SYNTHESIS AND *IN VITRO* ANALYSIS OF BORON DOPED 45S5 BIOGLASS PASTE USED FOR DENTINE HYPERSENSITIVITY TREATMENT

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I certify that in my opinion the thesis submitted by Razan ALMNAWER titled "SYNTHESIS AND *IN VITRO* ANALYSIS OF BORON DOPED 45S5 BIOGLASS PASTE USED FOR DENTINE HYPERSENSITIVITY TREATMENT" is fully adequate in scope and in quality as a thesis for the degree of Master of Science.

APPROVAL

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"I declare that all the information within this thesis has been gathered and presented in accordance with academic regulations and ethical principles and I have according to the requirements of these regulations and principles cited all those which do not originate in this work as well."

Razan ALMNAWER

ABSTRACT

M. Sc. Thesis

SYNTHESIS AND *IN VITRO* ANALYSIS OF BORON DOPED 45S5 BIOGLASS PASTE USED FOR DENTINE HYPERSENSITIVITY TREATMENT

Karabük University
Institute of Graduate Programs
The Department of Biomedical Engineering

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There are many professionally applied (in-office) and over-the-counter (OTC) products and methods currently available for the treatment of Dentin hypersensitivity (DH). DH is a short, sharp pain that arises from exposed dentin in response to various stimuli. However, bioactive glasses in toothpaste compositions have recently been recommended as a viable option for treating DH. In this thesis, 45S5 Bioactive glass (BG) and Boron (4.6 moles) doped bioactive glass (B-BG) were successfully synthesized using the sol-gel method. The prepared materials were characterized using a Scanning electron microscope (SEM) with Energy-dispersive X-ray spectroscopy (EDX), Transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) techniques. The BG and B-BG materials were in good match with the standard phase of BG. The incorporation of B reduced the particle size of BG. *In vitro* degradation analysis of BG and B-BG materials was evaluated in PBS for 14 days. The leaching profile of Ca, Si, P, Na and B ions were measured using inductively coupled plasma mass spectrometry (ICP-MS) and it was observed

that incorporation of B increased the degradation rate of BG materials. In this study, the use of BG and B-BG paste for DH treatment was evaluated *in vitro*. About 75, third molar teeth were collected (with ethical permission) from Karabuk Oral and Dental Training and Research hospital. The teeth were cleaned, cut into disc shapes and then treated with phosphoric acid to open the dentinal tubules. The teeth in disc shape were washed with BG, B-BG paste and several commercial desensitizing toothpastes Sensodyne Repair & Protect (Novamin), Colgate Sensitive Pro-Relief (Arginine), Ipana Pro expert Clinic Line Sensitive (Sodium Fluoride) and Placebo for 14 days (two times per day), then the teeth were stored in artificial saliva (AS). Before and after washing, the teeth in disc shape were examined using XRD, SEM and EDX. The discs washed by BG, B-BG and Novamin pastes showed that the dentinal tubules were entirely covered by the calcium phosphate layer and partially covered when the discs were cleaned by Arginine, Sodium Fluoride and Placebo. For three days, the BG, B-BG and Novamin pastes were evaluated for calcium phosphate layer deposition and acid challenge. The results showed that the dental tubules were covered in 3 days and the results were comparable between the tooth pastes we used. The B-BG paste showed better resistance to acid than pure BG and Novamin pastes. The obtained results demonstrated that the B-BG paste holds a promising future for its inclusion in toothpaste formulation as an effective method for treating DH.

Keywords **:** Dentin hypersensitivity, tubules occlusion, bioactive glass, boron doped bioactive glass.

Science Code **:** 92503

ÖZET

Yüksek Lisans Tezi

DENTİN HİPERSENSİTİVİTE TEDAVİSİNDE KULLANILAN BOR KATKILI 45S5 BİYOGLAS MACUNU SENTEZİ VE *IN VITRO* ANALİZİ

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Dentin aşırı duyarlılığı (DH) tedavisi için profesyonel olarak uygulanan (ofis içi) ve tezgah üstü (OTC) birçok ürün ve yöntem mevcuttur. DH, çeşitli uyaranlara yanıt olarak açığa çıkan dentinden kaynaklanan kısa, keskin bir ağrıdır. Bununla birlikte, diş macunu bileşimlerindeki biyoaktif camlar, son zamanlarda DH tedavisi için uygun bir seçenek olarak önerilmiştir. Bu tezde, sol-jel yöntemi kullanılarak 45S5 Biyoaktif cam (BG) ve Bor (4.6 mol) katkılı biyoaktif cam (B-BG) başarıyla sentezlenmiştir. Hazırlanan materyaller, Taramalı elektron mikroskobu (SEM) ile Enerji-dağılımlı X-ışını spektroskopisi (EDX), Geçirgenlik elektron mikroskobu (TEM), X-ışını kırınımı (XRD) ve Fourier dönüşüm kızılötesi (FTIR) teknikleri kullanılarak karakterize edildi. BG ve B-BG malzemeleri, BG'nin standart fazıyla iyi bir uyum sağladı. B'nin dahil edilmesi, BG'nin parçacık boyutunu azalttı. BG ve B-BG malzemelerinin *in vitro* bozunma analizi, Fosfat tamponlu tuzu (PBS) içinde 14 gün boyunca değerlendirildi. Ca, Si, P, Na ve B iyonlarının liç profili, endüktif olarak eşleştirilmiş plazma kütle spektrometrisi (ICP-MS) kullanılarak ölçüldü ve B'nin dahil edilmesinin, BG

malzemelerinin bozunma hızını arttırdığı gözlemlendi. Bu çalışmada, DH tedavisi için BG ve B-BG macunun kullanımı *in vitro* olarak değerlendirilmiştir. Karabük Ağız ve Diş Eğitim ve Araştırma hastanesinden yaklaşık 75 adet üçüncü büyük azı dişi (etik izinle) toplanmıştır. Dişler temizlendi, disk şeklinde kesildi ve daha sonra dentin tübüllerini açmak için fosforik asite maruz bırakıldı. Disk şeklindeki dişler, BG, B-BG macunu ve birkaç ticari duyarsızlaştırıcı diş macunu Sensodyne Repair & Protect (Novamin), Colgate Sensitive Pro-Relief (Arginin), Ipana Pro uzmanı Clinic Line Sensitive (Sodyum Florür) ve Plasebo ile 14 gün boyunca (günde iki kez) yıkandı, ardından dişler yapay tükürükte (AS) saklandı. Yıkama öncesi ve sonrası disk şeklindeki dişler XRD, SEM ve EDX kullanılarak incelendi. BG, B-BG ve Novamin macunları ile yıkanan diskler, dentin tübüllerinin tamamen kalsiyum fosfat tabakası ile kaplandığını, disklerin Arginin, Sodyum Florür ve Plasebo ile temizlendiğinde ise kısmen kaplandığını gösterdi. Üç gün boyunca BG, B-BG ve Novamin macunları kalsiyum fosfat tabakası birikimi ve asit yüklemesi bakımından değerlendirildi. Sonuçlar, diş tübüllerinin 3 günde kaplandığını ve sonuçların kullandığımız diş macunları arasında karşılaştırılabilir olduğunu gösterdi. B-BG macunu, saf BG ve Novamin macunlara göre aside karşı daha iyi direnç göstermiştir. Elde edilen sonuçlar, B-BG macununun, DH tedavisi için etkili bir yöntem olarak diş macunu formülasyonuna dahil edilmesi için umut verici bir geleceğe sahip olduğunu göstermiştir.

Anahtar Kelimeler : Dentin aşırı hassasiyeti, tübül oklüzyonu, biyoaktif cam, bor katkılı biyoaktif cam.

Bilim Kodu **:** 92503

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SYMBOLS AND ABBREVITIONS INDEX

SYMBOLS

°C : The degree Celsius

Cm : Centimeter

nm : Nanometer

μm : Micrometer

2θ : The diffraction angle

ABBREVITIONS

AS : Artificial saliva

BG : Bioglass

B-BG : Boron doped bioactive glass

CAF : Coronally advanced flap

DH : Dentine hypersensitivity

EDJ : Enamel-dentine junction

EDX : Energy dispersive X-ray spectroscopy

FHA : Fluorinated hydroxyapatite

FTIR : Fourier transform infrared

Gluma : Glutaraldehyde and methacrylate

CAF : Coronally advanced flap

CTG : Connective tissue graft

h : Hour

HA : Hydroxyapatite

ICP-MS : Inductively Coupled Plasma Mass Spectrometry

keV : Electronvolt

Mwg : Mean weight gain

Mwl : Mean weight loss

OTC : Over the counter

PBS : Phosphate-buffered saline

Ppb : Parts per billion

RNA : Ribonucleic Acid

SBF : Simulated Body Fluid

SEM : Scanning electron microscope

Std : Standard deviation

TEM : Transmission electron microscopy

TEOS : Tetra Ethyl Ortho Silicate

TEP : Triethyl Phosphate

VAS : Visual analogue scale

XRD : X-ray Diffraction

PART 1

INTRODUCTION

Dentin hypersensitivity (DH) is one of the foremost persistent complaints heard in dental practice [1]. It appears to peak between the ages of 20 and 50, especially 30-39, affecting up to 57 % of patients [2]. The predominance of DH among women is significantly higher [1], most as a rule affecting canines and premolars of both arches [3]. Holland defined DH as "short, sharp pain arising from exposed dentin in response to stimuli that are usually thermal, evaporative, tactile, osmotic, or chemical and that cannot be attributed to any other form of dental defect or or pathology" [4-6]. Many oral health experts emphasize that other dental defects should be excluded before diagnosing DH because other conditions may lead to similar symptoms[7]. Several theories have been proposed to explain the mechanisms of dentine hypersensitivity pain. Currently, most of the researches is based on hydrodynamic theory as the promise for DH clinical treatment [8]. As shown in (Figure 1.1.) the relief of any pain or discomfort from DH would be focused on the occlusion of the open dentinal tubules, which would restrict or limit the flow of fluid through dentin [9, 10]. DH can be considered a condition of pain that can restrict patients' everyday activities, such as the desire to eat food or drink beverages. Dental practitioners need to develop techniques for managing DH, enhancing people's quality of life. There are several over-the-counter (OTC) items and professionally applied (in-office) methods accessible for the treatment of DH. However, toothpastes have been supported as a potential long-term option for DH management [11]. The ideal toothpaste for treating DH needs to reduce dentin permeability quickly and maintain occlusive effects in the oral environment [12, 13].

Bioactive glass (BG) and glassy ceramics are widely considered one of the most effective clinical treatments for bone, dentin and enamel regeneration [14].

Recently, 45S5 BG has been suggested to treat DH by precipitating hydroxyapatite (HA) within patent dentinal tubules when it came in contact with a biological fluid [15]. Moreover, efforts have increased to improve the mechanical properties, bioactivity and chemical stability of 45S5 BG by combining it with other biomaterials. In this respect, boron was an interesting chemical element that significantly affected the structural, biological and mechanical properties of glasses. Boron improves the mechanical properties of glass and its capability to hydrolysis by generating B-OH bonds in water, which in turn stimulates bioactivity [16].

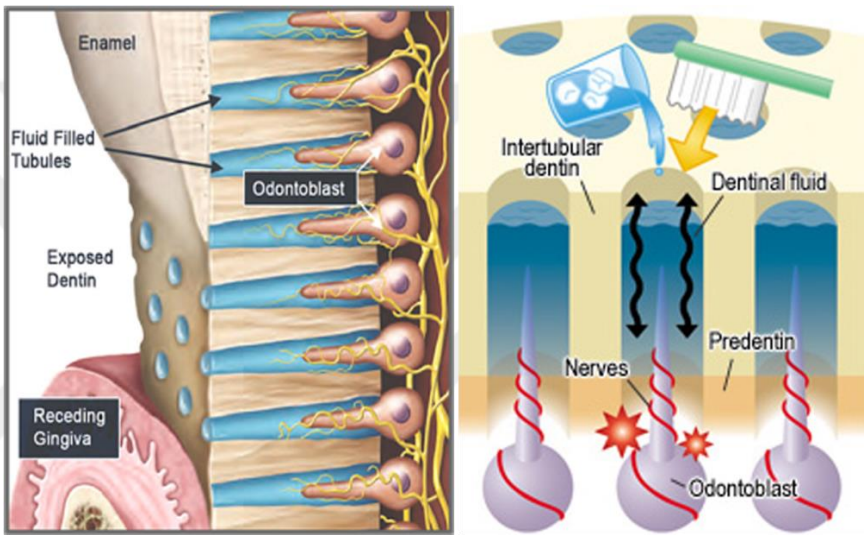


Figure 1.1. Schematic view of fluid movement through the dentinal tubules [17].

1.1 ANATOMY OF THE TOOTH AND DENTIN-PULP COMPLEX

(Figure 1.2.) below explains the anatomy of tooth parts. It is essential to understand the characteristic features of enamel, dentine and pulp to obtain a strong knowledge of the effect of tooth structure in dentine hypersensitivity.

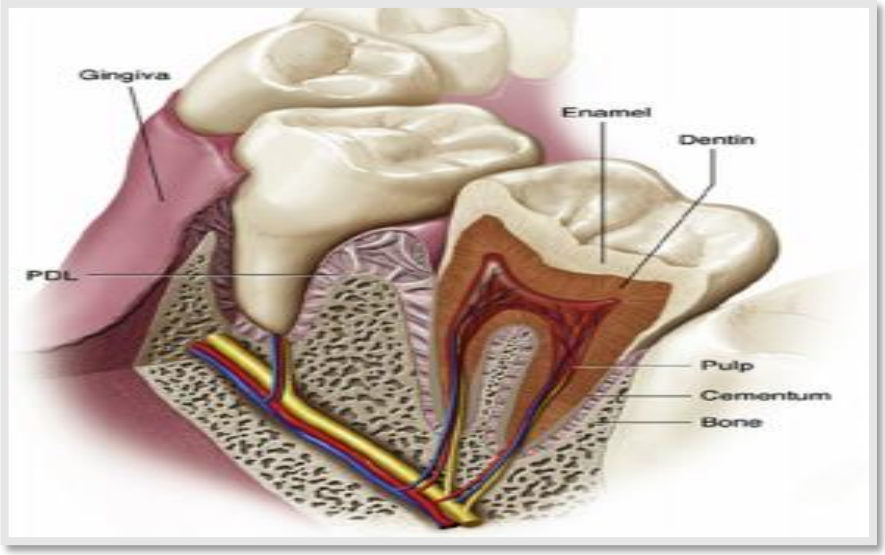


Figure 1.2. Schematic explanation of anatomy of dentin-pulp complex [18].

1.1.1 Enamel

Enamel, is the outer layer of the tooth, is the human body's hardest and most strongly mineralized tissue. The position of the enamel is shown in (Figure 1.2.) It consists of inorganic calcium phosphate forming mineral crystals of HA (about 96-97% in weight) surrounding by organic phase, primarily composed of proteins like amelogenins and enameling, lactates carbohydrates, lipids, citrates, lactates and water [19]. Enamel provides a solid and wear-resistant protective cover for the dentine and the pulp. This enables the tooth to resist chewing forces during regular days [20].

1.1.2 Dentine

Dentine is a mineralized tissue that forms the majority of teeth; it is physically located between the outer enamel surface and the inner pulp chamber [21]. Dentin comprises 65 % inorganic, 35 % organic (mainly type I collagen) and water [22]. As illustrated in (Figure 1.3.) Dentine itself contains dentinal tubules that spread through the dentin's total thickness from the pulp to the dentin-enamel junction (EDJ). The number and diameter increase towards the pulp [23, 24]. Dentinal tubules involve odontoblastic process, protein combination, collagen fiber [25]. These tubules provide the dentin with a degree of permeability that can contribute to the sensation of pain and the

response of the pulp to the oral environment as it allows fluid movement through the dentin [24, 26].

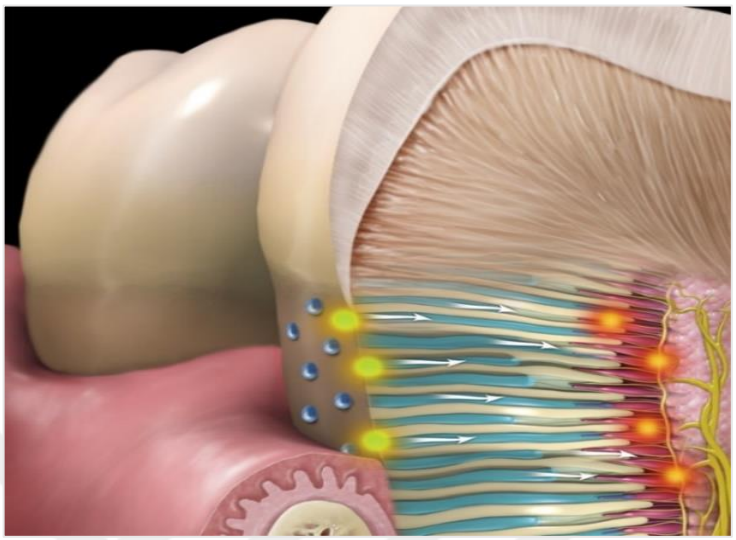


Figure 1.3. Schematic explanation of fluid movement through dentin tubules [27].

1.1.3 Dental Pulp

The dental pulp is a highly developed connective tissue located directly under the dentin layer within the tooth's center, surrounded by dense mineralized tissue [28]. It is penetrated a system of vascular blood and lymph vessels with periodontal ligament-derived nerve bundles. The presence of odontoblasts uniquely characterizes the pulp in addition to fibroblasts, defensive cells and extracellular matrix [23]. Pulp is closely related to dentin as it helps in the formation and nutrition of dentin [29]. Any biochemical and pathological responses in one tissue will affect the other [20] and innervate and defend the teeth.

1.2 DENTINE HYPERSENSITIVITY

DH is a common oral complaint that affects adults. It is one of the most painful and difficult to cure chronic dental conditions [30]. Recent research suggests that hypersensitivity prevalence varies from 10% to 30 % of the population [31, 32]. DH is defined as a short, sharp pain that arises from exposed cervical dentin in response to many stimuli that are typically thermal, evaporative, tactile, osmotic, or chemical and

cannot be attributed to any other form of dental pathology or disease [33]. DH symptoms are described by the sudden occurrence of extreme and short-duration pain; it has been identified as a chronic disease with acute episodes[34].

DH etiology may hardly ever be defined as a direct result of a single factor, which is usually a multifactorial situation [35]. The incidence of DH is associated with exposure of the dentine surface to the oral environment, allowing fluid movement within the tubules and the patency of the dentinal tubules. Gingival recession, abrasion, erosion and attrition are considered the main factors in the development of DH [1, 34, 36]. Although the specific cause of DH is still unknown, many researchers believe that the hydrodynamic theory explains the symptoms. In general, the literature states that DH affects premolars and canine teeth [34].

Over recent decades, several theories have been presented to explain the mechanism causing DH. These are the direct innervation hypothesis, the odontoblast receptor theory and the hydrodynamic theory [30]. However, Hydrodynamic theory is the most widely accepted mechanism to explain DH [8, 37]. This is based on the existence and movement of fluid inside dentinal tubules; this fluid flow, in turn, activates nerve endings at the end of dentinal tubules or the pulp–dentine complex, leading to neural discharge producing pain [38, 39].

1.3 MANAGEMENT OF DENTIN HYPERSENSITIVITY

Treatment of a patient with DH should be based on the correct diagnosis of the condition, with a comprehensive clinical history, examination and the exclusion of other causes of dental pain [40]. DH has been managed using a variety of therapeutic methods. The treatment of DH has been categorized according to the type of delivery as professionally administered therapy applied in the dental clinic or in-office and self-administered treatment used by the patient at home [41]. To allow safe use, OTC desensitizing agents are often based on components with the same active ingredients as in-office agents but at a lower concentration. They are usually inexpensive and can simultaneously treat generalized DH affecting several teeth [36]. A wide range of office-based agents are available to treat complex hypersensitivity conditions with immediate pain relief. These can include protective varnishes, calcium compounds,

oxalates, resins and adhesives, restorative materials and laser treatments. In particular, the efficiency of the Nd: YAG and Er: YAG lasers in blocking dentinal tubules has been demonstrated in many studies [42-44]. At home, desensitizing agents include mouthwash, chewing gum and toothpaste[38]. However, The most common type of OTC desensitizing agent is toothpastes [30]. Desensitizing toothpaste was considered easy to use, noninvasive and cost-effective of these treatments [45].

The other category is based on the method of action (Figure 1.4.), which may be divided into two categories: dentinal tubule occluding agents, which prevent the hydrodynamic mechanism of pain stimulation (Figure 1.4. (A)) and nerve desensitizers, which block the neurological response to pain signals in the pulp (neuroblockers) (Figure 1.4. (B)) [39, 46]. Dentine tubule occlusion can decrease dentin permeability and sensitivity by altering fluid flow in dentinal tubules and forming a protective layer on the dentin surface [12, 47].

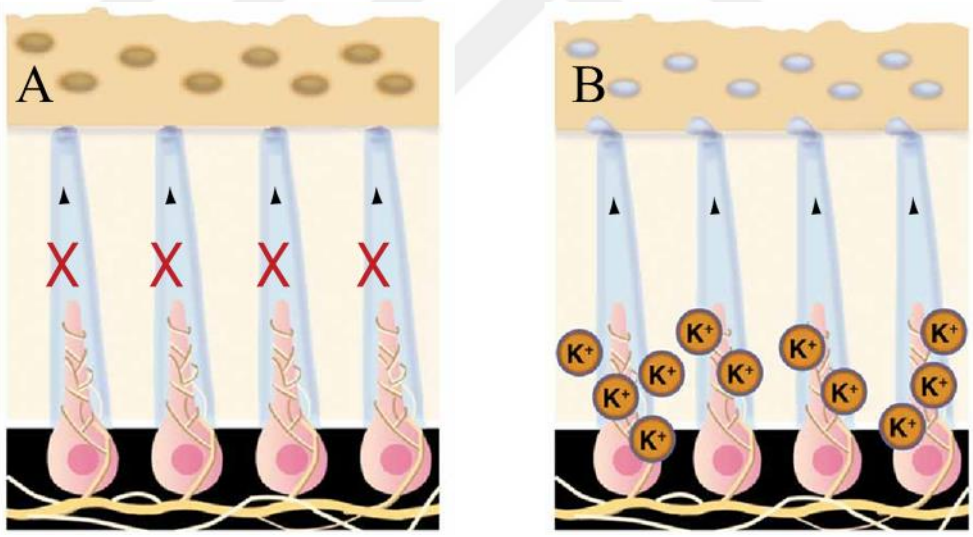


Figure 1.3 4. Schematic explanation of DH treatment A) Occlusion of the dentinal tubules [38, 48], B) Nerve desensitizers [31].

1.3.1 Dentin Hypersensitivity Toothpastes

Toothpaste is the most popular treatment used in the management of DH [34]. A wide range of agents and compounds have been researched and evaluated to determine their effectiveness in relieving DH symptoms.

The ideal desensitizing agent should have a rapid onset of action, be effective for long periods, be painless, be non-irritant to the pulp, be easy to use and not discolor the tooth [38]. Toothpastes are the most cost-effective way to use desensitizing in-home treatments and regulatory agencies classify them based on the chemicals in the formulation [38, 48]. Several treatments are being developed to treat DH, but there is no perfect product that can cure the condition. Toothpastes are widely recommended due to their low cost, convenience and ability to be used at home. Although pain relief usually takes four to eight weeks [30].

There are two types of toothpaste to treat DH on the market: analgesic toothpaste and dentin tubule occluding toothpaste [48]. The first type is potassium-containing toothpaste, which depolarizes nerve conduction, preventing pain signals from reaching the brain. The second type is toothpaste containing compounds like strontium chloride, stannous fluoride, calcium sodium phosphosilicate and arginine, which occludes exposed dentine tubules and reduces fluid movement within the tubules during external stimulation to achieve a desensitizing effect [38, 48].

Although it has been widely indicated that some toothpastes are able to reduce dentin permeability and block the dentinal tubules, exposure to acidic substances and immersion in saliva can reverse the decrease in permeability caused by desensitizing agents, remove the smear layer and open the dentinal tubules [49, 50]. As a result, the ideal toothpaste for DH should decrease dentine permeability and keep the occlusion of the tubules in the face of acid challenges and saliva immersion [51].

1.3.2 The Active Ingredients In Dentin Hypersensitivity Toothpaste

Many toothpaste formulations with various active ingredients are being developed to treat DH. Potassium salts, such as KNO_3, are the most common active ingredient that has been used in DH toothpaste [52] as shown in (Figure 1.4. (A)) can produce a high extracellular K^+ level, limiting nerve cell membrane re-polarization and blocking impulse transmission without producing pulp changes [31]. However, the other active ingredients are based on occlusion of dentinal tubules. Fluoride compounds can occlude dentin tubules and precipitate calcium fluoride crystals within them [36] to

increase dentin resistance to acid attack by remineralization [48]. Stannous fluoride also has the potential to occlude the tubules by forming SnF_2 and CaF_2 and producing a protective layer on the tooth surface by creating Sn-Na hexametaphosphate through a reaction of the Sn^{2+} ion with Na, Ca and P components [31, 53]. Toothpaste containing arginine also showed a reduction in DH. Arginine, an amino acid in saliva, forms a barrier in dentinal tubules that prevents fluid flow when combined with calcium carbonate and phosphate [54].

Strontium chloride and strontium acetate formulations have been used as active ingredients in DH toothpaste for years [55]. The capacity of strontium salts to penetrate the dentine and produce strontium apatite, which may occlude the dentine tubules, is considered responsible for their occluding action [56]. Calcium oxalates can precipitate crystals inside dentine tubules and create a protective layer on the dentin surface [57]. Several studies have confirmed the efficacy of calcium hydroxide [58] and calcium phosphate [48, 59] in treating DH as calcium is essential for remineralization. It is the main component of HA [48, 59].

Recently, bioactive glass such as Calcium sodium phosphosilicate or Novamin has been advocated in toothpaste formulations as an effective long-term solution for DH management [12, 38, 60]. The contact of Novamin with the oral environment leads to the release of calcium and phosphate ions from it and the deposition of a thick layer consisting of calcium and phosphate that mechanically blocks the dentinal tubules and reduces the flow of fluids inside the dentin [60]. This layer crystallizes into hydroxyapatite, which is structurally similar to the teeth's mineral content and resistant to acidic challenges [60].

1.3.3 Bioactive Glass

Biological active glass materials have received significant interest in medical and dental use [15]. They have developed and taken on many applications since the development of 45S5 Bioglass (BG) by Dr. Larry Hench [61, 62]. The BG has excellent bioactivity, osteoconductivity and osteostimulative properties [63]. Bioactive glasses are composed of specific amounts of silicon, calcium, sodium and

phosphorus oxides (SiO_2, CaO, P_2O_5 and Na_2O) [63]. By introducing BG into the oral environment, BG produces a negative charge on the surface, allowing it to attach to type I collagen fibers in the dentin tubules [64]. The binding of bioactive glass molecules to the dentin begins by exchanging sodium ions in the glass with hydrogen ions from body fluids, allowing the pH value to rise. Calcium and phosphate ions eventually move from the glass, creating a top layer abundant in calcium-phosphate under which a layer becomes gradually rich in silica due to the loss of sodium, calcium and phosphate ions. Regarding the silica-rich layer and the small size of bioactive glass particles [65, 66]. This observation encouraged the researchers to consider using bioactive glass particles as a potential desensitizing agent for DH treatment.

1.3.4 Boron Doped Bioglass

It is known that a change in BG composition by substitution and/or fusion of different ions can regulate their biological activity [67]. BG containing B_2O_3 have received much attention in recent years because of their potential effects and various biomedical applications, such as osteogenesis, angiogenesis, soft tissue regeneration, improving coating adherence and enhancing mechanical properties [68]. As a trace element, boron is essential for bone physiology and its combination with BG enhances its rapid and complete ability to convert to HA when immersed in body fluid. Boron doped bioglass (B-BG) has never been studied for occluding dentinal tubules to eliminate or treat DH. Still, many studies in bone and hard tissue engineering have been confirmed the effectiveness of this composition in the rapid transformation to a HA layer [68, 69]. Since dentin has many similarities to bone [15], B-BG can be proposed as a formulation to dentin tubule occlusion and treat DH.

1.4 PROBLEM STATEMENT

The feeling of pain in daily life is familiar among hypersensitivity sufferers, which significantly affects their quality of life [70, 71]. Therefore, a successful treatment method must relieve symptoms and reduce or eliminate DH in the shortest possible time. After reviewing the literature, it becomes clear that most products designed to eliminate or relieve the symptoms of DH are primarily in the form of toothpaste that

can be used at home. The active ingredients in these toothpastes may take a long time to build up a thick enough layer on the surface of the dentin to block the dentinal tubules and the treatment must be maintained constantly to be successful. In addition, most of these treatments are not resistant to acid challenges. They are easily removed when exposed to an acidic environment, causing the opening of the tubules and more excellent permeability in the dentin.

1.5 OBJECTIVES

The objectives and aims of this Master Thesis were:

- To synthesize BG and B-BG particles and determine the effectiveness of these materials, which are administered in the form of toothpastes on occluding dentinal tubules as a means to eliminate the effects of DH.

- To evaluate the efficacy of many commercial desensitizing toothpastes based on different active ingredients in occluding the exposed dentinal tubules.

- To compare the results of dentin samples treated with bioactive glass formulations to dentin samples treated with different formulations in addition to the control group.

- To verify the rapid deposition and formation of HA layer on the surface of the dentin samples caused by the incorporation of boron with BG particles, which provides the immediate elimination of DH

- To provide evidence of the applicability of boron as an effective treatment for complete occlusion of the exposed dentinal tubules and covering the dentin surface with deposits resistant to acidic challenges present in the oral environment, providing a long-term effect.

1.6 SIGNIFICANCE OF THE STUDY

The importance of the thesis lies in the production of B-BG, a new biocompatible material in the field of treating DH, especially in blocking dentinal tubules. It can be commercially available on the market at a low price. It has the same results as in-office products, such as acid resistance and long-term treatment, but it is safe, easy to use and accessible to all individuals.

PART 2

LITERATURE REVIEW

DH has received significant attention from researchers and healthcare professionals. This literature review proposes to examine the publications of recent years, current methods used and potential future methods of DH management. They were numerous and often at odds, linked to various DH treatment products and mechanisms. Search engines such as Google Scholar, Web of Science, Scopus, Research Gate and PubMed have been used to find relevant, recently published literature. In recent years, the increasing interest of researchers in studying DH and blockage of open dentinal tubules has been observed in recent years. According to Scopus, documents by year analysis shows an increase in articles and studies published in recent years about occluding dental tubules, as shown in (Figure 2.1.) This increase can be attributed to the availability of sufficient references for studies and the development of techniques and methods required for treatment.

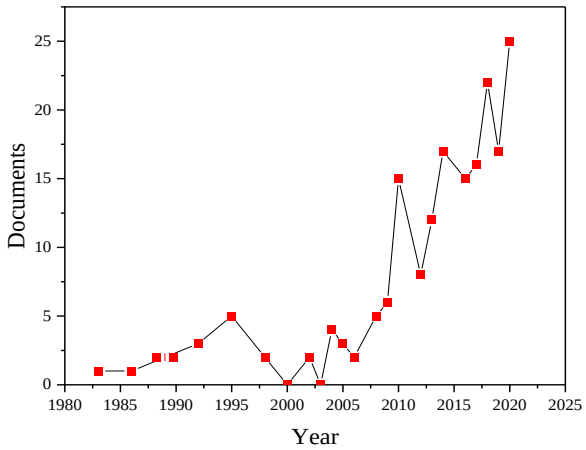


Figure 2.1. Publications rate over years of studies related to the dentine hypersensitivity and tubules occlusion.

2.1 MECHANISMS OF DENTIN HYPERSENSITIVITY

The literature discusses three main theories that have been suggested to explain the mechanism of DH pain. These theories are the theory of direct nerve innervation, odontoblast receptors and hydrodynamics [72].

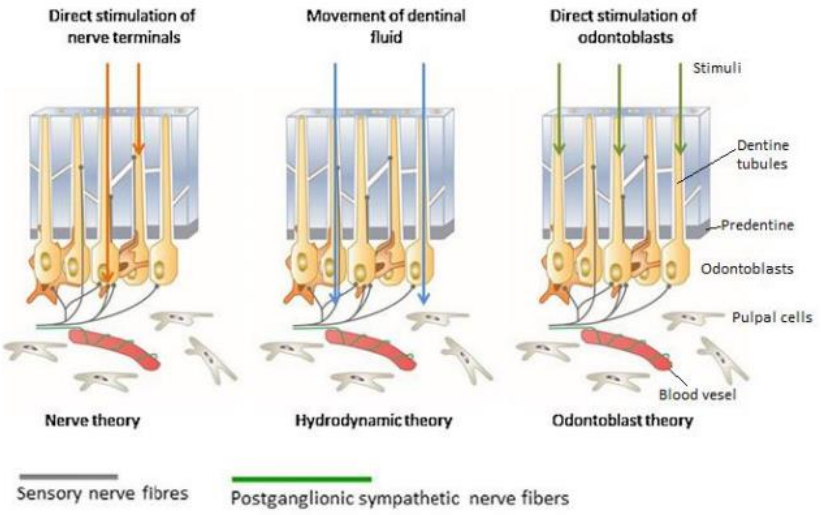


Figure 2.2. Schematic description of the three main theories of dentine sensitivity clarification [73].

2.1.1 Direct Nerve Innervation

The principle of nerve or direct innervation, shown in (Figure 2.2.) indicates that nerve endings pass directly through the pulp into the dentine and extend to the dentin-enamel junction, transferring pain directly through the nerves through some mechanical stimulus [74]. This theory considered that the whole length of tubules contains free nerve endings. But modern electron microscopy shows that nerve fibers exist only at the inner dentine, mainly at the pulpal horns [75]. Therefore, do not expand to DEJ, the dentin's most sensitive region. It is also possible for the newly formed teeth to be sensitive. However, studies have shown that the parietal layer of nerves responsible for transmitting the feeling of pain from the pulp of the tooth does not mature until the teeth erupt [34]. This raises doubts whether this is the leading cause of DH.

2.1.2 The Odontoblast Receptor Theory

The theory of the odontoblast receptor indicates that odontoblasts themselves work as pain receptors and thus transfer signals through synaptic junctions to the pulpal nerves [76]. This was not supported by direct microscopy as the odontoblastic mechanisms spread only to a limit of one half of the length of the dentinal tubule, a gap can be located between the process and the dentinal fluid-filled tubule wall, as well as the odontoblast cellular matrix cannot induce neural impulses [38]. Studies have shown that odontoblasts are matrix-forming cells and are not classified as excitable cells since no synapses between nerve endings and odontoblasts have been developed [34].

2.1.3 Hydrodynamic Theory

The hydrodynamic theory is the most popular theory regarding the cause of DH. suggested first in the 19th century by Gysi *et al.*, after creating a hypothesis of external fluid movement along dentinal tubules rising after the proper stimuli were applied, eliciting a reaction in the pulpal nerves [77]. In a sequence of experiments, the hydrodynamic theory was supported throughout the 1960s as the primary interpretation of DH by Brännström and his colleagues [34]. The hypothesis indicates that fluid movement caused by stimulation within dentinal tubules is responsible for the activation of nociceptors in the pulp and the corresponding activation of neurons reported as pain [38, 78]. The increased fluid movement causes a shift of pressure in the dentine, which stimulates myelinated A-β and some interdental A-δ nerves and unmyelinated C-fibers, either at the pulp-dentine boundary or inside the dentinal tubules When DH happens, the acute pain felt in a particular area is primarily due to A-δ nerves.

In vivo experiments have illustrated the significance of the patentability of dentinal tubules in promoting the passage of fluid within the tubules by opening a narrow cavity and evaluating changes in hydrostatic force during stimulation application. However, this technique requires patients who are able to perform the experiment to be recruited, which may not be appropriate since the findings may not be repeated outside the oral cavity [79].

Microscopically, multiple experiments have found that the characteristics interact directly with the degree of hypersensitivity. These characteristics have included dentinal tubule patency, radius, density and depth of the open dentinal tubules [80]. The number of tubules per unit area in sensitive teeth is around 8 times higher than that observed in non-sensitive teeth. The tubular diameter is 2 times higher than that observed in non-sensitive teeth [76, 80]. Addy *et al.,* have confirmed that variation in tubule diameter is probably the most critical factor since fluid flow is equal to the fourth power of the radius, doubling the diameter of the tubule results in a 16-time rise in fluid flow [1]. Furthermore, inflammation of the pulp could play an essential role in increasing the degree of pain and reducing the threshold of pain, thereby exposing dentin might becoming hypersensitive [81].

2.2 TREATMENT OF DENTINE HYPERSENSITIVITY

There is an interestingly wide range of DH control treatment options. Chemical or physical agents have been used to either desensitize the nerve or occlude the exposed dentinal tubules [82, 83]. The most effective management method is placing a topically applied agent, either by a dental specialist or at home by the patient. It is recognized that several parameters constitute an optimal desensitizing agent. This includes not irritating the pulp, being relatively painless to administer, being easy to apply, prompt intervention, being permanently successful and not discoloring the teeth.

In general, patient reactions are very variable and therefore, clinical experiences are primarily dependent on the pain level of the patient [40, 84]. Theories for the DH process are closely related to the anatomy and histology of the dentin-pulpal complex. Besides that, odontoblast cells produce the collagen matrix of dentin (majority Type I) and are essential in the process of mineralization; odontoblasts are decisively associated with the formation and regeneration of dentin [85]. The dentin macrostructure includes a group of tubules surrounded by hypermineralized tissue: dentin peritubular, Serum-like substance and an odontoblast cell process are found in the dentin tubule imaged by scanning electron microscope (SEM) (Figure 2.3. (A)). Ultrastructure research has supported sensory nerves' near spatial similarity to the odontoblast (process and cell body) [23].

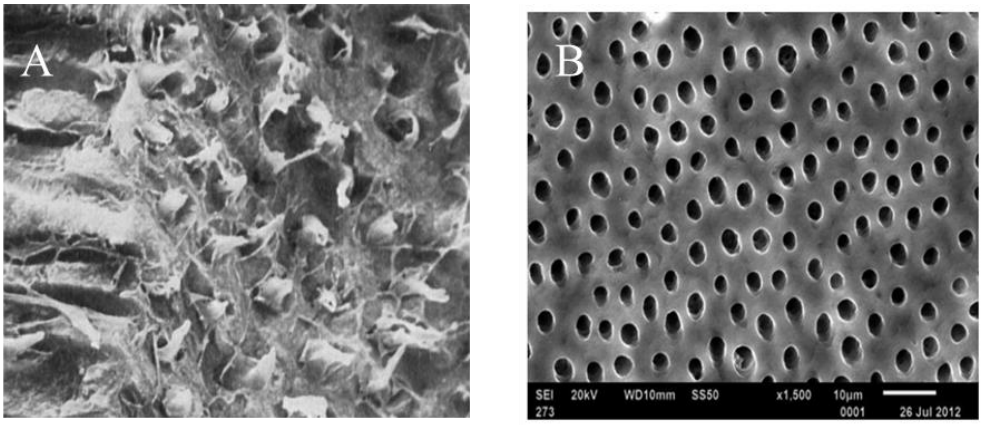


Figure 2.3. A) SEM image of exposed dentin containing odontoblast process [86], B) SEM image of open dentinal tubules [87].

Diameter size, patency status and the number of open tubules are all possible factors that distinguish sensitive teeth from non-sensitive teeth. The sensitive teeth DH have a higher number of patent tubules, about 8 times per unit area, than non-sensitive teeth [80]. This patency (or lack of patency) is proposed to be linked to acid demineralization and remineralization inability. The exposed dentin tubules can be occluded due to reactive sclerosis, secondary and tertiary dentin [88]. Reactive sclerosis occurs from hyper mineralization of the dentin around the tubules, i.e., the dentin located close to the odontoblastic process and the deposition of saliva or fluid minerals within the tubules [89]. As shown above in (Figure 2.3. B)), the nature of largely open dentin tubules has been confirmed in many studies by SEM examination of exposed dentin [80]. Pol *et al.,* indicated that tooth hypersensitivity often occurs due to exposure to dentinal tubules after cervical erosion or abrasion, excessive dietary acid consumption, or periodontal treatment [90]. DH can also be initiated by abrasive tooth brushing and the use of abrasive toothpaste [90]. These activities can weaken or destroy tooth tissue and lead to anatomical features in the enamel/cementum junction and/or enamel region or cement loss. They can also remove layers of protective plaque, exposing dentinal tubules to stimuli in the oral environment [91].

The critical pH for enamel erosion is 4.5 or less [92], resulting in dentinal tubule exposure and eventual DH. However, at a normal pH within the oral environment, calcium and phosphate levels are supersaturated in saliva in the form of HA. In acute

hypersensitivity attacks, calcium deposits in saliva help block the exposed dentinal tubules [93]. The formula in (2.1) defines the equilibrium for HA dissolution:

$$Ca_{10}(PO_4)_6(OH) \leftrightarrow 10Ca^{2+} + 6PO_4^{3-} + 2OH^- \quad (2.1)$$

If the oral environment's pH decreases, PO_4^{2-} is converted to HPO_4^{3-} or $H_2PO_4^{3-}$ and OH^- is neutralized to form water. Under these circumstances, concerning free calcium and phosphate, saliva will no longer be supersaturated and exposed dentinal tubules will not be easily blocked; therefore, hypersensitivity will continue [93].

It is crucial to consider the individual causative factors of DH In order to avoid propagation of the problem. DH control techniques almost exclusively depend on treatment requirements, with formulations of toothpaste being designed to protect the exposed dentine and/or block the dentinal tubules [94]. As shown in (Figure 2.4.) the permeability and fluid movement of exposed, open dentinal tubules have provided a favored mechanism for dentin stimulus transmission. The occlusion of these dentinal tubules has been established as a possible way of reducing discomfort associated with sensitive teeth [47].

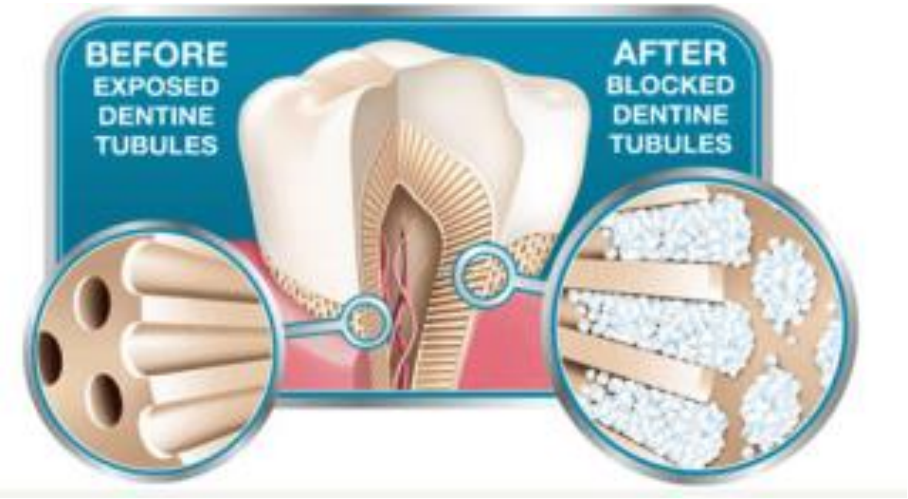


Figure 2.4. Schematic view of treating DH by occlusion open dentinal tubules [17].

Basically, as shown in Table (2.1.), treatment for DH can be divided by type of delivery into professional or in office, or OTC or home use. The other classification depends on the action used and could be divided into two main types. The dentinal tubules'

occluding agents obstruct the hydrodynamic mechanism of the pain stimulus and the desensitizers of the nerves to inhibit the neuronal responses to pain stimuli (neuronal blockers) [36]. OTC desensitizing agents are usually based on the same chemical compounds as in-office agents, but with a lower dosage to allow safe use and appear to be economical. They can treat generalized DH that affects many teeth simultaneously.

2.2.1.1 Professionally Applied Treatment

Professional treatment can provide an acceptable method in order to minimize the level of DH. Laser therapy, primers, adhesive resin bonding, varnishes, sealants, traditional glass ionomer cement and resin-reinforced glass ionomer have all been reported to demonstrate different levels of effectiveness for dentine hypersensitivity management, with some evidence supporting their usage [95-97]. However, some indicate that they are inefficient [98, 99].

2.2.1.2 Adhesive Resin Bonding

Many studies have indicated that applying adhesive resin materials leads to the dentinal tubules' occluding by forming a hybrid layer and resin tags [36]. It facilitates a micro-mechanical interlocking mechanism by a hybridization process. In addition to the presence of functional monomers, they have the ability to combine chemically with Ca^{2+} of the residual HA that remains within the submicron hybrid substrate [100]. In an early study, Brannstrom demonstrated that resins and adhesives materials can seal the dentin tubules and avoid the transfer of hydrodynamic impulses to the pulpal nerve complex. Utilizing professionally applied polymer-based substances, including resins and dentin bonding agents, the formulation of a thin film coating creates an artificial smear layer to cover open tubules [101].

The use of such self-etching adhesive systems is a recent development for managing DH. According to Türkün, instead of using a traditional total-etch technique, the treatment of enamel and dentin with self-etching adhesive systems avoids collagen network breakdown. In addition, no etching, washing and drying are needed for the

self-etching primers so that the possible risk of over-etching and over-drying of the dentin is removed [102]. In another study, a new generation of dentin bonding agents (HEMA/GA) includes desensitizing agents introduced for the treatment of DH. These new products caused coagulation and precipitation of serum proteins within the dentin tubules and the formation of resin tags [103]. However, this method has been used in specific and localized cases of DH rather than the general condition [40].

Burke and Malik proposed covering the tubules with either a binding agent or sealing resin. This can occur through the use of dense polymer resin consisting mainly of glutaraldehyde and (hydroxyethyl) methacrylate (for example, Gluma), which is a biological fixative that can occlude the dentin tubules by coagulation of the salivary proteins inside the tubules [104]. This approach reveals positive results instantly. However, these results are limited to established sore spots and are minimized if the polymer peels off [31]. Furthermore, Gluma has to be used solely under the recommendation of a specialist because glutaraldehyde is toxic and its effectiveness depends on the skill of a clinician [31, 105]. Clinically, this choice of therapy typically arises after the exhaustion of at-home techniques.

2.2.1.3 Varnishes and Precipitants

Professionally applying sodium fluoride varnishes has been used for DH treatment in many studies [106]. These varnishes comprise fluoride-based natural resin structures that are highly sticky to the tooth's surface, simple to apply and relatively cheap [54]. Fluoride varnishes have been developed to improve the effectiveness and substantivizes of fluoride when in contact with the tooth surface in order to allow for a steady and consistent release of fluoride [78]. Since some researchers show an apparent enhancement of the surface enamel with such a varnish due to the occlusion of the tubules by the resin rather than the effect of the fluoride [76]. However, a study comparing two different varnishes has reported that Cervitec varnish is more effective than Gluma varnish in decreasing dentine hypersensitivity [107].

In another study, researchers manufactured varnishes containing potassium chloride (KCl) and fluorinated HA (FHA). SEM images obtained from this study showed that

the dentin tubules were occluded in the FHA varnish group. The KCl-FHA varnish can release potassium ions and reduce the hydraulic conductivity of the dentine discs and can therefore be a practical treatment choice for DH. Over time, FHA has the capacity to occlude dentine tubules; however, the samples have not been exposed to an acidic challenge or any other food and drink that may affect the clinical environment [108].

2.2.1.4 Laser Treatment

Laser application was believed to operate either by recrystallizing dentin to create a glazed, non-porous surface that partially or wholly occludes dentine tubules or by disrupting the neural transmission in the dentine fluid by coagulating proteins and thereby decreasing permeability and preventing the passage of fluid [44]. Alternatively, the use of lasers could be the basis of another possible future treatment for dentine hypersensitivity. The efficacy of two forms of lasers in people with sensitive teeth was compared by research by Ladalardo *et al.*, (2004) and their discomfort was assessed before and during therapy. It was confirmed that a 660 nm red laser had greater desensitizing efficacy than the 830nm infrared laser [109]. This was confirmed by other studies, as it indicated that low-energy lasers, including GaAlAs diode lasers, with a wavelength ranging between 780 and 900 nm, operate at the neural level. In contrast, medium-energy lasers, including the Nd: YAG laser and CO_2 and Er: YAG, contribute to narrowing and occlusion of the dentinal tubules, causing desensitization [110].

A meta-analysis has shown that lasers Er: YAG, Nd: YAG and GaA1A tend to be an efficient treatment choice, but data heterogeneity indicates that further research is required [42]. Diode laser treatment has recently been shown to impede 68.9 % of all tested tubules, demonstrating its potential utility in DH treatment [111]. Some researchers formulated a potassium binoxalate gel and measured the effectiveness with laser or alone with Visual Analogue Scale (VAS) after cold air and hot water relaxation. The data was carried out directly, 3-6-9 months later, with the aid of an electron microscope. Although the gel tends to be a legitimate assist for its micro-crystals penetration, laser therapy is greater in longevity for dentinal tubular occlusion [44]. However, the SEM analysis demonstrates the actual efficacy of these lasers. The

occlusion proportion tends to be complete and the dentinal tubule diameter is diminished. In-office DH laser treatments have some drawbacks relative to traditional therapies (i.e. high cost, the difficulty of usage, reducing efficiency over time, etc.) that restrict their therapeutic effectiveness [36].

2.2.1.5 Iontophoresis Treatment

One such DH treatment approach may be based on iontophoresis. Iontophoresis was first used to treat DH in the early 1960s [112]. It uses a low-amperage direct electrical current to deliver ions or ionized drugs into tissues. This system acts on the theory of repelling charges and attracting opposite directions. Since adding a sufficiently charged electrical current, ionized drugs may be pushed through the tissue. It clarifies the local use of medications in condensed form through iontophoresis [111]. Patil *et al.,* performed a study to evaluate and compare the efficacy of iontophoresis with 0.33% NaF gel and diode laser alone in dentinal tubular occlusion. However, it has been observed that the diode laser application provided better results compared to the iontophoresis on dental tubules occlusion [111].

2.2.1.6 Periodontal Plastic Surgery

Another type of care for DH is surgical root coverage, which is known as a soft-tissue pedicle graft and a free soft-tissue graft [113]. Surgical treatments obstruct the uncovered dentinal tubules, enhancing esthetics in the sensitive parts of gingival recession [114, 115]. There are a wide variety of periodontal plastic surgery treatments for protecting the uncovered root surface, including a semilunar flap, pedicle graft, or coronally advanced flap (CAF), which can be associated with a subepithelial connective tissue graft (CTG) [116]. Although these treatments can seal exposed dentinal tubules, there is not enough empirical evidence to suggest that cervical DH is predictably decreased by surgical root coverage methods [116].

2.2.1.7 Corticosteroids

Anti-inflammatory agents such as corticosteroids have been recommended for use to treat dentine hypersensitivity. However, they are not especially useful by studies. Although it is hypothesized that these agents may induce mineralization leading to tubule occlusion, this opinion has not yet been confirmed and the feasibility of using such agents has been challenged [117].

Considering that most professionally formulated desensitizing agents appear to minimize or remove pain in the DH treatment process, it is still not obvious which treatment method is the safest. A meta-analysis of research that has evaluated the effectiveness of chemical and physical occlusion agents, nerve desensitizing agents, laser treatment, or combined management showed that all of them were effective relative to placebo control [118]. However, there is insufficient evidence about professionally administered agents to prove which agent's superior effectiveness, so it was hard to determine and suggest one agent for DH treatment relative to another [35].

2.2.2 Over The Counter Products

The primary clinical modalities used for pain management in DH therapy are the change of fluid flow in the dentinal tubules and the improvement or blockage of the pulpal nerve reaction [119]. The most popular preparations used in the treatment of DH are toothpastes. A wide range of agents and formulations have been tested and proved to characterize their effectiveness for DH symptom relief. There is an interestingly wide range of DH control treatment options. Chemical or physical agents have been used to either desensitize the nerve or occlude the exposed dentinal tubules [82, 83].

2.2.2.1 Potassium Nitrate

Many studies have documented the effectiveness of potassium nitrate in controlling dentine hypersensitivity [120]. Potassium nitrate has been used in toothpaste as a desensitizing agent to inhibit neuronal transmission of pain signals [121]. Such

controlled studies found that potassium nitrate was able to desensitize dentine for up to 4 months at a concentration of 5 % in a low abrasive toothpaste compared with the control paste. In bioadhesive gels, 5% and 10% potassium nitrate have also been productive in decreasing DH [122]. However, the Cochrane study of toothpaste-containing potassium nitrate, expressed in a meta-analysis of 6 studies, could not provide clear evidence supporting the effectiveness of potassium nitrate toothpaste in dentine hypersensitivity treatment [123].

Potassium-containing formulations of toothpaste intended to heal sensitive teeth may also have an analgesic impact. Potassium saline, for example, is responsible for preserving extracellularly elevated potassium ion levels, blocking repolarization of the nerve cell membrane and inhibiting impulse transmission. In short, to minimize DH symptoms, potassium nitrate has been suggested to act by blocking neural transmission. In humans, however, there is insufficient clinical evidence to confirm the mode of action of potassium ions DH reducing. Many studies have shown that toothpaste containing calcium sodium phosphosilicate or NovaMin® can more successfully cover dentinal tubules than potassium salts, as shown in clinical tests and *in vitro* studies immersing these materials in artificial saliva (AS) [48, 59, 124].

It has been reported that the combination of potassium, fluoride stannous and silica in toothpaste is more effective in pain reduction than only potassium-containing toothpaste [125]. Some toothpaste occludes dentine tubules in order to reduce DH pain. However, this occlusion can be temporary, depending on the active substance in toothpaste and susceptible to acid dissolution [126]. Finally, the use of potassium nitrate as an essential ingredient in treating DH has been investigated in various studies. However, it does not block the dentinal tubules [127]. Instead, the potassium ions in the solution were suggested to act by blocking the synaptic connections between nerve cells, reducing nerve excitation and related discomfort [128].

2.2.2.2 Fluoride Formulations

Toothpaste formulations usually focus on fluoride (to protect against caries), an abrasive portion that considers the cleaning power, substances that prevent bacterial

development and other ingredients [48, 59, 129]. Fluoride is a substance that can help in enamel remineralization, but there is little evidence of its effect on DH. However, it has been shown that fluoride in toothpaste can cause precipitation on the surface of the dentine and cover the dentin tubules, enhancing resistance to an acidic challenge [48]. However, the precipitation can also involve silica particles that may block the dental tubules rather than fluoride ions. Several oral health care companies now favor fluoride in most toothpastes due to its improved positive effects on the elimination of caries.

Numerous fluoride compounds have been documented to manage dentine hypersensitivity, including sodium fluoride, sodium silico-fluoride, stannous fluoride (SnF_2) and sodium monofluorophosphate. More recent studies indicate that in terms of fluoride accumulation on the teeth, sodium fluoride is preferable to sodium monofluorophosphate [76]. Fluorides reduce dentin permeability, likely by insoluble calcium fluoride deposition within the dentinal tubules [36, 130].

Suge *et al.*, has reported the ammonium hexafluorosilicate as a desensitizing agent. The precipitation of a combination of calcium fluoride and fluoridated apatite can provide a high level of dentinal tubule occlusion [131, 132]. Many researchers have discussed the daily usage of fluoride in glycine compounds [133]. However, other studies have shown that concentrated glycine may cause pain due to its high osmolarity resulting in water being drawn out from the tubules, leading to hypersensitivity by itself. On the other hand, although varnish-containing fluoride has been suggested as a solution to treat DH, its activity is temporary and usually lasts only a few hours (h) [133].

Several studies have shown different results regarding the evaluation of chloride toothpaste. Various *in vitro* experiments have reported that a thin crystalline layer has been precipitated on the surface of the dentine that can be quickly washed away; however, other studies have indicated positive impacts of the products containing chloride on DH relief [108, 134]. When comparing formulations of toothpastes that contain chlorine and fluoride, there were contradictory results reported in the published literature, with many studies confirming that there is no difference in the effectiveness of the two products; however, other studies reported that there were differences in

favor of chloride-containing toothpastes. *In vitro* studies and studies using dentin cross-sections have indicated that no occluding of dentin tubules was observed after treatment with toothpaste containing SnF_2 [126].

2.2.2.3 Oxalate Salts

Oxalate salts have been widely used in the treatment of DH. Calcium oxalate crystals have historically been shown to precipitate inside dentine tubules and thus work as desensitizing agents by occluding the dentine tubules. Cruz *et al.*. has indicated creating a successful layer by using oxalates to block open dentinal tubules [57]. Oxalates, such as 3% monohydrogen-monopotassium oxalate, have the additional advantage of relative acid insolubility, making them highly resistant to treatment dissolution. Just 3% monohydrogen-monopotassium oxalate was shown to reduce DH [57]. Potassium oxalate (Protect, Butler Inc.) and ferric oxalate are some examples of industrial oxalate schemes (Sensodyne, Block Drug) [135].

The efficiency of oxalates to decrease DH compared to placebo agents, professionally applied resins such as Gluma®, Diamine silver fluoride, Seal and Protect® and lasers have been studied. The findings revealed contradictory effectiveness where oxalate decreased discomfort to a certain degree relative to controls in some situations [96, 136-138], and fewer than the controls In other situations [139, 140]. Recently a self-administered oxalate substance has shown to be applied as a gel strip. Using these adhesive strips based on oxalate leads to significant and durable crystalline formation within the dentinal tubule lumen. The application of oxalate through a gel strip has reduced a 70% of fluid movement via dentine specimens. This reduction was still evident after 30 d, suggesting substantial benefits for the treatment of DH [141]. Although oxalates tend to be an effective tubule occlusion agent, the surface residue that forms the layer is gradually dissolved by saliva; therefore, repeated re-application is needed frequently.

2.2.2.4 Strontium Salts

Many published clinical studies confirm the effectiveness of incorporating strontium salts in desensitizing pastes, which helps the patient understand the symptoms related to DH. An example of a commercially used dentifrice using strontium salts is Sensodyne Rapid Relief (GlaxoSmithKline, United Kingdom), with 8 % strontium acetate in silica base (and sodium fluoride). The occlusion of tubules formed by strontium acetate has not significantly doped the dietary acid challenge [142]. Other clinical studies have reported decreasing patients' pain perception after using strontium-containing toothpaste [143].

More recent research has verified the tubule-occluding effects of a toothpaste containing strontium acetate in situ. Still, it has indicated that the dentifrice's silica abrasive portion could be partially responsible for desensitizing impacts [144]. In addition, toothpaste formulations may include strontium stannous and calcium phosphate, which can form physical barriers that can occlude the dentine tubules. These processes exist on the dentine surface by precipitating insoluble metal components. Stannous chloride has also been reported to be effective in occluding dentin tubules. In contrast, NovaMin®, especially when exposed to citric acid and AS, has been reported to be more effective than strontium chloride and OTC toothpaste [145]. Other studies also discussed the possibility of Stannous fluoride in occluding dentin tubules by forming SnF_2 and CaF_2. Additionally, strontium chloride has been reported to block dentin tubules [48].

2.2.2.5 Arginine and Calcium Carbonate

Arginine and calcium carbonate compositions have been produced to treat DH problems depending on salivary glycoprotein tubule occlusion naturally present biological mechanism. Saliva transfers calcium and phosphate close to dentin tubules to cause occlusion and develop a stable salivary glycoprotein with calcium and phosphate. These results assisted industrial research and production of a formulation containing arginine, an amino acid that is positively charged at physiological pH; bicarbonate, which acts as a pH buffer; and calcium carbonate, which functions as a

calcium source. The occlusion of patent tubules in this research is confirmed by SEM and atomic force microscopy [54, 146]. Studies assessing the effectiveness of 8% arginine-calcium carbonate-fluoride-containing toothpaste in minimizing DH symptoms indicated improvement over 2% potassium ion-based toothpaste in an 8-week study [147]. In addition, Arginine dentifrice-containing formulations benefit from offering rapid relief of DH in topical applications [148]. Published studies have demonstrated that arginine/calcium carbonate is an essential agent for DH reduction and should be treated as the gold standard for DH management [54, 119, 149].

2.2.2.6 Calcium Hydroxide

Several studies have confirmed the efficacy of calcium hydroxide in treating DH [58]. Pashley *et al.*, found that when Ca $(OH)_2$ was applied on dentin, there was a rise in the concentration of calcium ions originating from calcium hydroxide within the tubules. This physical obstruction facilitated a decrease in dentin permeability [58, 150]. Romano *et al.*, confirmed that calcium hydroxide paste obtained considerably higher scores for tubule occlusion than only carbon dioxide laser irradiation [58]. The exact mechanism of action of calcium hydroxide is unclear, but it is proposed that it can obstruct dentinal tubules or facilitate peritubular dentin formation. It is suspected that the high pH induces proteins coagulation in the odontoblastic process, resulting in the closing of the tubules by deposition of these proteins, reducing hydraulic conductance [151]. However, Its observed necrosis of gingiva tissues is an opposing point of calcium hydroxide [152]. In addition, Zand *et al.*, reported that the depth of penetration into the dentin tubules of nanoparticle calcium hydroxide is far greater than that of standard calcium hydroxide[153].

2.2.2.7 Calcium Phosphate

The formulation of toothpaste can also promote the formation of calcium phosphate leading to intratubular mineralization [48, 59]. Moreover, the use of calcium phosphate materials has been found promising for treating DH. As it was the main component of HA, calcium is also essential for remineralization in tooth restorations, with apatite as a source of calcium phosphate that can be used in dental applications (e.g., HA and

fluorapatite). Deposition of HA on the surface of exposed dentin can lead to blockage of dentinal tubules. It has been shown that calcium phosphate may occlude the dentinal tubules without inhibiting spontaneous remineralization of the tooth surface [19, 108]. However, tubule occlusion may occur naturally through natural remineralization processes by saliva and dentin sclerosis through secondary dentin formation. There is also a defensive feature of saliva against tooth wear. The biofilm layer has been reported to facilitate remineralization and reduce mineral depletion. Therefore, using some of these dental products may help protect dentin from increasing its resistance to mechanical and chemical attacks. Increasing the mineral density is one means of growing dentine surface resistance to wear by acid erosion and abrasion; additionally, the occlusion of dentin tubules with a mineral additive such as calcium and phosphate toothpaste will enhance the dentine's acid resistance [129, 145].

2.2.2.8 Calcium Sodium Phosphosilicate

Calcium sodium phosphosilicate based toothpaste has also been proven effective in DH management [35]. *In vitro* experiments have shown that calcium, phosphate and silica together occlude tubules and tend to resist water and acid. Calcium phosphate and silicate deposit into dentine collagen, creating precipitates on the dentine surface and inside the dentine tubules [154-157]. Randomized clinical trials evaluating the effectiveness of the treatment of Calcium sodium phosphosilicate, potassium nitrate and stannous fluoride for DH control showed that while all three agents had treatment effectiveness, Calcium sodium phosphosilicate had more substantial and significant improvements in pain relief [158]. Recent research has demonstrated that toothpaste-containing Calcium sodium phosphosilicate can mineralize dentin and occlude dentin tubules and these obstructions can overcome dietary acid challenges [159].

2.3 BIOACTIVE GLASS

Bioactive materials have received significant interest in medical and dental use, especially in cases of bone defects. They have developed and taken on many applications since the development of 45S5 BG by Dr. Larry Hench [61, 62]. Before the discovery of BG, biomaterials were usually supposed to be inert when being in

contact with body fluids and anatomical structures; however, 45S5 BG changed this concept by offering an alternative to graft materials that were active and bonded to bone and released biologically active ions to facilitate osteogenesis [61], as they have the ability to generate HA and stimulate osteogenesis in physiological systems [62]. The BG has excellent bioactivity, osteoconductivity and osteostimulative properties. Therefore they have been used in bone-related biomedical applications such as bone grafts or fillers, dentistry, craniomaxillofacial applications and implants coatings [63]. Although the 45S5 BG was discovered in the late 1960s, the implant's first clinical application was a mid-ear bone replacement for the treatment of hearing impairment [61]. BG found its way to dentistry after several years. The application of bioactive glasses in edentulous patients was associated with bone replacement implants to provide a more secure ridge for denture construction. It has also been used as a bone repair tool for periodontal disorders and bone defects [62]. In 1988 the endosseous ridge maintenance implant was the first commercial application of dental implants [61]. Since many studies have confirmed the significant similarity between dentin and bone in terms of tissue composition (i.e., HA), several researchers have discussed the effectiveness of biocompatible BG as a material to incorporate into toothpaste formulations a tubular occluding [19, 160]. BG has been widely used in dental tissue engineering and dental implants as a coating material. Compared to BG synthesized using melting methods, BG produced using the sol-gel method has numerous benefits, including improved control over composition, size and shape and increased surface area [161].

By introducing the glass to aqueous solutions, the bonding of bioactive glass particles to bone starts with the exchange of sodium ions in the glass with hydrogen ions from the body fluids, allowing the pH value to rise. Calcium and phosphate ions eventually move from the glass, creating a top layer abundant in calcium-phosphate, under which a layer becomes gradually rich in silica due to the loss of sodium, calcium and phosphate ions [65] [66]. The presence of the silica-rich layer and the small size of the bioactive glass particles encouraged the researchers to consider using bioactive glass particles as a potential desensitizing agent for DH treatment. Bioactive glasses are composed of specific amounts of silicon, calcium, sodium and phosphorus oxides (SiO_2, CaO, P_2O_5 and Na_2O) [63].

Cruz *et al.,* summarized the mechanism of BG dentinal tubular occlusion as BG particles react when they are in interaction with a biological fluid like saliva and three processes occur (1) leaching and silanol creation, (2) breakdown of the glass network and (3) precipitation. An effective mechanism for occluding dentin tubules is precipitation. Releasing ions from BG leads to forming a layer composed of calcium and phosphate, which can mechanically block the Dentinal tubules and lower fluid movement within the dentine. This layer is crystallized into HA and the presence of silica can enhance HA maturation. Bioactive glass induces an osteoblast cell cycle in the bone, resulting in rapid cell proliferation and differentiation [60].

Many studies have confirmed the importance of silica in bioactive glass formulations. In most bioactive glasses, silica is the main constituent and serves as a nucleation site after dissolution to precipitation dissolved calcium and phosphate ions to create HA. Silica also defines the solubility and amount of active ions and functions as a former network, stabilizing the system [15, 65]. The breakdown of the BG silicate network releases the ionic constituents into a solution, producing in the immediate environment a supersaturated solution, resulting in the eventual nucleation of an amorphous calcium-phosphate layer that converts into bone-like apatite, thereby producing a tightly bound interface between the implant and the actual bone [162].

An *in vitro* study conducted by Wang *et al.,* showed that applying Novamin containing toothpaste on exposed dentine specimens causes occlusion of most open dentinal tubules due to the formation of different-sized particles embedded in a smear-like layer on the surface of the dentin and within the dentinal tubules. The decrease in dentine permeability was 81.5 %, while distilled water alone decreased the fluid flow by 70%. It was also found that these positive effects were better than toothpaste containing arginine [12].

Mahmood *et al.,* also discussed the mechanism of occluding exposed dentinal tubules using a toothpaste based on 45S5 BG (Novamin). This study confirmed the effectiveness of Novamin in eliminating DH. Large particles of BG (<90 μm) were used to provide a long-term release of Ca^{2+} and PO_4^3 ions within the saliva, leading to the formation of hydroxycarbonate apatite (HCA) on the dentine surface and within

the dentin tubules. Toothpaste containing BG has been shown to be more effective than other treatments that primarily use calcium carbonate due to HCA's lower acidic solubility than calcium carbonate. However, one potential drawback of 45S5 is that it has a very high hardness compared to the hardness of the thinner enamel at the cervical margins and the soft one near the DEJ. Thus, brushing teeth with 45S5 toothpaste can cause enamel erosion [59].

In a study aimed at evaluating the effects of BG on the exposed dentine surface, the original 45S5 bioactive glass was compared with a new toothpaste formula containing a doped BG* by substituting part of the abrasive silica content. It was indicated that replacing the silica component with different proportions of BG led to an increase in the surface coverage and tubules occlusion compared to the original formulas. In the same study, Macleans toothpaste alone showed better tubules occlusion than Macleans mixed with BG. Unlike Elmex Amine, which showed better results when combined with BG [160]. As a result, incorporating bioactive glass particles into a vehicle with suitable composition may be an effective desensitizing agent for the treatment of DH.

The research undertaken by Cruz *et al.*, aims to compare various bioactive glass formulations to confirm their effectiveness in a well-established laboratory model. Using a laboratory-synthesized 45S5 glass with different other glass formulations: (1) a mixed glass (fluoride and chloride), (2) BioMinF, (3) a chloride glass and (4) an amorphous glass of chloride. [60]. SEM images have improved coverage of mixed glass products, BioMinF and chloride glass compared to 45S5. However, after the acid challenge, the formed HA layer was rinsed again in varying proportions, with some tubules occluded remaining. At the same time, an amorphous chloride glass toothpaste displayed exceptional surface coverage. Many of the tubules are occluded by particles of varying sizes and after the acid challenge, the results were also positive because many of the dentin tubules were still blocked [60].

Curits *et al.,* has tested the effectiveness of a novel sol-gel nanobioglass and a bioglass derived from melt to occlude tubules and facilitate apatite formation. After treatment with a bio-glass powder derived from melting, calcium-phosphate coating with a distinct and unique platelet-like morphology characteristic of apatite formed. [163].

After treating with a nanobioglass slurry, rod-like growths located around open tubules and appearing to emerge from inside the tubules have been observed [163].

2.4 BORON DOPED BIOGLASS

Substantial research has focused on bioactive glasses with the increasing demand for biomaterials to replace and regenerate bone. As noted above, bioglasses are a promising material for their ability to precipitate HA upon contact with a physiological fluid, forming a solid bond to bone and soft tissue [65]. In recent years, there has been a significant focus on improving the biological activity of bioglasses and combining them with other biomaterials, such as biopolymers, to improve mechanical and physical properties, chemical stability and biological reactivity. Enhanced mechanical strength and toughness have been obtained, for example, by the creation of various glass or glass-ceramic compositions [164, 165]. An essential method for developing improved biological activity of BG is the inclusion of metallic ions in the glass structure, which alters the dissolution of these materials and their bioreactivity when submerged in biological fluids [166]. Metallic ions are essential components of human tissue and play an important role in controlling cell metabolism and regulating numerous biochemical reactions in the human body [167]. Elements like Si, Ca and P, found in bioactive silicate glasses like 45S5 Bioglass®, substantially stimulate osteogenesis and bone metabolism [168, 169]. Magnesium strontium, zinc and boron have also been demonstrated to affect osteoblast activity and stimulate angiogenesis [170]. In particular, the impact of incorporating boron into BG structure have been widely reported.

Boron is an ‘ultra-trace element' found in plants since 1857 and it was identified as an essential nutrient in plants in 1923 [171]. Several studies demonstrated that boron deprivation exacerbates bone deformities[161] . Boron stimulates wound healing *in vivo* , releases growth factors and cytokines, increases the Ribonucleic Acid (RNA) synthesis and increases extracellular matrix turnover [161, 172]. Boron is present in the human body in amounts ranging from 3 to 20 mg, with the most significant concentrations in bone, nails and hair [173]. The presence of boron affects several

metabolic processes and it interacts with calcium, vitamin D and magnesium and all are important in the bone metabolism process [174].

Numerous studies have confirmed that the presence of boron at certain levels is necessary for calcium metabolism. It has been proven that boron deficiency is detrimental to bone formation and growth processes. Boron is also essential to prevent excessive bone loss, expected as people become older [175]. Moreover, adding metal ions like boron to BG formulations improves their functionality and biological activity. Incorporating boron into BG increases their biodegradability and bioactivity [176]. Many investigations have reported that boron promotes the growth of human osteoblasts and mesenchymal stem cells [177, 178], osteogenic differentiation of pre-osteoblastic cells and osteogenic and odontogenic differentiation dental stem cells [176].

The addition of B_2O_3 has a considerable impact on glasses' thermal, mechanical and structural characteristics [179]. One of the early publications in this subject examined the effect of incorporating boron on the crystallization capacities and bioactive behavior of gel-derived glasses in the $CaO\text{-}P_2O_5\text{-}SiO_2$ system. The original composition (without any additives) was amorphous when it was derived by melting. However, when it was obtained using the sol-gel method, it revealed a crystalline HA ($Ca_5(PO_4)_3(OH)$) phase. It was found that adding 5 mol of boron to the original gel was sufficient to detect crystalline calcium silicate (Ca_2SiO_4) [180].

The researchers discovered that the borate glass had the greatest ultimate limiting value of weight loss, indicating that the reaction rate increased as the B_2O_3 amount increased. According to another relevant study, boron-doped gels $CaO\text{-}P_2O_5\text{-}SiO_2$ reacted similarly to the basic gel when immersed in Simulated Body Fluid (SBF). Boron didn't inhibit calcium solubility and after 5 days of immersion in SBF, a calcium phosphate layer formed on the boron-containing materials [180]. In a related study, mesoporous glasses made from the $CaO\text{-}B_2O_3\text{-}SiO_2$ system showed superior compositional homogeneity, textural characteristics and *in vitro* bioactivity than original sol-gel glasses with the same composition [181].

Huang *et al.*, reported the conversion of 45S5 BG, a borate equivalent of 45S5 glass (all SiO_2 replaced with B_2O_3) and two intermediate borosilicate BG to HA in a dilute (0.02 M) K_2HPO_4 solution at 37 °C [182]. Based on the results obtained in this study, the mechanisms by which borate (3B) and silicates (0B) are converted to HA in dilute phosphate solution are described in (Figure 2.5.) It has been reported that when a borate glass is immersed in a physiological solution, components such as Na_2O and B_2O_3 are released, forming Na^+ and BO_3^{3-} and the PO_4^{3-} in the solution interacts with Ca^{2+} to precipitate HA on the glass. The fast conversion of borate glass to HA is caused by the simultaneous dissolution of Na^+ and the phosphate solution's attack on the B-O network structure, resulting in the nucleation of HA (immediately). This is believed to be owing to structural changes in the glass network as B_2O_3 concentration increases, as boron ([BO_3] trihedra) cannot ultimately form a three-dimensional network as Si can. The porous structure of the precipitated HA allows for facile ionic transfer. The continuance of the dissolution–precipitation processes causes the resulting layer to thicken from the particle's surface inward. As a result, the dissolution-precipitation operations continue until the borate glass is wholly converted to HA. When it comes to silicate-based bioglass, it forms a porous SiO2-rich gel layer and separates the precipitated HA layer from the shrinking glass core. For the reaction to proceed, ions must dissolve from the glass core and diffuse through the SiO2-rich layer. In this study, although the initial concentration of PO_4^{3-} in solution was high enough to react with all Ca^{2+} in the glass to form HA, the reaction was effectively stopped before complete conversion to HA. According to these reactions, it can be concluded that borate glasses have lower chemical stability and a higher dissolution rate than silicate glasses.

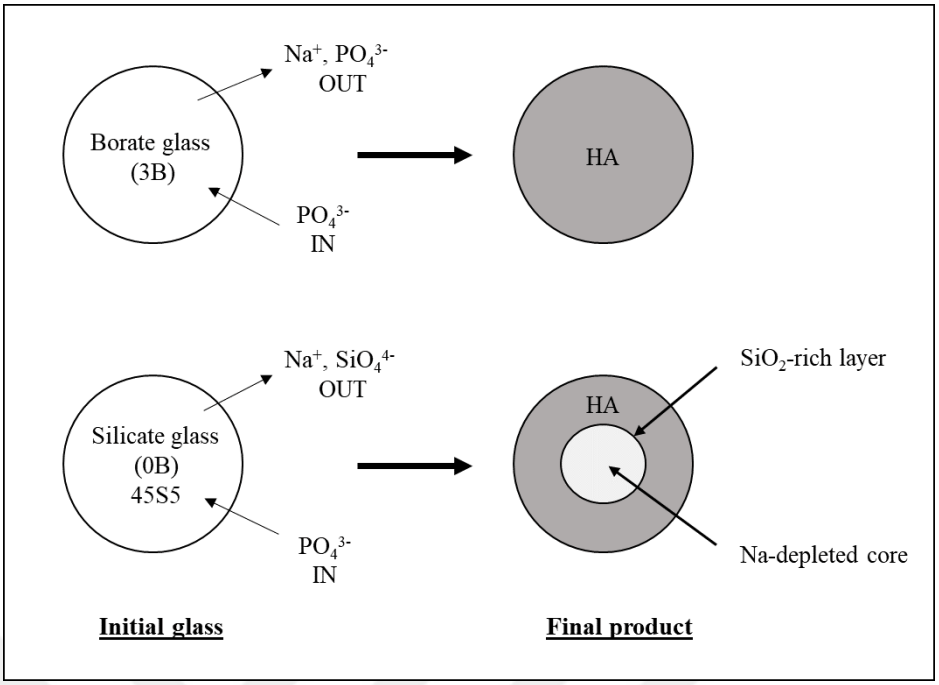


Figure 2.5. Schematic diagram showing the mechanisms for converting borate glass and 45S5 silicate glass to HA in a dilute phosphate solution [182].

Notably, the borate glass particles (3B) were wholly converted to HA in less than 4 d, while the 45S5 (0B) glass particles and two intermediate borosilicate formulations (1B and 2B) were partially converted, only even after 70 d.

It was also reported that the reaction rate and weight loss versus reaction time during immersion in phosphate solution increased with increasing the B_2O_3:SiO_2 ratio of glasses. After the 2400 h interaction for the 3B samples, the observed weight loss was >50%, while it was just < 10% for the 0B and 1B samples. The weight loss curve of the 3B sample exhibited a significant tendency to increase with time after 2400 h of immersion, indicating that the dissolving and conversion processes would probably continue until the glass reacted almost entirely to create calcium-phosphate [69].

Moreover, bioglasses doped with B_2O_3 have received much attention in recent years because of their rapid and full potential to convert to HA when immersed in SBF and decreased chemical durability, making them ideal scaffolds materials in tissue engineering. According to several observations in the literature, adding boric oxide to BG has several beneficial biological effects, including initiating wound healing *in vivo*

and improving the extracellular matrix [183]. This is consistent with other studies where it has been confirmed that the faster degradation of the borate-based bioactive glass and its conversion to HA, which initiates at the surface of BG, will have a significant impact on the ability of the scaffolds ability to promote cell proliferation and therefore on their potential usage *in vivo* [184]. Several studies have been performed *in vivo* using borate glasses. It can be indicated that borate-based BG has been shown to be non-cytotoxic [183], biocompatible with cells and promote cell adhesion, proliferation and differentiation under dynamic conditions. Thus, these glasses have improved the ability to stimulate angiogenesis, soft-tissue infiltration and new bone tissue formation *in vivo*. All of the results indicated that borate glasses, in the form of powders, pellets, scaffolds and fibers have the ability to heal bone defects and enhance bone regeneration [170].

Although researchers have commonly used BG as a biomaterial in dental tissue engineering [185] and in treating dentine hypersensitivity [60], incorporating boron with BG is not widely used in this field. However, results from a study on the effects of boron incorporation with BG on human dental pulp stem cells showed that boron incorporation increased intracellular calcium levels [161, 176] and immunohistochemical analysis showed enhanced expression of dentin sylophosphoprotein, osteopontin and Collagen type I in the groups contain boron [161]. As a result, B-BG nanoparticles have the potential to be used as biomaterials in dental tissue engineering applications [161]. A similar study indicated that B-BG nanoparticles are a promising material used in regenerative dentistry. It was reported that incorporating B-BG enhanced the deposition of a Ca-P-rich layer on the scaffold surface and improved cell adhesion, migration and odontogenic differentiation [176].

Table 2.1 summarizes the classification of the common desensitizing agents applied for the treatment of DH. Some desensitizing agents are commercially available products and a dentist professionally uses others. The mode of action of all treatments is based on dentinal tubule occlusion, except for potassium salts, which are based on neuronal depolarization. Despite the difference in the mechanism of action of treatments between physical, mechanical and chemical, the effectiveness of the

desensitizing agent lies in the rapid response to eliminate pain and acid resistance in the oral environment.

Table 2.1. Treatment strategies for DH.

Treatment method	Mode of administration	Mode of action	Mechanism of action according to literature
adhesive materials a. restorative resins b. bonding agents c. varnishes d. repairing resin composites e. Glass ionomer cement	Professionally	Dentinal occlusion	A hybridization method can create a thin-film coating layer over open dentinal tubules through micro-mechanical interlocking**.** In addition to the involvement of functional monomers, they have the ability to chemically bind with the remaining HA calcium ions that are present within the sub-micron hybrid layer[100].
Laser	Professionally	Dentinal occlusion	Lasers act in the dentinal fluid by coagulating proteins and thus reducing permeability [186]. They may also produce an amorphous closed coating on the surface of the dentine, which tends to be due to partial melting down of the surface [187]. Generally, the laser is preferred to other relevant therapies in DH treatment.
Iontophoresis	Professionally	Dentinal occlusion	Iontophoresis therapy can desensitize hypersensitive dentin by forming secondary dentin after applying an electrical current or raising the penetration and concentration of fluoride ions into the dentin tubules. Therefore, occluding the tubules and increasing stimulus conduction [188].
Periodontal Plastic Surgery	Professionally	Dentinal occlusion	Surgical treatments occlude the uncovered dentinal tubules, enhancing esthetics in the sensitive parts of gingival recession.
Potassium salts	OTC	Nerve Desensitization	Potassium ions have been reported to inhibit the neuronal response to pain signals by spreading around the tubules and increasing the concentration of local extracellular potassium ions, thus preventing intra-dental nerve activity [189]**.**
Fluorides	OTC	Dentinal occlusion	Fluorides decrease the permeability of dentin tubules by calcium fluoride precipitation on the surface of dentine with enhancement resistance to an acidic challenge [48].

Treatment method	Mode of administration	Mode of action	Mechanism of action according to literature
Oxalates	OTC	Dentinal occlusion	Oxalates can occlude dentinal tubules and reduce the permeability of dentine by precipitation of Calcium oxalate crystals inside dentine tubules [57].
Strontium Salts	OTC	Dentinal occlusion	The primary mechanism of action of strontium formulations was shown to be one of tubular blockage Via the involvement of strontium to replace calcium in HA [190].
Arginine	OTC	Dentinal occlusion	Arginine is an amino acid present in saliva that forms a seal in dentinal tubules by combining with calcium carbonate and phosphate that blocks fluid flow [54].
Calcium Hydroxide	OTC	Dentinal occlusion	Applying $Ca(OH)_2$ on dentin cause a rise in the concentration of calcium ions originating from calcium hydroxide into dentinal tubules and this physical obstruction leads to decreasing in dentin permeability [58].
Calcium phosphate	OTC	Dentinal occlusion	Dentinal occlusion occurs by remineralization processes. Increasing the mineral density means increasing dentine surface resistance to wear and acid. [129, 145].
Calcium Sodium Phosphosilicate	OTC	Dentinal occlusion	Calcium phosphate and silicate deposit collagen into dentine, creating precipitates on the dentine surface and inside the dentine tubules [58, 150].
Bioglass	OTC	Dentinal occlusion	Releasing Ca^{2+} and PO_4^{3-} ions from bioglass leads to forming a layer composed of calcium and phosphate, which can mechanically block the Dentinal tubules and lower fluid movement within the dentine [60]. Silicate is an essential component in bioglass, which serves as a nucleus for calcium and phosphate deposition. Therefore, bioglass helps an appetite film to form, which enhances the occlusion of dentinal tubules [191].

PART 3

MATERIALS AND METHODS

3.1 MATERIALS

In this thesis, the materials used to synthesize pure BG, B-BG, AS, phosphate buffer saline (PBS) and the acids (used in treating the dentin surface) were used summarised in (Table 3.1.).

Table 3.1. List of materials used in the research project.

Preparation of 45S5 BG and B-BG nanoparticles.		
Material	Chemical formula	Company
Tetraethyl orthosilicate (TEOS)	$(C_2H_5O)_4Si$	Merck (USA)
Triethyl phosphate (TEP)	$(C_2H_5O)_3PO$	Merck (USA)
Nitric acid	HNO_3	Merck (USA)
Tetrahydrate calcium nitrate	$Ca(NO_3)_2$	Merck (USA)
Nitrate sodium	$NaNO_3$	Merck (USA)
Boric acid	H_3BO_3	Sigma-Aldrich (USA)
Ethanol	C_2H_6O	Sigma-Aldrich (USA)
Preparation of AS.		
Calcium chloride	$CaCl_2$	Merck (USA)
Potassium chloride	KCl	Merck (USA)
Potassium dihydrogen phosphate	KH_2PO_4	Merck (USA)
Tris (Hydroxymethyl)Aminomethane	$(HOCH_2)_3CNH_2$	Sigma-Aldrich (USA)
Preparation of PBS solution.		
Sodium chloride	NaCl	Merck (USA)
Potassium chloride	KCl	Merck (USA)
Potassium dihydrogen phosphate	KH_2PO_4	Merck (USA)
Preparation of PBS solution.		
Material	Chemical formula	Company
Sodium hydrogen phosphate	Na_2HPO_4	Merck (USA)
Chemicals used in surface treatment of dentine specimens.		
Phosphoric acid	H_3PO_4	Sigma-Aldrich (USA)
Citric acid	$C_6H_8O_7$	Sigma-Aldrich (USA)

3.2 METHODS

3.2.1 Bioactive Glass Manufacture

The 45S5 bioactive glass was manufactured following the protocol by Pirayesh *et al.,* [192] within the laboratory of Karabuk University Biomedical Department. The bioactive glass 45S5 is composed of (SiO_2: Na_2O: CaO: P_2O_5) (46.1: 24.4: 26.9: 2.6 mol%). Firstly (33.5 ml), tetraethyl orthosilicate $(C_2H_5O)_4Si$) (TEOS) was added to 1 M (2.25 mL) pure nitric acid (HNO_3) with (48.6 mL) of distilled water to make 20 g of gel-derived powder. The mixture was permitted to react by stirring for 60 minutes to hydrolysis the precursor during stirring. The following reagents were prepared by reacting separately for 45 minutes sequentially as: 0.017 mol (2.9 ml) of triethyl phosphate $(C_2H_5O)_3PO$, 0.085 mol (20.13 g) of tetrahydrate calcium nitrate ($Ca(NO_3)_2$) and 0.16 mol (13.52 g) of nitrate sodium ($NaNO_3$). The formulated transparent liquid was kept for 5 days at room temperature in a sealed container to form the gel. After that, the gel was stored in a sealed jar for 1 day at 70°C and then dried in a drying oven for 1 day at 120°C. Finally, the obtained product was stabilized at 700 °C in a benchtop muffle furnace for 2 h to extract residual nitrates. The prepared powder was ground for 15 minutes by a laboratory porcelain mortar pestle and then sieved by a sieve tool with 80 μm diameter. The procedures for synthesizing B-BG were the same as those detailed above. SiO_2 was partially replaced with B_2O_3 (4.64 g H_3BO_3 was added to (16.75 ml of TEOS to 4.64 g of B) for borate modification. Boric acid was utilized as a starting material to make B_2O_3. The preparation producer is summarized in (Figure 3.1.).

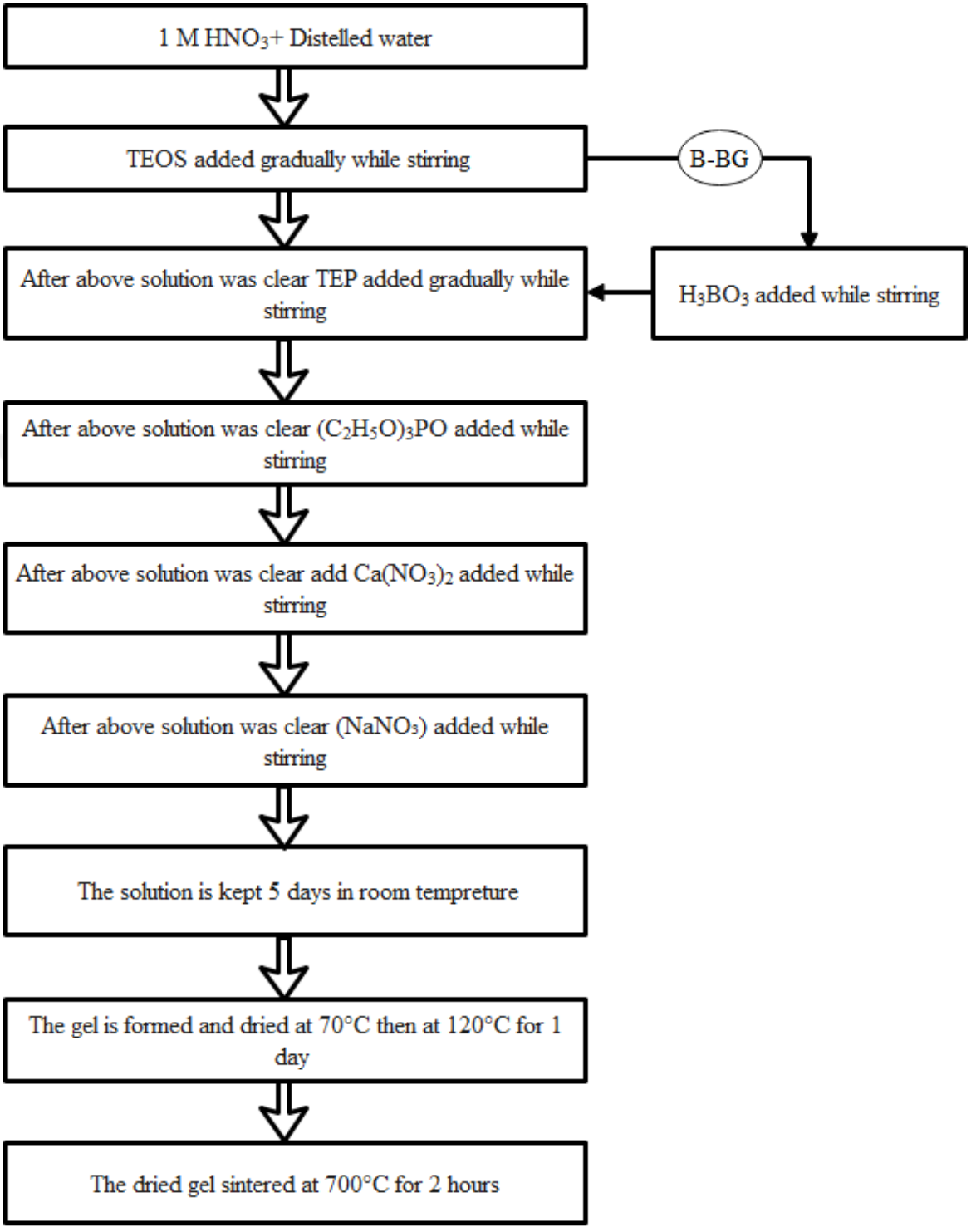


Figure 3.1. Flowchart for the preparation BG and B-BG materials.

3.2.2 Dentine Specimens Preparation

The preparation of the dentine samples is crucial for the accuracy of the dentinal tubules occlusion process. Extracted single-rooted human teeth were used for this experimental thesis. The teeth were collected from healthy adults after getting informed verbal consent under a protocol approved by the Ethics Committee of the Karabük University as y noninvasive clinical research (Karabük University 2021/590). The teeth were adequately washed with water no more than one month before their use at the laboratory. The teeth were completely padded in polymethyl methacrylate material from all sides before being cut. 150 Dentine discs were prepared by slicing 75 teeth. The teeth were initially cut (Figure 3.2. (A), then perpendicular to the long axis of the tooth above the cement-enamel junction (Figure 3.2. (B), using Secotom 50 (Struers, Denmark) cutting device by saw with direct water supply added to the blade. Each sample was cut with a thickness around 0.5 mm ±0.15-thick. Some unsuitable samples were excluded in order to achieve work quality. The dentine specimens were sectioned very carefully to prevent the appearance of enamel on the part nearest to the occlusal plane or the pulpal horns on the part closest to the cervical margin, as shown below.

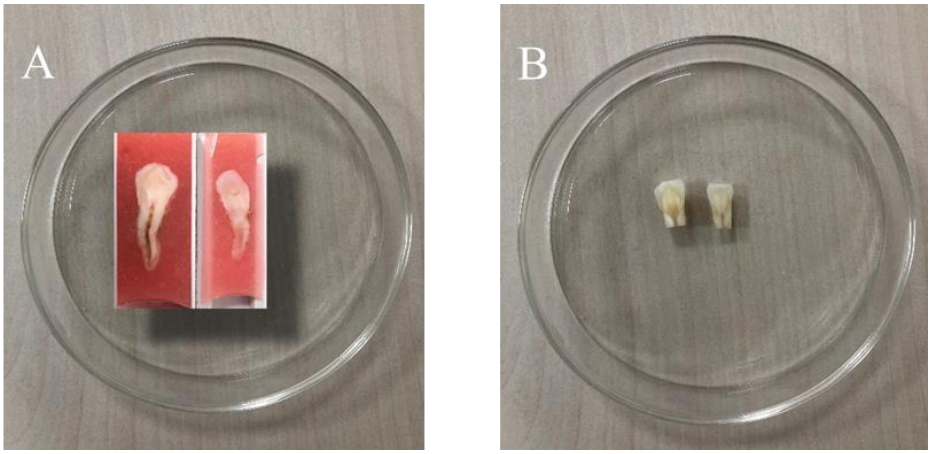


Figure 3.2. Longitudinal cross-section of dentin samples used in the experiment, A) after cutting vertically and B) horizontally.

3.2.3 Artificial Saliva and Toothpaste Application

AS was prepared in the university department's laboratory using the composition listed in (Table 3.2.) to provide an environment similar to the natural oral environment and store the teeth during the brushing process. The AS was prepared according to the protocol given by Wang [134]. The ingredients were weighed and added one after the other according to the order shown in (Table 3.2.) for one liter of distilled water with continuous stirring for half an hour for each ingredient. Then it was stored in a plastic container at a temperature of 4 °C until use in the experiment.

Table 3.2. The composition of AS storage solution.

Composition	Concentration (mmol/L)	Mass (g)
$CaCl_2$	1.5	0.166
KCl	50	3.72
KH_2PO_4	0.9	0.122
Tris	20	2.422

The consensus view on the DH mechanism supports the hydrodynamic theory that proposes that stimulation applied to the exposed dentinal tubules will lead to a transient change in pressure, leading to fluid movement in the tubules and subsequent activation mechanical receptors with associated pain sensation [38, 41, 78]. The toothpaste formulations used in this thesis work to block the dentine tubules and thus reduce the flow of fluid through them, which prevents the feeling of pain that travels from the tooth surface to the nerve.

Three different commercially produced toothpaste have been used against hypersensitivity, one toothpaste without anti-hypersensitivity ingredients, in Lab manufactured BG and B-BG particles. There were variations in inactive ingredients in all toothpaste listed in (Table 3.3.). The following toothpastes were used: 5%Novamine Toothpaste (Sensodyne Repair & Protect), 8% arginine toothpaste (Colgate Sensitive Pro-Relief), Sodium Fluoride toothpaste (Ipana Pro expert Clinic Line Sensitive) and toothpaste without anti hypersensitivity ingredients (Power Dent).

A 5 g of BG and B-BG particles were added to each 100 g of toothpaste separately. All ingredients have been weighed and mixed well in a plastic container. The selection of pastes in this thesis was based on previous studies that proved the effectiveness of the main active ingredient in the treatment of dentine hypersensitivity and the different methods of action to occlude the dentinal tubules. These studies relate, for example, that Arginine (Pro-Argine) is a viscous agent that physically clogs the open tubules [193], Calcium and sodium phosphosilicate (Novamin) forms a type of apatite when contact with an aqueous environment [163] and sodium fluoride can occlude the dentine tubules, enhancing resistance to an acidic challenge [48]. For two weeks, the specimens were brushed with undiluted toothpaste (approximately 1 g) manually for 2 minutes twice a day (at 10:00 and 17:00).

Table 3.3. Kinds of toothpaste used in the study.

#	Commercially product name	Components	Active ingredients	Company
1	Sensodyne Repair & Protect	Glycerin, PEG-8, Hydrated Silica, Calcium Sodium Phosphosilicate (NOVAMIN), Cocamidopropyl Betaine Sodium Methyl Cocoyl Taurate, Aroma, Titanium Dioxide, Carbomer, Sodium Saccharin, Limonene, Sodium Fluoride.	5%Novamin	Glaxo Smith Kline
2	Colgate Sensitive Pro-Relief	Calcium Carbonate, Aqua, Sorbitol, Arginine, Flavour , Poloxamer 407, Sodium Monofluorophosphate, Zinc Oxide, Cocamidopropyl Betaine, Benzyl Alcohol, Carmellose Gum, Zinc Citrate, Sodium Bicarbonate, Tetrasodium Pyrophosphate, Xanthan Gum, Sucralose, Saccharin Sodium, limonene, CI 77891.	8% arginine	Colgate-Palmolive
3	Ipana Pro expert Clinic Line Sensitive	Aqua, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, Carrageen, Aroma, Sodium Gluconate, Stannous Chloride, Xanthan Gum, Zinc Citrate, CI 77891, Sodium Hydroxide, Sodium Saccharin, Sodium Floride, Eugenol, Limonene.	Stannous Fluoride	Protector & Gamble Manufacturing
4	Power Dent	Aqua, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, Aroma, Cellulose Gum, Sodium Floride, Agar, Sodium Saccharin, Sodium Benzoate, Potassium Sobrate, Menthe Piperita Oil, Eugenol, Limonene, CI 77891,CI 42090.	No anti-sensitivity ingredient	Evyap Sabun

3.2.4 Experimental Design

The sectioning of dentine specimens from human teeth produces a smear layer in the dentine tubules and on the specimen's surfaces that affect dentine permeability. This smear layer consists of grinding debris produced by sectioning, with particles ranging in size from less than 0.5 μm to more than 15 μm [194]. To remove this smear layer and give the sample a standard thickness and surface morphology, the occlusal side of dentine specimens was sanded with 600-grit silicon carbide paper for 30 seconds [12], which led to more debris intrusion into the dentine tubules. Some researchers have suggested that a smear layer can help in occluding the dentinal tubules and reducing dentin permeability [195]. Therefore, this smear layer was consequently removed by exposing samples to 37% phosphoric acid (pH 7.4) solution for two minutes, then sonicated with the ultrasonic cleaner, which left the dentinal tubules completely open for treatment.

The specimens were equally distributed into seven groups for each treatment agent. Each contains eight dentine discs that are brushed with the different toothpastes and four subgroups according to the tests and analyses. All specimens were immersed in prepared AS, which changed every 24 h for 14 d.

Five discs from each group were prepared directly for SEM and EDX investigations, *in vitro* bioactivity evaluation and weight gain/loss measurements. One sample was prepared for X-ray diffraction analysis (XRD). All tests are performed before and after treatment for all samples. For two weeks, the dentine discs were brushed with Denta (soft) toothbrush.

The groups were distributed as follows: Group 1: phosphoric acid-etched specimens were brushed with Sensodyne Repair & Protect for 2 min; Group2 (Control 2): phosphoric acid-etched specimens were brushed with Colgate Sensitive Pro-Relief for 2 min; Group 3: phosphoric acid-etched specimens were brushed with Ipana Pro expert Clinic Line Sensitive for 2 min; Group 4: phosphoric acid-etched specimens were brushed with placebo Power Dent toothpaste for 2 min; Group 5: phosphoric acid-etched specimens were brushed with BG mixed with Power Dent toothpaste for 2 min;

Group 6: phosphoric acid-etched specimens were brushed with B-BG mixed with Power Dent toothpaste for 2 min; Group 7: phosphoric acid-etched specimens were brushed with AS for 2 min; Each specimen was brushed manually with undiluted toothpaste (approximately 1 g).

The SEM specimens were kept in AS (pH 7.4) for 24 h at 37 ºC and rinsed with deionized water, then treated with 6% citric acid (pH 1.5) for two minutes and rinsed in deionized water. The experimental design is summarized in (Figure 3.3.).

3.2.5 Statistical Analysis

Statistical analysis of the data was performed using statistical analysis system software known as IBM SPSS (Statistical Package for the Social Sciences) version 22 for windows. Analysis of Variance (ANOVA) one-way, Post-Hoc, Tukey's and homogeneity of variances tests were conducted to discover whether there are statistically significant differences in weight loss/gain depending on the test factor and treatment method and to evaluate the Ca/P ratio before and after applying test agents and after acid challenge. Differences between groups were considered significant at $p \leq 0.05$. The data were presented as a mean and standard deviation.

3.3 CHARACTERIZATIONS

3.3.1 Scanning Electron Microscope with Energy dispersive X-ray Spectroscopy Analysis

SEM analysis was used in conjunction with EDX to build a chemical profile of synthesized BG, B-GG and exposed surfaces and indicate the contents of the dentinal tubules. The implementation of SEM imaging for dentine tubule occlusion can provide an overview of the dentine surface morphology and quantifiable data where an image scoring system can be employed. SEM analysis was carried out using the Zeiss Ultraplus SEM machine (Zeiss, Germany). Five dentine discs were prepared for each treatment group for SEM/EDX investigation. The specimens were dehydrated in graded acetone, critical point dried and sputter-coated with gold-palladium in a

vacuum evaporator. Then, the specimens were examined under SEM at 10 &12 kV acceleration voltage. Standardized Micrographs of the dentine surface were acquired at a magnification of 2500 & 3000. Five images were acquired per disc. In addition, EDX detector attached to a SEM (Bruker XFlash 6|10, Billerica, USA) was used to identify Chemical element analyses of the dentine surface induced by the different treatments.

3.3.2 Transmission Electron Microscopy Analysis

Transmission electron microscope (TEM), (CM120, PEG, Philips) operating at 120 keV was used to examine the morphology and average size of the synthesized BG and B-BG particles. The TEM samples were ultrasonically dispersed in ethanol for 15 minutes before being placed in tiny droplets on carbon-coated copper grids for TEM analysis. To evaluate particle size distribution, measurements of particles size were made on 100 random locations in TEM images using image analysis software Image J.

3.3.3 Fourier Transform Infrared Spectroscopy Analysis

Fourier transform infrared spectroscopy (FTIR), (Bruker VERTEX 70v) was performed to identify functional groups and characterize covalent bonding in BG and B-BG particles. The chemical bonds in the molecules were determined by creating an infrared absorption spectrum. The spectra were obtained in reflectance mode in the mid-infrared region between 400 and 4000 cm^{-1}.

3.3.4 X-ray Diffraction Analysis

XRD analysis was used to characterize the synthesized BG, B-BG particles and crystalline materials deposited on dentine surface. Information on crystallinity, phases, crystallite size and other structural parameters is given. The XRD analysis is done with an X-ray source of Cu Kα radiation (30kV/20mA). The range of 2θ angles was from 10 to 90, at a step size of 0.02 and a step time of 1 s.

3.4 *IN VITRO* BIOACTIVITY

3.4.1 Ion Release Profiles

Inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7800 ICP-MS) analysis was performed to determine the concentrations of released ions from synthesized BG and B-BG particles in PBS at different periods. PBS (7.4 pH) was prepared in the laboratory [196]. The composition of PBS is shown in (Table 3.4.). Equal amounts of BG and B-BG particles were taken and immersed in falcon bottles containing 50 ml of PBS. The samples were incubated for 1,3,7 and 14daysat 37°C. The filtrate was collected and examined to determine the level of bioactivity of the prepared materials. The capacity of bioactive glass to generate HA in physiological environments is one of its most important properties. BG and B-BG powders were immersed in PBS to approximate how a solution containing physiological ion concentrations would react with the material to confirm that the glass promoted HA production. The dissolution and release of active ions from toothpastes directly affect the rate and stability of the active substance on the surface of the dentin, which in turn affects the physical and mechanical properties of these materials. Accordingly, dentine specimens were soaked in AS solution for seven d. The amounts of active ions leached and the changes in the mineral composition were investigated.

Table 3.4. Components of PBS.

Sample No	**Component**	**Amount**
1	NaCl	8 g
2	KCl	0.2 g
3	Na_2HPO_4	1.15
4	KH_2PO_4	0.2
5	distilled water	800 ml

3.4.2 Weight Measurement of The Deposited Layer

The specimens were weighed (n = 5 for each test factor) using an accurate analytical balance (Kern & Sohn, Germany) after 37% phosphoric acid immersion. They were dried at 40°C and their weights were taken after applying the treatment agents to the samples for 14 days to determine the amount of deposited layer on dentin samples. Then the samples were placed in closed plastic containers and AS was added (pH = 7.4) to the bottles and incubated at 37°C in a water bath for 10 d. The samples were taken from the containers, the excess water was removed by drying and their weight loss was measured according to the following formula in (3.2)

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} X100 \quad (3.2)$$

Weight loss measurements help evaluate the kinetics of the conversion process of BG and B-BG to HA in AS because the conversion reaction is associated with a loss in the mass of the glass. The results were compared and discussed

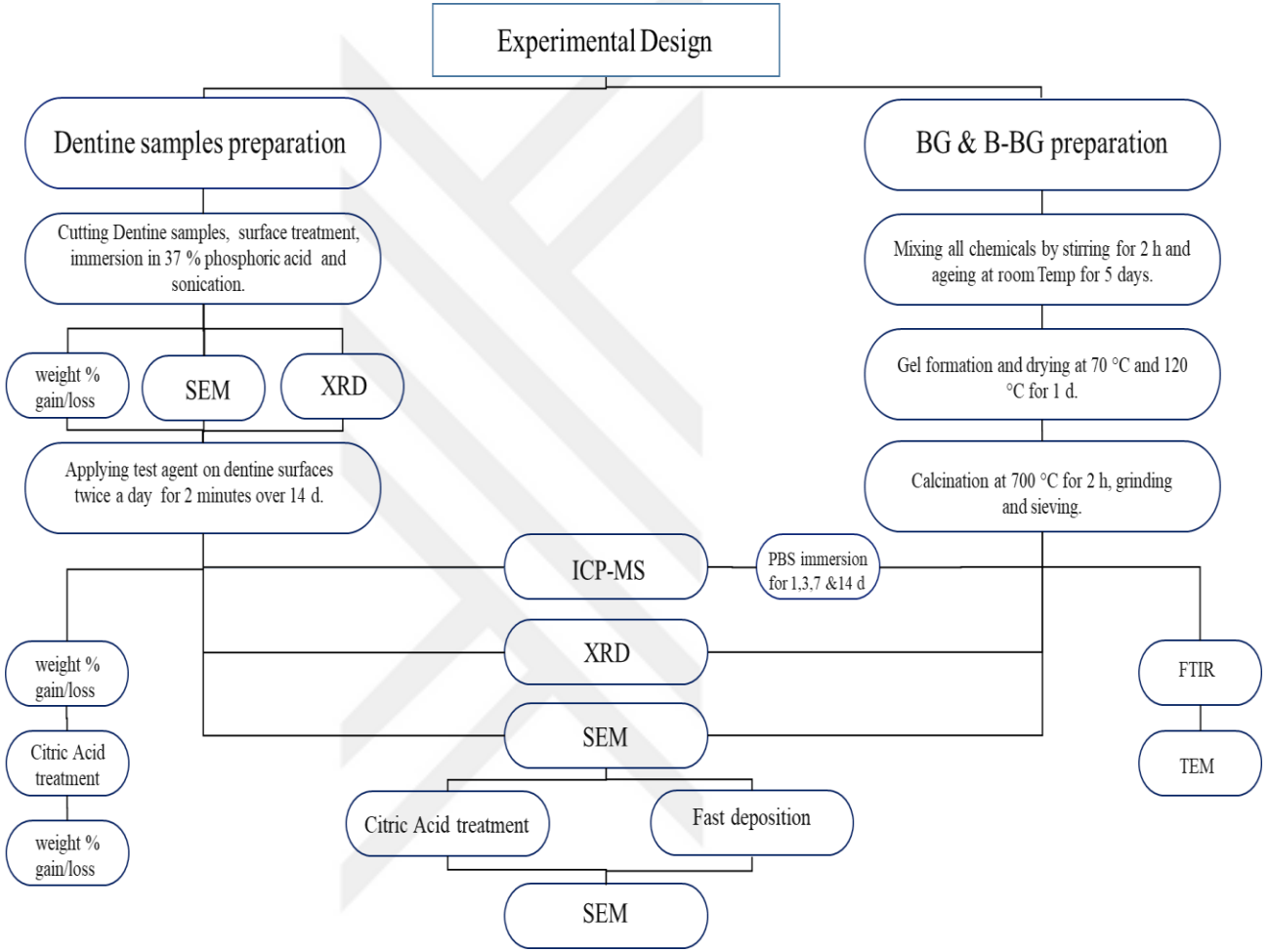


Figure 3.3. Summary of the experimental design.

PART 4

RESULTS AND DISCUSSION

4.1 BIOGLASS AND BORON-DOPED BIOGLASS: MICROSTRUCTURAL AND *IN VITRO* DEGRADATION ANALYSIS

4.1.1 Scanning Electron Microscope Analysis

SEM was a useful technic for obtaining high-magnification 3D images of the surface of glass particles. According to the SEM image (Figure 4.1. (A) & (B)). The as-prepared BG and B-BG particles had almost spherical shapes and homogenous size distribution. The size of BG and B-BG particles was about 200 nm and 50 nm, respectively. These results showed that both BG particles were also very fine and agglomerated. The sol-gel technique is predicted to form these sorts of particles. The addition of boron to the glass particles resulted in a reduction in particle size, which could be due to a faster gelation rate for B-BG than BG. This can be attributed to the rapid reaction between boric acid and TEOS and the formation of the H_3O^+ cation [197], which is expected to shorten the gelation time of solution, reducing the chance of colloidal particles accumulating and growing [198]. Reducing the particle size could increase the biological activity and make the particles more favorable for biomedical applications. The amount of bioactive glass and the particle size have been demonstrated to influence the glass's bioactivity. Several studies have found that glass with larger particles and a greater number of particles has lower biological activity than glass with smaller and medium-sized particles [179, 197, 199, 200]. In addition, the microstructures of B-BG particles were coarser than BG particles.

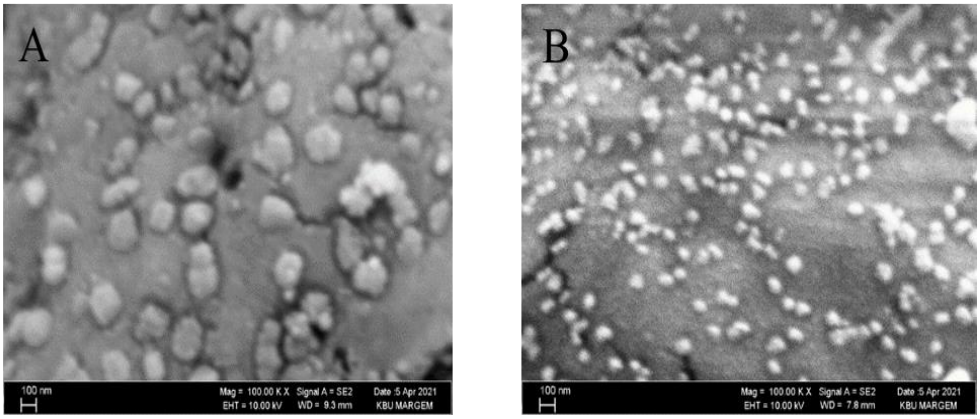


Figure 4.1 SEM images of (A) 45S5 BG and (B) B-BG particles synthesized by sol-gel method.

4.1.2 Transmission Electron Microscopy Analysis

The size and morphology of synthesized BG and B-BG particles were examined using TEM. TEM images of BG and B-BG surfaces were shown in (Figure 4.2 (A) and Figure 4.2 (B), respectively. Figure 4.2 (A i) shows the particle size distribution of the BG. The BG particle size was between 116 and 439 nm, with an average 258 nm. Furthermore, the particles' shapes do not appear to be spherical. Ellipsoidal particles with an agglomerated structure have also been detected, explaining the wide size distribution.

However, incorporating boron into the BG structure affected its particle size and resulted in a decrease in particle size below 100 nm. Several previous studies have proven the narrowing of BG particles after adding B_2O_3 [179, 197]. The Figure 4.2. (B i) showed the particle size distribution of the B-BG, from the schematic diagram, it showed that the BG particles were nano-sized, the particle size ranged from about 21 nm to 91 nm and more than 60% of the B-BG particles had **a** spherical shape. However, the average particle size was 59 nm.

The size of the BG particles can affect their biological activity. It has been shown that reducing the size of the bioactive glass particles to a smaller nanoscale leads to an increase in the interaction of the particles by releasing more ions into the surrounding environment [199]. Smaller nanoparticles are also known to enhance the number of

nucleation sites by raising the surface-to-volume ratio and thereby improving the crystallization kinetics of glasses with surface started crystallization [201]. in previous studies, It has been demonstrated that adding boron to bioglass decreased the specific surface area and increased the mesopores, resulting in a broader pore size range HA [197], as well as, the presence of boron resulted in a rapid formation of HA [197, 202, 203]. Moreover, it is clear from Figure 4.2 (A) and Figure 4.2 (B) that the BG and B-BG particles before application to the exposed dentin surface, both samples possess a smooth surface and after contact with the dentinal tubules and immersion in AS, SEM micrographs reveal the formation of apatite-like crystals that blocked the dentinal tubules and deposited on the surface.

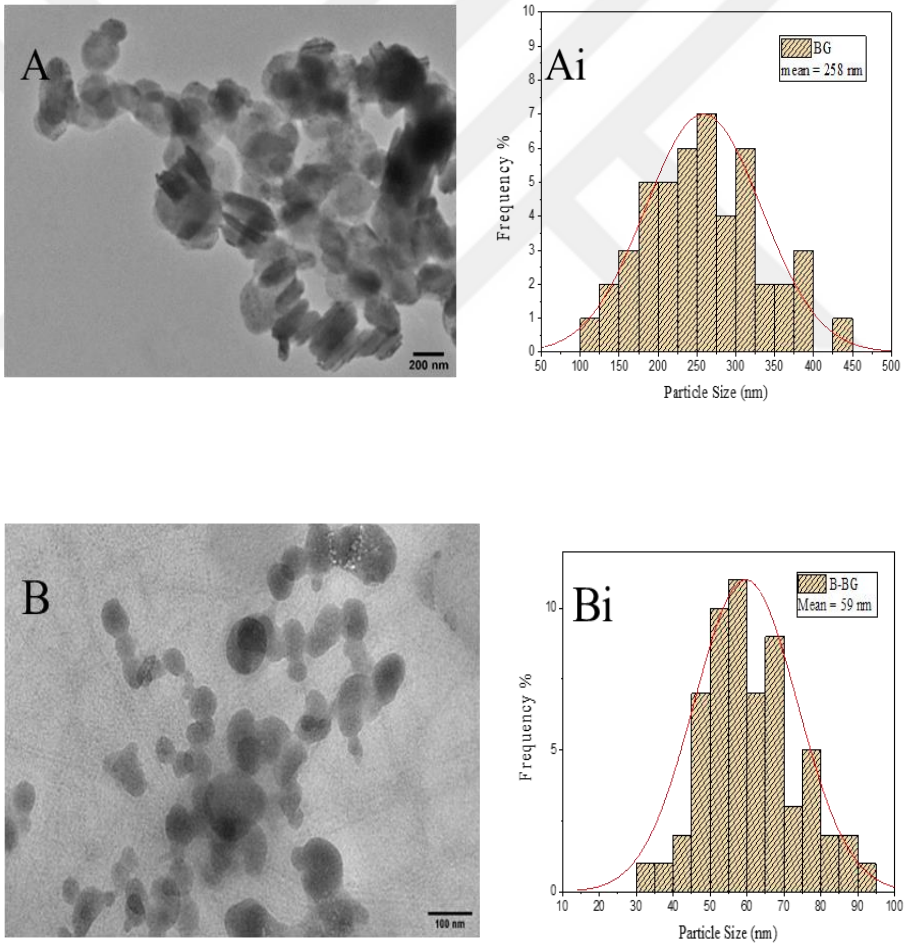


Figure 4.2. TEM images of (A) BG particles (A*i*) Particle size distribution diagram of BG particles, (B) B-BG particles, (B*i*) Particle size distribution diagram of B-BG particles.

4.1.3 X-ray Diffraction Analysis

The amorphous/crystalline phases of synthesized BG and B-BG nanoparticles produced by the sol-gel method were shown by XRD analysis. The broad dispersive band expresses the amorphous phase, while the sharp peaks show the crystalline nature of the produced crystalline bioactive glasses [204]. As crystallization is known to occur at temperatures around 620 ºC and above, BG with heat treatment at 500 ºC is predicted to be amorphous [205]. The prepared BG and B-BG powders were heated at 700° C. This explains the presence of the distinct sharp peak between 2θ= 31° – 34°. The addition of within glass resulted in shift the peaks towards high 2 θ values, which can be attributed to the decrease in lattice parameters.

Although it has been pointed out that B-BG crystallizes at a lower temperature than pure 45S5 BG, it causes a more solid and interlocking structure [183]. There was no new crystalline phase formation after doping boron.

The crystalline phase detected by XRD for BG and BPG was illustrated in (Figure 4.3.). The presence of peaks ((202), (121), (024), (220), (404), (424)) at (23.81°, 26.87°, 33.60°, 34.24°, 48.74°, 60.92°) respectively, reveled the primary formation of crystalline phase of $Na_4Ca_4(Si_6O_{18})$ (JPDCS- 00-075-1687). The peaks ((1 1 2), (0 2 2), (1 3 0), (0 2 3)) at (32.34°, 32.67°, 33.29°, 44.48°) confirmed the formation of the crystalline apatite-like phosphorus-rich phase of $Na_2Ca_4(PO4)_2SiO_4$ (JPDCS- 00-032-1053).However, other sodium-calcium silicates have also been reported as potential crystal phases derived from BG 45S5. These discrepancies could be due to several factors such as the shape of the raw powders, preparation method, particle size and heating conditions [206, 207].

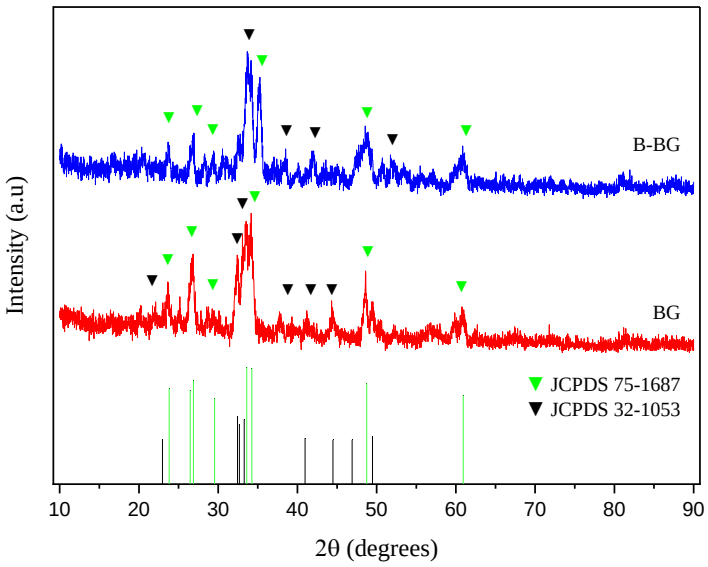


Figure 4.3. XRD patterns of BG and B-BG.

4.1.4 Fourier Transform Infrared Analysis

FTIR was used to identify the functional groups of 45S5 BG and B-BG. FTIR spectra of BG and B-BG indicated many spectral bands produced by various vibrational modes (tetrahedral, symmetric triangles and asymmetric units). As shown in (Figure 4.4.), FTIR of BG and B-BG are almost similar and revealed typical bioactive glass absorption bands. Even though glass is composed of several components, nearly all-glass forms create a similar-looking absorption band because the main glass structure has a Si-O backbone [208]. After incorporating boron with bioglass structure, the band intensities become weaker between 450 cm^{-1} and 1025 cm^{-1} region. This can be attributed to the minor structural changes in BG, proven by SEM and TEM results. Since B has lower molecule mass than Si, FTIR spectra of B-BG revealed shifting of the peaks toward lower wavenumber. The vibrations frequency is inversely proportional to the mass of the vibrating molecules. Therefore, B-BG had a higher wavenumber and vibration frequency than BG.

The absorption band observed at 456 cm^{-1} and 442 cm^{-1} for BG and B-BG, respectively, can be attributed to Si–O–Si bending vibration, which is commonly associated with amorphous glass formations [209]. Based on previous studies, the broad absorption band at 1015 cm^{-1} and 1023 cm^{-1} can be attributed to asymmetric

stretching of Si-O-Si groups in BG and B-BG, respectively [210, 211]. The bands at 840 and 930 cm^{-1} can be attributed to the asymmetric bending vibration of Si–O–Si; as reported in previous literature, the absorption bands at 520 cm^{-1} and 620 cm^{-1} correspond to the bending vibration of P–O in PO_4^{3-} function group [212].

The FTIR spectra of B-BG contain vibrational bands typical of the borate network, which is composed of triangular (BO_3) and tetrahedral (BO_4) groups. The asymmetric stretching vibrations of B-O in BO_4 units are active in the range 800–1200 $cm^{-1,}$ as proved by the presence of the strong bands at 1017 cm^{-1} and 1191 cm^{-1}. The high-frequency absorption between 1200 –1550 cm^{-1} can be attributed to BO_3 units, explaining the absorbing at this region. The bending of B-O-B vibration modes of different borate units comprises the mid region's low-frequency area (500 – 800 cm^{-1}) and the absorption band at 750 cm^{-1} exhibited this mode [210, 213, 214].

Additionally, the existence of absorption bands around 1725 cm^{-1} in the FTIR spectra of both samples proved the silicate glass's hygroscopic characteristics and this absorption band was correlated to the existence of bending vibration modes of O-H groups [210, 211]. Furthermore, the broadband at 1460 cm^{-1} was attributed to absorbed C–O functional groups [69].Since the BG and B-BG particles were prepared in air, CO_2 from the environment is likely to have dissolved into the solution, resulting in the substitution of CO_3^{2-} into the structure [182]. The bands at around 2180, 2320 and 3450 are related to different modes of water, O–H, or silanol groups [210].

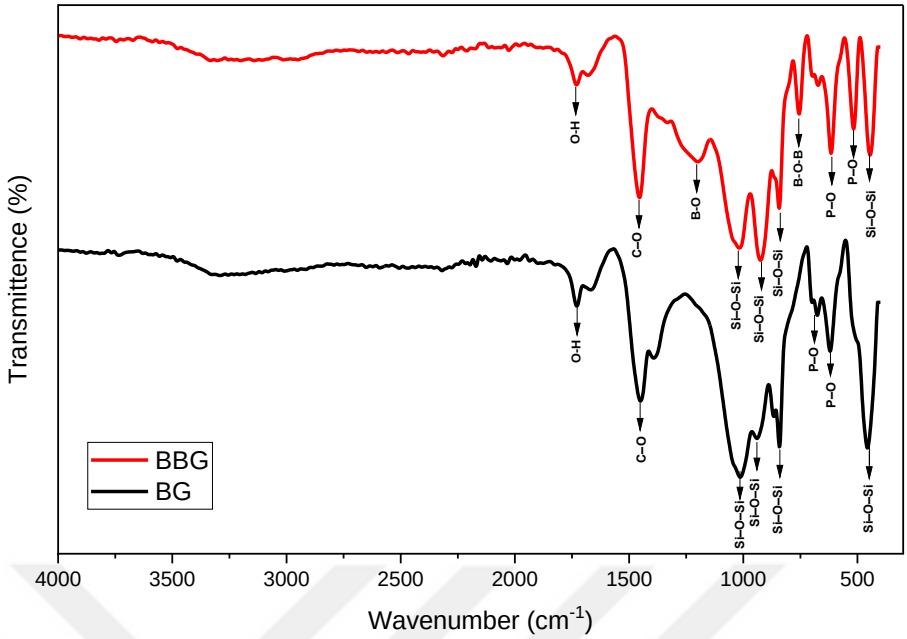


Figure 4.4. FTIR spectra of BG and B-BG

4.1.5 *In Vitro* Degradation Analysis

Bioactive glasses are reactive materials after coming into contact with body fluids or similar solutions. A sequence of events occur on their surface, starting with the partial dissolving of the glass surface, which prompts the formation of a silica-rich layer and subsequently the precipitation of amorphous calcium phosphate, which crystallizes in HA [215-217]. The chemical composition of BG plays a vital role in evaluating its bioactivity; adding or incorporating any other component to BG affects its biodegradation rates and mechanical properties [179].

ICP-MS test was conducted to examine the effect of B_2O_3 addition on BG biodegradation and dissolution behavior. This analysis was performed to test the ion release on the glass formulations at intervals of 1, 3, 7 and 14 d. 0.15 g of glass was added to 50 ml of PBS and incubated at 37 °C. A critical characteristic of bioactive glass is releasing ions into the surrounding environment and maintaining this release throughout the degradation period. Enhanced ion concentrations provide therapeutic effects such as increased osteogenic, antimicrobial and anti-inflammatory capabilities. ICP-MS tests were used to identify the ion release profiles of each element in the glass

structure. The release profiles of ion concentrations for Si, Ca, Na and Ca are shown below. It is evident that each ion concentration changes from 1 to 14 d. This oscillation indicates that the glass dissolution causes a continuous release of ions during the test period.

As determined by the ICP-MS technique, the changes in Si concentrations are shown in (Figure 4.5 (A)). The breakdown of the glassy network coincides with the release of silicon into PBS. On the first day of immersion in PBS, the bioactive glass exhibits a significant Si ion release 940638 parts per billion (ppb) and their concentration continued to increase gradually during the 3^{rd} and 7^{th} day (1632743 ppb and 1008224 ppb) to reach then to the saturation phase on the 14^{th} day (1264829 ppb). However, the amounts of silicon ions released from B-BG were not as high as in pure BG. The concentration of Si ions released from B-BG during 3^{rd} day was decreased from 540445 ppb to 128275 ppb. On the 7^{th} day of immersion, Si increased again to 351224 ppb and kept increasing sharply until the 14^{th} day (896190 ppb). This measured release profile might be related to the precipitation of insoluble salts containing Si, or it might be due to the newly produced apatite, which acts as a diffusion barrier, reducing Si release [218].

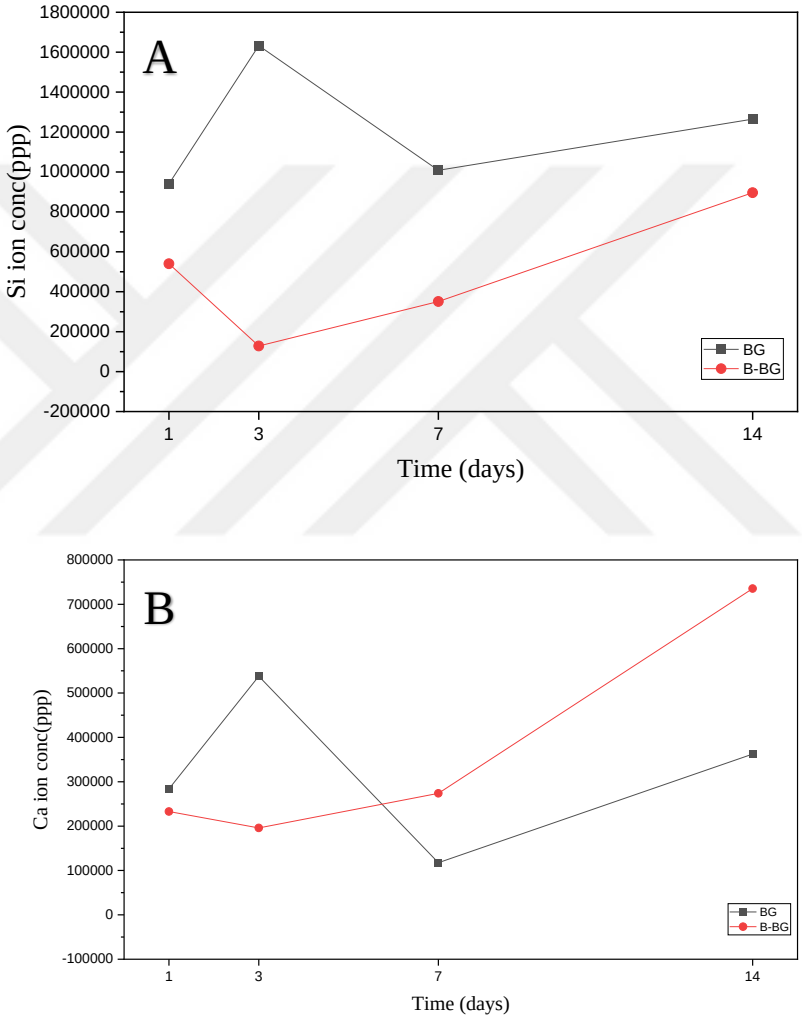
According to the results, the amount of Si ion concentrations in B-BG was consistently lower than that in pure BG, which can be attributed to the substitution of SiO_2 with B_2O_3. The boron concentration was 245361 ppb on the first day of immersion in PBS. After the 3rd d, the ion concentration increased to about 525848 ppb and was almost constant until the 14th d, indicating the dissolution of the boron network during 3 days of immersion (Figure 4.5. (E)). The borate network in B-BG is less durable than the silicate glass network. It is expected that the degradation of this glass will be controlled by breaking the borate network. (Figure 4.5.(C)) shows the concentration of phosphorous ions, amount of released ions decreased to the lowest level on the 3^{rd} day of immersion of B-BG in PBS from 312101 ppb to 96500 ppb, in accordance with the increase in boron. The fact that the phosphorous is low indicates the formation of a Ca-P layer on the glass [219].

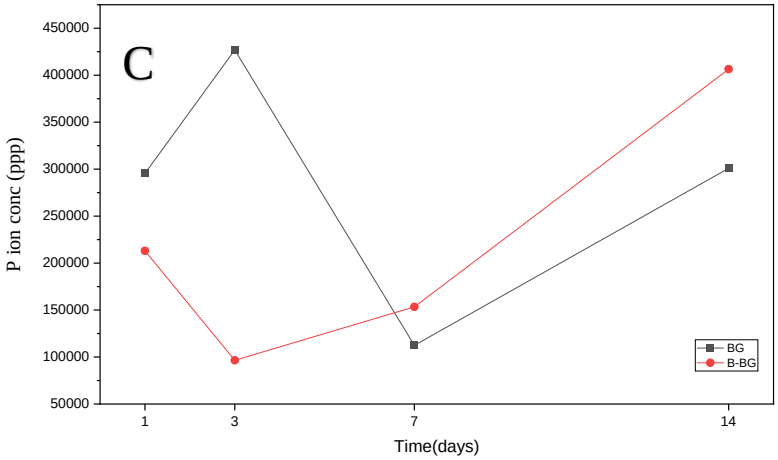
On the other hand, on the 14th day of immersion, the calcium concentration was almost twice the phosphorous concentration in the B-BG sample 735507 and 406374, respectively. In contrast, calcium and phosphorous ion concentrations were very close in the BG sample (362316 ppb and 300872 ppb) (Figure 4.5. (B)). Moreover, based on the current results and literature data, the boron ion concentrations released from B-BG in PBS were significantly below the toxic level [220-222]. The low rate of releasing boron ions made it biocompatible *in vivo* and non-toxic to surrounding hard or soft tissues. It should be considered for tissue engineering applications in humans [222].

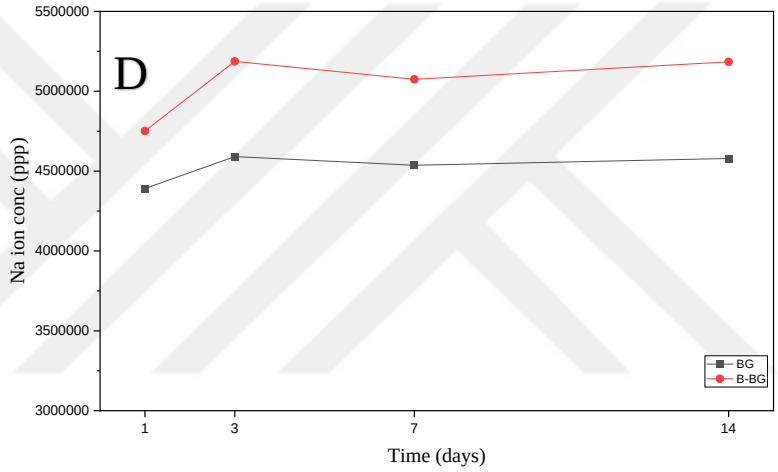
According to (Figure 4.5. (D)), the rate of Na ion concentrations was maintained during the immersion period. The amount released from B-BG was higher than that from pure BG (5057244 ppb and 4536485 ppb, respectively). This can be attributed to the decomposition of B-BG in phosphate solution accompanied by the release of glass modifiers, such as Na, K, Ca and Mg and the transformation of glass into a substance in the form of HA [184]. It has been discussed that immersion of borate glass in a physiological solution releases some components such as Na_2O and B_2O_3, forming Na^+ and BO_3^{3-} and the PO_4^{3-} in the solution interacts with Ca^{2+} to precipitate HA on the glass [170]. The fast conversion of borate glass to HA be caused by the simultaneous dissolution of Na^+ and the phosphate solution's attack on the B-O network structure, resulting in the nucleation of HA. [170, 184]. As shown in (Figure 4.5. (D)) and (Figure 4.5. (E)) the concentrations of boron and sodium ions remained relatively stable after 3 d, suggesting that they were fully dissolved.

The concentration of calcium ions over the immersion period was varied between low and high in both BG and B-BG due to the continuous degradation and precipitation process. As shown in (Figure 4.5. (B)), the Ca^{2+} concentrations of BG and B-BG leaching were 284085 ppb and 232866 ppb, respectively. Ca^{2+} are likely to be released quickly and replaced by H_3O^+ ions present in the surrounding environment. The level of Ca^{2+} concentration in B-BG on the 14th .day was high compared to BG, where it was 735507 ppb and 362316 ppb, respectively. Although Ca^{2+} can be precipitated in a stable type of calcium phosphate, several studies have shown that B-BGes release high Ca ion concentration than pure BG, Which may be explained by the weaker glass

structure generated by B_2O_3 replacement [219, 223]. The solubility of the bioactive glasses depends on their connection to the network [224]. As the B_2O_3 content increases, the connection of the glass network decreases, resulting in higher glass solubility [225]. As a result, the initial rise in Si, Ca and B concentrations and the decrease in P concentration were attributable to the degradation of particles and the formation of HA on the surfaces of particles [179].







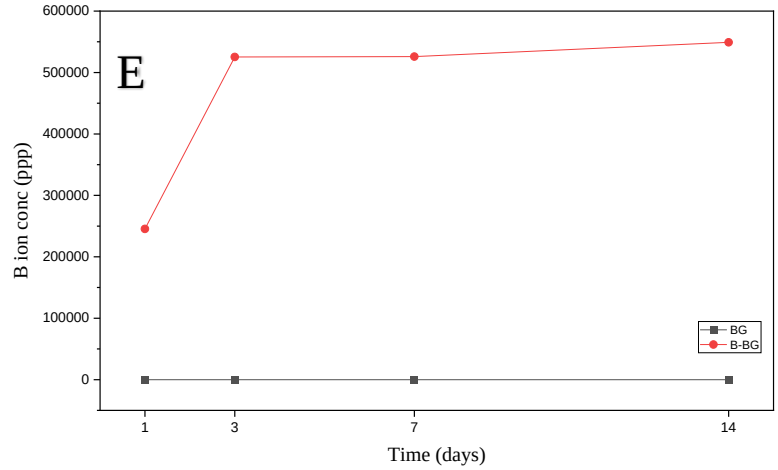


Figure 4.5. Ion release of (A) Si, (B) Ca, (C) P, (D) Na and (E) B from BG and B-BG as a function of immersion time.

4.2 MICROSTRUCTURAL ANALYSIS AND *IN VITRO* BIOACTIVITY OF DENTIN SPECIMENS

4.2.1 Scanning Electron Microscope Analysis with Energy Dispersive X-Ray Spectroscopy

SEM analysis aimed to determine the tubule occlusion efficacy of test agents when applied to dentine samples by examining features in untreated and treated samples. SEM enabled imaging of dentine tubules and EDX provided chemical analysis of points within the dentine to identify the composition of any residues. Dentine surfaces were free of smear layer and smear plugs after immersion of all specimens 2 minutes in 37 % phosphoric acid and virtually all of the dentinal tubules were opened entirely and the permeability was increased. Dentine was dense and homogeneous in the peritubular area. The opening of the tubules can be observed in (Figures 4.6. (A), 4.7. (A), 4.8. (A), 4.9. (A), 4.10. (A), 4.11. (A) and 4.12. (A)) for all samples. EDX analysis of acid-etched dentin samples revealed low values of Ca and P, due to the complete permeability of dentin tubules after application of phosphoric acid Figures 4.6. (B), 4.7. (B), 4.8. (B), 4.9. (B), 4.10. (B), 4.11. (B) and 4.12. (B).

According to SEM results, (Figures 4.6. (C) and 4.10. (C)) showed that after brushing the dentine samples in groups 1 and 5 for 14 days with a toothpaste containing a bioactive glass, the tubules were excluded with abrasives by creating a uniform layer on the surface of the dentin. The bioactive glass stimulates osteogenesis in physiological systems and tends to have an appropriate surface reaction. BG particles (Novamin) in Sensodyne Repair & Protect toothpaste and synthesized 45S5 BG particles appeared to deposit on the dentine surface. They interacted with calcium and phosphorous in the AS, occluding dentinal tubules physically [226].

When exposed to body fluids such as saliva, the active BG tends to precipitate hydroxycarbonate apatite, a supersaturated mineral concerning AS [12, 226, 227]. This action can be explained by when these test agents are applied to the surface of exposed dentin, the sodium ions (Na^+) of BG begin to exchange with hydrogen cations (H^+ or H_3O^+) in AS, allowing calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) to be released

from the particles. Therefore, these reactions could increase the pH value, which enhances and accelerate the formation of HA. This initial chain of reactions occurs within seconds of contact with saliva and the release of Ca^{2+} and PO_4^{3-} continues as long as the particles are in connection with an aqueous environment.

As these reactions and the precipitation of calcium and phosphate continue, this leads to the formation of a layer of calcium phosphate (Ca-P) on the surfaces of the dentin or in the tubules. This layer crystallizes into apatite hydroxycarbonate, which is chemically and structurally similar to biological apatite. Resulting in occlusion of the dentine tubules physically, which significantly reduces hypersensitivity [226, 228, 229]. These obtained results are consistent with previous studies by Wang *et al.,* [12, 134], who demonstrated that toothpaste-containing bioglass presents special clogging effects after brushing and immersion of AS. Similar results were obtained in a study by Burwell *et al.,* Who reported that Novamin has the ability to adhere to the surface of exposed dentin and interact with it forming a mineralized layer.on the surface [230].

Although bioglass (Novamin) is the active ingredient in Sensodyne Repair & Protect toothpaste, the 455S bioactive glass particles have shown better results in completely covering the dentin's surface and blocking the dentin the dentinal tubules. After treatment with Novamin and BG test agents, EDX analysis revealed a high content of Ca and P Figures 4.6. (D) and 4.10. (D), respectively. However, after the citric acid challenge, Novamin showed lower Ca and P elements levels while BG maintained Ca and P peaks at the same level (Figures 4.6. (F) and 4.10. (F) Respectively.

The samples treated with B-BGin group 6 showed complete occlusion of the dentinal tubules, which can be considered the best blockage ever among all groups. As shown in (Figure 4.11. (C)) a thick protective layer and large amounts of particles in agglomerates were observed covering the dentin surface completely. This remarkable increase can be attributed to the synergistic effect of both bioglass and boron. As a trace element, boron is essential for bone physiology. Its combination with BG enhances its rapid and complete ability to convert to HA when immersed in body fluid as AS [231].

When borate glass is immersed in AS, components such as Na_2O and B_2O_3 are released, resulting in Na^+ and BO_3^{3-} and PO_4^{3-} from solution reacting with Ca^+ to precipitate HA the glass. The reason for the fast conversion of borate glass into HA is that the dissolution of Na^+ and the attack on the structure of the B-O network by phosphate solution occurs simultaneously, which leads (instantly) to HA nucleation [170, 182].

The results showed an intensification of the deposition on the surface of the dentin with the incorporation of boron to the 45S5 bio-glass compared to the other groups. The HA deposition became more uniform throughout the surface of the dentin samples and the thickness of the deposition became more pronounced over time. This is consistent with high calcium peaks in the EDX spectra upon application of B-BG, which is probably due to the formation of calcium-containing deposits blocking almost all dentinal tubules (Figure 4.11. (D). B-BG was the only agent among the groups that showed significant resistance to citric acid challenge and this may be due to mineral deposits formed inside the dentinal tubules that settled and difficult to be removed by acid and this can be confirmed by the results of EDX analysis where the samples preserved high level of Ca and P even after acid challenge (Figure 4.11. (F). B-BG has never been studied by researchers in blocking dentinal tubules with the aim of treatment DH before. Still, the effectiveness of this compound in the rapid transformation into a HA layer in bone and hard tissue engineering has been confirmed in many studies [68, 69].

Dentin samples in group 2 were treated with Colgate Sensitive Pro-Relief toothpaste containing 8% Arginine, an amino acid positively charged at biological pH, bicarbonate a pH buffer and calcium carbonate as a calcium source [226]. As shown in (Figure 4.7. (C)), the occlusion of the dentinal tubules was less than in groups1, 5 and 6. However, the degree of occlusion is considered sufficient to relieve teeth sensitivity. Some abrasive particles from the toothpaste were left in the dentine tubules blocking them while the diameters of some tubules were narrowed. The reason behind occluding dentinal tubules when applying an arginine-based toothpaste is that arginine is physically absorbed on the calcium carbonate surface *in vivo* , producing a positively charged accumulation that can easily bind to the negatively charged dentin on the

exposed surfaces and inside the tubules [227, 232]. In addition, the pH of the accumulation of arginine and calcium carbonate is alkaline sufficiently to facilitate the precipitation of calcium and phosphate from saliva and dentin [146]. The interaction of arginine and calcium carbonate causes the precipitation of phosphate and arginine, calcium and carbonate on the exposed dentine surfaces and inside the tubules, resulting in impeding them [233].

Although the results of SEM showed a narrowing in the diameter of the tubules, there was no complete obstruction as with Novamin, BG and B-BG and the superiority of BG in occlusion dentinal tubules over arginine has been confirmed in many previous studies [234, 235]. These results are similar to a study obtained by Petrou *et al.*, who described dentinal tubule occlusion using the arginine and calcium carbonate technology as highly effective[146]. However, the results are considered reasonable compared to the Control group, where the partial blockage of the tubules can reduce the flow of fluids through them, resulting in the reduction of DH [12, 226].

Moreover, it is clear from TEM results, which the BG and B-BG particles before application to the exposed dentin surface, both samples possess a smooth surface. After contact with the dentinal tubules and immersion in AS, SEM micrographs reveal the formation of apatite-like crystals that blocked the dentinal tubules and deposited them on the surface. EDX showed lower Ca and P peaks after citric acid treatment and this result agrees with SEM showing that the particles on the surface were washed away, leaving the tubules open. (Figures 4.7. (E and F).

The narrowing of the diameter of the dentinal tubules can also be observed in dentin samples in group 3 which were treated with Ipana Pro sensitive toothpaste, as shown in (Figure 4.8. (C)), SEM results showed a partial occlusion of the dentinal tubules in the surface of the dentin, but failed to produce a complete occlusion. The effectiveness of this toothpaste in occluding the dentinal tubules has not been reviewed yet in the literature. Therefore, the active ingredient in its composition (NaF) was relied upon to interpret the results. This deposition of some particles is due to the active ingredient in the toothpaste, sodium fluoride (NaF). Fluoride can be used in toothpaste formulations

with the aim of remineralizing enamel, but evidence regarding its effect on eliminating tooth sensitivity is limited.

However, it has been shown that fluoride in toothpastes can create deposits on the dentin surface and block dentin tubules, enhancing acid resistance [60]. Although the everyday use of sodium fluoride prevents cavities, it can be a treatment option for decreasing DH. NaF interacts with calcium in the dentin, resulting in calcium fluoride crystals, which are deposited on the opening of the dentinal tubules [236].

According to Tosun *et al.,* these crystals have a small diameter. Applying NaF alone may not be sufficient to narrow the diameter of dentinal tubules and may require repeated treatments to obtain better results [237]. However, the mineral deposits on the dentine surface may help reduce the permeability. Still, they are not sufficient to occlude all dentinal tubules and the layer formed on the surface of the dentin is thin and easy to be rinsed. In addition, this toothpaste contains potassium nitrate, which is designed to deliver potassium ions to reduce nerve excitability in highly sensitive teeth. Besides, many studies confirmed that this agent has the ability to block dentinal tubules and reduce the permeability of dentin because silica and calcium contain abrasives instead of their effective clinical ingredients [238, 239]. According to SEM and EDX results, as shown in Figures 4.8. (E, F), group 3 specimens failed to show excellent resistance to citric acid challenge after the acid challenge. This can be attributed to the fact that in the presence of acids, calcium and fluorapatite can be easily dissolved at acidic pH [240] .

The samples in Group 4 (placebo) and Group 7 (control) showed the lowest occlusion of the dentinal tubules. However, the formation of some deposits on the surface of the dentin can be observed in (Figure 4.9. (C)). This can be attributed to that AS was sufficient to form a thin layer covering some dentinal tubules due to calcium and phosphate ions contained in its composition [13, 54]. Saliva acts as a natural reservoir for the regrowth of apatite and/or the intending of new nanocrystals and plays an essential role in the natural remineralization processes and reduces mineral loss, thus aiding in the naturally occurring blockage of the dentinal tubules [228].

These results were confirmed by EDX, which showed a high rate of calcium and phosphorous after treatment. However, this level did not last long after the acid challenge. As in Group 4 specimens, brushing the specimens continuously over 14 days may allow greater access of saliva to the dentinal tubules, which may promote tubular occlusion through the deposition of calcium and phosphate from the saliva [241]. However, Tubule occlusion may occur naturally through saliva and dentine sclerosis's normal remineralization processes through secondary dentine formation. Saliva also has a protective function against tooth wear. The layer has been reported to promote remineralization and reduce mineral loss [60].

SEM results showed that citric acid could partially remove the precipitated particles from the tubule orifices and dentine surfaces. The results indicate that the effectiveness of Novamin, BG and B-BG decreased after the citric acid treatment. The layer formed on the surface of dentin is more resistant to acid challenges than other groups and is mechanically robust. This finding is consistent with a study by Bakri *et al.*, analyzing the efficacy of dentine tubules occlusion in bioglass toothpaste. After the acid challenge, they concluded that bioglass-containing toothpastes shows better tubules occlusion than the groups containing arginine and fluoride [242]. In addition, the continuous release of calcium over time maintains the protective effects on the surface of the dentin and provides a constant blockage of the dentinal tubules.

The thickness of the layer formed on the surface of the dentin in Group 5 helped protect the dentinal tubules from partial exposure. Arginine was not resistant to acid attack because the positively charged agglomeration with calcium carbonate binds with the negatively charged dentin at the physiological pH [243].

Therefore, under acidic conditions, the charge will lose and this may cause agglomeration to separate from the dentin, even though the toothpaste contains bicarbonate to help prevent pH changes [54, 243] in group 3, where it is possible to notice the breakdown of the layer covering the tubules, due to the solubility of CaF_2 under low pH values. For the samples in group 4 and group 7, upon exposure to 6% citric acid challenge, most of the layer formed by AS was dissolved. However, there was evidence of occlusion of some tubules. These findings were similar to the results

obtained by the researchers, where they suggested that saliva can obscure patented dentinal tubules by delivering calcium and phosphate ions into the tubules and creating a surface protecting layer of salivary glycoprotein with calcium and phosphate. However, natural tubule occlusion is a slow mechanism and tubule plugging is easily removed by dietary 6% acid and physical attack, making it ineffective and unreliable in providing long-term DH relief [13, 54, 228]. Finally, upon exposure to citric acid challenge, most of the layers formed by test agents were dissolved, but group 6 was resistant with partial tubule occlusion.

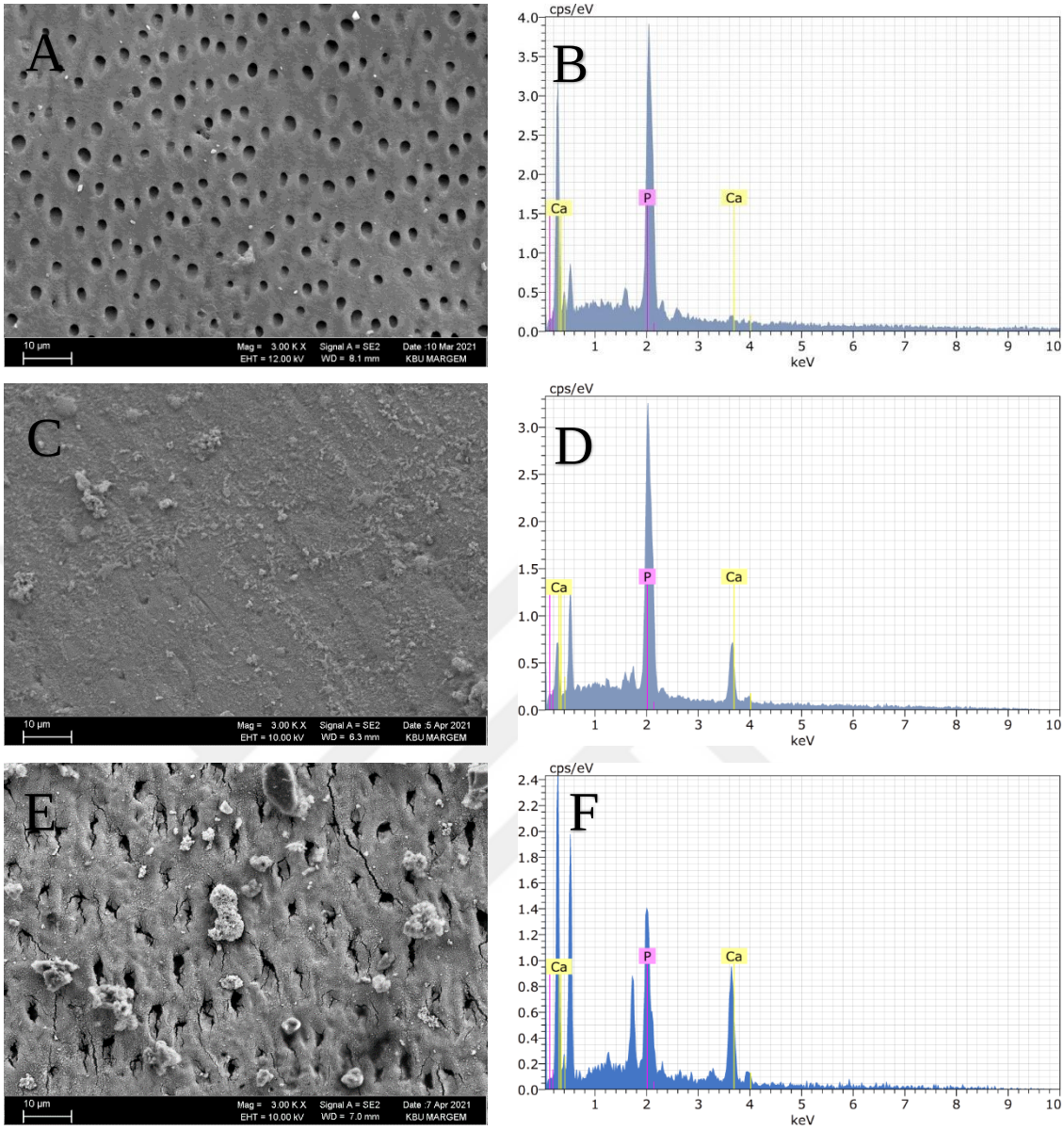


Figure 4.6. SEM images and EDX spectra of specimens in Group 1: (A) SEM image (B) EDX spectra of acid-etched dentine specimen. (C) SEM image (D) EDX spectra of dentin surface treated with Novamin. (E) SEM image. (F) EDX spectra of treated dentine surface after citric acid challenge.

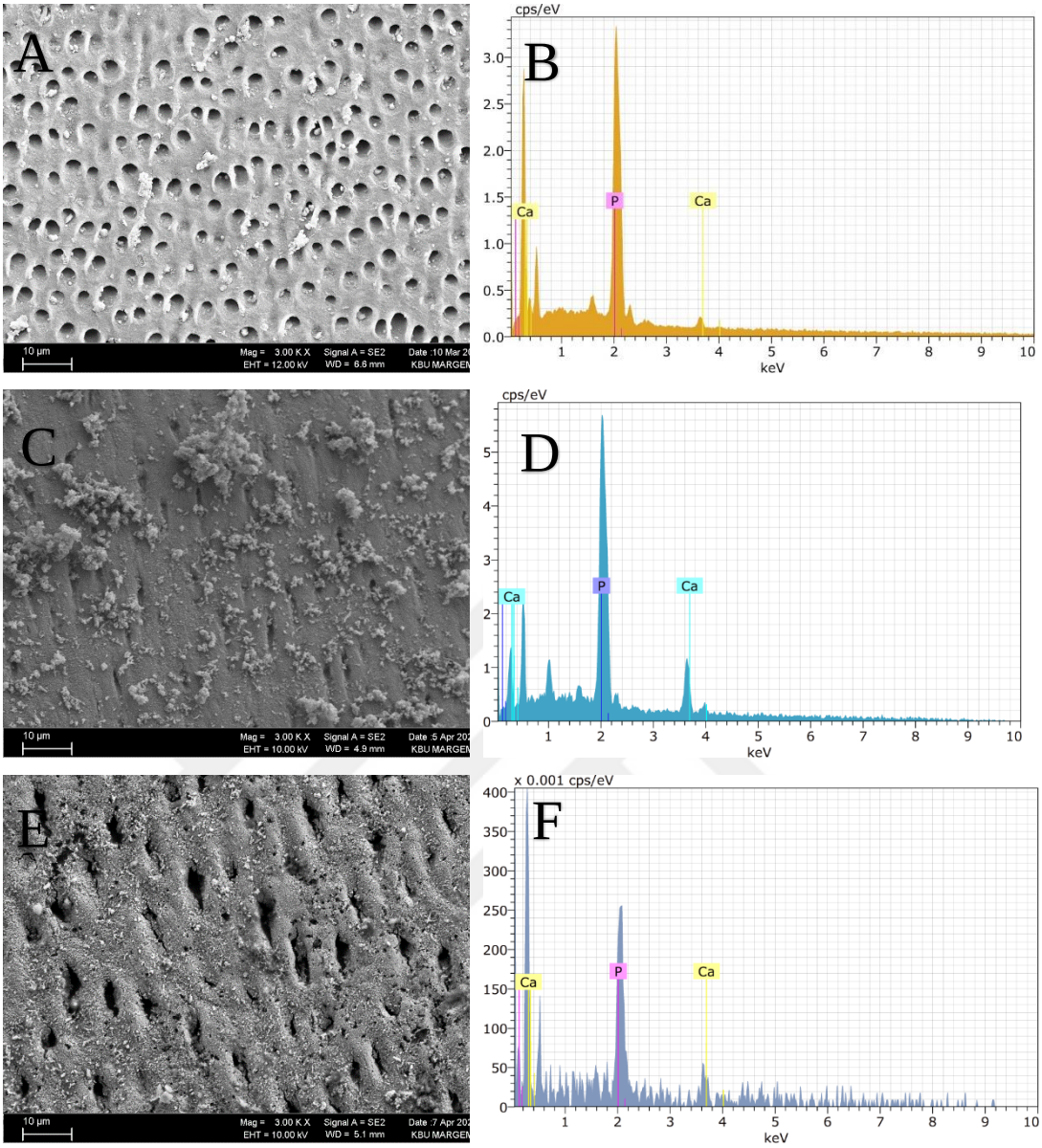


Figure 4.7. SEM images and EDX spectra of specimens in Group 2: (A) SEM image (B) EDX spectra of acid etched dentine specimen. (C) SEM image (D) EDX spectra of dentin surface treated with 8% Argenine. (E) SEM image. (F) EDX spectra of treated dentine surface after citric acid challenge.

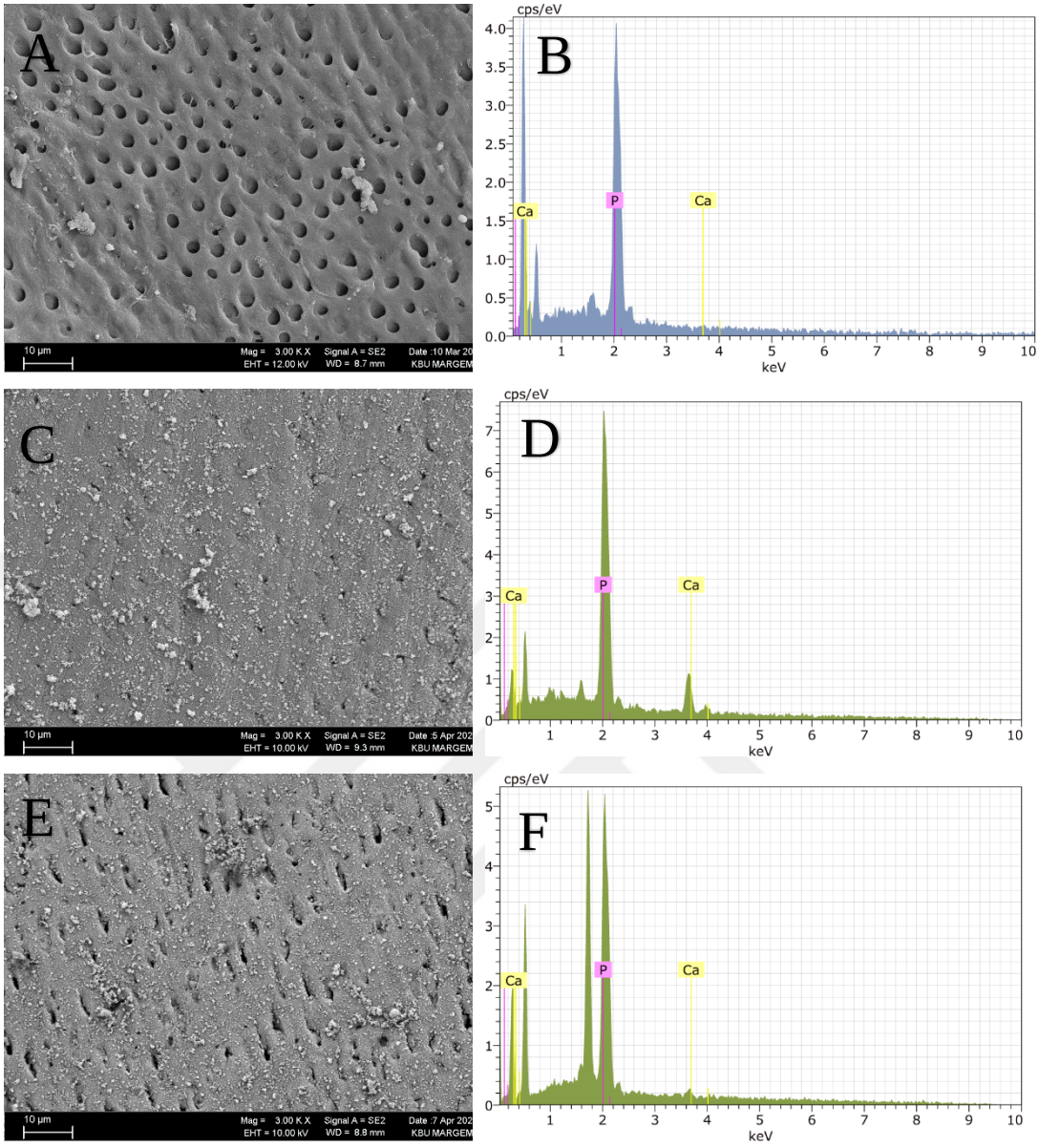


Figure 4.8. SEM images and EDX spectra of specimens in Group 3: (A) SEM image (B) EDX spectra of acid-etched dentine specimen. (C) SEM image. (D) EDX spectra of dentin surface treated with NaF. (E) SEM image (F) EDX spectra of treated dentine surface after citric acid challenge.

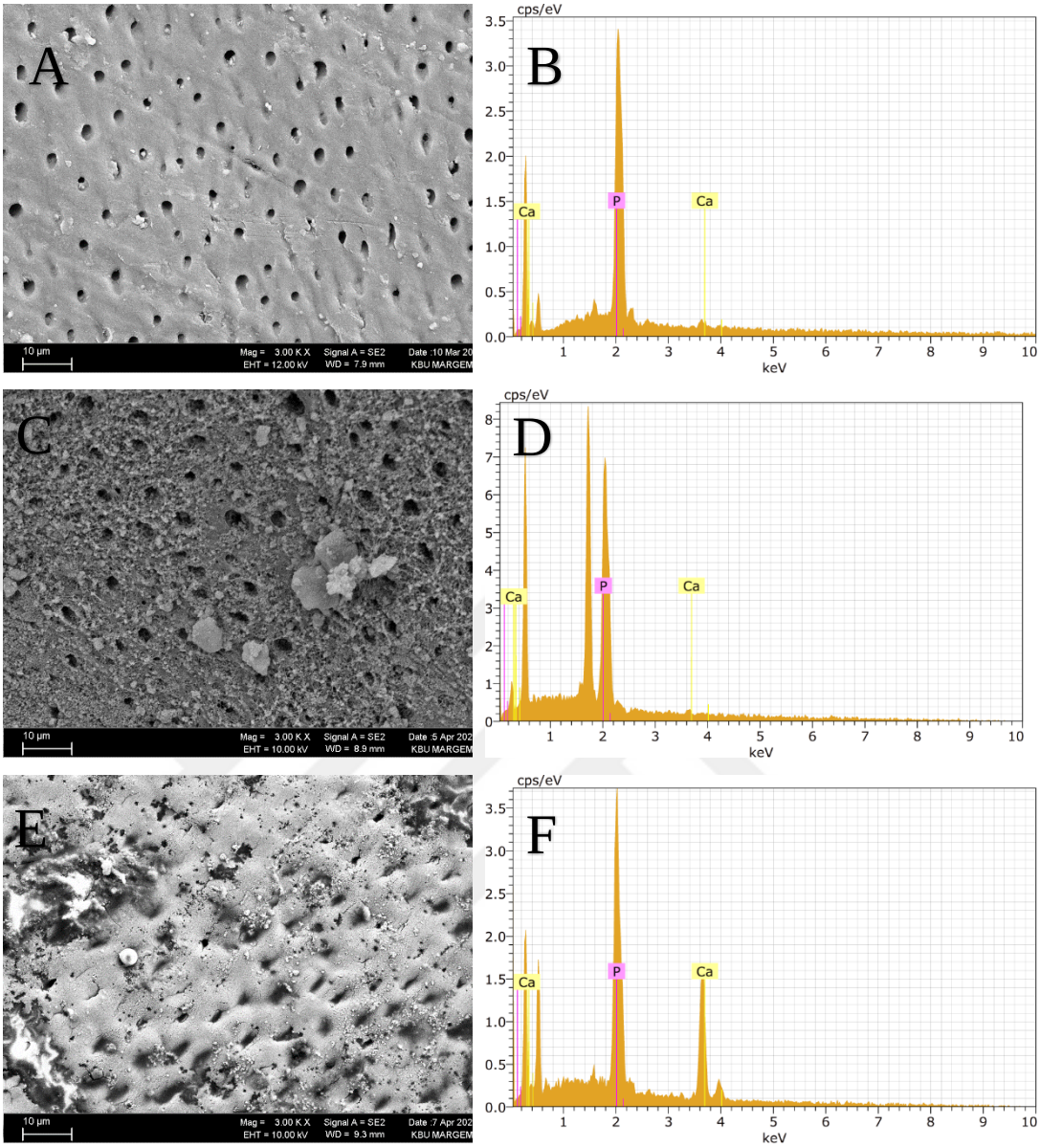


Figure 4.9. SEM images and EDX spectra of specimens in Group 4: (A) SEM image (B) EDX spectra of acid-etched dentine specimen. (C) SEM image. (D) EDX spectra of dentin surface treated with placebo. (E) SEM image (F) EDX spectra of treated dentine surface after citric acid challenge.

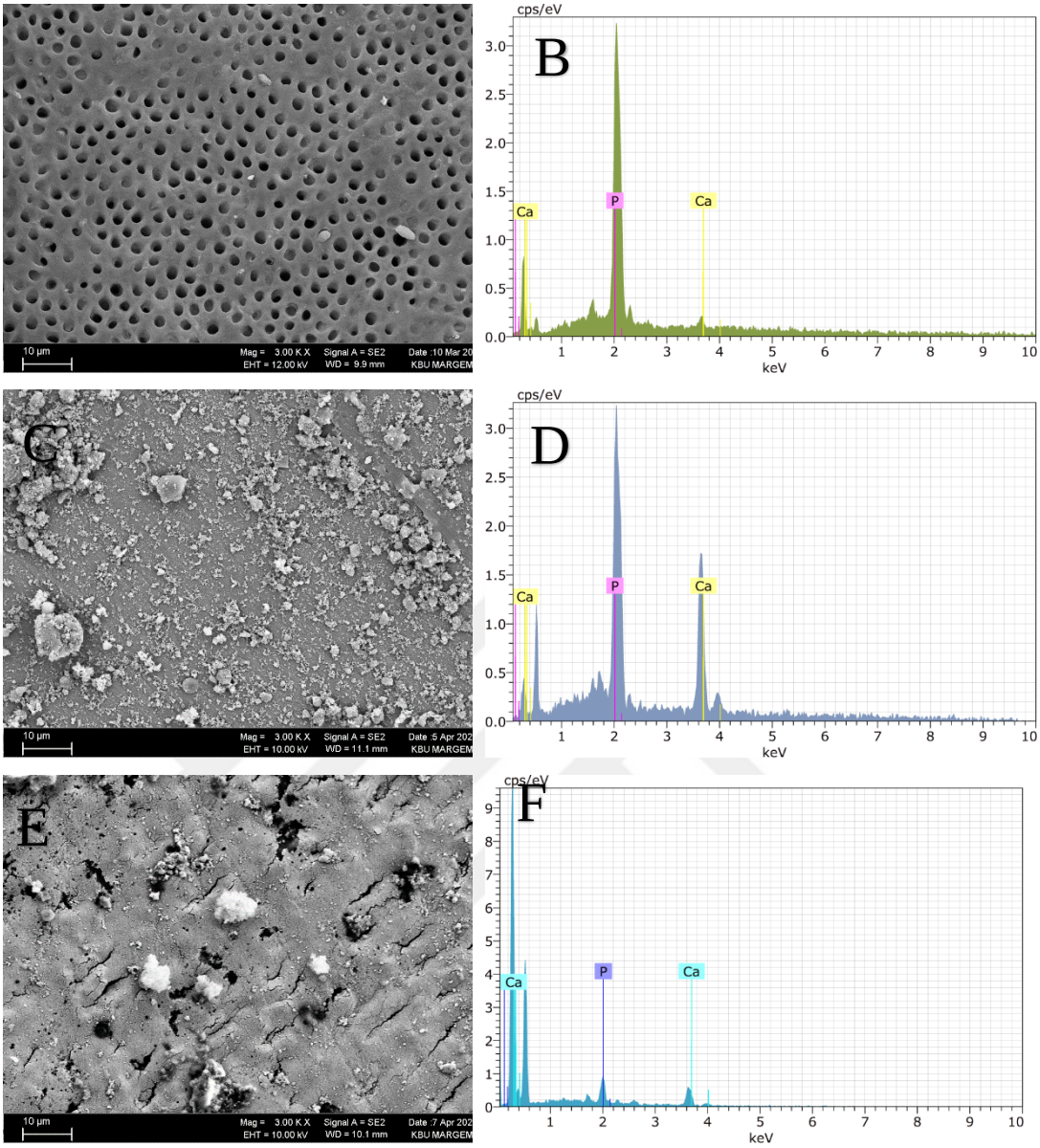


Figure 4.10. SEM images and EDX spectra of specimens in Group 5: (A) SEM image. (B) EDX spectra of acid-etched dentine specimen. (C) SEM image (D) EDX spectra of dentin surface treated with 45S5 BG. (E) SEM image (F) EDX spectra of treated dentine surface after citric acid challenge.

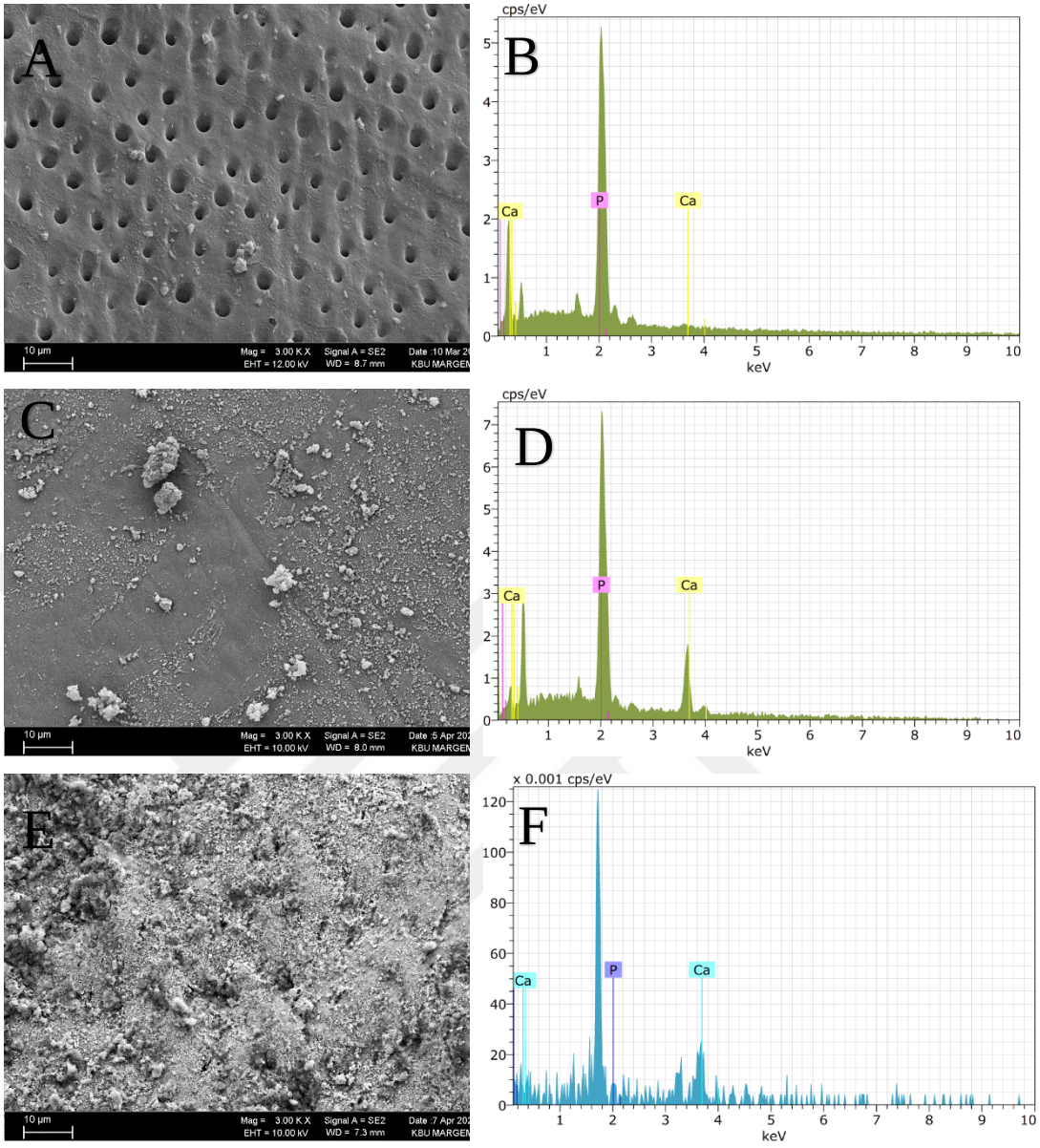


Figure 4.11. SEM images and EDX spectra of specimens in Group 6: (A) SEM image. (B) EDX spectra of acid-etched dentine specimen. (C) SEM image (D) EDX spectra of dentin surface treated with B-BG (E) SEM image (F) EDX spectra of treated dentine surface after citric acid challenge.

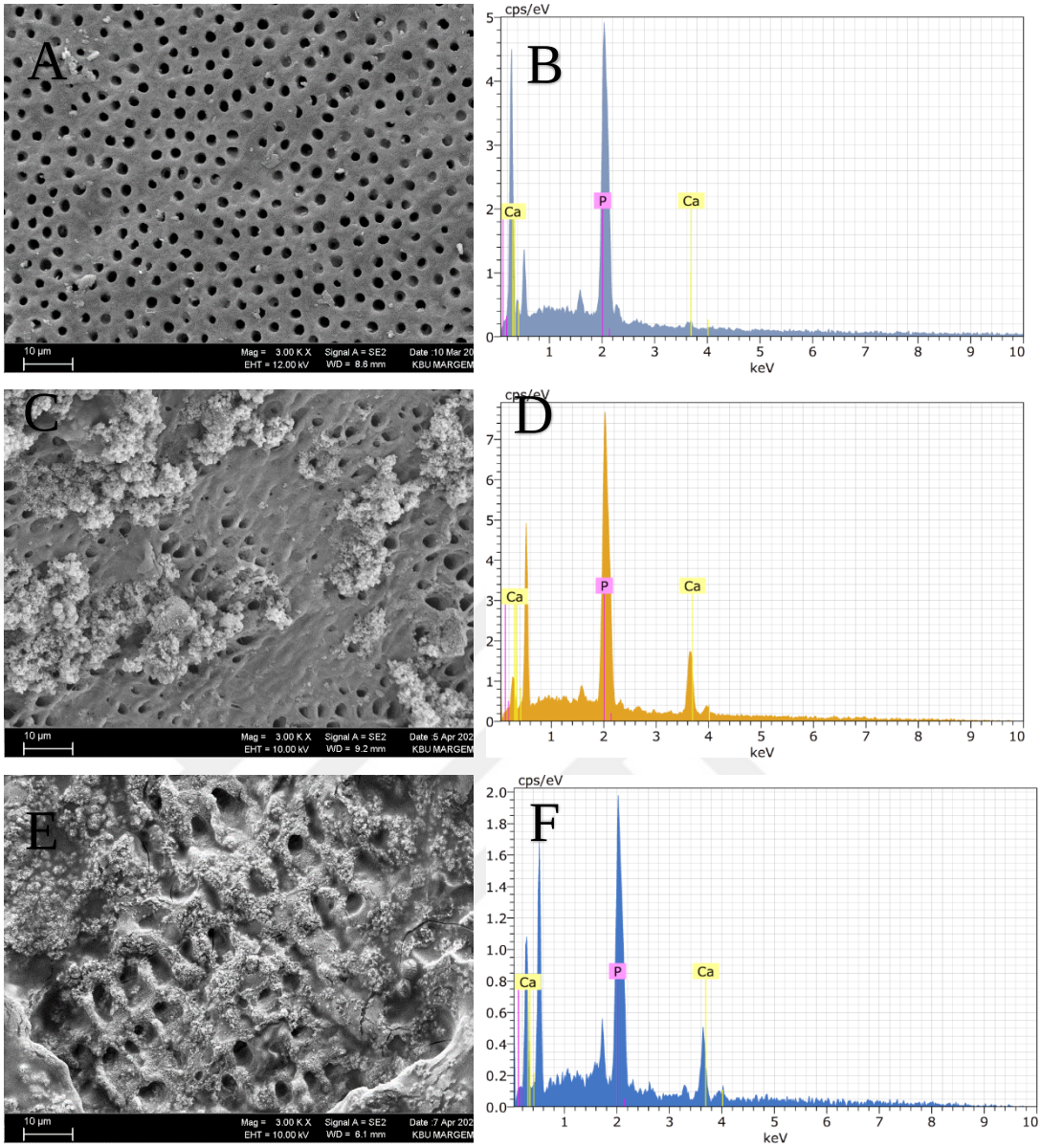


Figure 4.12. SEM images and EDX spectra of specimens in Group 7: (A) SEM image. (B) EDX spectra of acid-etched dentine specimen. (C) SEM image (D) EDX spectra of dentin surface brushed with AS. (E) SEM image (F) EDX spectra of treated dentine surface after citric acid challenge.

4.2.2 X-ray Diffraction Analysis

XRD is also used to identify the mineral phase of the deposited minerals on the dentine surface and to observe the crystallinity differences between samples before and after treatment. Ca, P, O and C, as well as the trace elements Mg and Na, make up the majority of dentin[244]. The literature identified the mineral phase of the dentin as a calcium phosphate with an apatite structure [245]. It was indicated that dentin is

composed of HA and minor whitlockites [244]. However, the association of many minor and trace elements with biological appetites has prompted more research and differing viewpoints.

According to the results, the XRD patterns of dentin samples before and after treatment were almost similar and their diffraction peaks matched with several phases of calcium phosphate. However, all samples were matched with standard HA diffraction peaks (JCPDS-00-009-0432). The diffraction peaks of crystalline apatite were mainly observed at 25.9°, 31.8°, 32.9° and 46.7° of 2θ and were connected with Miller indices (0 0 2), (2 1 1), (3 0 0) and (2 2 2), respectively [246].

According to the XRD spectrum shown in (Figure 4.13.) the Novamin sample's diffraction peak showed significant similarity with standard HA diffraction peaks with the total agreement with SEM results. The Peak (002) was shifted toward higher 2θ indicating the change in crystal lattice parameters [247]. The decrease in lattice parameters and crystallite size is observed in (Table 4.1.) and approved by broadening reflections in the XRD pattern after treatment. The diffraction peaks after treatment were sharper and higher intensity in small amounts, increasing the intensity resulting in increased crystallinity.

After brushing dentine samples with a group 2 test agent (arginine), the intensity of the diffraction peak (211) was decreased in agreement with the reduction of crystallinity (22.62 %). The low crystallinity is due to the presence of CO_3^{2-} ions in the structure of arginine, which can be restricted the crystallization of the HA phase [248]. The peak (210) in the pattern after treatment can be observed in (Figure 4.14.).

The diffraction peaks of the group 3 XRD pattern (Figure 4.15.) were a little sharper after treatment and the peak at 21.8° was shifted to 25.8° due to the reduction in the lattice parameters. As shown in (Figure 4.16.), for group 4, there was no significant difference between the diffraction patterns before and after treatment. This can be attributed to the fact that the placebo treatment had no apparent effect on the surface of dentine.

XRD patterns of the samples treated with BG and B-BG are shown in (Figure 4.17. and Figure 4.18.). The change of the diffraction peaks with lower intensity indicated that the change of surface samples was from crystalline to amorphous, which can be attributed to the amorphous nature of BG. It was reported that silica in solution slows the growth of HA crystals. Therefore, the formed layer will be restricted in crystallization due to the silica, which acts as a barrier to crystallization against another phase by limiting the atomic arrangement [249].

However, group 6 showed higher crystallinity than group 5 which means that it is more biologically active and can convert to HA in less time. The diffraction Peak (211) was less sharp and broader, which can be explained by the small size of crystallites. In addition, According to previous investigations, the reduction of particle size could cause an increase in the specific surface area, which improves the interaction between the deposited material and the physiological solution and thus enhances the kinetics of biomechanical reactions.

The XRD pattern also revealed an increase in the crystallization of the surface of dentin in the control group (Figure 4.19.), in agreement with the results of SEM, which showed that the AS itself is able to form a layer of calcium phosphate can partially cover the open dentinal tubules.

Table 4.1. Lattice parameters, degree of crystallinity and Crystallite size of dentin samples before and after treatment.

Group #	Treatment	Lattice parameters			Crystallinity	Crystallite size (nm)
		a (Å)	b (Å)	c (Å)		
Group 1	Before treatment	9.441	9.441	6.88	64.56 %	19.275 nm
	After treatment	9.415	9.415	6.879	72.40 %	19.367 nm
Group 2	Before treatment	9. 426	9. 426	6.865	61.81 %	30.86 nm
	After treatment	9.44	9.44	6.86	48.99 %	21.875 nm
Group 3	Before treatment	9. 415	9. 415	6.879	56.39 %	32.20 nm
	After treatment	9. 418	9. 418	6.879	61.13 %	28.84 nm
Group 4	Before treatment	9.4410	9.4410	6.8770	60.21 %	22.57 nm
	After treatment	9.4410	9.4410	6.8770	71 %	25.440 nm
Group 5	Before treatment	9.416	9.416	6.874	65.07 %	20.82 nm
	After treatment	9.424	9.424	6.879	47.33 %	18.188 nm
Group 6	Before treatment	9.416	9.416	6.874	68.32 %	19.175 nm
	After treatment	9.418	9.418	6.884	52.64 %	16.9 nm
Group 7	Before treatment	9.4190	9.4190	6.8810	45.95 %	21.270 nm
	After treatment	9.4190	9.4190	6.8810	59.26 %	19.988 nm

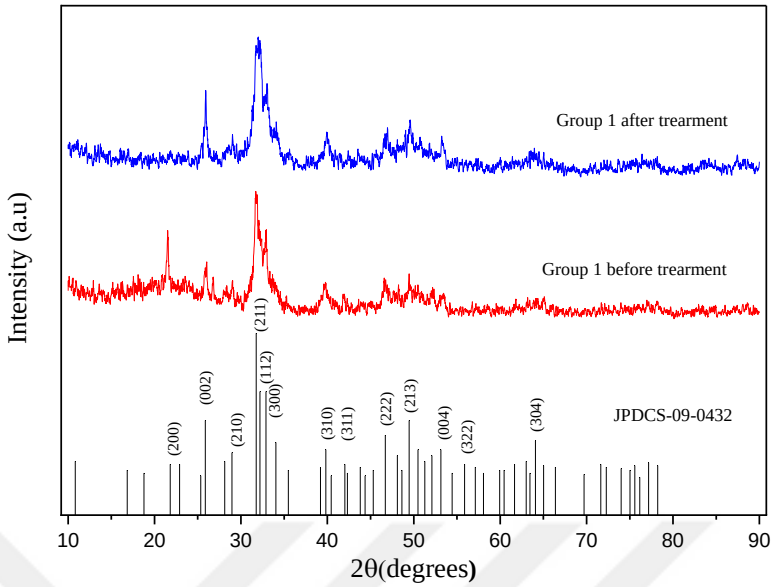


Figure 4.13. XRD spectra of the dentin surface before treatment and precipitates formed on dentin after treatment with Arginine.

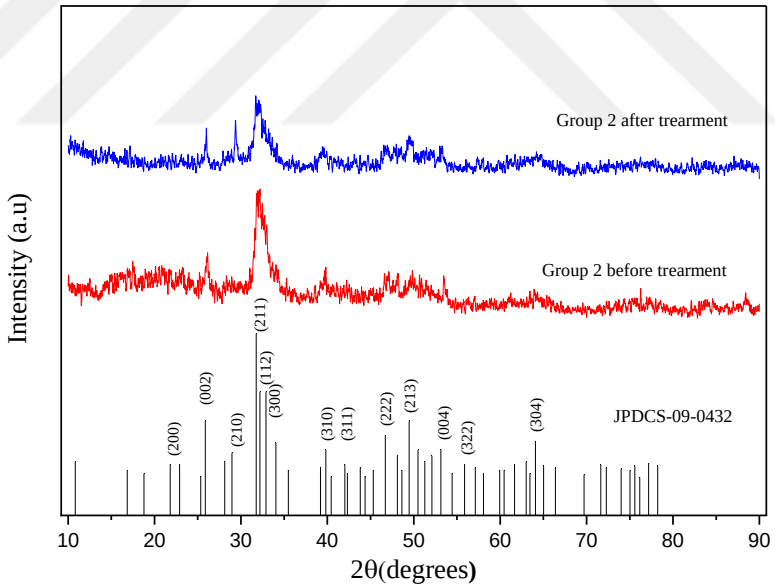


Figure 4.14. XRD spectra of the dentin surface before treatment and precipitates formed on dentin after treatment with Arginine.

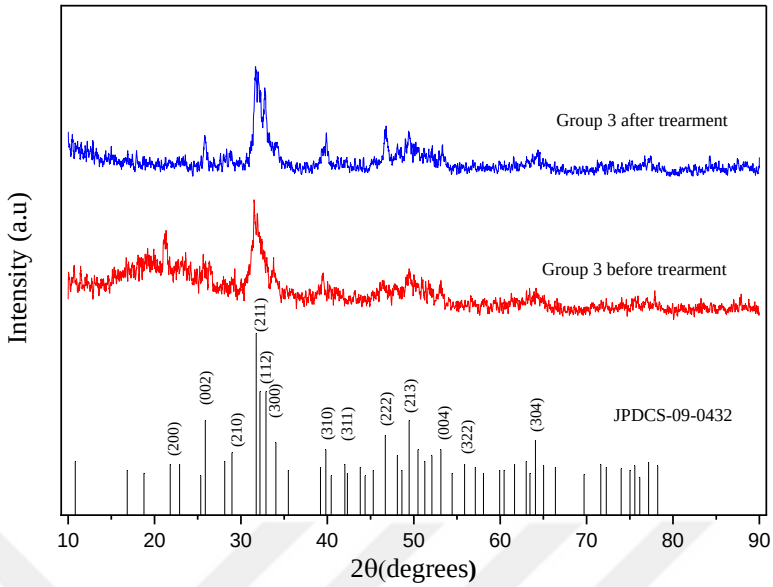


Figure 4.15. XRD spectra of the dentin surface before treatment and precipitates formed on dentin after treatment with Sodium Florid.

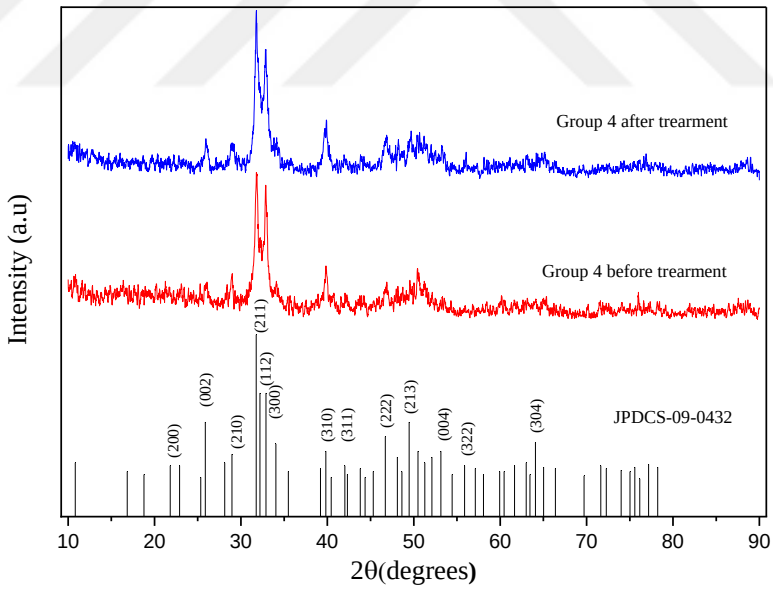


Figure 4.16. XRD spectra of the dentin surface before treatment and precipitates formed on dentin after treatment with placebo agent.

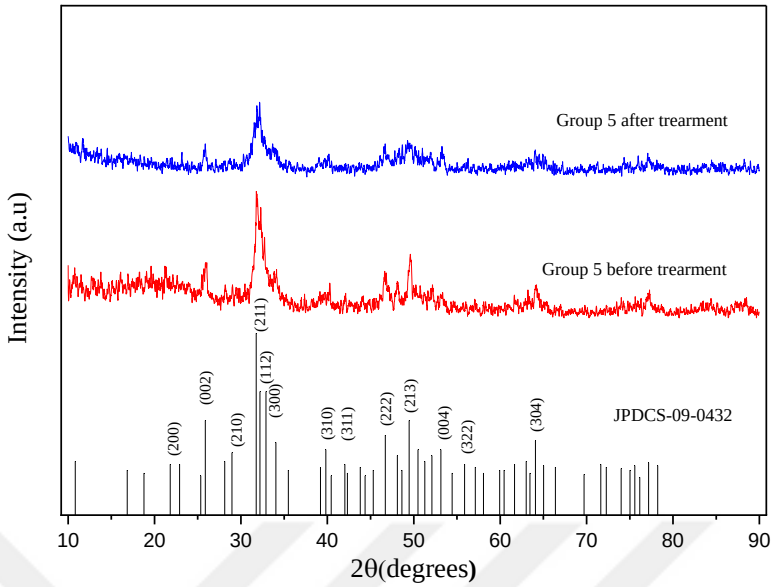


Figure 4.17. XRD spectra of the dentin surface before treatment and precipitates formed on dentin after treatment with BG particles.

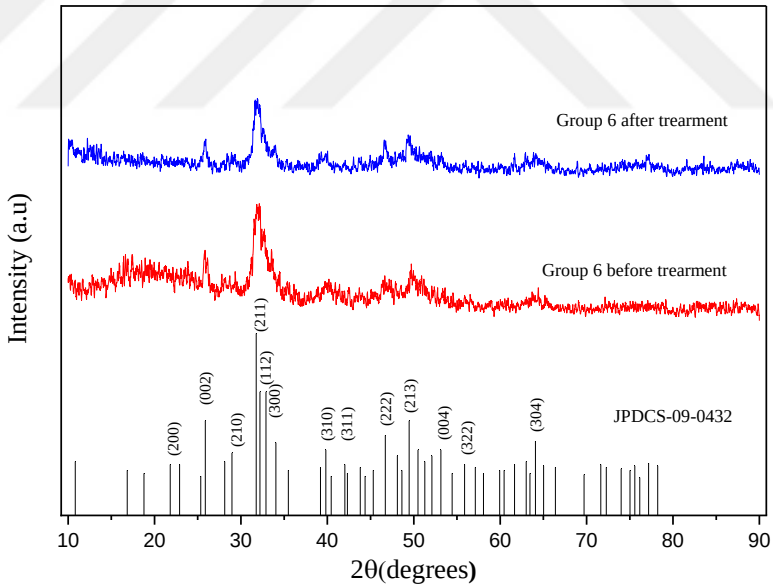


Figure 4.18. XRD spectra of the dentin surface before treatment and precipitates formed on dentin after treatment with B-BG particles.

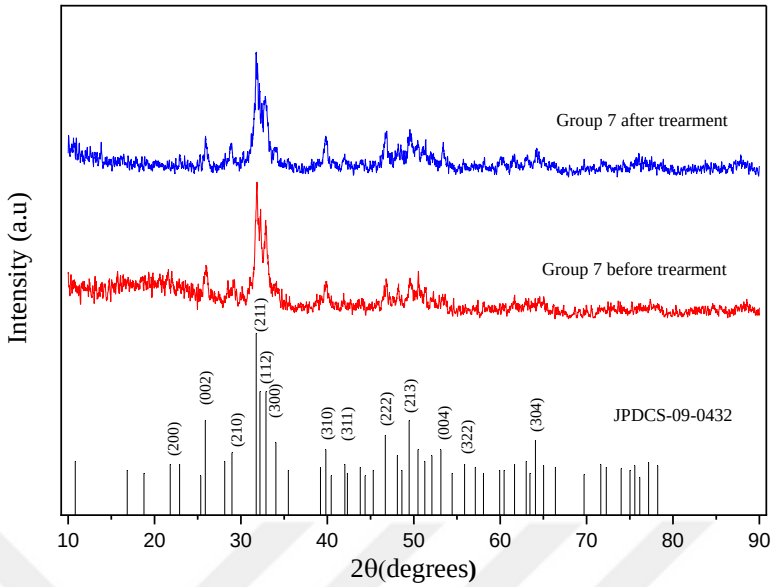


Figure 4.19. XRD spectra of the dentin surface before treatment and precipitates formed on dentin after treatment with AS.

4.2.3 *In Vitro* Bioactivity Analysis

4.2.3.1 Ion Release Profiles

The leaching of ions from the deposited layer on dentin samples was compared and test agents' bioactive behavior was evaluated**.** While the treated dentin samples are immersed in AS, the particles deposited on the surface are attacked by the surrounding environment. The ions are released from the particles to precipitate in a layer that clogs the dentinal tubules. As a result, AS containing calcium and phosphate can play a role in increasing sedimentation over time, which may disturb the accurate measurement of ionic leaching. Thus, the measurement of ion concentration release will be more sensitive in citric acid**.** After completing 14 days of treatment, the dentin samples were immersed in 6% citric acid for two minutes to evaluate the release of ion concentrations under acidic conditions (Figure 4.20.) Groups 2 and 3 had the highest rate of calcium ions releasing, indicating the fast dissolution of the precipitated layer in an acidic environment, agreeing with SEM results. The release of calcium ions concentrations in the rest of the groups was almost similar. The release of calcium ions

concentrations in groups 1, 5 and 6 can be attributed to the thickness of the calcium-phosphate-rich layer deposited on the surface of the dentin.

It was evident that the rate of release of sodium ions in group 3 is the highest since the test agent used in the treatment contains sodium. The other groups were largely similar in the rate of releasing sodium ions concentrations, except for group 4, in which the release of sodium ions was the lowest.

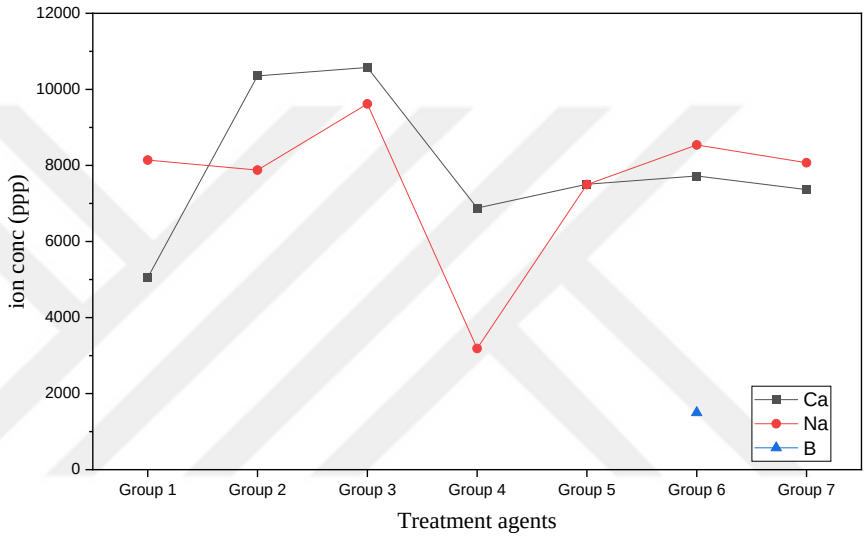


Figure 4.20. Ion release of Ca, Na and B from dentin sample groups under citric acid immersion for 2 minutes.

4.2.3.2 *In Vitro* Deposition and Degradation Analysis

The deposition and degradation of the formed layer/ particles on the surface of dentin were assessed as a function of the time of immersion of the samples in AS with an initial pH of 7.4 at 37 °C, as described in detail elsewhere. The weight changes were used to monitor the deposited layer on the treated dentin surface and the durability of the precipitations under immersion in As. After removing the AS at specific times, dentine specimens were dried at 50°C and weighed with repetition for all samples.

The mean values of weight are shown in (Table 4.2.). One-Way Analysis Of Variance (ANOVA) was conducted to discover statistically significant differences in weight change depending on the test factor and treatment method. According to the results

of the ANOVA test, can observe the statistically significant differences in weight gain/loss according to a variable of the treatment method, where the value of F was 21.849 with a significance value of 0.000 < 0.005 for weight gain and was 9.385 with a significance value of 0.001 < 0.005. This means there were statistically significant differences in both cases. (Figure 4.21. (A)**),** showed that the mean weight gain of the B-BG containing group is higher than the other groups and in (Figure 4.21. (B)), the weight loss was lower than the rest of the groups.

Table 4.2 Mean values of weight gain\loss according to Anova test.

Group #	Mwg*	Std*	F	Sig	Mwl*	Std*	F	Sig
Group 1	2.960	0.2600	21.8	0.00	0.163	0.0116	9.3	0.00
Group 2	2.153	0.3371			1000	0.3035		
Group 3	1.983	0.3200			0.863	0.3190		
Group 4	1.730	0.2862			0.713	0.0681		
Group 5	3.023	0.2650			0.127	0.0473		
Group 6	3.617	0.1834			0.130	0.0964		
Group 7	1.940	0.1229			0.190	0.3538		
Total	2.487	0.7407			0.4552	0.4109		

Mwg*: Mean weight gain.
Std*: Standard deviation.
Mwl*: Mean weghit loss.

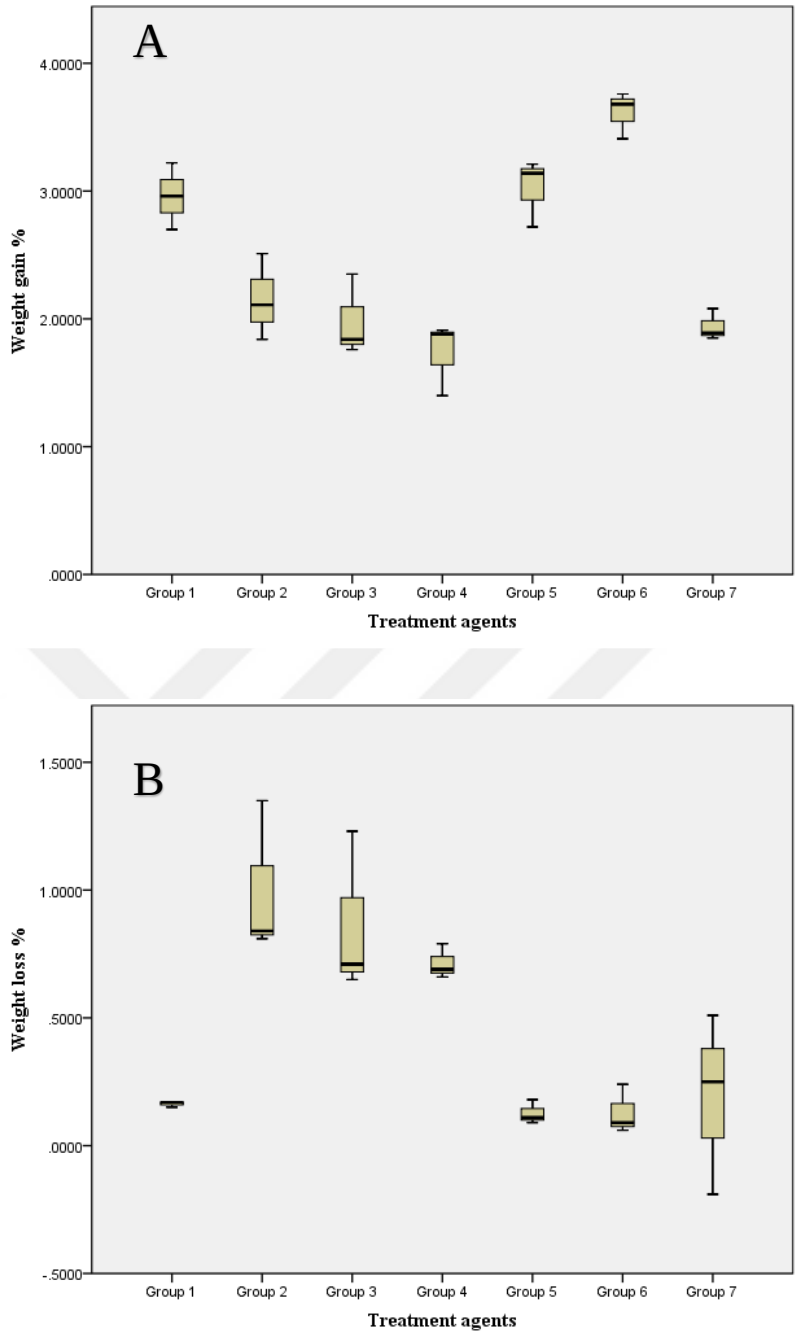


Figure 4.21. (A) Weight gain of dentin saples after treatment. (B) Weight loss of after immersion 10 days in AS.

In order to find out the amount of the differences between the samples, the dimensional comparisons were tested. There were significant differences in weight gain on the dentin surface depending on the treatment agent. The difference between the Novamin, BG and B-BG groups was not statistically significant ($P > 0.005$), indicating that a layer of close thickness blocked the dentinal tubules. While the difference between

these groups containing BG formulations and group 3, groups 4 and 7 was highly statistically significant to other groups ($P > 0.005$). The increasing significance of weight in these groups could be attributed to the advantage of the strong bioactivity of BGes in body fluids, as salts from the liquid medium (AS) preferentially shifted the equilibrium between salts towards HA formation [16]. These results are consistent with SEM and XRD results. All dentinal tubules were blocked with a thick layer. However, the weight gain analysis showed that the boron-containing group could deposit the most significant possible amount of particles on the dentin surface. The placebo group (group 4) showed the slightest increase in weight and there were no statistically significant differences between group 2, group3 and group 7 ($P > 0.005$).

The current study results indicate the weight loss after immersion of dentin samples in AS for 10 d. There were statistically significant differences between group 2 and group 1, group 5, group 6 and 7 (Sig 0.021, 0.016, 0.016 and 0.027, respectively). The differences were not statistically significant between group 2 and group 3 and group 4 (0.995 and 0.848, respectively). Although group 3 and group 4 were not statistically significant with group 2, there were statistically significant differences in weight loss between them and groups 1, 5, 6 and 7. Statistical analysis of mean weight loss revealed significant differences ($P < 0.05$) between group 1, group 5, group 6 and group 7. Weight loss can be attributed to dissolved soluble salts in AS. The reason for maintaining or slightly decreasing weight may be the balance between salt dissolution and calcium phosphate deposition on the dentin surface.

Weight measurements showed a noticeable increase in all samples after applying the test agents for 14 days due to the deposition of a layer of particles covering the surface of the dentin, which was confirmed by SEM results. The superiority of Group 6 can be observed in residue, as the weight of samples increased significantly after treatment due to the continuity of the dissolution–precipitation processes cause thickening of the deposited layer on the surface. All samples were weighed after 10 days of immersion in the AS to measure the rate of degradation and the effectiveness of the applied agents over time. Surprisingly, groups 1, 5, 6 and 7showed similar amount of weight loss because AS contains calcium and is able to deposit a layer on the surface over time. However, Groups 2, 3 and 4 had higher levels of dissolution, especially group 2.

Finally, Group 6 showed high stability, with little weight loss compared with the other groups. (Figure 4.22.).

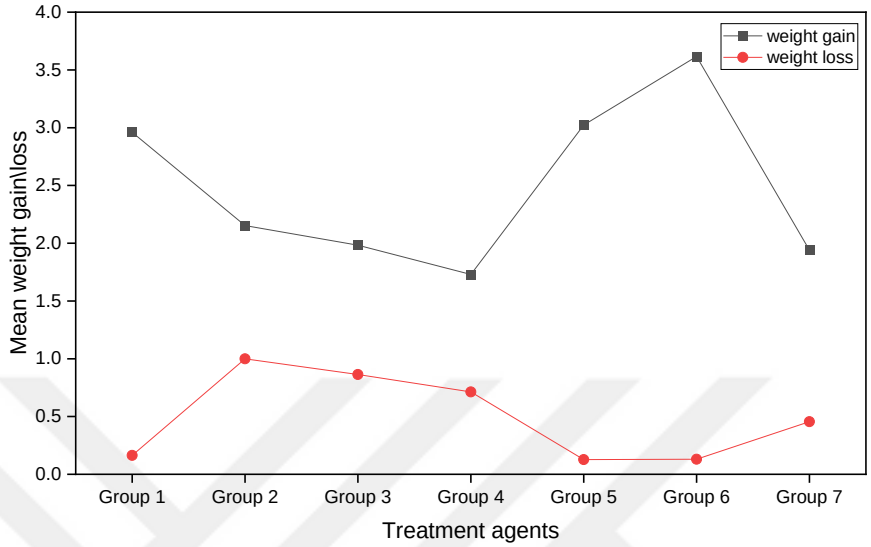


Figure 4.22. Estimated marginal means of weight gain/loss of treatment agents.

4.3 FAST DEPOSITION EVALUATION

According to SEM and EDX results, groups 1, 5 and 6 showed significant occlusion of the dentinal tubules and a significant increase in mineral deposition on the dentin surface compared to the other test groups. Therefore, the effectiveness of these three groups was tested in the short term (for two minutes over three d) under the same conditions mentioned previously in the treatment for the long term to compare their ability of rapid deposition and to block the dentinal tubules in a short time.

It was observed from (Figure 4.23. (B, C, D)), the presence of a layer deposited on the surface of the dentin in all samples. However, group 6 outperformed groups 1 and 5 in forming a homogeneous layer that blocked all the dentinal tubules. In groups 1 and 5, ion exchange reactions occur between the BG and AS, which leads to the formation of a layer rich in SiO_2 on the surface of the glass, followed by the deposition of a layer consisting of amorphous calcium and phosphate on SiO_2-rich layer, which is expected to crystallize into HA. However, despite its excellent bioactivity, bioactive glass 45S5 converts to HA slowly and incompletely [250].

The rapid conversion from applied test agent to calcium phosphate layer on the dentin surface is a criterion for evaluating its biological activity. Group 6 excelled in forming a thicker calcium phosphate layer on the surface of the dentin in a short period. This is because the addition of boron to the BG causes structural changes in the glass network, leading to lower chemical durability and a faster dissolution rate than the original glass [170]. Several studies confirmed this by indicating the rapid biodegradation and enhanced bioactivity of boron-containing bioglass [68, 69, 231]. The glass used in this group mainly consists of a borate network due to the high content of B_2O_3 to SiO_2. This network has lower chemical stability than the 45S5 network and its degradation can be controlled by breaking the borate network [69]. This leads to the rapid diffusion of borate ions in the solution, thus promoting the deterioration process .

These results were confirmed by the ICP-MS analysis, where the boron network was decomposed on the third d and phosphorous ion concentrations were reduced concerning calcium ions. However, bioactive glass 45S5 converts to HA slowly and incompletely [250]. Many recent studies proved this as they confirmed that partial or complete replacement of SiO_2 in silicate 45S5 with B_2O_3 resulted in bioactive glass formulations with lower chemical stability, resulting in faster and more complete conversion of the glass to HA [202, 251].

As the degradation rate of the bioactive glass increases, the release rate of degradation agents from the glass will increase. The mechanism for converting the glass to HA may differ from that of the original bioactive glass. All three groups tested showed comparable results concerning the degree of tubules occlusion. The best rapid deposition and occlusion of the dentinal tubules in a short time resulted from the addition of boron to the BG network. BB is a promising substance for treating sensitive teeth and for eliminating pain in a short period of time.

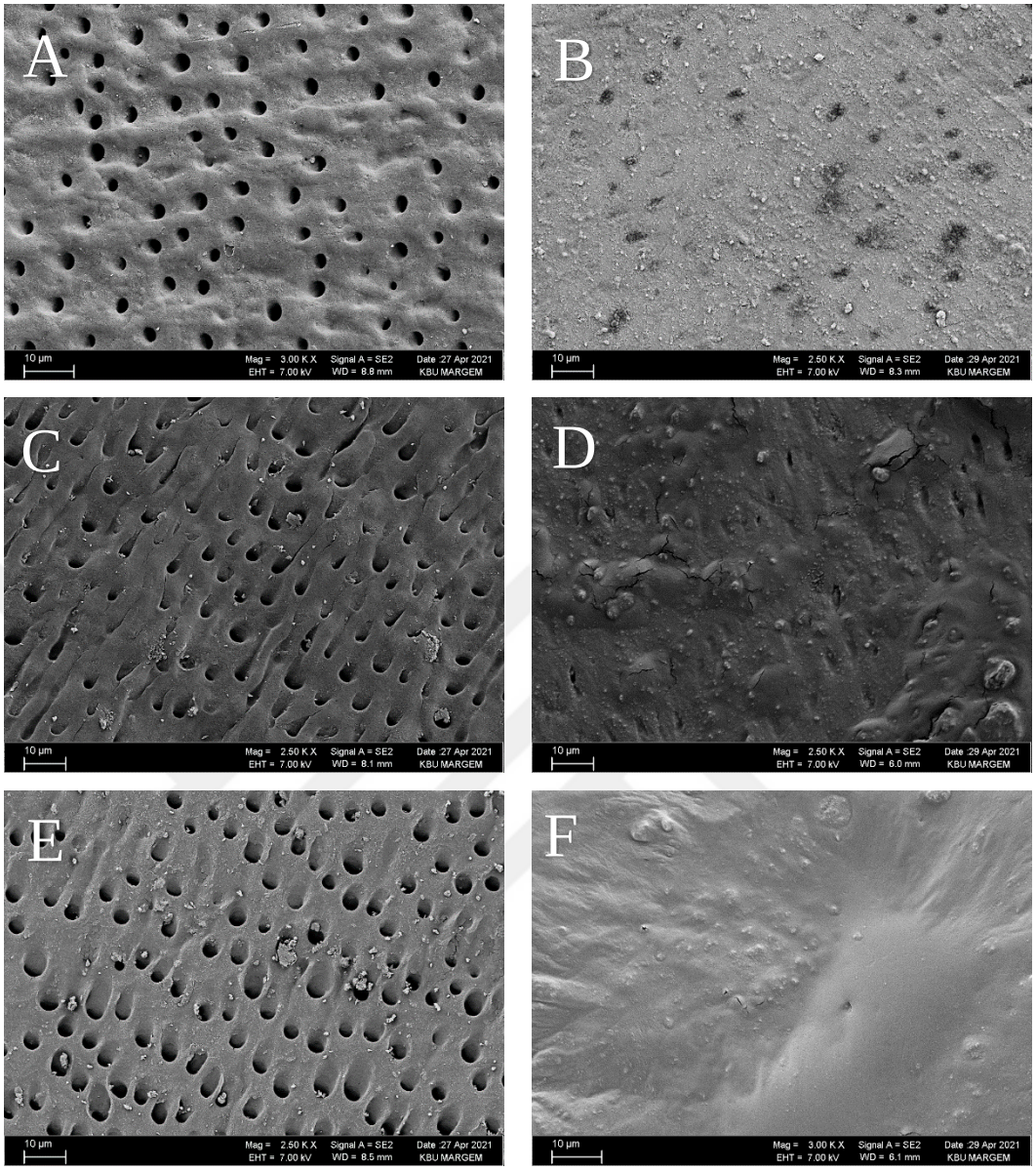


Figure 4.23. SEM image of (A) acid etched and (B) treated with Novamin dentin specimens. (C) SEM image of acid etched and (D treated with BG dentin specimens. (E) SEM image of acid etched and (F) treated with B-BG dentin specimens.

PART 5

CONCLUSION

In this thesis, pure and boron-doped bioactive glass was characterized and compared with different formulations to examine their *in vitro* effects on dentinal tubule occlusion in order to reduce DH. The results indicated a decrease in dentin permeability and narrowing in tubule orifices by forming nano-crystals within the dentinal tubules and resistance to the acid challenge after brushing the dentin with different treatment formulations. However, the results of this study provided conclusive evidence that toothpaste formulations containing bioactive glasses (Novamin, 45S5 BG and B-BG) had high efficiency in occluding dentin tubules compared to the other formulations. In particular, it was found that treatment with BG formulations produced effective dentinal tubular occlusion and could form a layer of HA covering the entire dentinal surface, characterized by an acid-resistant that significantly reduced the hydraulic conductivity of fluid flow through the dentinal tubules. The nano-size of the synthetic BG particles was an influential factor for the diffusion of particles through the dentinal tubules.Furthermore, the B-BG group showed enhanced AS and acid resistance and increases in calcium levels in specific sites. The addition of boron to the BG structure confirmed the improvement of the deposition on the surface of the dentin and the transformation into a thick layer in a long and short time. The samples of the B-BG group gained the most significant amount of weight after treatment. At the same time, the rate of degradation after immersion in AS for 10 days was similar between Novamin, BG and B-BG and AS groups. This provides evidence that BG formulations effectively clogged the tubules over a long period, even after immersion in AS. It is noteworthy that the AS was able to partially block the dentinal tubules and reduce permeability, according to the analysis results. However, this degree of occlusion is not considered sufficient to eliminate DH.

In conclusion, the present *in vitro* study results support the growing evidence in the published literature that toothpaste formulations containing bioactive glasses occlude the dentin tubules and have a solid acid-resisting capacity. Moreover, B-BG can be proposed as a promising effective biomaterial used in hypersensitivity toothpastes. However, *in vivo* studies are required to investigate the efficacy of the prepared formulations in the clinic. In addition, more quantitative and functional investigations are needed to improve the saliva and acids resistance of future hypersensitivity toothpastes to increase the occlusive effect in treating DH.

REFERENCES

1. Addy, M., "Dentine hypersensitivity: new perspectives on an old problem", ***International Dental Journal***, 52 (S5P2): 367–375 (2002).

2. Gillam, D. G., Chesters, R. K., Attrill, D. C., Brunton, P., Slater, M., Strand, P., Whelton, H. and Bartlett, D., "Dentine hypersensitivity–guidelines for the management of a common oral health problem", ***Dental Update***, 40 (7): 514–524 (2013).

3. Porto, I. C. C. M. and rade, A. K. M. and Montes, M. A. J. R., "Diagnosis and treatment of dentinal hypersensitivity", ***Journal Of Oral Science***, 51 (3): 323–332 (2009).

4. Vijay, G., "Dentine Hypersensitivity: A review of treatment modalities.", ***Oral Health (0974-3960)***, 16 (7): (2020).

5. Dababneh, R. H., Khouri, A. T. and Addy, M., "Dentine hypersensitivity—An enigma? A review of terminology, mechanisms, aetiology and management", ***British Dental Journal***, 187 (11): 606–611 (1999).

6. Longridge, N. N. and Youngson, C. C., "Dental pain: dentine sensitivity, hypersensitivity and cracked tooth syndrome", ***Primary Dental Journal***, 8 (1): 44–51 (2019).

7. Chu, C. H., Lui, K. S., Lau, K. P., Kwok, C. M. and Huang, T., "Effects of 8% arginine desensitizing paste on teeth with hypersensitivity", ***J Dent Res***, 89: (2010).

8. Tarbet, W. J., Silverman, G., Stolman, J. M. and Fratarcangelo, P. A., "Clinical evaluation of a new treatment for dentinal hypersensitivity", ***Journal Of Periodontology***, 51 (9): 535–540 (1980).

9. Nasser, N., Jayaram, P., Raju, P. and Chatterjee, A., "Comparative Evaluation of Commercially Available of Three Desensitizing Toothpaste On Dentinal Tubule Occlusion-An In-vitro SEM Study", .

10. Joao-Souza, S. H., Scaramucci, T., Borges, A. B., Lussi, A., Carvalho, T. S. and Aranha, A. C. C., "Influence of desensitizing and anti-erosive toothpastes on dentine permeability: An in vitro study", ***Journal Of Dentistry***, 89: 103176 (2019).

11. Creeth, J., Gallob, J., Sufi, F., Qaqish, J., Gomez-Pereira, P., Budhawant, C. and Goyal, C., "Randomised clinical studies investigating immediate and short-term efficacy of an occluding toothpaste in providing dentine hypersensitivity relief", ***BMC Oral Health***, 19 (1): 1–9 (2019).

12. Wang, Z., Sa, Y., Sauro, S., Chen, H., Xing, W., Ma, X., Jiang, T. and Wang, Y., "Effect of desensitising toothpastes on dentinal tubule occlusion: a dentine permeability measurement and SEM in vitro study", ***Journal Of Dentistry***, 38 (5): 400–410 (2010).

13. Shaikh, K., Pereira, R., Gillam, D. G. and Phad, S., "Comparative Evaluation of Desensitizing Dentifrices containing BioMin®, Novamin® and Fluoride on Dentinal Tubule Occlusion before and after a Citric Acid Challenge–A Scanning Electron Microscope in-vitro Study.", ***Journal Of Odontology***, (2018).

14. Tirapelli, C., Panzeri, H., Soares, R. G., Peitl, O. and Zanotto, E. D., "A novel bioactive glass-ceramic for treating dentin hypersensitivity", ***Brazilian Oral Research***, 24: 381–387 (2010).

15. da Cruz, L. P. D. and de Campos Tuñas, I. T., "Bioactive glass as a treatment option for dentin hypersensitivity", ***Rev. Bras. Odontol***, 75: e1125 (2018).

16. Furlan, R. G., Correr, W. R., Russi, A. F. C., da Costa Iemma, M. R., Trovatti, E. and Pecoraro, É., "Preparation and characterization of boron-based bioglass by sol− gel process", ***Journal Of Sol-Gel Science And Technology***, 88 (1): 181–191 (2018).

17. Elmarakby, W. M. A. A. M., Alkhanani, M. J. and Alghabban, A. S., "How to Deal with Cervical Tooth Sensitivity", .

18. Yamamoto, A., Sakai, K., Matsubara, K., Kano, F. and Ueda, M., "Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury", ***Neuroscience Research***, 78: 16–20 (2014).

19. Davis, H. B., Gwinner, F., Mitchell, J. C. and Ferracane, J. L., "Ion release from and fluoride recharge of a composite with a fluoride-containing bioactive glass", ***Dental Materials***, 30 (10): 1187–1194 (2014).

20. Nanci, A., "Ten Cate's Oral Histology-e-Book: Development, Structure and Function", ***Elsevier Health Sciences***, (2017).

21. Lynnerup, N. and Klaus, H. D., "Fundamentals of human bone and dental biology: structure, function and development", Ortner's Identification of Pathological Conditions in Human Skeletal Remains, ***Elsevier***, 35–58 (2019).

22. Lee, E.-S., Wadhwa, P., Kim, M.-K., Jiang, H. B., Um, I.-W. and Kim, Y.-M., "Organic Matrix of Enamel and Dentin and Developmental Defects", Human Teeth–Structure and Composition of Dental Hard Tissues and Developmental Dental Defects, ***IntechOpen***, (2021).

23. Carda, C. and Peydro, A., "Ultrastructural patterns of human dentinal tubules, odontoblasts processes and nerve fibres", ***Tissue and Cell***, 38 (2): 141–150 (2006).

24. Orchardson, R. and Cadden, S. W., "An update on the physiology of the dentine–pulp complex", ***Dental Update***, 28 (4): 200–209 (2001).

25. Pashley, D. H., "Mechanisms of dentin sensitivity.", ***Dental Clinics of North America***, 34 (3): 449–473 (1990).

26. Pashley, D. H., "Dentine permeability and its role in the pathobiology of dentine sensitivity", ***Archives of Oral Biology***, 39: S73–S80 (1994).

27. Felix, J. and Ouanounou, A., "Dentin Hypersensitivity: Etiology, Diagnosis and Management.", ***Compendium of Continuing Education in Dentistry (Jamesburg, NJ: 1995)***, 40 (10): 653–657 (2019).

28. Yu, C. and Abbott, P. v, "An overview of the dental pulp: its functions and responses to injury", ***Australian Dental Journal***, 52: S4–S6 (2007).

29. Ghannam, M. G., Alameddine, H. and Bordoni, B., "Anatomy, head and neck, pulp (tooth)", (2019).

30. Mohammed, A. S. A. and Ofuonye, I. L. N., "Dentine hypersensitivity: review of a common oral health problem", ***Journal of Dental and Craniofacial Research***, 2 (2): 16 (2017).

31. Bartold, P. M., "Dentinal hypersensitivity: a review", ***Australian Dental Journal***, 51 (3): 212–218 (2006).

32. Ye, W., Feng, X. and Li, R., "The prevalence of dentine hypersensitivity in Chinese adults", ***Journal of Oral Rehabilitation***, 39 (3): 182–187 (2012).

33. West, N. X., Lussi, A., Seong, J. and Hellwig, E., "Dentin hypersensitivity: pain mechanisms and aetiology of exposed cervical dentin", ***Clinical Oral Investigations***, 17 (1): 9–19 (2013).

34. Davari, A. R., Ataei, E. and Assarzadeh, H., "Dentin hypersensitivity: etiology, diagnosis and treatment; a literature review", ***Journal of Dentistry***, 14 (3): 136 (2013).

35. West, N., Seong, J. and Davies, M., "Dentine hypersensitivity", ***Erosive Tooth Wear***, 25: 108–122 (2014).

36. Orchardson, R. and Gillam, D. G., "Managing dentin hypersensitivity", ***The Journal of The American Dental Association***, 137 (7): 990–998 (2006).

37. UZEL, İ. and KESİM, B., "Güncel Restoratif ve Profitez Çalışmaları I", (2019).

38. Miglani, S., Aggarwal, V. and Ahuja, B., "Dentin hypersensitivity: Recent trends in management", ***Journal Of Conservative Dentistry: JCD***, 13 (4): 218 (2010).

39. Bubteina, N. and Garoushi, S., "Dentine hypersensitivity: a review", ***Dentistry***, 5 (330): 1122–2161 (2015).

40. Addy, M. and Dowell, P., "Dentine hypersensitivity-A review: Clinical and in vitro evaluation of treatment agents", ***Journal Of Clinical Periodontology***, 10 (4): 351–363 (1983).

41. Pereira, J. C., de Carvalho Sales-Peres, S. H., Francisconi-dos-Rios, L. F., Calabria, M. P., Ishikiriama, S. K., Gillam, D. G. and Wang, L., "Current and novel clinical approaches for the treatment of dentin hypersensitivity", Dentine Hypersensitivity, ***Springer***, 101–132 (2015).

42. Sgolastra, F., Petrucci, A., Severino, M., Gatto, R. and Monaco, A., "Lasers for the treatment of dentin hypersensitivity: a meta-analysis", ***Journal Of Dental Research***, 92 (6): 492–499 (2013).

43. Borges, A., Barcellos, D. and Gomes, C., "Dentin hypersensitivity-etiology, treatment possibilities and other related factors: a literature review", ***World Journal Of Dentistry***, 3 (1): 60–67 (2012).

44. Talesara, K., Kulloli, A., Shetty, S. and Kathariya, R., "Evaluation of potassium binoxalate gel and Nd: YAG laser in the management of dentinal hypersensitivity: a split-mouth clinical and ESEM study", ***Lasers In Medical Science***, 29 (1): 61–68 (2014).

45. Hu, M.-L., Zheng, G., Zhang, Y.-D., Yan, X., Li, X.-C. and Lin, H., "Effect of desensitizing toothpastes on dentine hypersensitivity: A systematic review and meta-analysis", ***Journal Of Dentistry***, 75: 12–21 (2018).

46. Creeth, J. E., Goyal, C., Qaqish, J., Maclure, R. and Holt, J. S., "Efficacy of an occluding toothpaste on dentinal hypersensitivity over 14 days", ***BDJ Open***, 7 (1): 1–8 (2021).

47. Kim, S. Y., Kim, E. J., Kim, D. S. and Lee, I. B., "The evaluation of dentinal tubule occlusion by desensitizing agents: a real-time measurement of dentinal fluid flow rate and scanning electron microscopy", ***Operative Dentistry***, 38 (4): 419–428 (2013).

48. Maldupa, I., Brinkmane, A., Rendeniece, I. and Mihailova, A., "Evidence based toothpaste classification, according to certain characteristics of their chemical composition", ***Stomatologija***, 14 (1): 12–22 (2012).

49. Jain, P., Reinhardt, J. W. and Krell, K. v, "Effect of dentin desensitizers and dentin bonding agents on dentin permeability.", ***American Journal Of Dentistry***, 13 (1): 21–27 (2000).

50. Pereira, J. C., Segala, A. D. and Gillam, D. G., "Effect of desensitizing agents on the hydraulic conductance of human dentin subjected to different surface pre-treatments-an in vitro study", ***Dental Materials***, 21 (2): 129–138 (2005).

51. Gandolfi, M. G., Silvia, F., Gasparotto, G. and Carlo, P., "Calcium silicate coating derived from Portland cement as treatment for hypersensitive dentine", ***Journal Of Dentistry***, 36 (8): 565–578 (2008).

52. Pashley, D. H., "Dentin permeability, dentin sensitivity and treatment through tubule occlusion", ***Journal Of Endodontics***, 12 (10): 465–474 (1986).

53. Hooper, S. M., Newcombe, R. G., Faller, R., Eversole, S., Addy, M. and West, N. X., "The protective effects of toothpaste against erosion by orange juice: studies in situ and in vitro", ***Journal Of Dentistry***, 35 (6): 476–481 (2007).

54. Cummins, D., "Recent advances in dentin hypersensitivity: clinically proven treatments for instant and lasting sensitivity relief.", ***American Journal Of Dentistry***, 23: 3A-13A (2010).

55. Markowitz, K., "The original desensitizers: strontium and potassium salts.", ***The Journal Of Clinical Dentistry***, 20 (5): 145–151 (2009).

56. Saeki, K., Marshall, G. W., Gansky, S. A., Parkinson, C. R. and Marshall, S. J., "Strontium effects on root dentin tubule occlusion and nanomechanical properties", ***Dental Materials***, 32 (2): 240–251 (2016).

57. Cunha-Cruz, J., Stout, J. R., Heaton, L. J., Wataha, J. C. and PRECEDENT, N., "Dentin hypersensitivity and oxalates: a systematic review", ***Journal Of Dental Research***, 90 (3): 304–310 (2011).

58. Romano, A. C. C. C., Aranha, A. C. C., da Silveira, B. L., Baldochi, S. L. and de Paula Eduardo, C., "Evaluation of carbon dioxide laser irradiation associated with calcium hydroxide in the treatment of dentinal hypersensitivity. A preliminary study", ***Lasers In Medical Science***, 26 (1): 35–42 (2011).

59. Mahmood, A., Mneimne, M., Zou, L. F., Hill, R. G. and Gillam, D. G., "Abrasive wear of enamel by bioactive glass-based toothpastes", ***Am J Dent***, 27 (5): 263–267 (2014).

60. da Cruz, L. P. D., Hill, R. G., Chen, X. and Gillam, D. G., "Dentine tubule occlusion by novel bioactive glass-based toothpastes", ***International Journal Of Dentistry***, 2018: (2018).

61. Baino, F., Hamzehlou, S. and Kargozar, S., "Bioactive glasses: where are we and where are we going?", ***Journal Of Functional Biomaterials***, 9 (1): 25 (2018).

62. Hench, L. L., "The story of Bioglass®", ***Journal Of Materials Science: Materials In Medicine***, 17 (11): 967–978 (2006).

63. Goh, Y.-F., Alshemary, A. Z., Akram, M., Kadir, M. R. A. and Hussain, R., "In vitro study of nano-sized zinc doped bioactive glass", ***Materials Chemistry And Physics***, 137 (3): 1031–1038 (2013).

64. Samueli, A., Hill, R. and Gillam, D. G., "Bioactive Glasses in the Management of Dentine Hypersensitivity: A Review", ***Dental Health: Current Research***, (2017).

65. Hench, L. L., Splinter, R. J., Allen, W. C. and Greenlee, T. K., "Bonding mechanisms at the interface of ceramic prosthetic materials", ***Journal Of Biomedical Materials Research***, 5 (6): 117–141 (1971).

66. Litkowski, L. J., Hack, G. D., Sheaffer, H. B. and Greenspan, D. C., "Occlusion of dentin tubules by 45S5 Bioglass®", ***Bioceramics***, 10: 411–414 (1997).

67. Xia, L., Ma, W., Zhou, Y., Gui, Z., Yao, A., Wang, D., Takemura, A., Uemura, M., Lin, K. and Xu, Y., "Stimulatory effects of boron containing bioactive glass on osteogenesis and angiogenesis of polycaprolactone: in vitro study", ***BioMed Research International***, 2019: (2019).

68. Lu, X., Kolzow, J., Chen, R. R. and Du, J., "Effect of solution condition on hydroxyapatite formation in evaluating bioactivity of B2O3 containing 45S5 bioactive glasses", ***Bioactive Materials***, 4: 207–214 (2019).

69. Yao, A., Wang, D., Huang, W., Fu, Q., Rahaman, M. N. and Day, D. E., "In vitro bioactive characteristics of borate-based glasses with controllable degradation behavior", ***Journal Of The American Ceramic Society***, 90 (1): 303–306 (2007).

70. Strassler, H. E., Drisko, C. L. and Alexander, D. C., "Its Inter-Relationship to Gingival Recession and Acid Erosion", ***Inside Dentistry***, 12–34 (2008).

71. Bekes, K. and Hirsch, C., "What is known about the influence of dentine hypersensitivity on oral health-related quality of life?", ***Clinical Oral Investigations***, 17 (1): 45–51 (2013).

72. Addy, M., "Dentine hypersensitivity: definition, prevalence, distribution and aetiology", ***Tooth Wear And Sensitivity***, 239–248 (2000).

73. Solé-Magdalena, A., Martínez-Alonso, M., Coronado, C. A., Junquera, L. M., Cobo, J. and Vega, J. A., "Molecular basis of dental sensitivity: The odontoblasts are multisensory cells and express multifunctional ion channels", ***Annals Of Anatomy-Anatomischer Anzeiger***, 215: 20–29 (2018).

74. Irvine, J. H., "Root surface sensitivity: a review of aetiology and management", ***Journal Of The New Zealand Society Of Periodontology***, (66): 15–18 (1988).

75. Frank, R. M. and Steuer, P., "Transmission electron microscopy of the human odontoblast process in peripheral root dentine", ***Archives Of Oral Biology***, 33 (2): 91–98 (1988).

76. West, N. X., "Dentine hypersensitivity", ***Dental Erosion***, 20: 173–189 (2006).

77. Gysi, A., "An attempt to explain the sensitiveness of dentine", ***Brit J Dental Sci***, 43: 865–868 (1900).

78. Cunha-Cruz, J., Wataha, J. C., Zhou, L., Manning, W., Trantow, M., Bettendorf, M. M., Heaton, L. J. and Berg, J., "Treating dentin hypersensitivity", ***The Journal Of The American Dental Association***, 141 (9): 1097–1105 (2010).

79. Ahlquist, M., Franzén, O., Coffey, J. and Pashley, D., "Dental pain evoked by hydrostatic pressures applied to exposed dentin in man: a test of the hydrodynamic theory of dentin sensitivity.", ***Journal Of Endodontics***, 20 (3): 130–134 (1994).

80. Absi, E. G., Addy, M. and Adams, D., "Dentine hypersensitivity: a study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentine", ***Journal Of Clinical Periodontology***, 14 (5): 280–284 (1987).

81. Pashley, D. H., "How can sensitive dentine become hypersensitive and can it be reversed?", ***Journal Of Dentistry***, 41: S49–S55 (2013).

82. Kolker, J. L., Vargas, M. A., Armstrong, S. R. and Dawson, D. v, "Effect of desensitizing agents on dentin permeability and dentin tubule occlusion", ***Journal Of Adhesive Dentistry***, 4: 211–222 (2002).

83. Arrais, C. A. G., Chan, D. C. N. and Giannini, M., "Effects of desensitizing agents on dentinal tubule occlusion", ***Journal Of Applied Oral Science***, 12: 144–148 (2004).

84. Scherman, A. and Jacobsen, P. L., "Managing dentin hypersensitivity: what treatment to recommend to patients", ***The Journal of The American Dental Association***, 123 (4): 57–61 (1992).

85. Holland, G. R., "Morphological features of dentine and pulp related to dentine sensitivity", ***Archives of Oral Biology***, 39: S3–S11 (1994).

86. Brännström, M. and Garberoglio, R., "The dentinal tubules and the odontoblast processes a scanning electron microscopic study", ***Acta Odontologica Scandinavica***, 30 (3): 291–311 (1972).

87. Amaechi, B. T., "Dental Erosion and Its Clinical Management", ***Springer***, (2015).

88. Tay, F. R. and Pashley, D. H., "Resin bonding to cervical sclerotic dentin: a review", ***Journal of Dentistry***, 32 (3): 173–196 (2004).

89. Baig, M., Cook, R., Dennington, S., Gee, M., Pratten, J. and Wood, R., "Understanding the effects of tooth brushing using an abrasive dentifrice on the wear of enamel", (2017).

90. Cartwright, R. B., "Dentinal hypersensitivity: a narrative review", ***Community Dent Health***, 31 (1): 15–20 (2014).

91. West, N., Addy, M. and Hughes, J., "Dentine hypersensitivity: the effects of brushing desensitizing toothpastes, their solid and liquid phases and detergents on dentine and acrylic: studies in vitro", ***Journal of Oral Rehabilitation***, 25 (12): 885–895 (1998).

92. Benjakul, P. and Chuenarrom, C., "Association of dental enamel loss with the pH and titratable acidity of beverages", ***Journal of Dental Sciences***, 6 (3): 129–133 (2011).

93. Touyz, L. Z., "The acidity (pH) and buffering capacity of Canadian fruit juice and dental implications.", ***Journal (Canadian Dental Association)***, 60 (5): 454–458 (1994).

94. Addy, M., "Introduction and overview: statement of the problem", Dentine Hypersensitivity, ***Springer***, 1–10 (2015).

95. Duran, I. and Sengun, A., "The long-term effectiveness of five current desensitizing products on cervical dentine sensitivity", ***Journal of Oral Rehabilitation***, 31 (4): 351–356 (2004).

96. Pamir, T., Dalgar, H. and Onal, B., "Clinical evaluation of three desensitizing agents in relieving dentin hypersensitivity", ***Operative Dentistry***, 32 (6): 544–548 (2007).

97. Veitz-Keenan, A., Barna, J. A., Strober, B., Matthews, A. G., Collie, D., Vena, D., Curro, F. A. and Thompson, V. P., "Treatments for hypersensitive noncarious cervical lesions: a Practitioners Engaged in Applied Research and Learning (PEARL) Network randomized clinical effectiveness study", ***The Journal Of The American Dental Association***, 144 (5): 495–506 (2013).

98. Mehta, D., Gowda, V. S., Santosh, A., Finger, W. J. and Sasaki, K., "Randomized controlled clinical trial on the efficacy of dentin desensitizing agents", ***Acta Odontologica Scandinavica***, 72 (8): 936–941 (2014).

99. Vieira, A. H. M., Passos, V. F., de Assis, J. S., Mendonça, J. S. and Santiago, S. L., "Clinical evaluation of a 3% potassium oxalate gel and a GaAlAs laser for the treatment of dentinal hypersensitivity", ***Photomedicine And Laser Surgery***, 27 (5): 807–812 (2009).

100. Pei, D., Liu, S., Huang, C., Du, X., Yang, H., Wang, Y. and Deng, D., "Effect of pretreatment with calcium-containing desensitizer on the dentine bonding of mild self-etch adhesives", ***European Journal Of Oral Sciences***, 121 (3pt1): 204–210 (2013).

101. Bra, M. and Johnson, G., "Effects of various conditioners and cleaning agents on prepared dentin surfaces: a scanning electron microscopic investigation", ***The Journal of Prosthetic Dentistry***, 31 (4): 422–430 (1974).

102. Türkün, Ş. L., "Clinical evaluation of a self-etching and a one-bottle adhesive system at two years", ***Journal of Dentistry***, 31 (8): 527–534 (2003).

103. Qin, C., Xu, J. and Zhang, Y., "Spectroscopic investigation of the function of aqueous 2-hydroxyethylmethacrylate/glutaraldehyde solution as a dentin desensitizer", ***European Journal Of Oral Sciences***, 114 (4): 354–359 (2006).

104. Femiano, F., Femiano, R., Lanza, A., Festa, M. V., Rullo, R. and Perillo, L., "Efficacy of diode laser in association to sodium fluoride vs Gluma desensitizer on treatment of cervical dentin hypersensitivity. A double blind controlled trial", ***Am J Dent***, 26 (4): 214–218 (2013).

105. Cilli, R., Prakki, A., de Araújo, P. A. and Pereira, J. C., "Influence of glutaraldehyde priming on bond strength of an experimental adhesive system applied to wet and dry dentine", ***Journal Of Dentistry***, 37 (3): 212–218 (2009).

106. Sethna, G. D., Prabhuji, M. L. and Karthikeyan, B. v, "Comparison of two different forms of varnishes in the treatment of dentine hypersensitivity: a subject-blind randomised clinical study", ***Oral Health Prev Dent***, 9 (2): 143–150 (2011).

107. Owoturo, O. E., Loto, A. O., Awotile, A. O., Oyapero, A. and Ebigwei, S. O., "Comparison of two different forms of varnishes in the treatment of dentine hypersensitivity: A subject-blind clinical study in Lagos State university teaching hospital", ***Archives of Medicine And Health Sciences***, 5 (2): 191 (2017).

108. Lochaiwatana, Y., Poolthong, S., Hirata, I., Okazaki, M., Swasdison, S. and Vongsavan, N., "The synthesis and characterization of a novel potassium chloride-fluoridated hydroxyapatite varnish for treating dentin hypersensitivity", ***Dental Materials Journal***, 2014–2102 (2015).

109. Ladalardo, T. C. C. G. P., Pinheiro, A., Campos, R. A. de C., Brugnera Júnior, A., Zanin, F., Albernaz, P. L. M. and Weckx, L. L. M., "Laser therapy in the treatment of dentine hypersensitivity", ***Brazilian Dental Journal***, 15: 144–150 (2004).

110. Asnaashari, M. and Moeini, M., "Effectiveness of lasers in the treatment of dentin hypersensitivity", ***Journal of Lasers in Medical Sciences***, 4 (1): 1 (2013).

111. Patil, A. R., Varma, S., Suragimath, G., Abbayya, K., Zope, S. A. and Kale, V., "Comparative evaluation of efficacy of iontophoresis with 0.33% sodium fluoride gel and diode laser alone on occlusion of dentinal tubules", ***Journal of Clinical and Diagnostic Research: JCDR***, 11 (8): ZC123 (2017).

112. Aghanasini, S., Rajeshwari, H. R., Basavarajappa, M. D., Sapna, N., Phadke, P. v and Sravani, K., "Novamin versus Sodium Flouride Iontophoresis: The “Salvage Crew” to the Rescue", .

113. Lindhe, J., Karring, T. and Lang, N. P., "Clinical Periodontology and Implant Dentistry", ***Blackwell***, (2003).

114. Hwang, D. and Wang, H., "Flap thickness as a predictor of root coverage: a systematic review", ***Journal of Periodontology***, 77 (10): 1625–1634 (2006).

115. Chambrone, L., Sukekava, F., Araújo, M. G., Pustiglioni, F. E., Chambrone, L. A. and Lima, L. A., "Root-coverage procedures for the treatment of localized recession-type defects: A Cochrane systematic review", ***Journal of Periodontology***, 81 (4): 452–478 (2010).

116. Douglas de Oliveira, D. W., Oliveira-Ferreira, F., Flecha, O. D. and Gonçalves, P. F., "Is surgical root coverage effective for the treatment of cervical dentin hypersensitivity? A systematic review", ***Journal of Periodontology***, 84 (3): 295–306 (2013).

117. Sarapur, S. and Shilpashree, H. S., "Dentin hypersensitivity: a review", ***International Journal of Dental Clinics***, 4 (1): (2012).

118. Shiau, H. J., "Dentin hypersensitivity", ***Journal of Evidence Based Dental Practice***, 12 (3): 220–228 (2012).

119. Cummins, D., "The efficacy of a new dentifrice containing 8.0% arginine, calcium carbonate and 1450 ppm fluoride in delivering instant and lasting relief of dentin hypersensitivity.", ***The Journal of Clinical Dentistry***, 20 (4): 109–114 (2009).

120. Sharma, S., Shetty, N. J. and Uppoor, A., "Evaluation of the clinical efficacy of potassium nitrate desensitizing mouthwash and a toothpaste in the treatment of dentinal hypersensitivity", ***Journal Of Clinical And Experimental Dentistry***, 4 (1): e28 (2012).

121. Andini, S., Masulili, S. L. C. and Kemal, Y., "Toothpaste containing sodium bicarbonate, sodium fluoride and potassium nitrate for dentine hypersensitivity with periodontal inflammation", (2018).

122. Frechoso, S. C., Menéndez, M., Guisasola, C., Arregui, I., Tejerina, J. M. and Sicilia, A., "Evaluation of the efficacy of two potassium nitrate bioadhesive gels (5% and 10%) in the treatment of dentine hypersensitivity. A randomised clinical trial", ***Journal Of Clinical Periodontology***, 30 (4): 315–320 (2003).

123. Poulsen, S., Errboe, M., Mevil, Y. L. and Glenny, A., "Potassium containing toothpastes for dentine hypersensitivity", ***Cochrane Database Of Systematic Reviews***, (3): (2006).

124. Acharya, A. B., Surve, S. M. and Thakur, S. L., "A clinical study of the effect of calcium sodium phosphosilicate on dentin hypersensitivity", ***Journal of Clinical And Experimental Dentistry***, 5 (1): e18 (2013).

125. Schiff, T., Zhang, Y. P., DeVizio, W., Stewart, B., Chaknis, P., Petrone, M. E., Volpe, A. R. and Proskin, H. M., "A randomized clinical trial of the desensitizing efficacy of three dentifrices.", ***Compendium of Continuing Education In Dentistry. (Jamesburg, NJ: 1995). Supplement***, (27): 4–10 (2000).

126. Arnold, W. H., Prange, M. and Naumova, E. A., "Effectiveness of various toothpastes on dentine tubule occlusion", ***Journal of Dentistry***, 43 (4): 440–449 (2015).

127. Hodosh, M., "A superior desensitizer—potassium nitrate", ***The Journal of The American Dental Association***, 88 (4): 831–832 (1974).

128. Markowitz, K. and Kim, S., "Hypersensitive teeth. Experimental studies of dentinal desensitizing agents.", ***Dental Clinics of North America***, 34 (3): 491–501 (1990).

129. Markowitz, K. and Pashley, D. H., "Discovering new treatments for sensitive teeth: the long path from biology to therapy", ***Journal of Oral Rehabilitation***, 35 (4): 300–315 (2008).

130. Arısu, H. D., Dalkıhç, E. and Üçtaşlı, M. B., "Effect of desensitizing agents on the microtensile bond strength of a two-step self-etch adhesive to dentin", ***Operative Dentistry***, 36 (2): 153–161 (2011).

131. Suge, T., Kawasaki, A., Ishikawa, K., Matsuo, T. and Ebisu, S., "Effect of ammonium hexafluorosilicate on dentin tubule occlusion for the treatment of dentin hypersensitivity.", ***American Journal of Dentistry***, 19 (4): 248–252 (2006).

132. Suge, T., Kawasaki, A., Ishikawa, K., Matsuo, T. and Ebisu, S., "Ammonium hexafluorosilicate elicits calcium phosphate precipitation and shows continuous dentin tubule occlusion", ***Dental Materials***, 24 (2): 192–198 (2008).

133. Hansen, E. K., "Dentin hypersensitivity treated with a fluoride-containing varnish or a light-cured glass-ionomer liner", ***European Journal of Oral Sciences***, 100 (6): 305–309 (1992).

134. Wang, Z., Jiang, T., Sauro, S., Pashley, D. H., Toledano, M., Osorio, R., Liang, S., Xing, W., Sa, Y. and Wang, Y., "The dentine remineralization activity of a desensitizing bioactive glass-containing toothpaste: an in vitro study", ***Australian Dental Journal***, 56 (4): 372–381 (2011).

135. Vachiramon, V., Vargas, M. A., Pashley, D. H., Tay, F. R., Geraldeli, S., Qian, F. and Armstrong, S. R., "Effects of oxalate on dentin bond after 3-month simulated pulpal pressure", ***Journal of Dentistry***, 36 (3): 178–185 (2008).

136. Merika, K., HeftitArthur, F. and Preshaw, P. M., "Comparison of two topical treatments for dentine sensitivity.", ***The European Journal of Prosthodontics And Restorative Dentistry***, 14 (1): 38–41 (2006).

137. Gillam, D. G., Newman, H. N., Davies, E. H., Bulman, J. S., Troullos, E. S. and Curro, F. A., "Clinical evaluation of ferric oxalate in relieving dentine hypersensitivity", ***Journal of Oral Rehabilitation***, 31 (3): 245–250 (2004).

138. Erdemir, U., Yildiz, E., Kilic, I., Yucel, T. and Ozel, S., "The efficacy of three desensitizing agents used to treat dentin hypersensitivity", ***The Journal of The American Dental Association***, 141 (3): 285–296 (2010).

139. Camilotti, V., Zilly, J., Busato, P. do M. R., Nassar, C. A. and Nassar, P. O., "Desensitizing treatments for dentin hypersensitivity: a randomized, split-mouth clinical trial", ***Brazilian Oral Research***, 26: 263–268 (2012).

140. Vora, J., Mehta, D., Meena, N., Sushma, G., Finger, W. J. and Kanehira, M., "Effects of two topical desensitizing agents and placebo on dentin hypersensitivity.", ***American Journal of Dentistry***, 25 (5): 293–298 (2012).

141. Hare, T. C., Zsiska, M., Boissy, Y., Dufresne, T. E., Nurre, J. H., Chmielewski, P. A. and Drake, P. A., "Immediate and Durable Effects of an Oxalate Strip on Human Dentin In Vitro.", ***Compendium of Continuing Education In Dentistry (Jamesburg, NJ: 1995)***, 37 (1): e6–e12 (2016).

142. Olley, R. C., Pilecki, P., Hughes, N., Jeffery, P., Austin, R. S., Moazzez, R. and Bartlett, D., "An in situ study investigating dentine tubule occlusion of dentifrices following acid challenge", ***Journal of Dentistry***, 40 (7): 585–593 (2012).

143. Docimo, R., Perugia, C., Bartolino, M., Maturo, P., Montesani, L., Zhang, Y., DeVizio, W., Mateo, L. and Dibart, S., "Comparative evaluation of the efficacy of three commercially available toothpastes on dentin hypersensitivity reduction: an eight-week clinical study", (2011).

144. Banfield, N. and Addy, M., "Dentine hypersensitivity: development and evaluation ofamodel in situ to study tubulepatency", ***Journal of Clinical Periodontology***, 31 (5): 325–335 (2004).

145. Pillai, V. P. and Neelakantan, P., "Desensitizing toothpastes for treatment of dentin hypersensitivity", ***International Journal of PharmTech Research***, (2013).

146. Petrou, I., Heu, R., Stranick, M., Lavender, S., Zaidel, L., Cummins, D., Sullivan, R. J., Hsueh, C. and Gimzewski, J. K., "A breakthrough therapy for dentin hypersensitivity: how dental products containing 8% arginine and calcium carbonate work to deliver effective relief of sensitive teeth", ***Journal of Clinical Dentistry***, 20 (1): 23 (2009).

147. Docimo, R., Montesani, L., Maturo, P., Costacurta, M., Bartolino, M., DeVizio, W., Zhang, Y., Cummins, D., Dibart, S. and Mateo, L., "Comparing the efficacy in reducing dentin hypersensitivity of a new toothpaste containing 8.0% arginine, calcium carbonate and 1450 ppm fluoride to a commercial sensitive toothpaste containing 2% potassium ion: an eight-week clinical study in Rome, Italy", (2009).

148. Schiff, T., Delgado, E., Zhang, Y. P., DeVizio, W., Cummins, D. and Mateo, L. R., "The clinical effect of a single direct topical application of a dentifrice containing 8.0% arginine, calcium carbonate and 1450 ppm fluoride on dentin hypersensitivity: the use of a cotton swab applicator versus the use of a fingertip.", ***The Journal of Clinical Dentistry***, 20 (4): 131–136 (2009).

149. Yang, Z., Wang, F., Lu, K., Li, Y. and Zhou, Z., "Arginine-containing desensitizing toothpaste for the treatment of dentin hypersensitivity: a meta-analysis", ***Clinical, Cosmetic and Investigational Dentistry***, 8: 1 (2016).

150. Pashley, D. H., "Sensitivity of dentin to chemical stimuli", ***Dental Traumatology***, 2 (4): 130–137 (1986).

151. ALJAMMALI, Z. M., "SURVEY ON TYPES OF DENTAL AMALGAM IN MEDICINE", .

152. de Bruyne, M. A., de Moor, R. J. and Raes, F. M., "Necrosis of the gingiva caused by calcium hydroxide: a case report.", ***International Endodontic Journal***, 33 (1): 67–71 (2000).

153. Zand, V., Mokhtari, H., Hasani, A. and Jabbari, G., "Comparison of the penetration depth of conventional and nano-particle calcium hydroxide into dentinal tubules", ***Iranian Endodontic Journal***, 12 (3): 366 (2017).

154. Cummins, D., "Advances in the clinical management of dentin hypersensitivity: a review of recent evidence for the efficacy of dentifrices in providing instant and lasting relief", ***Journal of Clinical Dentistry***, 22 (4): 100 (2011).

155. Joshi, S., Gowda, A. S. and Joshi, C., "Comparative evaluation of NovaMin desensitizer and Gluma desensitizer on dentinal tubule occlusion: a scanning electron microscopic study", ***Journal of Periodontal & Implant Science***, 43 (6): 269–275 (2013).

156. LaTorre, G. and Greenspan, D. C., "The role of ionic release from NovaMin®(calcium sodium phosphosilicate) in tubule occlusion: an exploratory in vitro study using radio-labeled isotopes", ***Journal Of Clinical Dentistry***, 21 (3): 72 (2010).

157. Layer, T. M., "Development of a fluoridated, daily-use toothpaste containing NovaMin technology for the treatment of dentin hypersensitivity", ***J Clin Dent***, 22 (3): 59–61 (2011).

158. Sharma, N., Roy, S., Kakar, A., Greenspan, D. C. and Scott, R., "A clinical study comparing oral formulations containing 7.5% calcium sodium phosphosilicate (NovaMin), 5% potassium nitrate and 0.4% stannous fluoride for the management of dentin hypersensitivity.", ***The Journal of Clinical Dentistry***, 21 (3): 88–92 (2010).

159. Jones, S. B., Parkinson, C. R., Jeffery, P., Davies, M., Macdonald, E. L., Seong, J. and West, N. X., "A randomised clinical trial investigating calcium sodium phosphosilicate as a dentine mineralising agent in the oral environment", ***Journal of Dentistry***, 43 (6): 757–764 (2015).

160. Gillam, D. G., Tang, J. Y., Mordan, N. J. and Newman, H. N., "The effects of a novel Bioglass® dentifrice on dentine sensitivity: a scanning electron microscopy investigation", ***Journal Of Oral Rehabilitation***, 29 (4): 305–313 (2002).

161. Rad, R. M., Alshemary, A. Z., Evis, Z., Keskin, D., Altunbaş, K. and Tezcaner, A., "Structural and biological assessment of boron doped bioactive glass nanoparticles for dental tissue applications", ***Ceramics International***, 44 (8): 9854–9864 (2018).

162. Kokubo, T., Kim, H.-M., Kawashita, M. and Nakamura, T., "REVIEW Bioactive metals: preparation and properties", ***Journal of Materials Science: Materials In Medicine***, 15 (2): 99–107 (2004).

163. Curtis, A. R., West, N. X. and Su, B., "Synthesis of nanobioglass and formation of apatite rods to occlude exposed dentine tubules and eliminate hypersensitivity", ***Acta Biomaterialia***, 6 (9): 3740–3746 (2010).

164. Kaur, G., Pandey, O. P., Singh, K., Homa, D., Scott, B. and Pickrell, G., "A review of bioactive glasses: their structure, properties, fabrication and apatite formation", ***Journal Of Biomedical Materials Research Part A: An Official Journal Of The Society For Biomaterials, The Japanese Society For Biomaterials and The Australian Society For Biomaterials And The Korean Society For Biomaterials***, 102 (1): 254–274 (2014).

165. Desimone, D., Li, W., Roether, J. A., Schubert, D. W., Crovace, M. C., Rodrigues, A. C. M., Zanotto, E. D. and Boccaccini, A. R., "Biosilicate®–gelatine bone scaffolds by the foam replica technique: development and characterization", ***Science And Technology Of Advanced Materials***, (2013).

166. Hoppe, A., Güldal, N. S. and Boccaccini, A. R., "A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics", ***Biomaterials***, 32 (11): 2757–2774 (2011).

167. Fraga, C. G., "Relevance, essentiality and toxicity of trace elements in human health", ***Molecular Aspects of Medicine***, 26 (4–5): 235–244 (2005).

168. Maeno, S., Niki, Y., Matsumoto, H., Morioka, H., Yatabe, T., Funayama, A., Toyama, Y., Taguchi, T. and Tanaka, J., "The effect of calcium ion concentration on osteoblast viability, proliferation and differentiation in monolayer and 3D culture", ***Biomaterials***, 26 (23): 4847–4855 (2005).

169. Beck, G. R., Zerler, B. and Moran, E., "Phosphate is a specific signal for induction of osteopontin gene expression", ***Proceedings of The National Academy of Sciences***, 97 (15): 8352–8357 (2000).

170. Balasubramanian, P., Buettner, T., Pacheco, V. M. and Boccaccini, A. R., "Boron-containing bioactive glasses in bone and soft tissue engineering", ***Journal of The European Ceramic Society***, 38 (3): 855–869 (2018).

171. Warington, K., "The effect of boric acid and borax on the broad bean and certain other plants", ***Annals of Botany***, 37 (148): 629–672 (1923).

172. Dzondo-Gadet, M., Mayap-Nzietchueng, R., Hess, K., Nabet, P., Belleville, F. and Dousset, B., "Action of boron at the molecular level", ***Biological Trace Element Research***, 85 (1): 23–33 (2002).

173. Moseman, R. F., "Chemical disposition of boron in animals and humans.", ***Environmental Health Perspectives***, 102 (suppl 7): 113–117 (1994).

174. Chapin, R. E., Ku, W. W., Kenney, M. A. and McCoy, H., "The effects of dietary boric acid on bone strength in rats", ***Biological Trace Element Research***, 66 (1): 395–399 (1998).

175. Nielsen, F. H., "Studies on the relationship between boron and magnesium which possibly affects the formation and maintenance of bones.", ***Magnesium and Trace Elements***, 9 (2): 61–69 (1990).

176. Moonesi Rad, R., Pazarceviren, E., Ece Akgün, E., Evis, Z., Keskin, D., Şahin, S. and Tezcaner, A., "In vitro performance of a nanobiocomposite scaffold containing boron-modified bioactive glass nanoparticles for dentin regeneration", ***Journal of Biomaterials Applications***, 33 (6): 834–853 (2019).

177. Wu, C., Miron, R., Sculean, A., Kaskel, S., Doert, T., Schulze, R. and Zhang, Y., "Proliferation, differentiation and gene expression of osteoblasts in boron-containing associated with dexamethasone deliver from mesoporous bioactive glass scaffolds", ***Biomaterials***, 32 (29): 7068–7078 (2011).

178. Ciftci, E., Köse, S., Korkusuz, P., Timuçin, M. and Korkusuz, F., "Boron containing nano hydroxyapatites (Bn-HAp) stimulate mesenchymal stem cell adhesion, proliferation and differentiation", (2015).

179. Yang, X., Zhang, L., Chen, X., Sun, X., Yang, G., Guo, X., Yang, H., Gao, C. and Gou, Z., "Incorporation of B2O3 in CaO-SiO2-P2O5 bioactive glass system for improving strength of low-temperature co-fired porous glass ceramics", ***Journal Of Non-Crystalline Solids***, 358 (9): 1171–1179 (2012).

180. Laczka, M., Cholewa-Kowalska, K., Laczka-Osyczka, A., Tworzydlo, M. and Turyna, B., "Gel-derived materials of a CaO-P2O5-SiO2 system modified by boron, sodium, magnesium, aluminum and fluorine compounds", ***Journal Of Biomedical Materials Research***, 52 (4): 601–612 (2000).

181. Xiu, T., Liu, Q. and Wang, J., "Comparisons between surfactant-templated mesoporous and conventional sol–gel-derived CaO–B2O3–SiO2 glasses: Compositional, textural and in vitro bioactive properties", ***Journal Of Solid State Chemistry***, 181 (4): 863–870 (2008).

182. Huang, W., Day, D. E., Kittiratanapiboon, K. and Rahaman, M. N., "Kinetics and mechanisms of the conversion of silicate (45S5), borate and borosilicate glasses to hydroxyapatite in dilute phosphate solutions", ***Journal Of Materials Science: Materials In Medicine***, 17 (7): 583–596 (2006).

183. Dey, P., Pal, S. K., Banerjee, I. and Sarkar, R., "Effect of addition of B 2 O 3 to the sol-gel synthesized 45S5 bioglass", ***Journal Of The Australian Ceramic Society***, 56: 1309–1322 (2020).

184. Liu, X., Pan, H., Fu, H., Fu, Q., Rahaman, M. N. and Huang, W., "Conversion of borate-based glass scaffold to hydroxyapatite in a dilute phosphate solution", ***Biomedical Materials***, 5 (1): 015005 (2010).

185. Polini, A., Bai, H. and Tomsia, A. P., "Dental applications of nanostructured bioactive glass and its composites", ***Wiley Interdisciplinary Reviews: Nanomedicine And Nanobiotechnology***, 5 (4): 399–410 (2013).

186. Cuy, J. L., Mann, A. B., Livi, K. J., Teaford, M. F. and Weihs, T. P., "Nanoindentation mapping of the mechanical properties of human molar tooth enamel", ***Archives Of Oral Biology***, 47 (4): 281–291 (2002).

187. Kumar, N. G. and Mehta, D. S., "Short-term assessment of the Nd: YAG laser with and without sodium fluoride varnish in the treatment of dentin hypersensitivity–a clinical and scanning electron microscopy study", ***Journal of Periodontology***, 76 (7): 1140–1147 (2005).

188. Gillam, D. G. and Newman, H. N., "Iontophoresis in the treatment of cervical dentinal sensitivity--a review.", (1990).

189. Närhi, M., Jyväsjärvi, E., Virtanen, A., Huopaniemi, T., Ngassapa, D. and Hirvonen, T., "Role of intradental A-and C-type nerve fibres in dental pain mechanisms", ***Proc Finn Dent Soc***, 88 (Suppl 1): 507–516 (1992).

190. Minkoff, S. and Axelrod, S., "Efficacy of strontium chloride in dental hypersensitivity", ***Journal of Periodontology***, 58 (7): 470–474 (1987).

191. Forsback, A., Areva, S. and Salonen, J., "Mineralization of dentin induced by treatment with bioactive glass S53P4 in vitro", ***Acta Odontologica Scandinavica***, 62 (1): 14–20 (2004).

192. Pirayesh, H. and Nychka, J. A., "Sol–gel synthesis of bioactive glass-ceramic 45S5 and its in vitro dissolution and mineralization behavior", ***Journal Of The American Ceramic Society***, 96 (5): 1643–1650 (2013).

193. Yuan, P., Lu, W., Xu, H., Yang, J., Liu, C. and Xu, P., "In vitro dentin tubule occlusion by an arginine-containing dentifrice.", ***American Journal Of Dentistry***, 32 (3): 133–137 (2019).

194. Violich, D. R. and Chandler, N. P., "The smear layer in endodontics–a review", ***International Endodontic Journal***, 43 (1): 2–15 (2010).

195. Hasheminia, S. M., Birang, R., Feizianfard, M. and Nasouri, M., "A comparative study of the removal of smear layer by two endodontic irrigants and Nd: YAG Laser: a scanning electron microscopic study", ***International Scholarly Research Notices***, 2012: (2012).

196. Dulbecco, R. and Vogt, M., "Plaque formation and isolation of pure lines with poliomyelitis viruses", ***Journal of Experimental Medicine***, 99 (2): 167–182 (1954).

197. Zheng, K., Fan, Y., Torre, E., Balasubramanian, P., Taccardi, N., Cassinelli, C., Morra, M., Iviglia, G. and Boccaccini, A. R., "Incorporation of boron in mesoporous bioactive glass nanoparticles reduces inflammatory response and delays osteogenic differentiation", ***Particle & Particle Systems Characterization***, 37 (7): 2000054 (2020).

198. El-Kady, A. M., Ali, A. F. and Farag, M. M., "Development, characterization and in vitro bioactivity studies of sol–gel bioactive glass/poly (L-lactide) nanocomposite scaffolds", ***Materials Science and Engineering: C***, 30 (1): 120–131 (2010).

199. Brunner, T. J., Stark, W. J. and Boccaccini, A. R., "Nanoscale bioactive silicate glasses in biomedical applications", ***Preface XV List of Contributors XIX***, (2009).

200. Vichery, C. and Nedelec, J.-M., "Bioactive glass nanoparticles: from synthesis to materials design for biomedical applications", ***Materials***, 9 (4): 288 (2016).

201. Erasmus, E. P., Johnson, O. T., Sigalas, I. and Massera, J., "Effects of sintering temperature on crystallization and fabrication of porous bioactive glass scaffolds for bone regeneration", ***Scientific Reports***, 7 (1): 1–12 (2017).

202. Liang, W., Rahaman, M. N., Day, D. E., Marion, N. W., Riley, G. C. and Mao, J. J., "Bioactive borate glass scaffold for bone tissue engineering", ***Journal of Non-Crystalline Solids***, 354 (15–16): 1690–1696 (2008).

203. Gorustovich, A. A., López, J. M. P., Guglielmotti, M. B. and Cabrini, R. L., "Biological performance of boron-modified bioactive glass particles implanted in rat tibia bone marrow", ***Biomedical Materials***, 1 (3): 100 (2006).

204. Du, R. L., Chang, J., Ni, S. Y., Zhai, W. Y. and Wang, J. Y., "Characterization and in vitro bioactivity of zinc-containing bioactive glass and glass-ceramics", ***Journal of Biomaterials Applications***, 20 (4): 341–360 (2006).

205. Desogus, L., Cuccu, A., Montinaro, S., Orru, R., Cao, G., Bellucci, D., Sola, A. and Cannillo, V., "Classical Bioglass® and innovative CaO-rich bioglass powders processed by Spark Plasma Sintering: A comparative study", ***Journal of the European Ceramic Society***, 35 (15): 4277–4285 (2015).

206. Grasso, S., Chinnam, R. K., Porwal, H., Boccaccini, A. R. and Reece, M. J., "Low temperature spark plasma sintering of 45S5 Bioglass®", ***Journal Of Non-Crystalline Solids***, 362: 25–29 (2013).

207. Clupper, D. C. and Hench, L. L., "Crystallization kinetics of tape cast bioactive glass 45S5", ***Journal of Non-Crystalline Solids***, 318 (1–2): 43–48 (2003).

208. Derrick, M. R., Stulik, D. and Landry, J. M., "Infrared Spectroscopy in Conservation Science", ***Getty Publications***, (2000).

209. Carvalho, S. M. de, Martins, T. and Pereira, M. de M., "Fluorine-containing bioactive glass spherical particles synthesized by sol-gel route assisted by ultrasound energy or mechanical mixing", ***Materials Research***, 23: (2020).

210. Ouis, M. A., Abdelghany, A. M. and ElBatal, H. A., "Corrosion mechanism and bioactivity of borate glasses analogue to Hench's bioglass", ***Processing And Application Of Ceramics***, 6 (3): 141–149 (2012).

211. Goh, Y.-F., Alshemary, A. Z., Akram, M., Kadir, M. R. A. and Hussain, R., "In-vitro characterization of antibacterial bioactive glass containing ceria", ***Ceramics International***, 40 (1): 729–737 (2014).

212. Li, Y., Cai, S., Xu, G., Shen, S., Zhang, M., Zhang, T. and Sun, X., "Synthesis and characterization of a phytic acid/mesoporous 45S5 bioglass composite coating on a magnesium alloy and degradation behavior", ***RSC Advances***, 5 (33): 25708–25716 (2015).

213. Pal, M., Roy, B. and Pal, M., "Structural characterization of borate glasses containing zinc and manganese oxides", ***Journal of Modern Physics***, 2011: (2011).

214. Zhou, Y., Yang, Y., Huang, F., Ren, J., Yuan, S. and Chen, G., "Characterization of new tellurite glasses and crystalline phases in the TeO2–PbO–Bi2O3–B2O3 system", ***Journal of Non-Crystalline Solids***, 386: 90–94 (2014).

215. Hench, L. L., "Chronology of bioactive glass development and clinical applications", (2013).

216. Martin, R. A., Twyman, H., Qiu, D., Knowles, J. C. and Newport, R. J., "A study of the formation of amorphous calcium phosphate and hydroxyapatite on melt quenched Bioglass® using surface sensitive shallow angle X-ray diffraction", ***Journal of Materials Science: Materials in Medicine***, 20 (4): 883–888 (2009).

217. Jones, J. R., "Reprint of: Review of bioactive glass: From Hench to hybrids", ***Acta Biomaterialia***, 23: S53–S82 (2015).

218. Sarin, J., Björkvik, L., Hiltunen, M., Hupa, L., Pulkkinen, J. and Vallittu, P. K., "The effect of fibrin sealant on bioactive glass S53P4 particles–pH impact and dissolution characteristics in vitro", ***Journal Of Science: Advanced Materials And Devices***, 1 (4): 482–487 (2016).

219. Arango-Ospina, M., Hupa, L. and Boccaccini, A. R., "Bioactivity and dissolution behavior of boron-containing bioactive glasses under static and dynamic conditions in different media", ***Biomedical Glasses***, 5 (1): 124–139 (2019).

220. Demirci, S., Kaya, M. S., Doğan, A., Kalay, Ş., ALTIN, N. Ö., YARAT, A., AKYÜZ, S. H. and Şahin, F., "Antibacterial and cytotoxic properties of boron-containing dental composite", ***Turkish Journal Of Biology***, 39 (3): 417–426 (2015).

221. Durand, L. A. H., Góngora, A., López, J. M. P., Boccaccini, A. R., Zago, M. P., Baldi, A. and Gorustovich, A., "In vitro endothelial cell response to ionic dissolution products from boron-doped bioactive glass in the SiO 2–CaO–P 2 O 5–Na 2 O system", ***Journal of Materials Chemistry B***, 2 (43): 7620–7630 (2014).

222. Jung, S., Day, D. E., Brown, R. F. and Bonewald, L., "Potential toxicity of bioactive borate glasses in-vitro and in-vivo", (2013).

223. Lepry, W. C. and Nazhat, S. N., "Highly bioactive sol-gel-derived borate glasses", ***Chemistry of Materials***, 27 (13): 4821–4831 (2015).

224. Nandi, S. K., Kundu, B. and Datta, S., "Development and applications of varieties of bioactive glass compositions in dental surgery, third generation tissue engineering, orthopaedic surgery and as drug delivery system", ***Biomaterials Applications for Nanomedicine***, 4: 69–116 (2011).

225. Maheswaran, A., Hirankumar, G., Heller, N., Karthickprabhu, S. and Kawamura, J., "Structure, dielectric and bioactivity of P 2 O 5–CaO–Na 2 O–B 2 O 3 bioactive glass", ***Applied Physics A***, 117 (3): 1323–1327 (2014).

226. Shah, S., Shivakumar, A. T., Khot, O., Patil, C. and Hosmani, N., "Efficacy of NovaMin-and Pro-Argin-containing desensitizing dentifrices on occlusion of dentinal tubules", ***Dental Hypotheses***, 8 (4): 104 (2017).

227. Hongal, S., Torwane, N. A., Hiremath, V. and Jain, M., "A comparative evaluation of the effect of four desensitizing agents (strontium chloride, potassium nitrate, pro-argin, bioactive glass) on dentinal occlusion: An ex-vivo SEM study", .

228. Chen, C. L., Parolia, A., Pau, A. and Celerino de Moraes Porto, I. C., "Comparative evaluation of the effectiveness of desensitizing agents in dentine tubule occlusion using scanning electron microscopy", ***Australian Dental Journal***, 60 (1): 65–72 (2015).

229. Narongdej, T., Sakoolnamarka, R. and Boonroung, T., "The effectiveness of a calcium sodium phosphosilicate desensitizer in reducing cervical dentin hypersensitivity", ***The Journal Of The American Dental Association***, 141 (8): 995–999 (2010).

230. Burwell, A., Jennings, D. and Greenspan, D. C., "NovaMin and dentin hypersensitivity--in vitro evidence of efficacy.", ***The Journal of Clinical Dentistry***, 21 (3): 66–71 (2010).

231. Fu, Q., Rahaman, M. N., Fu, H. and Liu, X., "Silicate, borosilicate and borate bioactive glass scaffolds with controllable degradation rate for bone tissue engineering applications. I. Preparation and in vitro degradation", ***Journal of Biomedical Materials Research Part A***, 95 (1): 164–171 (2010).

232. García-Godoy, F., "Dentin hypersensitivity: the effects of an argenine-calcium carbonate and fluoride desensitizing dentifrice", ***American Journal of Dentestry***, (2010).

233. Cummins, D., "Dentin hypersensitivity: from diagnosis to a breakthrough therapy for everyday sensitivity relief", ***Journal of Clinical Dentistry***, 20 (1): 1 (2009).

234. LOPES, R. M., TURBINO, M. L., ZEZELL, D. M., Scaramucci, T. and ARANHA, A. N. A. C. C., "The effect of desensitizing dentifrices on dentin wear and tubule occlusion", ***American Journal Of Dentistry***, (2015).

235. Bae, J., Kim, Y. and Myung, S., "Desensitizing toothpaste versus placebo for dentin hypersensitivity: A systematic review and meta-analysis", ***Journal of Clinical Periodontology***, 42 (2): 131–141 (2015).

236. Petersson, L. G., "The role of fluoride in the preventive management of dentin hypersensitivity and root caries", ***Clinical Oral Investigations***, 17 (1): 63–71 (2013).

237. Tosun, S., Culha, E., Aydin, U. and Ozsevik, A. S., "The combined occluding effect of sodium fluoride varnish and Nd: YAG laser irradiation on dentinal tubules—A CLSM and SEM study", ***Scanning***, 38 (6): 619–624 (2016).

238. Nathoo, S., Delgado, E., Zhang, Y. P., DeVizio, W., Cummins, D. and Mateo, L. R., "Comparing the efficacy in providing instant relief of dentin hypersensitivity of a new toothpaste containing 8.0% arginine, calcium carbonate and 1450 ppm fluoride relative to a benchmark desensitizing toothpaste containing 2% potassium ion and 1450 ppm fluoride and to a control toothpaste with 1450 ppm fluoride: a three-day clinical study in New Jersey, USA.", ***The Journal of Clinical Dentistry***, 20 (4): 123–130 (2009).

239. James, J. M., Puranik, M. P. and Sowmya, K. R., "Dentinal tubule occluding effect of potassium nitrate in varied forms, frequencies and duration: An in vitro SEM analysis", ***Journal of Clinical And Diagnostic Research: JCDR***, 11 (8): ZC06 (2017).

240. Berkathullah, M., Farook, M. S. and Mahmoud, O., "The effectiveness of remineralizing agents on dentinal permeability", ***BioMed Research International***, 2018: (2018).

241. Pradeep, A. R. and Sharma, A., "Comparison of clinical efficacy of a dentifrice containing calcium sodium phosphosilicate to a dentifrice containing potassium nitrate and to a placebo on dentinal hypersensitivity: a randomized clinical trial", ***Journal of Periodontology***, 81 (8): 1167–1173 (2010).

242. Bakri, M. M., Hossain, M. Z., Razak, F. A., Saqina, Z. H., Misroni, A. A., Ab-Murat, N., Kitagawa, J. and Saub, R. B., "Dentinal tubules occluded by bioactive glass-containing toothpaste exhibit high resistance toward acidic soft drink challenge", ***Australian Dental Journal***, 62 (2): 186–191 (2017).

243. Seong, J., Macdonald, E., Newcombe, R. G., Davies, M., Jones, S. B., Johnson, S. and West, N. X., "In situ randomised trial to investigate the occluding properties of two desensitising toothpastes on dentine after subsequent acid challenge", ***Clinical Oral Investigations***, 17 (1): 195–203 (2013).

244. Hanlie, H., Liyun, T. and Tao, J., "The crystal characteristics of enamel and dentin by XRD method", ***Journal of Wuhan University Of Technology-Mater. Sci. Ed.***, 21 (1): 9–12 (2006).

245. LeGeros, R. Z., "Calcium phosphates in oral biology and medicine", ***Monographs in Oral Sciences***, 15: 109–111 (1991).

246. Huang, C.-L., Fang, W., Huang, B.-R., Wang, Y.-H., Dong, G.-C. and Lee, T.-M., "Bioactive glass as a nanoporous drug delivery system for teicoplanin", ***Applied Sciences***, 10 (7): 2595 (2020).

247. Alshemary, A. Z., Akram, M., Goh, Y.-F., Tariq, U., Butt, F. K., Abdolahi, A. and Hussain, R., "Synthesis, characterization, in vitro bioactivity and antimicrobial activity of magnesium and nickel doped silicate hydroxyapatite", ***Ceramics International***, 41 (9): 11886–11898 (2015).

248. Bang, L. T., Long, B. D. and Othman, R., "Carbonate hydroxyapatite and silicon-substituted carbonate hydroxyapatite: synthesis, mechanical properties and solubility evaluations", ***The Scientific World Journal***, 2014: (2014).

249. Taherian, M., Rojaee, R., Fathi, M. and Tamizifar, M., "Effect of different sol-gel synthesis processes on microstructural and morphological characteristics of hydroxyapatite-bioactive glass composite nanopowders", ***Journal of Advanced Ceramics***, 3 (3): 207–214 (2014).

250. Hamadouche, M., Meunier, A., Greenspan, D. C., Blanchat, C., Zhong, J. P., la Torre, G. P. and Sedel, L., "Long-term in vivo bioactivity and degradability of bulk sol-gel bioactive glasses", ***Journal Of Biomedical Materials Research: An Official Journal Of The Society For Biomaterials, The Japanese Society for Biomaterials and the Australian Society for Biomaterials and the Korean Society For Biomaterials***, 54 (4): 560–566 (2001).

251. Huang, W., Rahaman, M. N., Day, D. E. and Li, Y., "Mechanisms for converting bioactive silicate, borate and borosilicate glasses to hydroxyapatite in dilute phosphate solution", ***Physics And Chemistry of Glasses-European Journal of Glass Science and Technology Part B***, 47 (6): 647–658 (2006).

APPENDIX A.

COMPARISONS OF WEIGHT GAIN\LOSS BETWEEN GROUPS

Table Appendix A1. Multiple comparisons between weight gain of dentin samples after treatment.

Dependent Variable	(I) treatment agents	(J) treatment agents	Mean Difference (I-J)	Sig.
Weight gain after treatment for 14 d	Group 1	Group 2	0.8066667	0.088
		Group 3	0.9766667*	0.026
		Group 4	1.2300000*	0.004
		Group 5	-0.0633333	1.000
		Group 6	-0.6566667	0.231
		Group 7	1.0200000*	0.019
	Group 2	Group 1	-0.8066667	0.088
		Group 3	0.1700000	0.995
		Group 4	0.4233333	0.692
		Group 5	-0.8700000	0.057
		Group 6	-1.463333*	0.001
		Group 7	0.2133333	0.982
	Group 3	Group 1	-0.9766667*	0.026
		Group 2	-0.1700000	0.995
		Group 4	0.2533333	0.959
		Group 5	-1.0400000*	0.017
		Group 6	-1.6333333*	0.000
		Group 7	0.0433333	1.000
	Group 4	Group 1	-1.2300000*	0.004
		Group 2	-0.4233333	0.692
		Group 3	-0.2533333	0.959
		Group 5	-1.2933333*	0.003
		Group 6	-1.8866667*	0.000
		Group 7	-0.2100000	0.984
	Group 5	Group 1	0.0633333	1.000
		Group 2	0.8700000	0.057
		Group 3	1.0400000*	0.017
		Group 4	1.2933333*	0.003
		Group 6	-0.5933333	0.331
		Group 7	1.0833333*	0.012
	Group 6	Group 1	0.6566667	0.231
		Group 2	1.4633333*	0.001
		Group 3	1.6333333*	0.000
		Group 4	1.8866667*	0.000
		Group 5	0.5933333	0.331
		Group 7	1.6766667*	0.000
	Group 7	Group 1	-1.0200000*	0.019
		Group 2	-0.2133333	0.982
		Group 3	-0.0433333	1.000
		Group 4	0.2100000	0.984
		Group 5	-1.0833333*	0.012
		Group 6	-1.6766667*	0.000

Table Appendix A2. Multiple comparisons between weight loss of dentin samples after immersion in AS.

Dependent Variable	(I) treatment agents	(J) treatment agents	Mean Difference (I-J)	Sig.
Weight loss after immersion in AS for 10 d	Group 1	Group 2	-0.8366667*	0.021
		Group 3	-0.7000000	0.069
		Group 4	-0.5500000	0.225
		Group 5	0.0366667	1.000
		Group 6	0.0333333	1.000
		Group 7	-0.0266667	1.000
	Group 2	Group 1	0.8366667*	0.021
		Group 3	0.1366667	0.995
		Group 4	0.2866667	0.848
		Group 5	0.8733333*	0.016
		Group 6	0.8700000*	0.016
		Group 7	0.8100000*	0.027
	Group 3	Group 1	0.7000000	0.069
		Group 2	-0.1366667	0.995
		Group 4	0.1500000	0.993
		Group 5	0.7366667	0.051
		Group 6	0.7333333	0.052
		Group 7	0.6733333	0.086
	Group 4	Group 1	0.5500000	0.225
		Group 2	-0.2866667	0.848
		Group 3	-0.1500000	0.993
		Group 5	0.5866667	0.172
		Group 6	0.5833333	0.176
		Group 7	0.5233333	0.272
	Group 5	Group 1	-0.0366667	1.000
		Group 2	-0.8733333*	0.016
		Group 3	-0.7366667	0.051
		Group 4	-0.5866667	0.172
		Group 6	-0.0033333	1.000
		Group 7	-0.0633333	1.000
	Group 6	Group 1	-0.0333333	1.000
		Group 2	-0.8700000*	0.016
		Group 3	-0.7333333	0.052
		Group 4	-0.5833333	0.176
		Group 5	0.0033333	1.000
		Group 7	-0.0600000	1.000
	Group 7	Group 1	0.0266667	1.000
		Group 2	-0.8100000*	0.027
		Group 3	-0.6733333	0.086
		Group 4	-0.5233333	0.272
		Group 5	0.0633333	1.000
		Group 6	0.0600000	1.000

APPENDIX B.

ETHIC APPROVAL

Tarih ve Sayı: - E.38103

T.C.
KARABÜK ÜNİVERSİTESİ REKTÖRLÜĞÜ
Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu

Sayı : E-77192459-050.99-38103
Konu : 2021/590 Nolu Karar

Sayın Dr. Öğr.Üyesi Ammar Zeidan ALSHEMARY

Girişimsel Olmayan Klinik Araştırmalar Etik Kurulumuza sunmuş olduğunuz **"The Effects Of Various Bioglass Formolations Effectivness On Dentine Tubulus Occlusion. Dentin Tübüt Tıkanıklığına Çeşitli Biyoglass Formolasyonlarının Etkileri"** başlıklı araştırma projeniz amaç, gerekçe, yaklaşım ve yöntemle ilgili açıklamaları açısından Girişimsel Olmayan Klinik Araştırmalar Etik Kurul yönergesine göre incelenmiş olup etik açıdan uygun olduğuna oy birliği ile karar verilmiştir.

Bilgilerinize rica ederim.

Prof. Dr. Orhan ÖNALAN
Kurul Başkanı

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Figure Appendix B 1. Ethical approval

RESUME

Razan ALMNAWER completed first, elementary and high school education in Syria. She started undergraduate program in University of Karabuk Department of Medical Engineering in 2019. Then, she started M Sc. Education in Karabük University Department of Medical Engineering.