014) indicated that the hydroxyapatite has extremely small size particles that play two important roles in remineralization first, it increases surface area allowing the nanoparticles to interact with other particles and the structure of the enamel. The second role, filling the micropores left by the demineralization process, forming a superficial coating of synthetic enamel.

The study by Singh et al. (2017) showed that n-HA-based materials, such as Apagard and Aclaim, were the most effective in preventing demineralization. These results were followed by fluoride-based materials, such as Colgate Total and Clinpro Tooth Crème. Our study results also support the concept that HA-based materials perform better.

De Carvalho et al. (2014), studied the effectiveness of CPP-ACP, n-HA paste, and fluoride varnish. Enamel blocks were distributed into four groups. An artificial enamel lesion was established. A pH cycle was done for 7 days. KHN values were measured before the challenge, following an initial enamel lesion, and after the challenge. Following the cariogenic challenge, KHN values were considerably more in the n-HA group compared to the varnish group. In the CPP-ACP group, KHN did not rise. Which agrees our study results.

In consistent with our study, Hamed et al (2024) studied the effectiveness of Fluoridated Amorphous Calcium Phosphate (F-ACP) and Biomimetic Nano-Hydroxyapatite (Biom-nHA) for enamel remineralization; they found that both the (Biom-n-HA) cream and the (F-ACP) varnish showed significant results for enamel remineralization and no significant difference among them. It could be due to the presence of fluoride which plays a significant role in remineralization based on our study, as the EP (fluoridated ACP-based) group has the highest values followed by the RP (fluoridated HA-based) group with no significant variance among the groups.

In comparison between application methods of finger and electric toothbrush it found that at baseline, there was no statistically significant variance among groups ( $p = 0.058$ ). In contrast, after demineralization it found a significant variance among finger and toothbrush groups ( $p = 0.006$ ), as the average value of the finger group is (237.2). The average value of the brush group is (213.7) despite that no application or use of the material, this can interpreted as tooth response to acidic challenges is affected by various elements, including its structure and contenents (Zero and Lussi, 2005). The minerals found in dental tissues are imperfect forms of HA. When the mineralized crystals form during the hard tissue formation, “impurity” ions from tissue fluids are incorporated into HA crystals, leading to impurities. These ions replace ions in the HA crystal structure.  $\text{CO}_3^-$  for  $\text{PO}_4^-$  or  $\text{OH}^-$ , and  $\text{Na}^+$  and  $\text{Mg}^{2+}$  for  $\text{Ca}^{2+}$  are the main substituents. Due to differences in size, charge, or both between the impure HA and native HA ions, the impurity ions cause strain into the HA’s crystal structure, thus, increasing solubility (Shellis et al., 2014).

The groups for fingers and toothbrushes do not differ statistically significantly at baseline. Moreover, After demineralization, there is a significant variance among groups, due to the the structural composition of the enamel that affects its response the acidic challenges (Amaechi and Loveren, 2013).

At the remineralization phase, there was no statistically significant variance among finger and toothbrush groups. This result coincides with Singh et al.’s study (2017), In their study, they used two application methods finger and toothbrush and found there is no significant variance among the application methods. These findings support the acceptance of the null hypothesis which states that there is no statistically significant

difference between toothbrush and finger application methods in remineralization of early enamel lesion. This facilitates the application of the materials either by the parents or the patient himself and improves patient compliance with the treatment.

When comparing all groups together there is a statistically significant variance in the baseline, demineralization, and remineralization phases ( $p < 0.001$ ). The 3 groups are different from each other. The baseline mean value is 334.5, the demineralization mean value is 223.9, and the remineralization mean value is 345.2. These findings support the rejection of the null hypothesis which states that there is no statistically significant variance among baseline, demineralization, and remineralization phases.

Hence, the microhardness's mean value of all samples in the current study at baseline (334.5) is higher than after the demineralization (223.9) and is consistent with earlier research by Lata et al. (2010), where the surface microhardness at baseline was in range of 254 – 363 and drops after demineralization to be in range of 162-183. The surface microhardness increased after remineralization compared to after demineralization in agreement with Jabbarifar et al.'s study (2011), as after demineralization microhardness of the enamel decreased significantly, and after remineralization, its microhardness recover significantly.

Furthermore, the remineralization group has the highest microhardness value compared to the others, this result is like the study of Esfahani et al. (2011), they found that the mean value after the treatment for one-month was significantly more than the microhardness value at the baseline ( $P < 0.001$ ).

Remineralization has the highest value because there was an increased absorption of phosphate, calcium, fluoride, and ions on the surface of the enamel, the mean values after remineralization were considerably greater than the mean values after demineralization in the experimental groups because of the high ion concentration, the tooth's surface develops a reservoir coating that helps to form larger, stronger, and comparatively more stable enamel crystals (Mohd Said et al., 2017).

In comparison between all 9 groups at baseline, it was found that there is a significant variance among them ( $p = 0.044$ ). The highest baseline mean value was for the

EP group (363), while the lowest value was for ZF and ZP groups (313.5) which satisfy the average hardness of enamel values ranging from (250-360) (Gutiérrez-Salazar and Reyes-Gasga, 2003).

As all specimens were subjected to a demineralization pH cycle, there will be mineral loss in all specimens (Salinovic et al., 2021), thus, in our study there is a significant variance between all groups. In pairwise comparison there is no significant variance between all groups except for ZF (201) and EP (255.2), this significant difference in surface microhardness; as mentioned previously due to the impurities in the structural contents of the enamel will affect its solubility during the acidic challenges (Amaechi and Loveren, 2013). All mean demineralization values are similar to those in the study of Sahiti et al. (2020) which has a mean microhardness value of 257.77.

There is a significant statistical variance among all groups after remineralization ( $p < 0.001$ ). These findings support to reject the null hypothesis which states that there is no statistically significant variance between all groups at remineralization.

The control group has the least microhardness mean value in comparison to all groups. There is no significant difference between the control group and ZF, and ZP groups with mean values (282.7), (311.2), and (321.4) respectively. This means that the ZF and ZP groups have the lowest remineralization potential compared to others regardless of their application methods (finger or toothbrush), this can be attributed to its composition which is ACP-based materials without fluoride, which supports our study result that non-fluoridated and ACP-based materials have the lowest remineralization potential. Similar results obtained in the study of de Carvalho et al. (2014). where they found that the lowest value was for the control group followed by CPP-ACP. No effect on surface microhardness by the CPP-ACP group.

When comparing the groups in pairwise we found that, between the control group and KF, KP, EF, EP, RP, and RF groups shows a significant variance among them. Similar to the study of Nourolahian et al. (2021), the mean microhardness was significantly lower in the control group (that did not have any treatment) compared to the other three remineralizing groups ( $P < 0.001$ ).

The highest microhardness mean value among all groups was in the EP group (378.1) which is fluoridated ACP-based material followed by the RP group (372.9) which is fluoridated HA-based material, despite our study results determining that HA-based materials have higher efficacy than ACP-based materials, this high value of microhardness for EP group attributed to the type of fluoride that affect the remineralization potential and raise the efficacy of ACP-based materials, it is valuable to discuss that EP group contains 0.45% stannous fluoride in contrast, the RP group contains sodium fluoride NaF (1450 ppm).

The same findings were established by West et al.'s in situ study (2019), they compared the NaF and triclosan dentifrice and the stannous fluoride dentifrice using a randomized in situ clinical experiment, A highly 0.454% stannous fluoride dentifrice, and a commercial toothpaste control containing 0.24% sodium fluoride and 0.3% triclosan (Colgate-Palmolive) were the products that participants were randomly assigned to receive treatment. Every study session lasted for ten days. For six hours every day, participants wore an intraoral appliance that held 2 polished human enamel specimens. They swished twice daily with the prescribed toothpaste slurry and four times daily with 250 mL of orange juice (25 mL per minute) spread out over ten minutes. To assess surface change, contact profilometry measurements were taken for every sample on day 10 and at baseline. It showed noticeably higher erosion protection efficacy of stannous fluoride. Although surface staining was a concern with early stannous fluoride formulations, stain-mitigating additives, including sodium hexametaphosphate can enhance extrinsic whitening in more modern formulations.

Both materials are fluoridated, according to our study results the HA-based material (RP) should have a higher microhardness value, in contrast, the ACP-based material (EP) win to have the highest among all and exceeds RP, we interpreted it as the type of fluoride affects the remineralization efficacy.

The highest values were EP>RP>KP. KP microhardness value is 371.1 (non-fluoridated HA-based material) following the RP microhardness value of 372.9 (fluoridated HA-based material) as in our study the fluoridated groups have significantly higher microhardness values, even though the microhardness values of two fluoridated and non-fluoridated HA-based materials are nearly the same, this can be interpreted that

HA-based materials have high remineralization efficacy even without fluoride, as the *in vitro* study conducted by Amaechi et al. (2020), where the specimens have demineralized by acidulated gel. They were randomly distributed to three remineralization groups: hydroxyapatite-based (Karex gelée, 15% HA, fluoride-free), fluoride-based (Elmex gelée, 12,500 ppm F), and artificial saliva (AS). Using a pH-cycling model, remineralization was carried out for 28 days while being stored in AS. Microradiography was used to measure mineral loss at baseline and after the test. In terms of remineralizing the initial caries lesion, hydroxyapatite-based gel (15% HA) was just as successful as fluoride-based gel (12,500 ppm F). While there was not a significant difference in remineralization between the two gels, the percentage mineral gain in both gels was considerably ( $p < 0.001$ ) higher than that of AS alone.

Even though there was no significant statistical variance among application methods, it can be noted that the highest values are from finger application groups (EP, RP, and KP). It is attributed to that the finger application method permits the material to be combined on the surface of the tooth while the toothbrush method distributes the material and the material can enter between the bristles which leads to loss of the material bulk.

The EP and ZF groups differ significantly, as these materials are ACP-based but it is clear the fluoride makes a big difference in remineralization potential. EP is fluoridated when compared to the ZF group which is non-fluoridated. This result agrees with the results of Srinivasan et al.'s study (2010), where after the 45 enamel specimens were eroded in a cola drink for eight minutes, the specimens were affixed to intraoral devices and worn by five participants. The samples underwent three distinct *in situ* remineralization procedures: Group I used CPP-ACP, Group II used CPP-ACP with 900 ppm fluoride, and Group III used saliva as a control. It has been shown that the microhardness values of the CPP-ACP pastes and the CPP-ACP pastes with 900 ppm fluoride statistically significantly differed. The synergistic effects of fluoride and CPP-ACP on the remineralization of demineralized enamel were verified by this investigation.

In the CPP-ACP remineralization process the ACP is thought to be localized on the tooth, where it functions as a reservoir for free calcium and phosphate ions, contributing to the remineralization. These ions inhibit demineralization and encourage

remineralization by preserving a condition of supersaturation to the hydroxyapatite. Fluoride and CPP-ACP together most likely produced a joint localization of calcium and phosphate ions with fluoride ions at the enamel surface, most likely as CPP-ACFP nano complexes (Srinivasan et al., 2010). By producing fluorapatite crystals, which have the advantage of increasing the microhardness of the enamel, promoting resistance to future demineralization, and enhancing the remineralization process (Farooq et al. 2021).

There is a significant variance between ZF and KP groups, KP has a higher microhardness mean value (371.1) compared to ZF mean microhardness values (311.2). As both groups are non-fluoridated, and by our study, this is attributed mainly to the main composition of the material, where the HA-based materials have significantly higher microhardness values compared to ACP-based materials. Our results are corroborated by Sebastian et al.'s (2022) study, which uses a microhardness test to assess and contrast the remineralization capacity of CPP-ACP, nano-hydroxyapatite, and calcium sucrose phosphate toothpaste on early enamel caries lesions. Enamel specimens were prepared and then, based on the remineralizing agent implemented, they were randomly assigned into four groups: group I was Control, group II was GC Tooth Mousse™ (CCP-ACP), group III was Acclaim™ (n-HA) non-fluoridated, and group IV was EnaFix (CaSP). The samples lost their mineral content. Following the application of a remineralizing agent, post-treatment KHN testing was carried out. EnaFix (CaSP) has the highest value followed by the Acclaim (n-HA) group had significant enamel surface microhardness compared to (CPP-ACP).

The mean microhardness value of the ZF group differs significantly from the RP group, with values of 311.2 and 372.9, respectively. Based on our study findings, the ZF is a non-fluoridated ACP-based material, and because of that it differs significantly from fluoridated HA-based materials. Bajaj et al. (2016), support our study findings, as they studied the efficiency of three several remineralization materials in remineralizing initial enamel lesions. They used three materials CPP-ACP, Tri-calcium-phosphate (TCP), and Hydroxyapatite (Remin Pro). They concluded that HA-based materials showed improved remineralization in comparison to CPP-ACP and TCP.

The morphological structure of enamel's hydroxyapatite crystals provides an analytical indicator of the tooth's tissue health. Scanning Electron Microscopy (SEM)

can produce high-resolution pictures that provide qualitative details on the samples' enamel prism morphology (Vitiello et al., 2022). SEM was first used in 1965, as it permit a comprehensive examination of the topographic structure of the enamel surface and the alteration that occurred (McMullan, 1995). So, in the current study, we use SEM to compare the morphological changes and remineralizing efficacy of four distinct commercially available agents on artificial enamel lesions following a 28-day treatment period. When examining the surface morphologies of initial enamel caries for in vitro investigations, SEM is the gold standard in many studies (Chen et al., 2013; Patil et al., 2013; Lelli et al., 2014; Raghu and Ananthakrishna, 2016; Vitiello et al., 2022; Tosco et al., 2023).

Based on Tosco et al. (2023), the CPP-ACP scanning electron micrographs showed almost complete surface morphology reorganization. Nonetheless, the material is deposited on the surface and some surface integrity is lost. In addition, its surface structures differed from those of the other groups, showing some material remnants and mild dissolution zones. The distribution of Ca and P components may be implied by this morphological non-homogeneity. The remineralized enamel with a normal appearance and restoration of the structure was seen in the SEM micrographs of the fluoridated-ACP group. This is in line with our research since non-fluoridated ACP-based materials have uneven surfaces with a loss of enamel surface integrity and some pores exhibiting areas of dissolution, while fluoridated ACP-based materials have complete surface coverage.

Zinc-HA group, which lacked fluoride, had a uniform and homogeneous enamel surface with obvious structural regeneration. Similar to the results of our study, which show that the non-fluoridated HA-based materials completely cover the enamel's surface without any porosity.

Based on Oliveira et al.'s study (2016), this study evaluated the influence of dentifrices containing (CPP-ACP) with and without fluoride on enamel remineralization. The specimen morphological analysis results in that CPP-ACP has more enamel irregularities and the treated surface showed a visible area of alteration of the enamel organic matrix, evidenced by the more apparent 'honeycomb pattern of demineralization'. In CPP-ACFP's SEM micrograph, it shows a more homogeneous smooth surface and decreased roughness compared to CPP-ACP. This is in agreement

with our study when comparing both ACP-based materials, as the non-fluoridated group shows rough and irregular surfaces with multiple porosities. while fluoridated ACP-based material, shows more coverage and homogeneity, despite the irregularities in the surface.

In the Vyavhare et al. study, they evaluate the potential use of CPP-ACP and nano-hydroxyapatite as fluoride substitutes in the remineralization of early carious lesions. Before demineralization, the typical anatomical enamel surface was observed by the SEM to have a smooth and intact surface. Following demineralization, a rough, uneven surface with noticeably higher porosities was visible. Demineralization in the form of a small honeycomb pattern was seen along with a multilayer surface of demineralization. In nano-hydroxyapatite surface there is a reduction in defects and porosity. A small demineralization honeycomb pattern was visible in the SEM image using CPP-ACP, suggesting that little to no remineralization took place on the surface.

Our research supports this, as the appearance of normal enamel surfaces was smooth and intact. Counterwise, In the demineralized surfaces, it was discovered that the surface layer of the enamel deteriorated and the micropores were exposed. The deterioration of the integrity of the enamel prisms and the fact that the prisms are more susceptible to dissolution in the cor part than the periphery caused the emergence of a honeycomb-like appearance in these regions. As in our study, the non-fluoridated ACP-based material has the least amount of remineralization, and in line with this study, little or no remineralization has occurred.

In an in vitro study comparing the effect of nano-HA with and without fluoride on early enamel caries, the surface morphological changes in the lesions were examined with SEM. Following the application of fluoridated n-HA toothpaste, it was noted that newly formed  $\text{CaF}_2$  crystals had coated the enamel surfaces. The remineralization that is taking place on the original enamel lesions is not uniformly smooth. In certain areas, tiny globules merge to generate bigger globules. But it was seen that there were gaps in a few tiny areas and that some of the micropores were not covered. The whole surface of the samples treated with hydroxyapatite toothpaste was found to be homogenous and the porous structure was fully covered. The covering thickness is greater than that of the surface cover seen following applications of fluoridated nano-HA toothpaste.

Additionally, it has been noted that certain areas experience mineral precipitation (Koçyiğit, 2015). In agreement with our study observations, we evaluated the enamel surfaces using n-HA+F toothpaste, we found that the entire surface coverage, non-homogeneous, and some globules on the surface was observed. There was some crystal agglutinations of various diameters had developed on the surface. In the non-fluoridated nano-HA based group homogenous complete coverage with mineral precipitation.



## 6. CONCLUSIONS

The findings of our experimental study the Evaluation of the Efficacy of Fluoridated and Non-Fluoridated Remineralization Agents Applied by Different Methods on Remineralization of Early Enamel Lesions:

### 1. According to results of the Vickers microhardness test (VHM):

At the baseline, following demineralization, and following remineralization groups, there is a statistically significant variance between them ( $p < 0.001$ ). This suggests that the process of demineralization and remineralization has been accomplished effectively.

The VHM microhardness means values of the toothbrush and finger application techniques do not differ statistically significantly upon remineralization and at baseline ( $p = 0.058$ ). The microhardness mean values following demineralization show a significant difference ( $p = 0.006$ ) due to HA structural impurities. Both methods are effective in remineralization.

There is a statistically significant variance between the remineralization values of the fluoridated and non-fluoridated groups, where the fluoridated groups has higher microhardness mean values.

There is a significant variance between the remineralization values of the Hydroxyapatite-based material and Amorphous calcium phosphate-based material groups, the HA-based materials have higher microhardness mean value.

A is a significant statistical variance between all groups at remineralization ( $p < 0.001$ ). As a pairwise comparison, there is a significant variance between the control group that has the lowest value, and KF, RF, RP, EF, KP, and EP. Also, there is a significant difference between the ZF group that has the lowest value, and RP, EP, and KP. There is no significant variance between the control group and ZF and ZP.

### 2. According to SEM analysis:

Upon analyzing the SEM results of the samples subjected to HA and HA with fluoride toothpaste, it was noted that both pastes generated an apatite layer that completely covered the surface micropores that were visible during demineralization. Nonetheless, it has been determined that the toothpaste group containing only n-HA contains notable small mineral precipitations above the apatite layer and that these accumulations may eventually serve as a calcium-phosphate reservoir to protect against acid attacks. While fluoridated n-HA has some globular aggregation of calcium fluoride on the surface with irregular and non-homogeneous mineral accumulation on the enamel surface and some gaps were not closed.

When analyzing the morphological appearance of ACP-based materials, the surface is non-homogenous and change in surface integrity. The non-fluoridated ACP-based materials, its surface was covered with non-homogenous and irregular calcium phosphate crystals that provide incomplete coverage of micropores and have rough surfaces. while compared to fluoridated ACP-based materials, almost the entire coverage of the surface, more homogeneous surface, and decreased roughness compared to the non-fluoridated group.

Based on the results we obtained from the Vickers microhardness test and SEM images, we think that the remineralization mechanism of HA-based materials is by the formation of a layer with high mineral density by apatite precipitation on the demineralized surface and repairs the crystal structure of the enamel using the mineral accumulation in this layer. In addition, we think that this layer can act as a mineral store against acid attacks in the long term and will inhibit demineralization.

On the other hand, the ACP-based materials remineralize the surface by the formation of a calcium phosphate in an amorphous form that is unstable thermodynamically which transforms into crystals, these crystals are distributed on the surface in an inhomogeneous pattern that changes the enamel surface integrity, thus incomplete closure of the surface porosity. The presence of fluoride has a significant impact in enhancing the remineralization, thus more covering of the surface in comparison to non-fluoridated ACP-based materials.

The limitations of our study:

Since the small size of the samples in our experimental study make it challenging to make definite findings. Until now there are no particular formulations for remineralization and no demineralization criteria for in vitro research. Since the remineralization process in the oral cavity is complex and dynamic, thus hard to simulate in the in vitro study.

The recommendations of our study are:

1. The remineralization agents in this study can be applied by finger and toothbrush and have similar efficacy, but the finger application has the higher value, which is more practical for the patient and caregiver.
2. The hydroxyapatite-based materials are recommended for adults and children in both forms fluoridated and non-fluoridated which have higher remineralization efficacy.
3. The fluoridated agents are preferred over non-fluoridated agents in ACP-based materials as non-fluoridated agents have the least efficacy compared to fluoridated ones.
4. The fluoridated HA and ACP-based materials are recommended and taking into consideration the type of fluoride it contains.

Based on this study's results, it is recommended to have future in vivo tests for the materials. More tests are needed for in vitro studies that give more information about the efficacy of remineralization agents for example Energy Dispersive X-ray (EDX), Micro-CT analysis, DIAGNOdent, Microradiography analysis.

## TÜRKÇE ÖZET

### GİRİŞ

Diş çürüğü, diyetten etkilenen biyofilmlerin aracılık ettiği kronik, önlenabilir bir hastalıktır. Zamanla fermente edilebilir karbonhidratların diş yüzeyinde birikmesi, bu çok faktörlü ağız hastalığının ana nedeni olan ağız biyofilminin dengesizliğine yol açar. Dişleri, biyofilmi ve karbonhidrat tüketimi olan herkeste sonuçta çürük lezyonları gelişmeyecektir. Çeşitli koruyucu ve risk değiştirici değişkenler diş çürüğünün gelişimini etkiler. Bazı bakteriler, enerji üretmek için organik asit yan ürünleri ve rafine karbonhidratları metabolize eder. Bu organik asitler biyofilmde yeterince uzun süre kalırsa pH'ı kritik bir değerin altına düşürebilirler (Ritter ve ark., 2019). Bu nedenle hidroksiapatit (HA)  $pH < 5,5$  olduğunda bakterilerin metabolik ürünler yoluyla salgıladığı asit veya asitli gıdalar tüketildiğinde çözünerek demineralizasyon sürecini başlatır (Khijmatgar ve ark., 2020). Demineralizasyon ve remineralizasyon arasındaki dinamik denge zamanla demineralizasyona doğru kaydığında mine çürükleri meydana gelecektir. Kısmen demineralize olmuş minenin yenilenmesi, biyofilmdeki pH nötr bir seviyeye ulaştığında ve çözünebilir kalsiyum ve fosfat konsantrasyonu dişetine kıyasla aşırı doygunlaştığında mümkündür (Nazari ve ark., 2012; Pitts ve ark., 2017). ; Ritter ve ark., 2019). Son zamanlarda çürük yönetimi, çürük lezyonlarının erken teşhisine yönelik yöntemlerin ve invaziv olmayan tedavi yöntemlerinin geliştirilmesine doğru kaymıştır. Erken çürük lezyonlarının remineralizasyon yoluyla tedavisi, değerli, invaziv olmayan bir tedavi yaklaşımı olarak kabul edilir. Halihazırda erişilebilen bir dizi tedaviyi kullanarak çürük ve beyaz nokta lezyonlarını remineralize etmek mümkün olabilir (Naveena ve ark., 2014). Bu ajanlar florlanmış ve florlanmamış ajanlar olabilir.

Bu in vitro çalışmanın amacı, üreticinin talimatlarına göre iki farklı şekilde (parmak ve fırça ile) uygulanan iki remineralizasyon materyalinin (her maddenin florlu ve florsuz formu vardır) erken mine lezyonları remineralizasyonu üzerindeki etkilerini değerlendirmektir. Bu çalışmanın, erken mine lezyonlarının remineralizasyonunda daha iyi sonuçlar elde etmek için malzemelerin bileşim açısından etkinliği ve optimal uygulama yöntemleri hakkında bilgi sağlaması beklenmektedir.

Bu çalışmadaki sıfır hipotezleri şunlardır:

1. Florürlü ve florürsüz gruplar arasında mikrosertlik değerleri açısından istatistiksel olarak anlamlı bir fark yoktur.

2. Diş fırçası grubu ile parmak uygulama grubu arasında istatistiksel olarak anlamlı bir fark yoktur.

3. Farklı malzeme grupları arasında istatistiksel olarak anlamlı bir fark yoktur.

4. Tüm gruplar arasında istatistiksel olarak anlamlı bir fark yoktur.

5. Başlangıç, demineralizasyon ve remineralizasyon grupları arasında istatistiksel olarak anlamlı bir fark yoktur.

## GENEL BİLGİLER

### Mine Yapısı

Mine, dişin anatomik kron kısmını kaplayan vücuttaki en sert dokudur. Diş minesini, insan klinik kronunu koruyan ve stresleri ve fizyo-kimyasal etkileri tolere edebilen, oldukça mineralize, en dışta, yarı saydam, damarsız, onarılamaz bir kaplamadır (Hu ve ark., 2007; Qamar ve ark., 2017). Diş minesinin kimyasal bileşimi, içeriğinin %80-95'inin inorganik ve geri kalan %1-2'sinin hacimce organik olmasıyla büyük ölçüde farklılık gösterir. İnorganik bileşen çoğunlukla hidroksiapatit kristalleridir (HA) (Robinson ve ark., 1995; Qamar ve ark., 2017). Olgun mine %20'nin üzerinde organik madde içeren diğer mineralize dokularla karşılaştırıldığında %1'den az organik madde içerir. Kemik, dentin ve sementteki kristallerin hacmi, mine kristalitlerinden bin kat daha azdır (Simmer ve Hu, 2001).

Mine, kimyasal bileşimi ve yapısı nedeniyle asit saldırılarına dentine göre daha dayanıklıdır. Bu, minenin %25 organik bileşene sahip dentinden %4 daha az organik bileşene sahip olmasıyla açıklanabilir. Ayrıca minenin dentinden daha büyük kristalleri vardır, bu da daha az yüzey alanına sahip olmasına ve asit saldırılarına karşı daha dayanıklı olmasını sağlar. Sonuç olarak çürük ilerlemesi ve demineralizasyon minede dentine göre daha yavaştır. Ayrıca benzer durumlarda mine, dentinden daha hızlı remineralize olur (Amaechi ve Loveren, 2013).

## **Demineralizasyon**

Mineden mineral kaybı teorik olarak plak pH'ındaki her düşüşle başlar, ancak bu çözünme her zaman gerçekleşmez. Çözünmenin gerçekleşmesi için plak sıvısındaki asit konsantrasyonunun mine içine nüfuz edecek kadar yüksek olması gerekir.

Biyofilm pH'ının belirli bir noktanın (kritik pH) altına düşmesi nedeniyle dişte demineralizasyon veya net mineral kaybı yaşanır; bu, minerallerin (kalsiyum ve fosfat) diştten biyofilme doğru hareket etmesine neden olur. demineralizasyon sürecini başlatarak dengeye ulaşır (Zero, 1999; Ritter ve ark., 2019; Khijmatgar ve ark., 2020).

## **Remineralizasyon**

Demineralize edilmiş diş bölgesinin tamamen restorasyonuna "remineralizasyon" adı verilmektedir. Klinik olarak beyaz nokta lezyonlarının ortadan kalkması ve aşınmış minenin tebeşir beyazı görünümü ile gözlenmiştir. Remineralizasyon konseptinin temeli, tükürüğün mine yüzeylerini yeniden mineralleştirme yeteneğini geliştirerek diş minesinden kaybedilen minerallerin yerine konulmasıdır (Abdelmegid ve ark., 2019). Böylece kısmen demineralize olmuş minenin yenilenmesi, biyofilmdeki pH nötr bir seviyeye ulaştığında ve çözünebilir kalsiyum ve fosfat konsantrasyonu dişteki kıyasla aşırı doygun hale geldiğinde mümkün olur (Ritter ve ark., 2019).

## **Çürük Tespitinde Kullanılan Yöntemler**

Diş kliniklerinde çeşitli teknikler kullanılarak çürük tespit edilir. Bu teknikler: Optik Koherens Tomografi, Elektriksel İletkenlik Ölçümü, Ultrasonik Yöntem, Geleneksel Yöntemler [Görsel-Dokunsal Yöntemler, Radyografik Yöntemler, Dijital Radyografiler], Çürük Tespit Boyaları, [Fiber Optik Transilüminasyon (FOTI), Dijital Fiber Optik Transillüminasyon dahil olmak üzere Geliştirilmiş Görsel Teknikler (DIFOTI)], Floresan Teknikleri [Lazer Floresan Yöntemi-DIAGNOdent, DIAGNOdent Pen, FACE], Kamera ve Floresan Teknik Kombinasyonları [Kantitatif Işık Efektli Floresan-QLF, Canary, Soprolife ve Soprocure, Lifedt, Saproimaging, VistaCam] olarak sıralanabilir.(Selwitz ve ark., 2007; Vinod ve ark., 2020).

## **Erken Mine Lezyonu**

Erken mine lezyonu, kavitasyondan önce meydana gelen diş çürüğü gelişiminin aşamasıdır ve kavitasyona uğramamış bir mine yüzeyi altında yüzey altı demineralizasyonu ile karakterize edilir (Sadıkoğlu, 2020). Bu lezyonların tipik beyazımsı görünümü, lezyon gövdesi içindeki artan gözenekliliğe bağlıdır. Sonuç olarak, "beyaz" terimi tipik olarak bu lezyonlara uygulanır. (Torres ve ark., 2011). Klinik olarak görülebilen ve muhtemelen geri dönüşlü olan ilk diş çürüğü türüdür. Kavitasyona uğramamış mine çürüğü lezyonları ve erken lezyonlar, başlangıçtaki mine lezyonlarının diğer isimleridir (Paula ve ark., 2017; Ritter ve ark., 2019).

### **Klinik Özellikler**

Başlangıçtaki mine lezyonunun klinik özellikleri şunlardır:

1. Kurduğunda, ışık özelliklerinin değişmesi nedeniyle normal yarı saydamlığın kaybıyla birlikte kireçli beyaz görünüm.
2. Sondalamayla kolayca zarar görebilen, çukurlardan ve çatlaklardan oluşan kırılğan bir yüzey tabakası.
3. Yüzey altındaki gözenekliliğin artması sonucunda renk değişikliği potansiyeli artmıştır.
4. Yeraltı yoğunluğunun azalması radyografik olarak, transillüminasyonla veya modern lazer cihazlarıyla tespit edilebilir.
5. Özellikle ileri remineralizasyon tedavilerinin kullanılmasıyla remineralizasyon potansiyeli; daha sonraki asit saldırılarına karşı direncin artırılması (Mount, 2005).

### **Erken Mine Lezyonlarına Tedavi Yaklaşımları**

Diş plağındaki biyofilm, diyet, hassas diş sert dokusu ve zaman, diş çürüğünün gelişimine doğrudan katkıda bulunan etiyolojik değişkenlerdir. Restoratif olmayan diş çürüğü tedavisi sırasında çürük etiyolojisi ve risk faktörleri dikkate alınmalıdır. Sonuç olarak, diş plağının azaltılması, çürük riskinin azaltılması, diş sert dokusunun korunması ve uzun süreli bakım, diş çürüklerinin onarıcı olmayan yönetiminin temel unsurlarıdır (Yu ve ark., 2021).

## **Plak Kontrolü**

Antimikrobiyal ajanların kullanımına ek olarak uygun diş fırçalama ve diş arası temizliği yoluyla ağız hijyeni talimatları ile sağlanır.

## **Çürük Riskinin Azaltılması**

Diş çürüğüne ilişkin risk faktörleri ve koruyucu faktörler belirlenerek ve kişiye özel tedavi planı oluşturularak hastanın diş çürüğü riski azaltılabilir. Orta ile yüksek çürük riski olan hastalar diyet yönetimini, hiposalivasyonun iyileştirilmesini, dolgu macunu veya reçine infiltrasyonunu, florür ürünlerinin konsantrasyonunu artırmayı ve florürü daha sık kullanmayı düşünebilir (Yu ve ark., 2021).

## **Diş Sert Dokusunun Korunması**

Kapatılamayan veya temizlenemeyen kaviteye sahip çürük lezyonlarının çoğunluğu restorasyonla tedavi edilmelidir. Aktif kavitesiz lezyonlar veya temizlenebilir aktif kaviteli lezyonlar invazif olmayan bir şekilde tedavi edilmelidir. Çürük lezyon dentinin dış üçte birlik kısmı dışındaki bir alanla sınırlıysa, invaziv olmayan tedaviler önerilir.

Çürük mine ve dentinin remineralize edilmesi restoratif olmayan tedavinin temel amacıdır. Dişleri oluşturan mineral, diş oluşumu sırasında yabancı maddeleri emebilir ve bu da mineralin çözünürlüğünü değiştirebilir. Kırletici maddeler demineralizasyon ve remineralizasyon sırasında giderilecektir. Mine, kendisini kaplayan diş plağının pH'ı genellikle kritik pH olarak bilinen 5,5'in altına düştüğünde çözülür. pH 5,5'in üzerine çıktığında yeniden mineralizasyon gerçekleşecektir ancak mineralin çözünürlüğünü artıran safsızlıklar dahil edilmeyecektir. Demineralizasyon ve remineralizasyon döngüleri, remineralizasyon demineralizasyona ayak uydurabildiği sürece daha yüksek kalitede mineraller üretecektir (Amaechi ve Loveren, 2013).

## **Uzun Süreli Takip**

Düzenli kontrol ziyaretleri ve uzun süreli takipler ağız sağlığının korunması ve çürüklerin kontrol altına alınması açısından çok önemlidir.

## Remineralizasyon Tedavis

Demineralizasyonu önlemek ve/veya remineralizasyonu teşvik etmek için florür veya kalsiyum bazlı tedaviler gibi topikal remineralizasyon ajanlarının uygulanması olağan bir uygulamadır. Tri-kalsiyum fosfat, kazein fosfopeptit-amorf kalsiyum florür fosfat (CPP-ACFP), kazein fosfopeptit-amorf kalsiyum fosfat (CPP-ACP) ve nano-hidroksiapatit, çürük lezyonların remineralize edilmesinde kullanılan yaygın kalsiyum bazlı ürünlerdir. İdeal bir remineralizasyon malzemesinin gereksinimleri şunlardır:

- Alt tabakalara yayılır veya kalsiyum ve fosfat sağlar alt tabakalar içine.
- Fazla kalsiyum sağlamaz
- Diştaşı oluşumunu desteklemez
- Asidik pH'ta çalışır
- Ağız kuruluğu hastalarında çalışır
- Tükürüğün demineralize edici özelliklerini artırır (Yu ve ark., 2021).

## Florürlü Remineralizasyon Sistemleri

Soi ve diğerleri (2013) dört florür etki modu tanımlamıştır. Florür, mine apatit kristalleri ile reaksiyona girerek oluşan ve asit saldırılarına HA kristallerine göre daha dayanıklı olan florapatit kristalleri ile öncelikle demineralizasyonu önler. İkincisi, florür, fosfat ve kalsiyum iyonlarını bir araya toplayarak remineralizasyonu teşvik eder ve bu da yeni florapatit kristallerinin büyümesini artırır. Üçüncüsü, bakterilerdeki glikolitik yolun önemli bir aracısının sentezini önler, bu da karyojenik bakterilerin aktivitesini baskılar. Dördüncüsü, Florür iyonları, remineralizasyonu teşvik etmek ve demineralizasyonu azaltmak için diş plağı, ağız mukozası ve diş sert dokusunda tutulur (Amaechi ve Loveren, 2013; Soi ve ark., 2013; Arifa ve ark., 2019).

Florür ürünleri, diş macunu, gargara, jel, köpükler, vernikler, solüsyonlar ve daha fazlasını içeren çeşitli formlarda gelir (Yu ve ark., 2021). Ayrıca florür, Sodyum florür (NaF), sodyum monoflorofosfat (Na<sub>2</sub>FPO<sub>3</sub>), amin florür (C<sub>27</sub>H<sub>60</sub>F<sub>2</sub>N<sub>2</sub>O<sub>3</sub>), kalay florür

(SnF<sub>2</sub>) veya bunların kombinasyonları dahil olmak üzere farklı kimyasal formlarda gelir (Amaechi ve Loveren, 2013).

Florür, sıklıkla macun veya jel olarak satılan ve ağız sağlığını korumak ve iyileştirmek için diş fırçasıyla birlikte kullanılan diş macunundaki ana elementtir. Florürün biyoyararlılığı, çürük önleme etkisi açısından çok önemlidir. Florürün çözünürlüğü ve yüzeye tutunması biyoyararlanımını belirleyen temel faktörlerdir. Diş macunu formülasyonlarında sodyum florür, kalay florür, amin florür ve sodyum monoflorofosfat dahil olmak üzere farklı florür bileşikleri kullanılmıştır (Miller ve ark., 2012).

Tipik diş macunu 1000-1100 ppm florür içerir, ancak bazı ülkeler normal diş macununda bulunandan daha yüksek (1500 ppm) ve daha düşük (500 ppm civarında) florür konsantrasyonuna sahip diş macunları sağlar. Avrupa'nın çeşitli ülkelerinde düşük florürlü diş macunları küçük çocuklar için tavsiye edilirken, daha düşük florür içerikli diş macunlarının diş çürüklerini önleyebileceğine dair bilimsel bir kanıt yoktur (Ercan ve ark., 2010). Buna karşılık, daha yüksek florür konsantrasyonuna sahip diş macunları daha iyi çürük önleme özelliği göstermiştir. Ayrıca, florürlü diş macunu kullanmanın ve günde iki veya daha fazla fırçalamanın, diş çürüklerini günde bir kez veya daha az monoflorofosfat fırçalamaya göre daha fazla azalttığı çok açıktır (Miller ve ark., 2012).

### **Florürsüz Remineralizasyon Sistemi**

Sert doku bakımı ve gelişimi açısından tükürüğün, yaşam boyunca biyolojik olarak kullanılabilen iyonların (kalsiyum ve fosfat) kaynağı olduğu kabul edilir. Böylece bu iyonların mineral eksikliği olan lezyonlara normal pH'ta difüzyonu sağlanır. Çeşitli çalışmalar beyaz nokta lezyonlarının remineralizasyonunun tükürük remineralizasyonundan etkilenmediğini gösterdiğinden, tükürüğün remineralizasyonu beyaz nokta lezyonlarını remineralize etmek için yetersizdir. Buna ek olarak; mineral kazanımı tükürükten lezyona doğru iyon konsantrasyonunun düşük olması nedeniyle yalnızca yavaş bir süreç ve yüzeyseldir (Philip, 2019). Yüksek florür risklerini azaltmak için düşük florür konsantrasyonlarına sahip verimli florür içermeyen remineralizasyon sistemlerine ihtiyaç vardır (Philip, 2019).

## **Kalsiyum Fosfat Bazlı Sistem**

Cochrane ve ark. satın alınabilecek çeşitli yeni kalsiyum fosfat remineralizasyon sistemlerini üç kategoriye ayırdı: kristalli kalsiyum fosfat sistemleri, stabilize edilmemiş amorf kalsiyum fosfat sistemleri ve stabilize edilmiş amorf kalsiyum fosfat sistemleri (Cochrane ve ark., 2010).

### **Stabilize edilmemiş kalsiyum fosfatlar (Amorf kalsiyum fosfat-ACP)**

Amorf kalsiyum fosfat ACP, aşırı miktardaki kalsiyum fosfat çözeltisinden çöken ilk katı fazdır. ACP, düzensiz yapısı nedeniyle vücut sıvılarıyla güçlü bir şekilde reaksiyona girer ve bu da hızlı apatit yeniden çökmesine neden olur. Oktakalsiyum fosfat veya apatitik ürünler gibi stabil kristalin fazlara kolaylıkla dönüşebilir. Biyomineralizasyonun geçici bir aşaması ve biyoapatitin öncüsü olarak işlev görür (Zhao ve ark., 2011; Arifa ve ark., 2019).

Stabilize edilmemiş amorf kalsiyum fosfat, fosfat tuzu ve kalsiyum tuzlarının intraoral olarak ayrı formda uygulandığı veya düşük su aktivitesine sahip bir üründe birleştirildiği ACP teknolojisinin temelidir. Tuzlar tükürükle temas ettiğinde parçalanarak fosfat ve kalsiyum iyonları açığa çıkar. Kalsiyum ve fosfat iyonlarının karıştırılmasının bir sonucu olarak, amorf kalsiyum fosfatın (ACP) veya florürün varlığında amorf kalsiyum florür fosfatın (ACFP) anında çökmesine yol açar. Bu fazlar (ACP ve ACFP), ağız içi ortamda son derece kararsız olma potansiyeline sahiptir ve hızla daha kristalli, termodinamik olarak stabil bir faza (hidroksiapatit ve florhidroksiapatit gibi) dönüşebilirler. Mine yüzey altı lezyonunun remineralizasyonunu teşvik etmek için, kalsiyum ve fosfat iyonlarına zamanla faz değişiminden önce anlık olarak erişilebilir olmalıdır (Cochrane ve ark., 2010).

## **Biyometik Remineralizasyon Sistemi**

### **Nanohidroksiapatit (nHA)**

Sentetik nanohidroksiapatit (nHA), form, yapı ve kristallik açısından minede bulunan apatit kristaline benzediğinden biyoyumlu ve biyoaktif materyaller arasında en iyilerden biri olarak kabul edilmektedir. Nanopartiküller bakterilerle, plak parçalarıyla ve

mine yüzeyleriyle güçlü bir bağ oluşturabilir. NHA'yı oluşturan minik parçacıklar nedeniyle bağlanma için çok daha geniş bir yüzey alanına sahiptir ve emaye yüzeyindeki küçük çatlakları ve delikleri düzeltmek için dolgu maddesi olarak kullanılabilir. Laboratuvarda gerçekleştirilen dinamik pH döngüsü testleri, nHA'nın erken mine lezyonlarını florür kadar etkili bir şekilde, hatta daha fazla etkili bir şekilde remineralize edebildiğini göstermiştir.

nHA'nın biyomimetik işlevini gerçekleştirdiği kesin süreç bilinmemektedir, ancak bazı çalışmalar bunu mine lezyonlarında veya kusurlarında apatit nanopartikülleri biriktirerek veya diş çevresinde yeni bir sentetik mine tabakası oluşturarak yaptığını öne sürmektedir. NHA, kalsiyum fosfat deposu görevi görerek ve mine minerallerinin fazlalığını koruyarak demineralizasyonu önler ve reinerlizasyonu destekler. Ayrıca, diş mine çürük lezyonunun tercihli remineralizasyonunun nötral koşullar altında nHA tarafından kolaylaştırıldığı gösterilmiştir; lezyonun tam remineralizasyonu gözlenmez.

## **GEREÇ ve YÖNTEM**

Bu çalışma, Van Yüzüncü Yıl Üniversitesi Diş Hekimliği Fakültesi Restoratif Diş Tedavisi Anabilim Dalı'nda, Van Yüzüncü Yıl Üniversitesi Klinik Araştırmalar Etik Kurulu onayı ile in vitro koşullarda gerçekleştirildi. Remineralizasyon etkinliğini değerlendirmek ve karşılaştırmak için mikrosertlik testi yöntemi kullanıldı. Taramalı elektron mikroskobu test yöntemi ise yüzey morfolojisini ve özelliklerini değerlendirir.

Bu invitro olarak yapılacak olan bu deneysel çalışma Van Yüzüncü Yıl Üniversitesi Diş Hekimliği Fakültesinde, çekilmiş 117 küçük azı ve büyük azı dişi üzerinde yapılacaktır. Dişler 24 saat dezenfekte edilip ve kullanılıncaya kadar 37 °C'de distile suda saklanacak. Kökler CEJ'nin 2 mm altından kesilecektir.

Mikroskop altında diş kronları çürük, leke veya çatlak yönünden değerlendirilecektir. Dişler, bukkal yüzeyleri kalıp yüzeyi seviyesinde olacak şekilde akrilik rezinin merkezine gömülecektir.

Her dişin bukkal yüzeyi vicker'in mikrosertlik testi ölçmek ve düz ve pürüzsüz bir yüzey sağlamak için, 1000 grit ve 1200 grit silisyum karbür aşındırıcı kağıtlarla hafifçe parlatılacaktır. Dişlerin bukkal yüzeyine 4×4 mm ölçülerinde stiker yapıştırılmış ve diğer

yüzeyler izole edilmek üzere iki kat oje ile boyanacaktır. Çıkartmalar çıkarılacak ve fazla malzeme distile su ile durulanacaktır.

Örneklem büyüklüğü 117, her grupta (n=13) 13 diştir. Dişler rastgele 8 deney grubu ve 1 kontrol grubuna ayrılacaktır. Erken mine lezyonları, mine örneklerinin 37 °C'de 8 saatlik aralıklarla dört aşındırıcı döngüye tabi tutulmasıyla gerçekleştirildi. Örnekler 2 dakika süreyle %0,3 sitrik asit çözeltisine (pH 2,3) daldırıldı, ardından gösterildiği gibi remineralizasyon çözeltisi olarak yapay tükürükte 2 saat süreyle bekletildi. Numuneler, her daldırmadan sonra 10 saniye damıtılmış suyla yıkandı ve ardından hafif bir kağıt havluyla kurutuldu. Her döngüde çözelti yenilendi. Lezyonun oluşumundan sonra sertlik değerleri ölçülene kadar geçen süre boyunca örnekler dehidrasyonu önlemek amacıyla distile su içerisinde bekletildi.

çalışma grupları aşağıdaki gibi 9 gruba ayrılmıştır:

KP: Parmakla uygulanan, florürsüz HA bazlı malzeme.

KF: diş fırçası uygulamasıyla florürsüz HA bazlı malzeme.

RP: parmakla uygulanan florürlü HA bazlı malzeme.

RF: diş fırçası uygulamasıyla florürlü HA bazlı malzeme.

EP: parmakla uygulanan florürlü ACP bazlı malzeme.

EF: diş fırçası uygulamasıyla florürlü ACP bazlı malzeme.

ZP: parmakla uygulanan, florürsüz ACP bazlı malzeme.

ZF: diş fırçası uygulamasına sahip, florürsüz ACP bazlı malzeme.

CB: yapay tükürükte tutulan kontrol grubu (tedavi edilmeden minerallerinden arındırılmış).

Remineralizasyon malzemeleri her gruba üreticinin talimatına göre 30 saniye süreyle uygulandı ve 150 saniye yüzeyde bırakıldı (toplam uygulama süresi 3 dakika).

Mikrosertlik testi demineralizasyondan önce ve sonra ve remineralizasyon tamamlandıktan sonra (28 gün süreyle ) her grupta on numune için yapılacaktır. Üç numune mine yüzeyinde mikroyapısal özellikleri değerlendirmek için taramalı elektron mikroskobu kullanılacaktır demineralizasyondan önce ve sonra ve remineralizasyondan sonra yapılacaktır.

## BULGULAR

Mikrosertlik testine göre:

1. Florürlü ve florürsüz gruplar karşılaştırıldığında, başlangıçta ( $p = 0,804$ ) ve demineralizasyon sonrasında ( $p = 0,722$ ) gruplar arasında anlamlı bir fark yoktur. Ancak florürlü ve florürsüz grupların remineralizasyon değerleri arasında istatistiksel olarak anlamlı bir fark vardır ( $p=0,002$ ). Florürlü gruplar daha yüksek ortalama mikrosertlik değerine sahiptir.

2. Hidroksiapatit bazlı malzeme (HA) grupları ile Amorf kalsiyum fosfat bazlı gruplar (ACP) arasında karşılaştırıldığında, başlangıçta ( $p=0,206$ ) ve demineralizasyon sonrasında ( $p=0,678$ ) gruplar arasında anlamlı bir fark yoktur. Remineralizasyonda istatistiksel olarak anlamlı fark vardır ( $p=0,027$ ). Hidroksiapatit bazlı malzeme grubunu daha yüksek ortalama mikrosertlik değerine sahiptir.

3. Parmakla uygulama yöntemi grupları ile diş fırçası ile uygulama yöntemi grupları karşılaştırıldığında gruplar arasında başlangıçta anlamlı bir fark yoktur ( $p=0,058$ ). Demineralizasyon sonrası mikrosertlik ortalama değerleri anlamlı farklılık göstermektedir ( $p=0,006$ ). Parmak grubunun ortalama değeri 237,2 fırça grubunun ortalama değeri 213,7'dir. Parmak ve fırça gruplarına ait remineralization değerleri arasında istatistiksel olarak anlamlı bir farklılık bulunmamıştır ( $p=0,118$ ).

4. Başlangıçta tüm gruplar arasında istatistiksel olarak anlamlı bir fark bulundu ( $p = 0,044$ ). İkili karşılaştırmalar sonucunda aralarında fark bulunmamaktadır. Demineralizasyonda tüm gruplar arasında istatistiksel olarak anlamlı bir fark bulundu ( $p = 0,013$ ). İkili karşılaştırmalar sonucunda ZF ile EP arasında fark bulunmaktadır. Diğer gruplar arasında hiçbir fark yoktur.

5. Remineralizasyona göre tüm gruplar arasında istatistiksel olarak anlamlı fark vardır ( $p<0,001$ ). Yapılan ikili karşılaştırmalar sonucunda CB grubu ile KF, KP, EP, EF, RF ve RP grupları arasında fark bulunmaktadır. CB grubu ile ZF ve ZP grupları arasında anlamlı bir fark yoktur. CB grubu ile EP, RP ve KP grupları arasında anlamlı fark vardır.

SEM analizine göre:

1. Florürlü diş macunu ile HA ve HA uygulanan numunelerin SEM sonuçları incelendiğinde, her iki macunun demineralizasyon sırasında görülebilen yüzeydeki mikro gözenekleri tamamen ve homojen bir şekilde kaplayan bir apatit tabakası oluşturduğu kaydedildi. Bununla birlikte sadece n-HA içeren diş macunu grubunun apatit tabakasının üzerinde kayda değer küçük mineral çökeltileri içerdiği ve bu birikimlerin sonuçta asit saldırılarına karşı koruma sağlayacak bir kalsiyum-fosfat deposu görevi görebileceği belirlendi.

2. ACP bazlı malzemelerin morfolojik görünümü analiz edildiğinde yüzeyin homojen olmadığı ve yüzey bütünlüğünde değişiklik olduğu görülmektedir. Florür içermeyen ACP bazlı malzemelerin yüzeyi, mikro gözeneklerin tam olarak kaplanmasını sağlayan ve pürüzlü yüzeylere sahip, homojen olmayan ve düzensiz kalsiyum fosfat kristalleri ile kaplanmış. Florürlü ACP bazlı malzemelerle karşılaştırıldığında, yüzeyin neredeyse tamamını kaplar, daha homojen bir yüzey sağlar ve florürsüz gruba göre pürüzlülük azalır.

## TARTIŞMA

Bu çalışmanın amacı, erken mine lezyonlarının remineralizasyonunda iki farklı uygulama yöntemiyle uygulanan florürlü ve florürsüz remineralizasyon ajanlarının etkinliğini değerlendirmektir.

Mevcut çalışmada florür, florür içermeyene kıyasla yüzey mikro sertliğini artırarak remineralizasyonda önemli bir rol oynadığı için bu sonuç, florür içeren diş macununun farklı seviyelerde olduğunu bulan Rirattanapong ve arkadaşlarının (2010) çalışmasıyla tutarlıdır. konsantrasyonları, florür içermeyen diş macununa göre önemli ölçüde daha fazla remineralizasyon sağladı. Dijkman ve arkadaşlarının (1990) çalışması da aynı sonuçlara sahiptir. Ayrıca Thimmaiah ve ark. (2019) çalışma sonucumuzu

destekler şekilde, (CPP-ACFP) Tri-kalsiyum fosfat ve florür içermeyen (n-HA)'in remineralizasyon kabiliyetini araştırmışlardır. Tüm numuneler hazırlandı ve demineralize edildi. Remineralizasyondan sonra Ca/P kütle yüzdesi, CPP-ACFP ve TCP ve ardından n-HA ile anlamlı derecede daha yüksekti.

Florürlü ACP bazlı materyallerin remineralizasyon etkinliğini önemli ölçüde arttırdığı çalışma sonucumuzu desteklediler. HA bazlı malzemeler ile ACP bazlı malzemeleri karşılaştırdığımızda florürün etkinliklerinde önemli bir rol oynadığı sonucuna varabiliriz. Böylece hidroksiapatit yerine fluorapatit oluşumu mine mikrosertliğini artıracaktır. Florürlü ACP bazlı materyalin en yüksek VHM değerine sahip olması, içerdiği florür tipi olan kalay florürün yanı sıra florürün de varlığı nedeniyle yorumlanabilir ve remineralizasyon etkinliğinin sodyum florüre kıyasla arttırılmasını öneriyoruz. Florlanmış hidroksiapatit bazlı malzeme içerir. Bu, West ve arkadaşlarının çalışmasıyla (2019) uyumludur; NaF ve triklosan diş macunu ile kalay florür diş macununu karşılaştırdılar. Kalay florürün belirgin şekilde daha yüksek erozyon koruma etkinliği olduğunu gösterdiler. Her ne kadar yüzey lekelenmesi ilk kalay florür formülasyonlarında bir sorun olsa da, sodyum heksametafosfat dahil leke azaltıcı katkı maddeleri daha modern formülasyonlarda dışsal beyazlatmayı arttırabilir. Her iki malzeme de florürlüdür, çalışma sonuçlarına göre HA bazlı malzemenin (RP) daha yüksek bir mikrosertlik değerine sahip olması gerekirken, ACP bazlı malzemenin (EP) en yüksek mikrosertlik değerine sahip olduğu ve RP'yi aştığı yorumunu yaptık. Çünkü florürün türü remineralizasyon etkinliğini etkiler.

Çalışmamız sonucunda HA bazlı materyallerin VHM değeri ACP bazlı materyallere göre anlamlı bir artış göstermektedir. Bu durum HA bazlı materyaller ile ACP-bazlı materyaller arasında istatistiksel olarak anlamlı bir fark olmadığını ifade eden sıfır hipotezinin reddini desteklemektedir erken mine lezyonunun remineralizasyonunda.

Bizim çalışmamızla tutarlı olarak Singh ve arkadaşlarının (2017) çalışmasında, n-HA bazlı materyaller olan Aclaim ve Apagard'ın demineralizasyonu önlemede etkili materyal olduğu, ardından Clinpro Tooth Crème ve Colgate Total florürün geldiği bulunmuştur. -bazlı malzemeler, bu, HA bazlı malzemelerin daha iyi performansa sahip olduğu çalışma sonuçlarımızla tutarlıdır.

De Carvalho ve ark. (2014), kazein fosfopeptid-amorf kalsiyum fosfat, n-HA macunu ve florür verniğinin etkinliğini araştırmıştır. KHN değerleri n-HA grubunda vernik grubuna göre oldukça yüksekti. CPP-ACP grubunda KHN yükselmedi. Bu, florür içermeyen ACP bazlı materyalin kontrol grubuna benzer şekilde düşük remineralizasyon etkinliğine sahip olması nedeniyle çalışma sonuçlarımızla uyumludur. Demineralizasyon sonrası parmak ve diş fırçası grupları arasında anlamlı fark bulunmuş ( $p=0,006$ ), parmak grubunun ortalama değeri (237,2), fırça grubunun ortalama değeri (213,7) olduğundan bu durum diş tepkisi olarak yorumlanabilir. asidik mücadeleye karşı yapısı ve bileşimi de dahil olmak üzere çeşitli faktörlerden etkilenir (Zero ve Lussi, 2005). Diş dokularında bulunan mineraller HA'nın kusurlu formlarıdır. Sert doku oluşumu sırasında mineralize kristaller oluştuğunda, doku sıvılarındaki "safsızlık" iyonları HA kristallerine dahil edilir ve bu da safsızlıklara yol açar. Bu iyonlar HA kristal yapısındaki iyonların yerini alır.  $PO_4^{3-}$  veya  $OH^-$  için  $CO_3^{2-}$  ve  $Ca^{2+}$  için  $Na^+$  ve  $Mg^{2+}$  ana ikame edicilerdir. Saf olmayan HA ve doğal HA iyonları arasındaki boyut, yük veya her ikisindeki farklılıklar nedeniyle, safsızlık iyonları HA'nın kristal yapısında gerilime neden olur ve böylece çözünürlük artar (Shellis ve ark., 2014).

Remineralizasyon aşamasında parmak ve diş fırçası grupları arasında istatistiksel olarak anlamlı fark saptanmadı ( $p=0,118$ ). Bu sonuç Singh ve arkadaşlarının (2017) yaptıkları çalışmada parmak ve diş fırçası olmak üzere iki uygulama yöntemi kullandıkları ve uygulama yöntemleri arasında anlamlı bir fark olmadığı sonucuna vardıkları çalışmayla örtüşmektedir. Bu bulgular, erken mine lezyonunun remineralizasyonunda diş fırçası ve parmak uygulama yöntemleri arasında istatistiksel olarak anlamlı bir fark olmadığını belirten sıfır hipotezin kabulünü desteklemektedir. Bu, materyallerin hem ebeveynler tarafından hem de hastanın kendisi tarafından uygulanmasını kolaylaştırır ve hastanın tedaviye uyumunu artırır.

Tüm gruplar birlikte karşılaştırıldığında başlangıç, demineralizasyon ve remineralizasyon aşamalarında istatistiksel olarak anlamlı fark vardır ( $p<0,001$ ). Bu sonuç, demineralizasyon sonrası ve remineralizasyon sonrası baz değer değerleri arasında anlamlı bir fark olmadığını belirten sıfır hipotezinin reddini desteklemektedir. Bu nedenle, mevcut çalışmadaki tüm numunelerin başlangıçtaki ortalama yüzey mikrosertliği (334,5), demineralizasyon sonrasına göre (223,9) daha yüksektir ve Lata ve

arkadaşlarının daha önceki araştırmalarıyla tutarlıdır. (2010), burada yüzey mikrosertliği başlangıçta 254 – 363 aralığındaydı ve demineralizasyondan sonra 162-183 aralığına düşüyor. Jabbarifar ve arkadaşlarının (2011) çalışmasıyla uyumlu olarak, remineralizasyon sonrası yüzey mikro sertliği, demineralizasyon sonrasına kıyasla arttı; demineralizasyondan sonra minenin mikro sertliği önemli ölçüde azaldı ve remineralizasyondan sonra mikro sertliği düzeldi. Esfahani ve arkadaşlarının (2011) çalışmasında olduğu gibi remineralizasyon grubu en yüksek mikrosertlik değerine sahiptir.

Başlangıçta 9 grup arasında karşılaştırıldığında aralarında anlamlı fark olduğu görüldü ( $p=0,044$ ). En yüksek başlangıç ortalama değeri EP grubunda (363), en düşük değer ise (250-360) aralığındaki ortalama mine sertliği değerlerini karşılayan ZF ve EF gruplarında (313,5) görüldü (Gutiérrez-Salazar ve Reyes -Gasga, 2003).

Tüm örnekler demineralizasyon pH döngüsüne tabi tutulduğundan tüm örneklerde mineral kaybı olacaktır (Salinovic ve ark., 2021), dolayısıyla çalışmamızda tüm gruplar arasında önemli bir fark vardır. İkili karşılaştırmada ZF (201) ve EP (255,2) dışındaki tüm gruplar arasında anlamlı bir fark bulunmazken, bu anlamlı fark yüzey mikrosertliğinde; daha önce de belirtildiği gibi minenin yapısal bileşimindeki safsızlıklar nedeniyle asidik zorluklar sırasında çözünürlüğünü etkileyecektir (Amaechi ve Loveren, 2013). Tüm ortalama demineralizasyon değerleri Sahiti ve arkadaşlarının çalışmasındaki değerlere benzer. (2020) ortalama mikrosertlik değeri 257,77'dir.

Remineralizasyon sonrası tüm gruplar arasında istatistiksel olarak anlamlı fark vardır ( $p<0,001$ ). Bu bulgular, remineralizasyonda tüm gruplar arasında istatistiksel olarak anlamlı bir fark olmadığını belirten sıfır hipotezinin reddini desteklemektedir.

Kontrol grubu tüm gruplara göre en düşük mikrosertlik ortalama değerine sahiptir. Kontrol grubu ile ZF ve ZP grupları arasında sırasıyla (282,7), (311,2) ve (321,4) ortalama değerleri ile anlamlı bir fark yoktur. Bu, ZF ve ZP gruplarının diğerlerine kıyasla en düşük remineralizasyon potansiyeline sahip olduğu anlamına gelir; bu, florür içermeyen ACP bazlı materyallerden oluşan bileşimine atfedilebilir, bu da florürsüz ve ACP bazlı materyallerin en düşük değere sahip olduğu çalışma sonucumuzu destekler. remineralizasyon potansiyeli. De Carvalho ve ark.'nın çalışmasında da benzer sonuçlar

elde edildi (2014). En düşük değerin kontrol grubu için olduğunu ve bunu CPP-ACP'nin takip ettiğini buldular. CPP-ACP grubunun yüzey mikrosertliği üzerinde etkisi yoktur.

Kontrol grubu ile KF, KP, EF, EP, RP ve RF grupları arasında ikili karşılaştırma sonucunda aralarında anlamlı fark olduğu görülmektedir. Nourolahianet ark. (2021), ortalama mikrosertlik, diğer üç demineralizasyon grubuna kıyasla (herhangi bir tedavi almayan) kontrol grubunda anlamlı derecede düşüktü ( $P<0,001$ ).

En yüksek değerler  $EP>RP>KP$  idi. RP mikrosertlik değeri 372,9'dan (florürlü HA bazlı malzeme) sonra KP mikrosertlik değeri 371,1 (florürsüz HA bazlı malzeme) olup, çalışmamızda florlu gruplar önemli ölçüde daha yüksek mikrosertlik değerlerine sahiptir. Florür içermeyen HA bazlı materyallerin birbirine çok yakın olması, Amaechi ve ark.'nın (2011) yaptığı in vitro çalışmada olduğu gibi, HA bazlı materyallerin florür olmadan da yüksek remineralizasyon etkinliğine sahip olduğu şeklinde yorumlanabilir. (2020), hidroksiapatit bazlı (Karex gelée, %15 HA, florür içermeyen), florür bazlı (Elmex gelée, 12,500 ppm F) ve yapay tükürük (AS) olmak üzere üç remineralizasyon grubunu inceliyor. Başlangıçtaki çürük lezyonunun remineralizasyonu açısından, hidroksiapatit bazlı jel (%15 HA), florür bazlı jel (12,500 ppm F) kadar başarılıydı.

Uygulama yöntemleri arasında istatistiksel olarak anlamlı bir fark bulunmasa da en yüksek değerlerin parmak uygulama gruplarında (EP,RP ve KP) görülmesi, parmak uygulama yönteminin malzemenin yüzeyde birleştirilmesine imkan vermesine bağlanmaktadır. Diş fırçası yöntemi malzemeyi dağıtırken, malzeme dişin yüzeyini kaplar ve malzeme kılların arasına girebilir, bu da malzeme kütlesinin kaybına neden olur.

Bu malzemeler ACP bazlı olduğundan EP ve ZF grupları arasında önemli bir fark vardır ancak florürün remineralizasyon potansiyelinde büyük bir fark yarattığı açıktır. Bu sonuç Srinivasan ve arkadaşlarının (2010) numunelerin üç farklı yerinde remineralizasyon prosedürüne tabi tutulduğu çalışmasının sonuçlarıyla uyumludur: Grup I CPP-ACP kullanıldı, Grup II 900 ppm florür ile CPP-ACP kullanıldı ve Grup III kontrol olarak tükürüğü kullanıldı. CPP-ACP pastaları ile 900 ppm florürlü CPP-ACP pastalarının ortalama mikrosertlik değerlerinin istatistiksel olarak anlamlı derecede farklı

olduğu gösterilmiştir. Florür ve CPP-ACP'nin demineralize minenin remineralizasyonu üzerindeki sinerjistik etkileri bu araştırmayla doğrulandı.

CPP-ACP remineralizasyon sürecinde ACP'nin dış yüzeyinde lokalize olduğu, burada serbest kalsiyum ve fosfat iyonları için bir rezervuar görevi görerek remineralizasyona katkıda bulunduğu düşünülmektedir. Bu iyonlar demineralizasyonu engeller ve hidroksiapatite aşırı doygunluk durumunu koruyarak remineralizasyonu teşvik eder. Mine yüzeyinde kalsiyum ve fosfat iyonlarının florür iyonları ile birlikte lokalizasyonu, büyük olasılıkla CPP-ACFP nano kompleksleri olarak, büyük olasılıkla CPP-ACP ve florürün bir kombinasyonundan kaynaklanmıştır (Srinivasan ve diğerleri, 2010). Minenin yüzey mikrosertliğini artırma, gelecekteki demineralizasyona karşı direnci artırma ve remineralizasyon sürecini geliştirme avantajına sahip olan fluorapatit kristalleri üreterek (Farooq ve ark. 2021).

ZF ve KP grupları arasında anlamlı bir fark vardır; KP, ZF ortalama mikrosertlik değerlerine (311,2) kıyasla daha yüksek bir mikrosertlik ortalama değerine (371,1) sahiptir. Her iki grup da florür içermediğinden ve bizim çalışmamıza göre bu, esas olarak malzemenin ana bileşimine atfedilir; burada HA bazlı malzemeler, ACP bazlı malzemelerle karşılaştırıldığında önemli ölçüde daha yüksek mikro sertlik değerlerine sahiptir.

Sebastian ve ark. (2022) üç grubun remineralizasyon potansiyelini değerlendirip karşılaştırırken bulgularımızı desteklemektedir: grup I: Kontrol, grup II: GC Tooth Mousse™ (CCP-ACP), grup III: Acclaim™ (n-HA) florürsüz ve grup IV: Mikrosertlik testi kullanılarak yapay mine çürüğü lezyonları üzerinde EnaFix (CaSP). Remineralizasyon işlemi tamamlandıktan sonra KHN testleri yapıldı. EnaFix (CaSP) en yüksek değere sahiptir ve bunu GC Tooth Mousse (CPP-ACP) ile karşılaştırıldığında daha yüksek mine yüzey mikrosertliğine sahip olan Acclaim (n-HA) grubu takip etmektedir.

ZF grubunun ortalama mikrosertlik değeri sırasıyla 311,2 ve 372,9 değerleriyle RP grubundan önemli ölçüde farklılık göstermektedir. Çalışma bulgularımıza göre ZF, florür içermeyen ACP bazlı bir malzemedir ve bu nedenle florürlü HA bazlı malzemelerden önemli ölçüde farklıdır. Bajaj ve ark. (2016), erken mine lezyonlarının

remineralizasyonunda üç farklı remineralizasyon ajanının etkinliğini inceleyerek çalışma bulgularımızı desteklemektedir. Üç malzeme CPP-ACP (GC diş köpüğü), Tri-kalsiyum-fosfat (Clinpro diş kremi) ve Hidroksiapatit (Remin Pro) kullandılar. HA bazlı malzemelerin CPP-ACP ve Tri-kalsiyum fosfatla karşılaştırıldığında daha iyi remineralizasyon gösterdiği sonucuna vardılar.

Minenin hidroksiapatit kristallerinin morfolojik yapısı, dişin doku sağlığının analitik bir göstergesini sağlar. Taramalı Elektron Mikroskobu (SEM), numunelerin mine prizma morfolojisi hakkında niteliksel ayrıntılar sağlayan yüksek çözünürlüklü resimler üretebilir (Vitiello ve diğerleri, 2022). Bu nedenle, mevcut çalışmada, 28 günlük bir tedavi periyodunun ardından yapay mine lezyonları üzerinde ticari olarak temin edilebilen dört farklı ajanın morfolojik değişikliklerini ve remineralizasyon etkinliğini karşılaştırmak için SEM'i kullanıyoruz. İn vitro araştırmalarda erken mine lezyonlarının yüzey morfolojileri incelenirken birçok çalışmada SEM altın standarttır (Chen ve ark., 2013; Patil ve ark., 2013; Lelli ve ark., 2014; Raghu ve Ananthakrishna, 2016). ; Vitiello ve ark., 2022; Tosco ve ark., 2023).

Tosco ve arkadaşlarına göre (2023), CPP-ACP'nin taramalı elektron mikrografları, yüzey morfolojisinin neredeyse tamamen yeniden düzenlendiğini gösterdi. Ancak yüzeyde malzeme birikintileri ve yüzey bütünlüğünde hafif kayıplar vardır. Ayrıca yüzey yapıları diğer gruplardan farklıydı; bazı malzeme kalıntıları ve hafif çözünme bölgeleri gösteriyordu. Florürlü-ACP grubunun SEM mikrograflarında normal görünümde ve yapıda restorasyona sahip remineralize mine görüldü.

Bu, florürsüz ACP bazlı malzemelerin, emaye yüzey bütünlüğü kaybıyla birlikte düzgün olmayan yüzeylere sahip olması ve bazı gözeneklerin çözünme alanları sergilediği, aksine florlanmış ACP bazlı malzemelerin tam yüzey kaplamasına sahip olması nedeniyle araştırmamızla uyumludur. Florür içermeyen Çinko-HA grubu, belirgin yapısal yenilenme ile birlikte düzgün ve homojen bir mine yüzeyine sahipti. Florür içermeyen HA bazlı malzemelerin mine yüzeyini gözeneksiz olarak tamamen kapladığını gösteren çalışmamızın sonuçlarına benzer.

Oliveira ve arkadaşlarının (2016) çalışmasına dayanarak, bu çalışmada florürlü ve florürsüz (CPP-ACP) içeren macunların mine demineralizasyonu üzerindeki etkisi

değerlendirilmiştir. Numune morfolojik analizi, CPP-ACP'nin daha fazla emaye düzensizliğine sahip olduğu ve işlenen yüzeyin, emaye organik matrisinde gözle görülür bir değişiklik alanı gösterdiği ile sonuçlanır; bu, daha belirgin 'bal peteği demineralizasyon modeli' ile kanıtlanır. CPP-ACP'nin SEM mikroskopiğinde, CPP-ACP'ye kıyasla daha homojen bir pürüzsüz yüzey ve daha az pürüzlülük görülmektedir. Bu, her iki ACP bazlı malzemeyi karşılaştırırken yaptığımız çalışmayla uyumludur çünkü florürlenmemiş grup, birden fazla gözenekliliğe sahip pürüzlü ve düzensiz yüzeyler gösterir. Florürlü ACP bazlı malzeme ise yüzeydeki düzensizliklere rağmen daha fazla kaplama ve homojenlik gösterir.

Vyavhare ve arkadaşları bu çalışmada, erken çürük lezyonlarının remineralizasyonunda CPP-ACP ve nano-hidroksiapatitin florür ikameleri olarak potansiyel kullanımını değerlendiriyorlar. Demineralizasyondan önce, tipik anatomik mine yüzeyinin pürüzsüz ve sağlam bir yüzeye sahip olduğu SEM tarafından gözlemlendi. Demineralizasyonun ardından, gözle görülür derecede yüksek gözenekliliğe sahip pürüzlü, düzgün olmayan bir yüzey görüldü. Çok katmanlı bir demineralizasyon yüzeyi ile birlikte küçük bir petek deseni şeklindeki demineralizasyon görüldü. CPP-ACP kullanılarak yapılan SEM görüntüsünde küçük bir demineralizasyon bal peteği deseni görüldü; bu, yüzeyde çok az veya hiç remineralizasyonun meydana gelmediğini gösteriyor.

Normal mine yüzeylerin görünümü pürüzsüz ve sağlam olduğundan araştırmamız bunu desteklemektedir. Bunun tersine, demineralize yüzeylerde ise minenin yüzey tabakasının bozulduğu ve mikro gözeneklerin açığa çıktığı gözlemlendi. Mine prizmalarının bütünlüğünün bozulması ve prizmaların çevreye göre kor kısmında çözünmeye daha yatkın olması bu bölgelerde bal peteği benzeri bir görünümün ortaya çıkmasına neden olmuştur. Çalışmamızda olduğu gibi florür içermeyen ACP bazlı materyal en az miktarda remineralizasyona sahiptir ve bu çalışmayla uyumlu olarak çok az remineralizasyon meydana gelmiş veya hiç oluşmamıştır.

n-HA, amin florür, sodyum florür ve iyonik kalsiyum içeren diş macunlarının başlangıç mine lezyonları üzerindeki etkisinin karşılaştırıldığı in vitro çalışmada, lezyonlardaki yüzey morfolojik değişiklikleri SEM ile incelenmiş ve her grubun benzersiz bir remineralizasyon şekli olduğu bulunmuştur. Hidroksiapatit diş macununun

oluşturduğu remineralizasyon yüzeyinin düzgün ve pürüzsüz olduğu; florürlü diş macununun oluşturduğu remineralizasyon düzensiz ve homojen olmayan bir tabakadır ve bazı mikro gözenekler kapanmamaktadır (Kinsun, 2007). Bu bulgularla uyumlu olarak, araştırmamızda florürlü (ACP veya HA) diş macununun uygulandığı yüzeyin bazı düzensizliklere sahip olduğu, pürüzlü olduğu ve küresel birikintilerle tam olarak kaplanamadığı tespit edildi.

Mine yüzeylerini nHA+F diş macunu ile değerlendirdiğimizde tüm yüzeyin homojen ve pürüzsüz olduğunu, yüzeyde çeşitli çaplarda kristal topaklanmalarının geliştiğini gördük. Vickers mikrosertlik testi ve SEM görüntülerinden elde ettiğimiz sonuçlara dayanarak HA bazlı malzemelerin remineralizasyon mekanizmasının, demineralize yüzeyde apatit çökmesiyle yüksek mineral yoğunluğuna sahip bir tabaka oluşması ve malzemenin kristal yapısını onarması şeklinde olduğunu düşünüyoruz. Bu tabakadaki mineral birikimini kullanan mine. Ayrıca bu tabakanın uzun vadede asit ataklarına karşı mineral deposu görevi görebileceğini ve demineralizasyonu engelleyeceğini düşünüyoruz.

Öte yandan ACP bazlı malzemeler, termodinamik olarak kararsız, kristallere dönüşen amorf formda kalsiyum fosfat oluşturarak yüzeyi yeniden mineralize eder, bu kristaller homojen olmayan bir düzende yüzeye dağılarak mine yüzey bütünlüğünü değiştirir. , dolayısıyla yüzey gözenekliliğinin tam olarak kapanmaması. Florürün varlığı, yeniden mineralizasyonun arttırılmasında önemli bir rol oynar, dolayısıyla florür içermeyen ACP bazlı malzemelerle karşılaştırıldığında yüzeyin daha fazla kaplanması sağlanır.

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T.C.  
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**Sağlık Bilimleri Enstitüsü**



**G19 –TEZ ORJİNALLİK RAPORU**

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|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|---------------------------|---------------------|
| <b>Tez Başlığı / Konusu</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Farklı Yöntemler ile Uygulanan Florürlü ve Florürsüz Remineralizasyon Ajanlarının Erken Mine Lezyonlarının Remineralizasyonu üzerine Etkilerinin Değerlendirilmesi |              |                           |                     |
| <b>İntihal taraması yapılan bölümler ve sayfa sayıları</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                    |              |                           |                     |
| Kapak sayfası                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Giriş                                                                                                                                                              | Ana bölümler | Sonuç bölümleri           | Toplam sayfa sayısı |
| 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 2                                                                                                                                                                  | 74           | 18                        | 96                  |
| İntihal taraması yapılan program                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                    |              | Taramanın yapıldığı tarih | Benzerlik oranı %   |
| Turnitin                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                    |              | 11/ 06/ 2024              | 12%                 |
| <b>*Uygulanan filtreler aşağıda verilmiştir:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                    |              |                           |                     |
| <div style="display: flex; flex-wrap: wrap;"><div style="width: 50%;">- Kabul ve onay sayfası hariç,</div><div style="width: 50%;">- Gereç ve yöntemler hariç,</div><div style="width: 50%;">- Teşekkür hariç,</div><div style="width: 50%;">- Kaynakça hariç,</div><div style="width: 50%;">- İçindekiler hariç,</div><div style="width: 50%;">- Alıntılar hariç,</div><div style="width: 50%;">- Simge ve kısaltmalar hariç,</div><div style="width: 50%;">- Tezden çıkan yayınlar hariç,</div><div style="width: 100%;">- 7 kelimedenden daha az örtüşme içeren metin kısımları hariç (Limit match size to 7 words)</div></div> |                                                                                                                                                                    |              |                           |                     |
| <p>Van Yüzüncü Yıl Üniversitesi Lisansüstü Tez Orijinallik Raporu Alınması ve Kullanılmasına İlişkin Yönergeyi inceledim ve bu yönergede belirtilen azami benzerlik oranlarına göre tez çalışmamın herhangi bir intihali içermediğini; aksinin tespit edileceği muhtemel durumda doğabilecek her türlü hukuki sorumluluğu kabulettiğimi ve yukarıda vermiş olduğum bilgilerin doğru olduğunu beyan ederim.</p> <p style="text-align: center;">Gereğini bilgilerinize arz ederim.</p> <p style="text-align: right;">Mays Marwan Abedalaziz ALREFAI</p> <p style="text-align: right;">İmza</p>                                       |                                                                                                                                                                    |              |                           |                     |
| <b>Öğrencinin Adı Soyadı</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Mays Marwan Abedalaziz ALREFAI                                                                                                                                     |              |                           |                     |
| <b>Anabilim Dalı</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Restoratif Diş Tedavisi                                                                                                                                            |              |                           |                     |
| <b>Öğrenci No</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 18930005021                                                                                                                                                        |              |                           |                     |
| <b>Programı</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <input type="checkbox"/> Yüksek Lisans <input checked="" type="checkbox"/> Doktora                                                                                 |              |                           |                     |
| <b>DANIŞMAN ONAYI</b><br>UYGUNDUR<br>Doç. Dr. Alperen DEĞİRMENCİ                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                    |              | <b>ENSTİTÜ ONAYI</b>      |                     |

