

A RAPIDLY-DISSOLVING SILICA-SILVER BIOACTIVE GLASS FOR CARIOSTATIC APPLICATIONS

by

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Abstract

In this study, bioactive glasses were designed for the purposes of reducing the incidence of dental caries and lesion formation by supplying the teeth with therapeutic ions which may support remineralization and provide an antibacterial effect against oral cavity bacteria. Three glasses were synthesised through the melt quench method: Si-Control ($\text{SiO}_2\text{-CaO-P}_2\text{O}_5\text{-Na}_2\text{O}$), Si-02 and Si-05, where 0.2% and 0.5% Ag_2O were substituted, respectively, for SiO_2 in the control glass. The glasses were then ground, characterized and dissolved in tris buffer solution ($\text{pH}=7.30$) for 6, 12 and 24 hours, with the pH rise of the solution being recorded (7.48 for Si-Control, and 7.66 for both Si-02 and Si-05 after 24 hours) and the ions that were released into the tris buffer solution quantified. Samples of each glass were subsequently embedded into non-fluoridated toothpaste and samples of the paste were used to brush resin-mounted lamb molars after a 1.0M HCl overnight demineralization challenge. Knoop microhardness measurements were recorded before and after brushing to determine the presence of remineralization on the surface of the teeth (Percent Surface Hardness Loss of 37%, 35% and 34% for Si-Control, Si-02 and Si-05 respectively after 24 hours). Four oral cavity bacterial strains were isolated through swabs of the inner cheek, gums and teeth surfaces of three volunteers, and placed on agar discs. 0.5g of each glass were placed onto the discs and the resultant inhibition zones were measured after 6, 12 and 24 hours. Si-05 consistently performed better than Si-02 on all strains and timeframes, while Si-Control exhibited no antibacterial effect at any time point.

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Introduction

Dental caries is among the most common preventable diseases worldwide [1]. It is caused by physiological issues such as poor salivary flow, poor dietary choices and inadequate oral hygiene [1]. 92% of adults aged between 20 and 64 years and 60 to 90% of children have been affected by caries at least once in their lifetime [2], [3]. Caries is the local dissolution of hard dental tissue by acids produced by bacteria in the mouth [1]. These bacteria, which exist within the plaque build up on teeth, process fermentable carbohydrates to produce weak acids [1], [4]. These acids cause calcium, carbonate and phosphate ions to leech out of the enamel and dentine, weakening the mineral and resulting in decay [1]. This process can be reversed by buffering the oral environment and restoring it to the original pH value; saliva is an effective natural buffer [5]. However, remineralization can only occur if pH is high enough or if there are enough calcium and phosphorous ions available in the mouth for uptake. Demineralization and remineralization constantly happen throughout the day. If the pH is not restored or if the ionic material needed for remineralization is not available, however, then demineralization can progress into lesions and cavities.

Cavities indicate advanced progression of the disease. Restorations such as amalgams, composites, glass ionomer cements and composite-cement hybrids are designed to simply refill the missing demineralized material. While these materials have been used for decades, they all have their drawbacks around their aesthetics, mechanical properties, cavity sealing and toxicity [6]–[10]. There exists a need for more proactive solutions which can prevent lesions from developing into cavities.

Bioactive glasses can be designed to deliver therapeutic ions which may bond with bone, encourage remineralization and provide antibacterial effects [11]–[16]. As they dissolve under acidic conditions, they act like 'smart' materials which activate under the same acidity with which caries is associated [17]. These glasses can be doped with ions such as strontium and fluoride which release as the glass degrades to promote remineralization [18]–[21], or antibacterial agents such as silver and zinc [22]–[26], to minimise further decay. While bioactive glasses have been used as components in glass ionomer cements (GICs) for decades, the idea of embedding glasses into other media for dental remineralization is a new approach [24], [27]–[29].

For the purposes of this project, a series of novel bioactive glasses were synthesized which were tailored to be a proactive solution to dental caries. These glasses were characterized through standard spectroscopic techniques, such as XRD, FTIR, SEM-EDX and DSC. The glasses were then tested for their efficacy as cariostatic agents and their remineralization potential on dentin.

1.0 Literature Review

1.1 Tooth

The tooth is composed of two major portions: the crown and the root. The crown is the portion of the tooth which protrudes from the gum-line and is coated with enamel, the hardest tissue in the human body [30]. Freshly formed enamel can be made of up to 30% protein but after it matures it becomes chiefly composed of mineral with less than 1% protein. The proteins primarily take the form of amelogenins; amino acid rich proline, histidine, leucine and glutamine. Up to 90% of the proteins in enamel are amelogenins. The other 10% are made up by proteins such as tuftelins and ameloblastins. The remaining protein tends to be found among enamel tufts, groups of hypo-mineralized crystalline defects of enamel which occur near the dentine-enamel junction[31].

1.1.1 Inorganic Tooth Structure

The root of the tooth buries itself into the jaw and holds the tooth in place. The root primarily consists of a mineral known as dentine which surrounds the pulp chamber. It is the pulp that provides the blood supply for the tooth in addition to containing the nerve endings [32]. Finally, the root is covered with a bone-like substance known as cementum. A diagram of the tooth can be found in Figure 1.

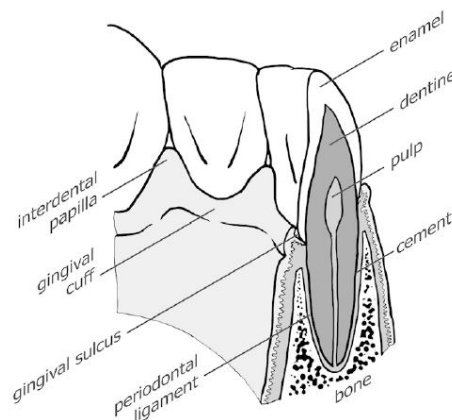


Figure 1: Cross-section of a tooth structure[32]

A tooth is made up mostly of inorganic material, ranging from 69% to 99% by weight. These minerals are typically different forms of apatite, following an idealized chemical formula of $\text{Ca}_5(\text{PO}_4)_3\text{X}$ where X is usually one of (OH^-) or F^- . This formula is often doubled in order to express the unit cell content [33]. Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), HA, is the main inorganic constituent of both bone and tooth. However, at different sites even within the same tooth, the mineral content can vary smoothly between fluoroapatite (FA) and HA. Fluoride ions are typically supplied through ingestion of drinking water and exposure to fluoridated toothpastes [32]. Table 1 below lists other ions commonly available for substitution in apatite crystals.

Table 1: Most commonly substituted ions available in apatites[32]

Apatite Ion	Substitutions available
Calcium (Ca^+)	Strontium (Sr^+) or Sodium (Na^+)
Phosphate (PO_4^{2-})	Orthophosphate (HPO_4^{2-}), Carbonate (CO_3^{2-}) or Hydrogen carbonate (HCO_3^-)
Hydroxyl (OH^-)	Chloride (Cl^-) or Carbonate (CO_3^{2-})

Some amount of inorganic materials exist in the dental tissues which are not in an apatite phase, but rather in other forms of crystalline calcium phosphate including whitlockite ($\beta\text{-Ca}_3(\text{PO}_4)$), brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), and octacalcium phosphate ($\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$). Non-apatite minerals form more commonly as amorphous calcium phosphates. These structures are usually unstable as their composition is highly variable which leads them to break down into apatite phases[32].

1.1.2 Enamel

Enamel is a very hard, highly mineralized and brittle tissue with a toughness similar to glass [34]. It is unique in the body due to its acellular nature [35] and its microstructure made up of rods composed of tightly packed carbonated HA crystals [36]. Rods have an approximately

keyhole shape and have a mean diameter of about 5 μm [37]. Figure 2 below shows a schematic drawing of an enamel rod. Each rod is aligned in parallel with other rods and run from the dentine-enamel junction towards the tooth surface [37]. They contain “fibre-like” apatite crystals that are orientated differently within the rod depending on if they are in the head or tail area of the rod.

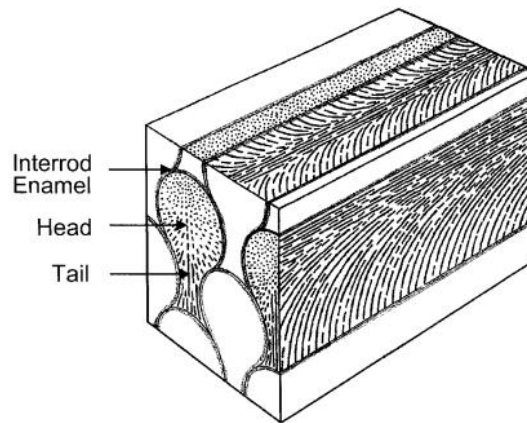


Figure 2: Diagram of enamel rod. The dashed lines on the section view represent the orientation of the apatite crystals[37].

Enamel crystallites are organized into rods separated by interrod enamel. Interrod enamel differs from rod enamel only by its crystal orientation. Rod crystals are orientated axially along the rods but interrod enamel can vary 40° to 65° from the rod direction [35].

1.1.3 Dentin

Dentin is a hard, elastic tissue which constitutes the majority of the tooth. It is found between the enamel layer and the pulp chamber and provides support for the brittle enamel to handle forces that result from mastication [38], [39]. Dentin is composed of four main phases: tubules, a mineralized matrix, collagen fibers with embedded apatite crystals and, finally, fluid, including water and plasma [38]. Tubules are small tracts which radiate away from the pulp chamber and traverse the bulk of the material. They can vary in size from 2.5 μm near the pulp chamber to 0.8 μm near the dentin-enamel junction. Collagen fibers are usually located perpendicular to tubules

but the two occasionally run in parallel[40]. Tubules have a strong influence on the fracture properties of dentin yet have little to no effect on elastic properties [40]. The walls of the tubules are made up of peritubular dentin, a highly mineralized tissue [41].

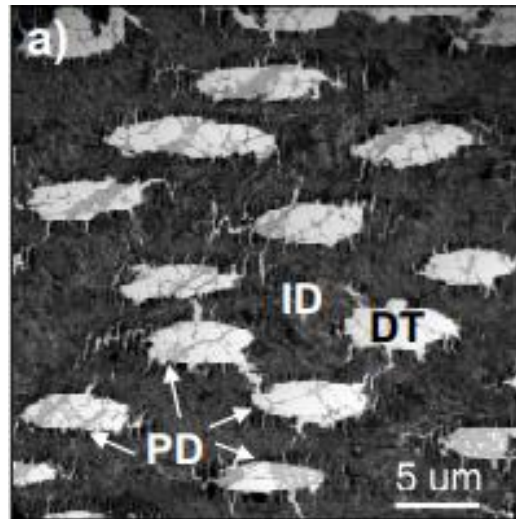


Figure 3: Images taken from BF-STEM of dentin tubules. (DT) represent dentinal tubules, (ID) represent intertubular dentin and (PD) represent peritubular dentin[41].

1.1.4 Cementum

Cementum lines the root up to the cervical or neck area of the tooth where the crown meets the root [32]. Cementum is not considered a primary bonding substrate, despite being identified as a source of microleakage contributing to high incidences of secondary caries [42], [43]. There are three main kinds of cementum: acellular afibrillar cementum, acellular extrinsic fiber cementum and cellular mixed stratified cementum.

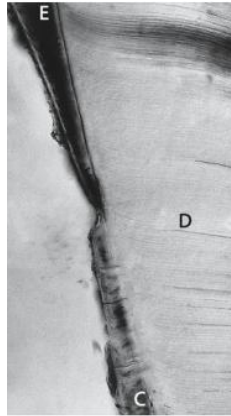


Figure 4: Adult human tooth light-micrograph at cervical tooth section. (E) represents enamel, (D) represents dentin and (C) represents cementum[42].

Acellular afibrillar cementum is a non-homogenous matrix chiefly found near the cement-enamel junction. It contains no collagen fibers, but is instead made up of multiple calcium layers [42]. Acellular extrinsic fiber constitutes the majority of cementum. It is characterized by a thin layer of mineral, about 50 μm thick during its functional life. A slow continuous process grows the matrix over the life of the tooth [42]. Cellular mixed stratified cementum is actually composed of layers of acellular extrinsic fiber cementum and cellular intrinsic fiber cementum one after another. Pure cellular intrinsic fiber cement grows significantly quicker than acellular cementum and is only deposited during the repair processes that follow root resorption [42]. It can reach mean thicknesses of 200 μm with the thickest layers found on the top third of the tooth. It performs the vital functions of maintaining the occlusion of teeth as well as repairing root surface areas which have undergone resorption [42].

1.1.5 Organic Tooth Structure

Organic materials are also present in the tooth. Approximately 20% of the weight of fresh bone and dentin is collagen, a fibrous protein that can be found in dentin, cementum, bone and, to a lesser extent, enamel [44]. It is a very tough structure and makes up about 30% of the proteins in the body[45]. It is formed by three different chains of amino acids which braid together in order

to form a larger spiral, as seen in Figure 5. These spirals can then be packed together into fibrils which range from 10nm in diameter to 200nm in diameter [32].

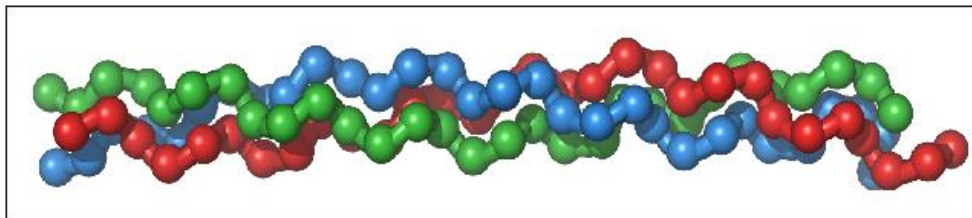


Figure 5: Triple helix form of collagen.[46]

Collagen is a stable protein which is not water soluble and is resistant to both bacterial and fungal attack [32]. It typically interweaves itself within the apatites to provide support and increase strength. Collagen was formerly believed to be absent in enamel, but Ail et al. showed that there are, in fact, small concentrations to be found [44]. Other than collagen, organic constituents are present as assorted proteins, lipids, peptides and other organic compounds. These are collectively referred to as ground substance and are usually amorphous [32].

1.1.6 Mechanical Properties of Teeth

The strength of tooth varies depending on which tooth the sample comes from, as well as the site on the tooth. This is due to the constant demineralization and remineralization processes affecting teeth. Table 2 details the findings of past studies explaining the strength of human enamel. Note that the strengths vary drastically based on the direction of the enamel rods.

Table 2: Previous studies on enamel strength adapted from Zaytsev and Panfilov (2014) [30]. Standard Deviations are included when available.

Author	Rod Direction	Ultimate Compressive Strength (MPa)	Young's Modulus (MPa)
Stanford et al. (1958)	Variable	277±12.4	47.5
	Across	194±15.2	30.3
	Along	134±27.9	8.96
Stanford et al. (1960)	Variable	261±41.4	46.2±4.8
	Across	250±29.6	32.4±4.1
	Along	94.5±32.4	9.65±3.45
	Along	126.9±30.3	11.0±2.1
Craig et al. (2010)	Along	384±85.3	84.1±6.2
	Along	372±56	77.9±4.8

Zaytsev and Panfilov suggest that the geometry of the enamel affects its strength [30]. In a study on 30 human molars, they found that the strength varied significantly based on the dimensions of the specimen tested; they show a relationship between Young's modulus, ultimate compressive strength, elasticity and plasticity with the ratio between sample height to sample length.

1.2 Dental Caries

One of the most common preventable diseases is caries, commonly referred to as tooth decay. Approximately 92% of adults aged 20 to 64 years old have had dental caries at least once in their permanent teeth. Furthermore, 23% of adults in the same age group have not had treatment for their decay. On average, this demographic is estimated to experience 3.28 decayed or missing permanent teeth [2]. Furthermore, the disease also affects 60% to 90% of children [3]. It can be caused by physiological issues, such as poor salivary flow and immunological conditions, poor

dietary choices and inadequate dental hygiene. In general, the causes of the disease can be summed up as in Figure 6.

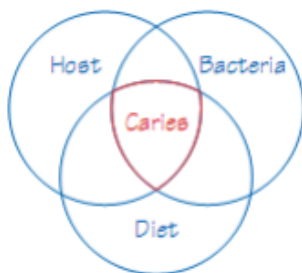


Figure 6: Factors contributing to dental caries [1]

Dental caries is defined as the localised decay of hard dental tissues by the acids which are produced from the bacterial processing of fermentable carbohydrates [1]. The disease originates in the biofilm formed by dental plaque [4]. Bacteria do not live in isolated cultures but in an aggregate network made up of extracellular material; this aggregate is called a biofilm [47]–[49]. The microorganisms consist of about 10% of the dry mass of this biofilm, while the extracellular matrix makes up 90% of its mass [47]. Plaque is the biofilm which accumulates on the surface of teeth in the oral cavity, consisting of over 500 different kinds of bacterial species and is affected by environmental conditions, such as saliva quantity and composition, as well as diet [1], [50]. Plaque formation is described in Figure 7. Fluoride is known to be a retardant in plaque activity [51]. Caries can occur in all portions of the tooth including the crown, root, cementum, dentin and enamel [1]. The disease eats away at material like dentin and enamel and causes carious lesions, commonly referred to as cavities [1]. Cavities are usually a sign of an advanced stage of the disease and are irreversible [1]. The cavity usually spreads into dentin towards the pulp chamber which can cause necrosis of the tooth and lead to periapical abscesses [1].

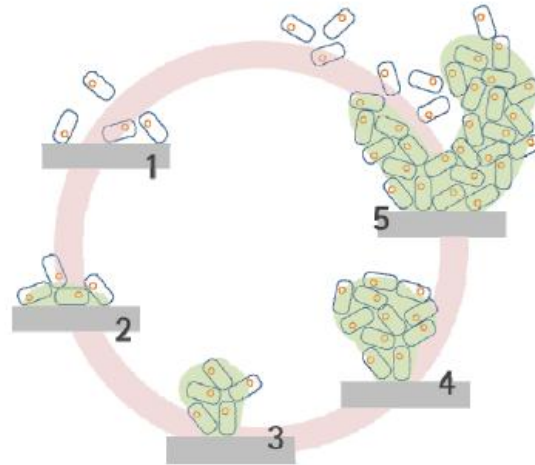


Figure 7: Biofilm formation. 1) Initial adhesion of bacterial cell to hard surface. 2) Induce adhesion through production of extracellular matrix, shown in green. 3) Develop biofilm structure. 4) Maturation of biofilm. 5) Cells break off from the biofilm and find new surface to proliferate. [49]

The engine by which the decay begins is through the processing of fermentable carbohydrates. Bacteria, primarily *streptococcus mutans*, *streptococcus sobrinus*, and *lactobacillus* consume these carbohydrates and produce a lactic acid [1]. Proteins and fats are seemingly not involved in cariogenic activity but the protein casein, found in milk, has been shown to have a protective effect [32]. The fermentation process is described by Figure 8.

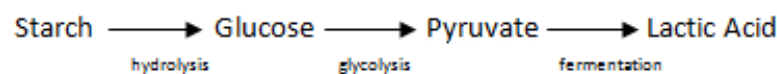


Figure 8: Fermentation process for Dental Caries [52]

The normal pH of the mouth is approximately 7.4 [1]. The weak acid produced by bacteria lowers the pH, resulting in a more acidic environment which can lead to demineralization; a process by which calcium, carbonate and phosphate ions begin to leech out of the enamel or the cementum [1], resulting in decay. This process can easily be reversed by the natural buffer action of the saliva, which restores the pH in the mouth. It can also be reversed through the consumption of calcium, phosphate and fluoride ions. Fluoride acts as a catalyst for the diffusion

of calcium and phosphate ions by the formation of fluoroapatites and fluoridated hydroxyapatites, as well as rendering the tooth structure more resistant to acid attack. The processes of demineralization and remineralization constantly happen throughout the day and can either result in cavitation of the tooth, repair of cavities in the tooth, or an equilibrium phase [5]. Figure 9 below shows the relationships between the two processes.

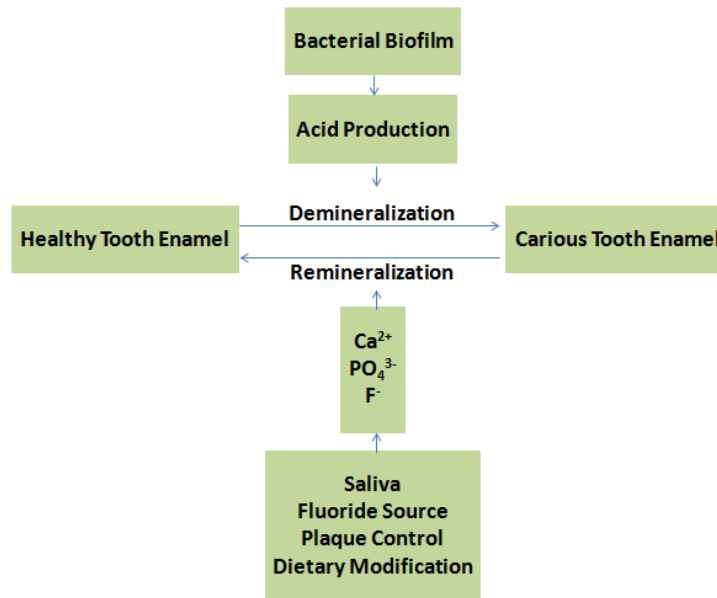


Figure 9: Diagram explaining the decay and repair of teeth [5]

1.2.1 Caries Complications

Left untreated, carious lesions are able to find their way into the pulp chamber of the tooth, which can lead to necrosis [1]. Other complications may include sinusitis, osteomyelitis, and swelling which can potentially block airways [53]. Bacterial infections can also affect other parts of the oral cavity, such as the gums and other supporting tissues which help hold the tooth. Diseases which can be caused by these bacteria include gingivitis and periodontitis [52]. Gingivitis is a condition caused by localized inflammation of the gums without the loss of supporting bones around the teeth, resulting in swollen, bleeding gums, superficial reddening of the gums, and halitosis [52]. Periodontitis affects the small bones which help hold the teeth in

place. Loss of these bones can result in abscesses and exposed teeth roots, as well as pain and swelling of the gums. It is often caused by chronic gingivitis [52].

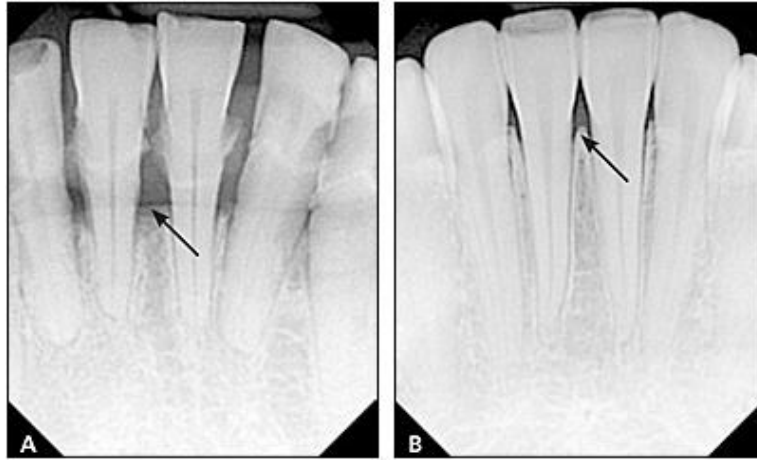


Figure 10: Bone loss from periodontitis on left vs. normal structure on right[52]

1.2.2 Dissolution of Teeth

Caries attacks are known to cause demineralization and dissolution of hard dental tissues. The solubility of dental tissues can increase rapidly as the host pH decreases. Larsen (1990) estimated that for pH values between 4-6, the solubility can change by a factor of 7-8 for every pH unit [54].

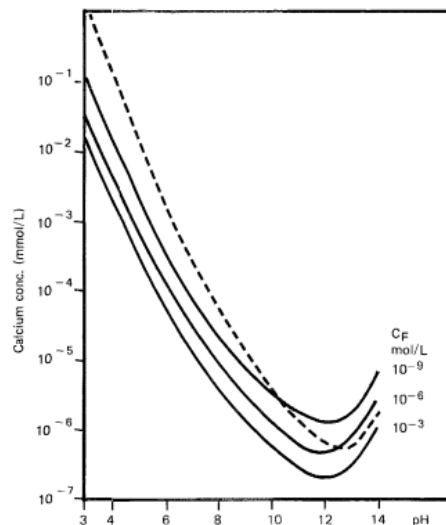


Figure 11: Solubility of Calcium ions versus pH. The dashed line represents hydroxyapatite while the solid lines represent fluorohydroxyapatite, with different concentrations of fluoride listed. [54]

The dissolution of valuable ions from the tooth can lead to a lower calcium to phosphate ratio, as well as a lower concentration of ions such as magnesium and carbonate [55]. These two ions are important as they are often used as an indicator of cariogenic activity. Early in the enamel attack, magnesium and carbonate tend to leech out of the mineral first. This explains the presence of magnesium rich and carbonate rich phases separate from the apatite [55]. Carbonate phases are believed to increase solubility and thereby increase caries risk. Generally, high carbonate and low fluorine phases represent a high caries risk while low carbonate and high fluorine phases represent low caries risk. *In vitro* experiments carried out by LeGeros *et al.* (1983) found that the depth of caries penetration was up to 33% higher in human enamel, which has a lower fluoride content, as compared to shark enamel, which has a higher fluoride content, over long exposure periods [56]. The study also found that the carbonate concentrations were higher in the human samples as opposed to the shark samples. Furthermore, there was up to a 50% difference in solubility when the powdered enamel samples were suspended in an acidic buffer solution with a pH of 5, with the human samples being more soluble. The study strongly suggested that both carbonate and fluoride ions have a significant impact on the solubility of apatite crystals: carbonate increases solubility while fluoride ions decrease solubility. LeGeros [56] also suggested that when both fluoride and carbonate phases are simultaneously present in apatite, the positive contributions of fluoride are more significant than the contributions of carbonate phases. However this phenomenon has not been fully investigated.

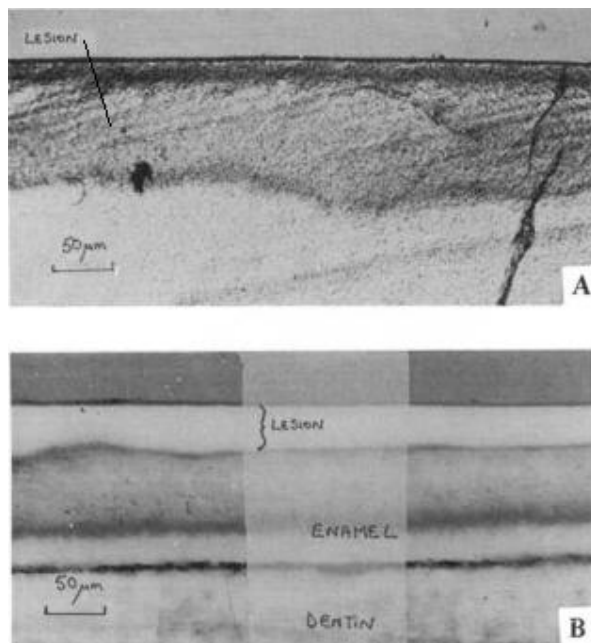


Figure 12: Artificial lesions produced in human enamel (A) and shark enamel (B). Human sample lesion penetration went up to 125 µm while shark samples penetrated to about 40 µm. [56]

Excessive fluoride concentrations can precipitate into CaF_2 instead of fluorapatite. CaF_2 is believed to create a barrier and act as a reservoir of fluoride for the enamel [57]. Mohammed *et al.* (2013) showed that the mineral may actually be detrimental to remineralization efforts as it can rob the apatite of valuable calcium ions [58]. The study found that above 45 ppm F concentration, CaF_2 begins forming in greater quantities and that at above 136 ppm F concentration, demineralization is no longer reduced as CaF_2 becomes the dominant phase.

1.2.3 Effect of Fluorine on Caries and Cariogenic Bacteria

Fluorine ions are widely regarded as effective cariostatic agents [1], [21], [59]. The mechanism by which they influence decay is not fully understood but there are some known effects that fluorine provides:

- The presence of small quantities of fluorine in the oral environment encourages remineralization. This is due to the fluorine ion replacing the less stable OH^- of

hydroxyapatite and acting as a catalyst for calcium and phosphate ions to diffuse more easily into the apatite crystals [21].

- Fluorapatite crystals are more resilient to acid attack, thereby decreasing enamel dissolution during acid attacks [21]
- Fluorine acts as a retardant for plaque development, inhibiting acid build-up by interfering with the function of several enzymes which are considered essential to the production of the acid in the first place. Studies by Bibby and Van Kesteren (1940) found that 1 ppm fluoride content was effective in inhibiting acid production, while 250 ppm fluoride concentration was required to inhibit bacterial growth [51]. Since the acid is the main cause of tooth decay, the prevention of acid buildup is considered one of the most important factors of fluorine presence[60].
- Fluorine ingestion also affects the fissures on tooth biting surfaces by reducing their depth. Shallower fissures are believed to reduce the ability of plaque to remain unperturbed. This reduces acid build up and prevents the occurrence of cavitation [60].

1.3 Restorations and Other Therapies

There are two main therapies available to treat advanced caries. One method is to extract the tooth, and thereby reduces pain. The other method is to fill the cavity so that bacteria may no longer reach the inner portions of the tooth and cause further damage. Untreated caries can result in many health complications; for example, growth, body weight and quality of life can be affected in preschool children with untreated caries and are at greater risk for adult caries [1], [61]–[64]. The most common filling materials are amalgams, resin-composites, glass ionomer cements (GICs) and compomers. Bioactive glasses and dentifrices can serve as cariostatic agents which can prevent the need for fillings in the first place [17].

1.3.1 Dental Amalgam

Historically, amalgam was the primary choice of material used as a filling in order to repair teeth. Amalgam is an alloy of mercury, silver, copper, tin, zinc and other trace metals, as per the particular manufacturer of the restoration. It is a highly economical option and has both high strength and wear resistance [65]; high-copper variants of the alloy last as long as 12 years [66] as they contain less of the gamma-2 phase from the Sn-Hg system. This phase causes progressive weakening of the alloy through corrosion and suffers from pitting [67]. Furthermore, fillings of amalgam were considered easy to use as they were not technique sensitive [65], [66].

Despite all this, the use of amalgam has waned for multiple reasons [66]. The most widely cited reasons were reports of mercury vapors leeching out and being consumed; in a study by Joselow *et al.* in 1968, it was shown that 14% of dental offices had mercurial vapors in excess of safe levels from the use and preparation of dental amalgam [68]. Another study by Gronka *et al.* found that one in seven dental offices had mercury contamination [9]. However, in 1973, a study by Lenihan, Smith and Harvey showed that there was "no evidence that the amount of mercury absorbed is harmful to the patient" [69]. Uçar and Brantley also reported that there is no evidence to support the claim of mercury release from amalgam fillings and neurological diseases [70]. The largest concern of amalgam was for patients allergic to mercury; these people represented 2 to 3% of the population [71]. From 1979 to 1990, amalgam filling use had declined by 38%, along with incidence of tooth decay. Reasons for their decline may include public perception of their safety; however, a larger factor for their decline has been due to a larger demand for aesthetics in restorative dentistry [10]. Table 3 below summarizes the advantages and disadvantages of amalgam fillings.

Table 3: Advantages and disadvantages of dental amalgam [65].

Advantages	Disadvantages
<ul style="list-style-type: none"> • Low cost • Easy to use • High compressive strength • Good wear properties 	<ul style="list-style-type: none"> • Poor aesthetics • Lack of bonding means tooth is not strengthened • Microleakage due to lack of bonding • Not as conservative of tooth structure and requires extensive preparation, such as undercutting

1.3.2 Resin Composites

Modern restorations are shifting from amalgam towards resin-composites [72]. In 1999, approximately 86 million resin composite fillings were installed compared to 71 million amalgams [10]. Composites are typically tooth colored and are able to adhere to the tooth surface chemically. Composites work through the use of an organic matrix which provides the continuous phase for the material while an inorganic filler enhances its mechanical properties. The matrix often contains an initiator meant to begin free radical polymerization [73].



Figure 13: The picture on the left shows an amalgam filling in perfect condition after 10 years [67]. The picture on the right shows a resin composite filling in perfect condition after three years[72]

Often, it is a photo initiator which causes polymerization when irradiated with blue light from a halogen lamp. The light is often in the region of 450-480 nm and produces highly-crosslinked networks which protect against dissolution, [73], [74]. They cure rapidly and at room

temperature, generating radicals by breaking double bonds in the monomer chains and creating a three-dimensional cross-linked polymeric network [73]. One of the most frequently used photo-initiators is camphorquinone/amine (CQ/amine) [75], [76]. The CQ absorbs the light and enters an excited state where it extracts a free hydrogen atom from a tertiary amine. This amine then becomes a radical and starts causing a chain polymerization by attacking carbon double bonds while the CQH is used to terminate the reaction when it recombines with the radical [75], [76]. It is important that the filler bonds to the matrix for the material to work. The filler particles are usually coated in a silane bonding agent in order to facilitate this [77]. This agent contains functional groups which can interact with the inorganic materials of the filler as well as monomers which can copolymerize with the matrix groups [7]. Generally, the filler constitutes 75-85% by weight while the matrix constitutes 15-25% by weight [78]. Table 4 below lists common matrix monomers as well as what most fillers are composed of.

Table 4: Common matrix monomers and filler constituents [79]

Matrix Compound		Filler Compound
Compound	Makeup	Metals, ceramics, glasses, polymers, etc. containing various compounds such as pigments, antioxidants, antimicrobials, oxide glasses with barium, strontium etc. for radiopacity
BIS-GMA	2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)-phenyl]-propane (C ₂₉ H ₃₆ O ₈)	
UDMA	1,6-bis(methacryloyloxy-2-ethoxycarbonylamino)-2,4,4-trimethylhexan (C ₂₃ H ₃₈ N ₂ O ₈)	
TEGDMA	Triethyleneglycol di-methacrylate (C ₁₄ H ₂₂ O ₆)	

1.3.3 Resin Composite Properties

The advantages that composites provide over traditional amalgam fillings are those of aesthetics and adhesion to tooth. The fillings are often tooth colored or close to tooth colored which makes them more desirable for use in anterior teeth. Unfortunately, composites suffer from low strength and elastic moduli, as well as poor wear properties compared with amalgams [6], [7]. However they are still the most popular material for dental restorations [80]. Enamel has a modulus of about 80 GPa while dentin has a modulus of about 18 GPa [7]. Most composites have an elastic modulus between 15 to 25 GPa [7]. Much work has been done on improving these materials in order to meet the mechanical needs imposed on them, such as minimizing shrinkage while also accounting for water sorption [81] and increasing fracture toughness [82], [83] and strength [84]–[86].

During polymerization, shrinkage can occur which puts an additional amount of stress onto the filling, as well as creating sites for microleakage [8]. These sites can then cause secondary caries to occur by allowing bacteria inside the cavity as well as cause pulpal inflammation. Shrinkage is a process inherent to the reaction itself as the polymer networks form [8], [87]. Furthermore, adhesion is considered unsatisfactory in cavity walls in the long term since bond strength would decrease with longer exposure to water [88]. Table 5 shows the bond strengths of composites to different media. Shrinkage may also exert stresses large enough to break the dentin-resin bonds, further opening up the cavity to microleakage effects [89]. Despite the shortcomings however, modern day composite fillings still have annual failure rates less than 9%, only marginally higher than dental amalgams which were found to have a 7% annual failure rate [90]. Table 6Table 5 shows the compressive strengths of five different composites.

Table 5: *In Vitro* Tensile Bond Strength of three different dental composites bonded to three different media. AC is an adhesive composite, TC is a traditional composite and BA is a bonding agent. Standard deviations are included in parentheses [91]. Compressive fracture strength and compressive fatigue strengths are averages of other materials, but do not include bonding agents [92].

Composite	Storage Conditions	Amalgam (MPa)	Dentin (MPa)	Enamel (MPa)	Compressive Fracture Strength	Compressive Fatigue Strength
AC	23° C	12.8 (1.6)	9.0 (0.9)	13.9 (5.6)	341.7 (13.6)	206.7 (8.1)
	Thermocycled	13.6 (2.6)	8.2 (3.6)	16.5 (8.6)		
AC+BA	23° C	21.8 (6.6)	14.2 (3.6)	15.6 (1.4)	N/A	N/A
	Thermocycled	26.2 (6.5)	13.9 (4.7)	18.3 (2.9)		
TC+BA	23° C	9.7 (3.7)	9.6 (4.7)	21.3 (5.1)	259.8 (8.18)	173.2 (5.38)
	Thermocycled	11.4 (3.7)	11.1 (3.4)	22.8 (8.0)		

As can be seen in the table above, composites bond better to enamel than to dentin, and even better to amalgam. Furthermore, while composites do exhibit some adhesion to dental media, bond strengths are greater when paired with luting cements. A higher bond strength is valuable in the clinical situation, as a strong bond between the restoration and the surrounding media is essential to prevent microleakage [91].

Masticatory loads are cyclical and so fatigue strengths are valuable in evaluating the life cycle of a restoration [92]. Evaluated under 10Hz, Table 5 **Error! Reference source not found.** above lists both the static compressive strength and the dynamic fatigue strength. While the upper limit to chewing frequency is reported to be 2Hz, 10Hz was selected because it has been shown to be a good simulation of the oral environment without internal heating of the sample and ensures all probable fatigue effects observed *in vivo* would occur *in vitro* [92], [93].

1.3.4 Resin Composite Polymerization Shrinkage

During the setting of resin-modified composite cements, a phenomenon known as polymerization shrinkage occurs. This is caused by monomers attempting to react and polymerize into polymers through the exchange of van der Waals bonds into covalent bonds [94]. The shrinkage can cause contractive stresses to be experienced in the material but it can also induce stresses and deformation of the surrounding tooth structure the cement is adhered to.

Such stresses can often be the underlying mechanism responsible for many restoration problems. The cement can often separate from the surrounding walls which results in microleakage. However, fractures can also be caused in surrounding tissues such as enamel and dentin as well as the fillings themselves [8]. If the surrounding tissues or the restoration do not have enough elastic compliance for the shrinking volume, some structure must eventually fracture in order to compensate [87]. This can create issues as the open spaces created by the restoration failure can result in secondary carious infections, pulpal irritation [8]. It is therefore a common practice in the dental field to employ the ‘sandwich technique’ [88] which involves the use of a luting cement to bond the restoration to the dental tissue while the composite is used for the bulk restoration.

Table 6: Regression analysis correlations indicating which variables relate to shrinkage and how significant an impact they have, averaged over 17 different commercially available resin composites [94]

Variables	Equation
Contraction Stress vs Tensile Modulus	$\sigma \text{ (MPa)} = -0.10 + 4.49E \text{ (GPa)}$
Contraction Stress vs Shrinkage	$\sigma \text{ (MPa)} = 25.00 - 3.74\epsilon \text{ (vol\%)}$
Shrinkage vs Tensile Modulus	$\epsilon \text{ (vol\%)} = 6.62 - 1.17E \text{ (GPa)}$
Shrinkage vs Filler Load	$\epsilon \text{ (vol\%)} = 8.65 - 0.097 \text{ filler\% (vol\%)}$

Table 6 lists the results of a regression analysis relating contraction, shrinkage and Young's Modulus, with the individual products tested listed in the appendix. As can be seen, there are strong correlations between each of the tested variables. Interestingly, the contraction stress, shrinkage, tensile modulus and filler load were correlated, despite the large variation in the number of products tested. Indeed, the 17 different commercial products varied in terms of resin type, filler size and load, and even including a system which was photo-initiated in the test [94].

Heliomolar (Ivoclar Vivadent, Schaan, Liechtenstein) demonstrated low shrinkage and low contraction stress, indicating superior performance compared to its competitors, as is made evident by its location below the regression line in Figure 14. **Error! Reference source not found..** This runs counter to the norm, in which low shrinkage is generally accompanied with high contraction stress [94]. Heliomolar accomplishes its result by using a microfilled resin with lower filler levels; the filler levels are close to 40% by volume. This suggests that the elastic modulus and contraction stress should be low while the shrinkage should be high. However, by including a filler which is pre-polymerized, a smaller shrinkage percentage can be obtained [94].

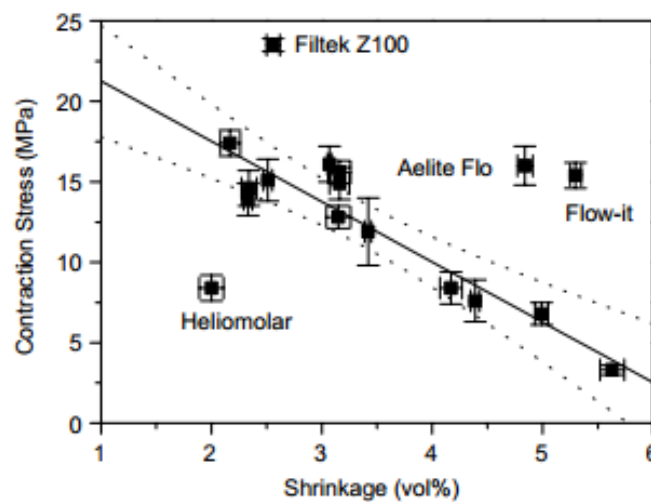


Figure 14: Polymerization Shrinkage vs Contraction Stress.[94]

Water sorption immediately following polymerization is known to relieve some of the residual stresses from shrinkage in composite restorations [8], [95]. Geometry also plays a large role in fractures stemming from shrinkage. When the ratio of the bonded surface of the restoration cement to free surface of the restoration cement is greater than 5, all shrinkage becomes uniaxial and can increase by up to 3 times more than expected [87]. In a study by Davidson, van Zeghbroek and Feilzer, it was found that a rigidly constrained cement had up to 25% less cohesive strength than a free shrinking cement after 20 minutes [8]. Stresses developing in luting cements were also observed and found to spike and then plateau. It is believed that this is due to the cements forming microcracks due to stresses in unset cement, which enlarges the free surface and thereby relieves stress, as per Figure 15 [96]. The microcracks relieve stress as it builds whilst increasing available free surface. As the setting process continues, however, the cement shrinks further and stress build up resumes. The presence of microcracks, however, would invariably result in premature failure as the cracks would serve as stress concentrators [96]. Furthermore, cariogenic bacteria could invade through the crack, rendering the filling useless as the patient would still suffer from dental decay.

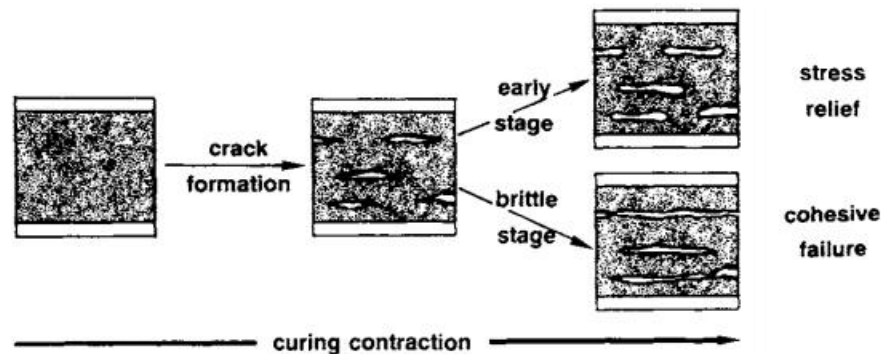


Figure 15: Microcracks form during the curing process due to contraction, which allows the stress to relieve itself. However if these cracks coalesce, they will lead to cohesive failure. [Davidson 1991]

1.3.5 Resin Composite Toxicity

The polymers in composites undergo degradation and erosion during their lifecycle. These processes break the chain into smaller segments as well as rob the polymer chain of material such as monomers or polymer fragments [79]. This can be caused by chemical, mechanical or thermal factors. Degradation usually happens due to hydrolysis or by attack from enzymes found in saliva. Erosion is usually caused by solvents, such as water, entering the bulk material. This can trigger chemical degradation which can result in the formation of pores in the microstructure. The material is then released through these pores [79]. Studies by Pearson and Longman and by Tanaka *et al.* found that a greater irradiation time correlated with less solubility and thus less degradation and erosion [97], [98]. However, Ferracane found a poor correlation between conversion of monomers to polymer and solubility in water but a much better correlation between a 50% ethanol and 50% water solution [84]. It is therefore believed that more than simply conversion to a polymer is significant in preventing elution. Further investigation by Lee *et al.* point to the type of solvent being significant and that ethanol was far more effective at penetrating the matrix and leeching out soluble chains than saliva [99]. It is estimated that between approximately 1.5% of material by weight is water soluble whilst 5-10 % of material by weight is soluble in organic solvents. Studies by Soderholm and Oysaed & Ruyter showed that barium containing glasses were more soluble than quartz or silica fillers in aqueous environments [100], [101]. From the matrices, the resins were found to release triethylene glycol dimethacrylate (TEG-DMA) in higher quantities than other comonomers[79]. TEG-DMA is known to show cytotoxic effects, and it may also break down during conversion to a monomer into more toxic components, including formaldehyde [102]. Clinical studies also suggest that TEG-DMA inhibits dentin remineralization through the interference of bonding agents [103].

1.3.6 Glass Chemistry and Structure

Glasses are defined by two major criteria: the lack of long-range repeating atomic arrangements and a time dependent glass transformation [104]. This glass transformation behaviour occurs over a temperature range known as the glass transformation range. Any amorphous material, including organic, inorganic and metallic which exhibit glass transformation behaviour can be described as a glass regardless of which technique produces them [104]. Techniques usually employed to form glasses include melt-quench, sol-gel, vapor deposition, and neutron irradiation of crystalline materials [104].

Silicate bioactive glasses are frequently studied for their biomedical applications, which include synthetic bone grafts and scaffolds [105], [106] as well as dental applications when used as part of glass polyalkenoate cements and composites [107]. Silicate bioactive glasses often contain silicon dioxide (SiO_2), calcium oxide (CaO), sodium oxide (Na_2O), and phosphorous pentoxide (P_2O_5) but can often contain chemical modifications by incorporating cations [105]. These cations can function as network formers or as network modifiers which provide useful effects such as modifying the mechanical and structural properties of the glass [108], [109], as well as offering therapeutic effects such as anabolic and antibacterial action [110]–[112].

The structure of glass formation is often discussed in accordance with Zachariasen's rules which have been called the random network theory [104], [113]. The rules are listed in Table 7. While they are not definitive as a structural model, they are useful for explaining glass formation behaviour, and have been expanded to include more complex glass formation models as well. These rules also do not necessarily describe glass formation but rather the formation of a continuous 3D network structure and can also be applied to crystalline material formation. In order to relate them more accurately to glass formation, a caveat must be added: the networks

described by these rules must be disrupted so that long-range periodicity does not occur [104]. These disruptions can occur in the form of variations in bond lengths, bond angles and rotation of structural units about their axes [104].

Table 7: Zachariasen's rules for glass formation [104]

Zachariasen's Rules for Glass Formation in Simple Oxides	
1.	Each oxygen atom is linked to no more than two cations
2.	The oxygen coordination number of the network cation is small
3.	Oxygen polyhedra share only corners and not edges or faces
4.	At least three corners of each oxygen polyhedron must be shared in order to form a 3D network
Modified Rules for Complex Glasses	
1.	The sample must contain a high percentage of network cations which are surrounded by oxygen tetrahedra or triangles
2.	The tetrahedra or triangles only share corners with each other
3.	Some oxygens are linked to only two network cations and do not form further bonds with any other cations

Silicate bioactive glasses are characterized by their SiO_4^{3-} tetrahedra, in which Si atoms are bound to four oxygen atoms while each oxygen atom is bound to two Si atoms. This results in a three dimensional (3D) network in which each SiO_4 tetrahedral unit is bound at the corners to other tetrahedral units, with cations balancing the charges [114]. The difference in the electronegativity of Si (electronegativity of 1.90) and O (electronegativity of 3.44) atoms allows for the formation of ionic Si-O-Si bonds, which form the backbone of the glass system [115], [116]. However, Si atoms can also bond to other species in the glass composition as dictated by electronegativity [105], [117]. Generally, when two atoms bond with different electronegativities, an electron flow goes from the lower electronegative atom to the greater electronegative atom [117]. It has also been shown that silica glasses experience a modification

to their network when other cations or alkali species are added, and that these cations find themselves near non-bridging oxygens [116]. This modification typically increases degradation as alkali concentration is increased, forming meta, pyro and ortho-silicates in order of increasing alkali concentration [116]. Units such as $[\text{SiO}_{4/2}]^0$, $[\text{SiO}_{3/2}\text{O}]^-$, $[\text{SiO}_{2/2}\text{O}_2]^{-2}$, $[\text{SiO}_{1/2}\text{O}_3]^{-3}$ and $[\text{SiO}_4]^{-4}$, which are present in silicate glasses are designated as Q_4 , Q_3 , Q_2 , Q_1 and Q_0 respectively, where the subscripts on the Q_n designate the number of bridging oxygens centered on a given Si atom and through which they are connected to other Si atoms in the glass structure [116]. The addition of glass former cations in silicate glasses will result in decreased solubility in these systems [116].

Transition metals can function as network formers or network modifiers, depending on their valences; oxygen atoms bound to highly charged cations, such as MoO_4 , cannot form bonds with Si and therefore must act as network modifiers [118]. While transition metals can form glasses, sometimes they are only able to do so in combination with other glass forming oxides [105]. For example, V_2O_5 can form glasses by forming chains of tetrahedra linked through two corners, while a fifth oxygen atom links to chains together to form double chains. Each Va atom therefore can be said to have five oxygen neighbours [105], [119], [120].

Glass dissolution is commonly described by one of two models: the "homogenous" model and the "heterogeneous" model [121]. In the homogeneous model, the liquid completely enters the particles and reacts throughout them at all times. The dissolution reaction is governed by a homogeneous first-order reaction. In the heterogeneous model, glass dissolution is driven by two steps: the first step involves alkali ions being replaced with hydronium ions (H_3O^+) from the solution with the square root of time [121]. The second step is the dissolution of the glass network which is linear with time [121]. Both models have been criticized as being inadequate.

There are deviations from the $t^{1/2}$ kinetics in the first stage of heterogeneous model which are affected by environmental pH and the extent of network hydrolysis [122], [123]. A clear transition from the first stage to the second is not always apparent due to the formation of a microporous surface hydrated layer formed by silica dissolution [122], [123]. This layer acts as a stiff gel which does not craze but plastically deforms to relieve induced tensile stresses. The hydrated surface layer is usually brittle and crazing causes etching (dissolution) of the silicate glass network [123].

1.3.7 Glass Ionomer Cements

Glass Ionomer Cements (GICs) were developed by Smith (1968) and refined by Wilson and Kent (1972) [107]. They are tooth-coloured materials which bond chemically to dental hard tissues and release fluoride for a relatively long period [124]. As acid-base cements, there are three essential ingredients for GICs: a polymeric water-soluble acid, a basic ion-leachable glass and water [125]. These are usually presented as an aqueous polymeric acid and a fine, acid-degradable glass powder which are mixed to form a viscous paste that sets rapidly [125]. They can also, however, be presented in other forms, such as with the acid and glass mixed in a powder and pure water starting the reaction, or with some of the acid mixed with the glass powder while the rest of the acid is in a dilute solution in water; these varying formulations do not appear to show any obvious effect on the final properties of the cement [125]. The International Organization for Standardization (ISO) classifies their proper name as "glass polyalkenoate cements" but glass-ionomer is recognized and widely accepted in the dentistry profession [126]. The GIC setting reaction is described in Figure 16.

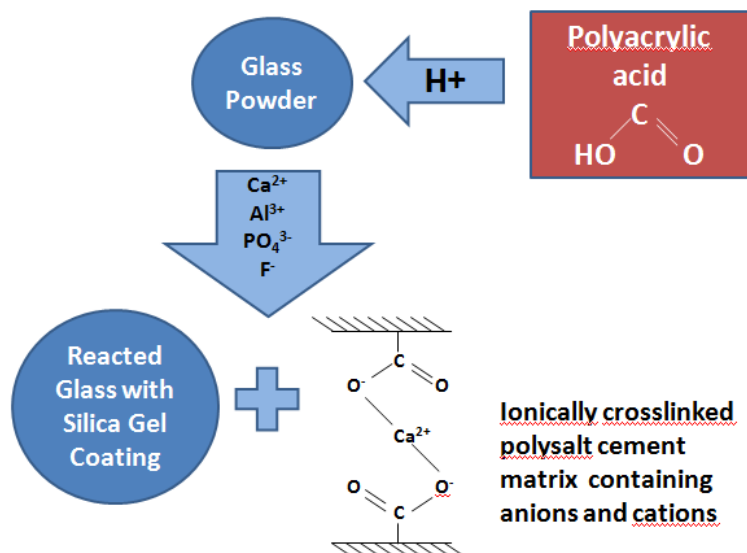


Figure 16: GIC setting reaction

Conventionally, GICs are made through an aqueous polyacrylic acid and a fluoroaluminosilicate glass component, which polymerise through an acid-base reaction [124], [125]. As metallic polyalkenoate salts begin to precipitate in the form of calcium acrylate and aluminum acrylate, gelation occurs until the cement sets [124], [127]. The final structure contains a substantial amount of unreacted glass which functions as a filler to reinforce the cement once set [125]. The carboxylic chains that form during the reaction can react with the calcium in hydroxyapatite in order to bond the cement to the tooth without the need for bonding agents [7], [128]. This is considered one of the greatest advantages of GICs as it allows the cement to be used as substitutes for missing bits of enamel and dentin during restoration procedures, instead of just a filling for cavities. If the glasses are ground smaller than $15\mu\text{m}$, the cements can also be used as luting cements in the "sandwich" technique [88], [127]. A luting cement is a lining of cement used to bond the dentin to the filling material, leveraging the superior bonding capability of the cement to create a greater seal and prevent microleakage [88].

1.3.8 Glass Ionomer Cement Mechanical Properties

GICs have many of the same advantages of composites. They boast good esthetics and adhesion to hard dental tissues. Frequently, a therapeutic bioglass is employed in mixing which releases beneficial ions, such as fluoride, calcium, and even antibiotic agents like zinc, silver and strontium [128], [129].

The major drawback of GICs is their lack of strength and toughness; they are weak when first set and unstable in water [128]. This is because the the water can act like a plasticizer which reduces compressive and flexural strength, as well as act as a solvent for the cements components [130], [131]. However, their compressive strengths can increase up to 400 MPa after one year [128]. Surface treatments of enamel and dentin were not found to produce significant differences in bond strength with enamel or dentin according to [132]. The mechanical properties of commercially available GICs were measured by Xie *et al.* (2000) after seven days and are listed in Table 8.

Table 8: Mean compressive strengths of commercially available GICs and resin-modified GICs. All strengths are expressed in MPa with standard deviations expressed in parentheses.[133]

Glass Ionomer Cement	Mean Compressive Strength (MPa)
Ketac-Bond	225.7 ± 7.3
α-Silver	176.0 ± 6.5
α-Fil	196.5 ± 8.3
Ketac-Silver	211.8 ± 3.2
Ketac-Fil	251.2 ± 10.0
Ketac-Molar	301.3 ± 10.1
Fuji II	202.0 ± 10.0
Vitremer (Tricure)	265.3 ± 7.5
Fuji II LC	306.2 ± 6.8
Photac-Fil	243.5 ± 7.9
Vitro Fil DFL GIC	54.00 ± 6.56
Vitro Fil LC resin-modified GIC	105.1 ± 17.29

To overcome their poor mechanical properties, resin-modified GICs were developed by adding methacrylate to the polyacrylic acid component. According to a 2007 study by Mallmann *et al.* , resin-modified GICs were found to be stronger in compression [134].**Error! Reference source not found.** Table 8 compares an unmodified GIC (Vitro Fil DFL GIC) with a resin modified version (Vitro Fil LC resin-modified GIC). This is likely due to the extra polymer links from the resin which produce a stronger material [133], [135].

1.3.9 Glass Ionomer Cements - Acid Phase

The most common polymeric acids are homopolymer poly(acrylic acid) or a 2:1 copolymer of acrylic acid and maleic acid [136]. The properties of the polymer affect the cements made from them. High molecular weight acids increase the strength of the resultant cement but increase the

viscosity, making them difficult to mix. An optimum weight for restorative cements is said to be an average molecular weight of 11,000 (number average) or 52,000 (mass average) [137]. Cements derived from homopolymers show increases in compressive strength over the first 4-6 weeks while cements derived from copolymer acids show an increase in compressive and flexural strength over the first few months of the cements lifetime before declining to an equilibrium value. This has been attributed to the progressive slow reactions stemming from the higher crosslink density of cements deriving from copolymers as compared with cements derived from homopolymers [127]. This higher crosslink density causes the cement to be more brittle, increasing sensitivity to surface flaws and other imperfections within the cement with aging [127].

1.3.10 Glass Ionomer Cements - Glass Phase

Glasses must be basic in order for the acid-base reaction to occur and salts to form [127]. While in principal many glass formulations could form salts, in practice, only aluminosilicate glasses with sodium calcium, fluoride and phosphate additives are used [125]. This is because pure silica glasses would form SiO_4 tetrahedra linked at the corners to form chains with no charge, since Si has a 4^+ charge. The addition of alumina results in similar geometry (AlO_4 units), but aluminum carries a formal 3^+ charge which does not counteract the negatively charged oxygen atoms as effectively, resulting in a basicity which is vulnerable to acid attack [125], [127]. Extra cations such as Na^+ , Ca^{2+} or Sr^{2+} balance the charge and break the continuous bridging oxygen network [125]. Glass chemistry will be discussed in greater detail in Chapter 1.6.

1.3.11 Glass Ionomer Cement Cariostatic Efficacy

A study by Aboush and Torabzadeh (1998) determined that cumulative fluoride release from GICs is up to five times higher than compomers and up to twenty-one times more than resin composites at the end of twelve months in deionized water [138]. Additionally, during a 24

month span, the fluoride release from GICs was five to six times higher than compomers. Table 9 summarizes the findings. Furthermore, Benelli *et al.* (1993) found that plaque formed on enamel blocks adjacent to GICs *in situ* contained six times more fluoride than plaque adjacent to resin composites after 28 days [139]. The same study also showed a greater microhardness at several depths in the enamel from the surface, as well as at some distance from the filling as compared to composite resins. This suggests a more potent remineralizing effect from GICs than from composite resins as the increased fluorine concentration can limit the cariogenic effects of *S. mutans*. Furthermore, the increased microhardness result at several depths demonstrates that the mineral phase of the enamel blocks have undergone significant remineralization.

Table 9: Fluoride release after twelve months and in twenty-four hours one year after curing from three different dental restoration materials. [138]

After twelve months ($\mu\text{g}/\text{cm}^2$)			During last 24 hour period of twelve-month test period ($\mu\text{g}/\text{cm}^2$)		
GIC	Compomer	Resin Composite	GIC	Compomer	Resin Composite
Fuji Cap II (GC International)	Dyract (De Trey Dentsply)	Tetric (Ivoclar Vivadent)	Fuji Cap II (GC International)	Dyract (De Trey Dentsply)	Tetric (Ivoclar Vivadent)
480 ± 42	87 ± 17	22 ± 2	1.3 ± 0.20	0.21 ± 0.04	0.23 ± 0.02

The cariostatic effect of GICs and composite resins have been compared. Ersin *et al.* (2006) and Levy and Jensen (1990) showed that there is no significant caries preventative difference on either primary or permanent teeth between the two materials [140], [141]. However, Mickenautsch *et al.* (2009) showed that permanent teeth restored with GICs have significantly less carious lesions after six years as compared to teeth restored with amalgam [142]. While the difference was not statistically significant in primary teeth, GICs were still favoured.

1.3.12 Polyacid-Modified Resin Composites (Compomers)

Poly*-acid-modified resin composites, commonly referred to as compomers, are a class of dental materials that are designed to provide the benefits of both resin composites and GICs [7]. The introduction of components from GICs into the resin composite as well as additional monomers with carboxylic acid groups means that as they mature, they take up small amounts of moisture to facilitate an acid-base reaction [7], [143]. This allows compomers to take in and release clinically significant amounts of fluoride [58–60]. A study by Asmussen and Peutzfeldt found that while compomers release relatively little fluoride upon setting compared to the ‘burst’ that GICs provide, the long term fluoride release rate of compomers is comparable to GICs [147]. This gives compomers a similar effect to GICs in their ability to act as fluoride reservoirs, albeit with smaller quantities of fluoride. It is believed that this is due to the fluoride being bonded to the filler particles rather than being free, making the compomer behave far more like a resin composite during the first phase of the setting process [148].

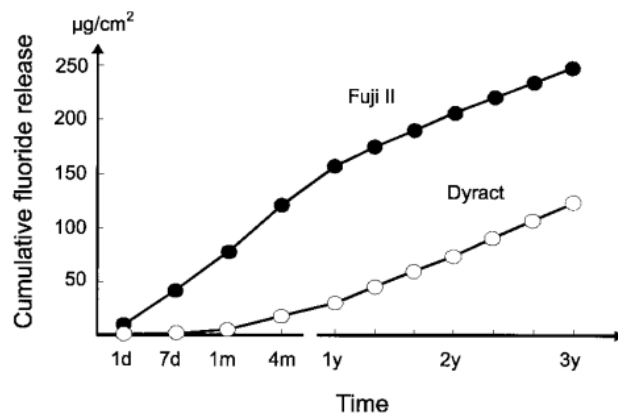


Figure 17: Cumulative fluoride release of a conventional GIC (Fuji II) vs a Compomer (Dyract) in distilled water over three years [147]

Compomers also boast good esthetics, high diametral compression and flexural strengths compared to resin composites and conventional GICs [6], [143]. They have better wear

properties than conventional GICs and resin composites [149], [150] but lack the ability to bond to hard dental tissues [7], [151].

1.3.13 Bioactive Glasses

Before the advent of bioactive materials, most implant devices would not bond with hard body tissues. Rather, the body would form scar tissue which would not allow the implant to adhere to the site [11]. Therefore, initial biomaterials were designed to be bio-inert in order to not provoke a reaction between the foreign body and the host site. Metallic implants were designed to have very high corrosion resistance due to the dangers of electrochemical dissolution; it was therefore suggested that dissimilar metals must never be placed in the same patient, as they can cause an electrolytic reaction [12]. Only Vitallium and surgical stainless steel implants were used as orthopaedic implants [11], [12], [152]. Polymeric implants were designed to prevent degradation [11], [12]. However, Hench and Greenlee changed the approach to biomaterials by developing a soda-lime-phosphate-silicate bioglass which was designed to bond with bone [11], [12], [153]. The materials were bioactive as they promoted adhesion to bone and bone regrowth [154].

1.3.14 Hench's 45S5 Bioactive Glass

A silica-based glass with network modifiers of Na_2O and CaO , along with an amount of P_2O_5 to simulate the Ca/P ratio of hydroxyapatite, 45S5 did not form scar tissue and instead bonded to femoral rat bone and could not be removed from the host site, as shown in Figure 18 [11], [12], [153]. Using transmission electron microscopy, it was found that the growing bone mineral had interlocked with collagen fibers in the bonded interface, suggesting a bond to bone. Furthermore, X-ray diffraction of the glasses in a calcium-phosphate solution showed the growth of biologically active hydroxyapatite crystal phases on the surface of the glass that were similar to the spectra of HA in living bone, again showing the bonding to bone [11], [153], [155]. These materials are commonly used as coatings for other implant devices to promote bonding to bone,

bone grafts in which they are used to fill bone defects, or as bulk implants to restore teeth [11], [156], [157].

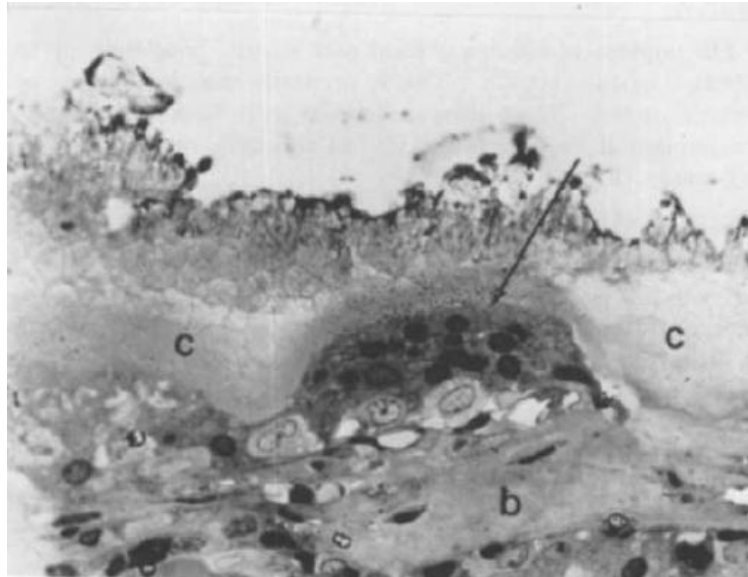


Figure 18: Bonding to bone between 45S5 glass and rat femur after 6 weeks. (c) represents the implant and (b) represents bone. The arrow points to osteoclasts forming at the interface between the remnants of the implant and the bone. Light microscopic image at x630 magnification [153]

Bioglasses are marketed under several different tradenames. The bioglass 45S5 has been altered to tailor it to several different applications including bone grafts (NovaBone, NovaBone Products, FL, USA), dentinal hypersensitivity and periodontal defects (NovaMin, GlaxoSmithKline, UK) [11], [158]. NovaMin is incorporated into dentifrices (Sensodyne Repair and Protect, GlaxoSmithKline, UK) to promote remineralization of enamel and treat dental hypersensitivity [159]. Calcium-phosphate crystals can plug up dentinal tubules, decreasing sensitivity and dentin permeability [160], [161].

45S5 remineralizes enamel by precipitating the calcium and phosphate ions onto the enamel surface layer. The calcium, sodium and phosphate ions are leached out of the glass network structure during an acid attack and go into aqueous solution in which the phosphate ions pick up and bond to the calcium ions to form calcium phosphate salts[160]. The enamel may also give up

calcium and phosphate ions to the aqueous solution [162]. These salts then precipitate onto the dentin surface as they dissolve out of the remaining silanol compounds. The silanol compounds are soluble in water and elemental analysis shows that only trace amounts of silica are found in the interaction layer [163], although it is believed that the silica may enhance remineralization for early cariogenic lesions, especially in dentin [13], [14], [164]. Some of the smaller crystals are able to penetrate the deeper sub-surface lesion through the porous outer enamel layer to saturate the lesion with calcium and phosphate ions while surface enamel will have a layer of brushite, an acidic calcium-phosphate salt form [160], [163]. They are then remineralized in to the surface and sub-surface of the enamel lesions [160]. The process is illustrated in Figure 19 below.

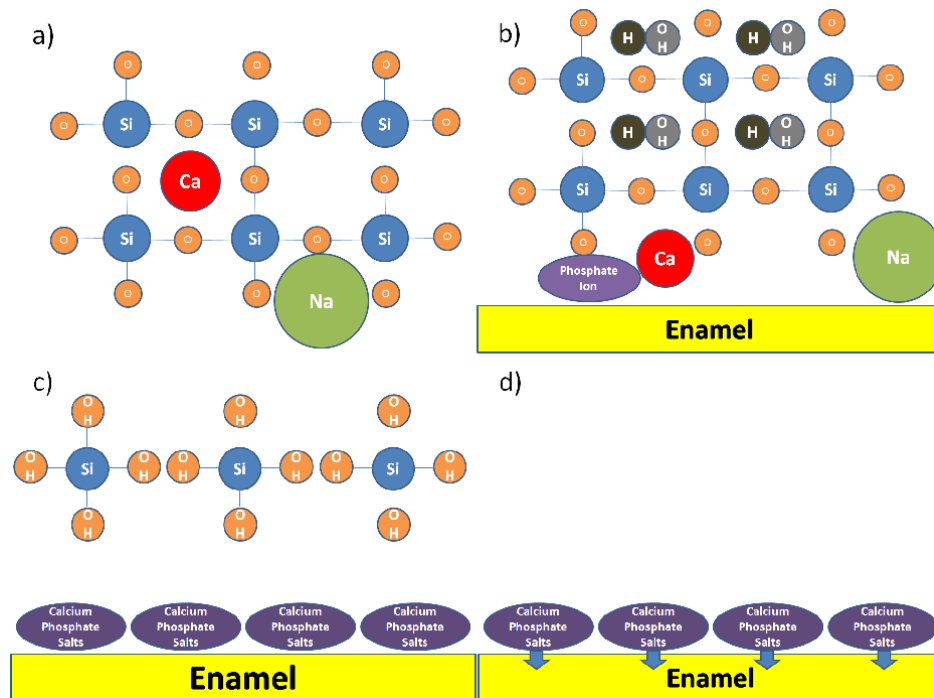


Figure 19: Suggested action of enamel remineralization by 45S5 bioglass. A) Structure of 45S5 bioglass. B) Calcium, phosphate and sodium ions leached out of network structure. Phosphate ions pick up calcium ions and form calcium phosphate salts. C) Calcium phosphate salts precipitate onto the enamel surface as they dissolve out of silanol compounds. D) Calcium phosphate salts begin to remineralize enamel in the surface and sub-surface of enamel lesions. [160]

1.3.15 Antibacterial Effects of Bioactive Glass

Bioactive glasses can also demonstrate antibacterial effects. Studies by Prabhakar and Kumar (2010) found that 45S5 was effective against *enterococcus faecalis*, a bacterial strain commonly associated with apical periodontitis, when compared with controls [15]. A similar study by Zehnder *et al.* (2004) found that the bioactive glass S53P4 was also effective against *e. faecalis* [16] when compared with controls. Apical periodontitis is an inflammation of the tip of the root area of a tooth due to infection of mixed, mostly anaerobic bacteria [165]. The addition of powdered dentin increased the antibacterial effect against many oral microorganisms after 24 hours and three days, although the effect reduced after five days. The antibacterial effect of bioactive glass is a function of the ability of the glasses to raise the pH of an aqueous solution [15]. Bacteria and host cells are not well suited to surviving in the higher pH environments. However, raising the pH in the host environment does not provide an immediate antibacterial effect like potassium iodide, sodium hypochlorite or chlorhexidine but rather a sustained, long-lasting, slow-acting effect [16]. The antibacterial effect of adding dentin powder is not fully understood but is believed to provide a source of calcium and phosphate ions, allowing the bioactive glass to "mineralize" the bacteria by forming Ca/P precipitates on the surface, destroying their cell integrity [16]. Adding powdered enamel did not significantly increase the antibacterial effect of bioactive glasses after 24 hours or three days, but did demonstrate an increased effectiveness after five days [15]. Furthermore, particle size was found to have a significant effect on the antibacterial action of bioactive glasses; the increased surface area makes the glass more reactive by exposing a greater portion of the glass to acid attack thus resulting in the release of more ions [166]. Additionally, Perioglass, commonly used as a bone graft material, has particle sizes near 20-63 μ m and exhibits a lower antimicrobial effect since the

glass cannot enter the deeper portions of the dentinal tubules, which can be full of microorganisms [166].

1.3.16 Remineralization Potential of Bioactive Glass

A study by Bakry *et al.* (2014) investigated the effect of NovaMin particles dissolved in a phosphoric paste on demineralized enamel [160]. Enamel slices were subjected to an erosion challenge and then treated with either fluoride gels for short and long term exposures or a bioglass paste, made by mixing 45S5 bioglass with 0.2 mL of 50wt% phosphoric acid (Wako Chemicals, Japan). They found that the sub-surface enamel lesions demonstrated significantly better micro-mechanical properties after treatment with the bioglass when compared with the control group and fluoride treatments at depths of 30 μm , 40 μm and 50 μm . Fluoride treatments improved remineralization at depths of 30 μm and long term exposure improved remineralization at depths up to 40 μm . However, the remineralization from bioglass treatments was more significant than the fluoride treatments, as evidenced in Figure 20. This is likely due to the fluoride ions combining with superficial layers of the enamel lesion, making it difficult for fluoride ions to penetrate any deeper in to the lesion [167], [168].

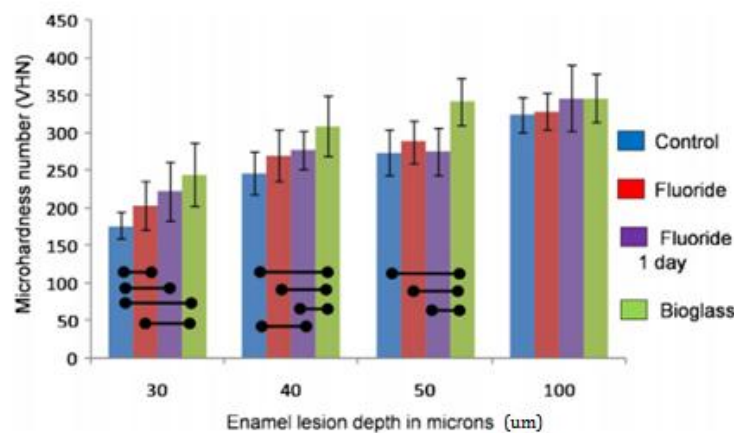


Figure 20: Cross-sectional microhardness at various enamel lesion depths when subjected to fluoride and bioglass treatments after erosion challenges. Connected bars are statistically significant ($p < 0.05$). [160]

However, a study by Diamanti *et al.* (2010) found that fluoridated dentifrices were more effective than NovaMin containing toothpastes at remineralizing dentin after an acid challenge [13]. Ten daily cycles of demineralization and remineralization cycles were applied to dentin slabs in a 30 minute demineralization phase followed by a four hour remineralization period. A second demineralization phase lasting another 30 minutes was then followed up by an overnight remineralization period lasting 16 hours. The dentin slabs were then submersed in toothpaste slurries consisting of 1:3 dentifrice to distilled water, where the dentifrices included three different concentrations of fluoride containing toothpaste (5000ppm F, 2800ppm F, 1450ppm F), a 7.5% NovaMin containing toothpaste as well as a non-fluoridated toothpaste as a control. Knoop hardness measurements were taken to determine the remineralization efficacy of each treatment. The fluoridated treatments showed the greatest hardness, with the 5000ppm F treatment the most effective at 28.85 ± 1.06 KHN, followed by the 2800ppm F at 26.48 ± 1.41 KHN, then the 1450ppm F at 23.96 ± 1.60 KHN and finally the 7.5% NovaMin treatment 21.62 ± 2.83 . The control measured in at 20.85 ± 1.78 KHN. It is suspected that the presence of silica near dentin precipitates into hydroxyl-carbonate apatite more favourably than fluor-hydroxyl apatite, forming a far more soluble crystal [169].

It can be surmised from the above studies that while bioactive glass therapies are better at remineralizing enamel, high fluoride treatments are better at remineralizing dentin. Fluoride ions precipitate in superficial layers on the enamel and are unable to penetrate deeper into lesions, making them ineffective at remineralizing them. In dentin remineralization tests however, fluoride performed better as it is able to reach the deeper levels it could not during enamel remineralization challenges. Additionally, the silica content in bioactive glasses favors the

formation of hydroxyl-carbonate apatite, which is a more soluble mineral when compared with fluor-hydroxyl apatite.

A study by Deng *et al.* found that the use of 45S5 bioactive glass during hydrogen peroxide whitening treatments helped reduce the microhardness loss of enamel [170]. Bioactive glass alone could not whiten teeth but hydrogen peroxide alone caused significant microhardness loss and morphological change of enamel. The symbiotic effect of bioactive glass in hydrogen peroxide took advantage of the alkalinity and accelerated the ionic leaching of the bioactive glass in order to offset the damage of bleaching therapies [170].

1.3.17 Dentifrices

Most dental therapies are generally reactive measures in that they are employed when lesions have grown into cavities. Ideally, therapies should be more proactive in that they address demineralized enamel before lesions can form. Dentifrices can be useful in impeding erosive tooth surface loss [171]. A dentifrice is a preparation which is generally used with a toothbrush in order to clean or polish teeth [172]. Modern dentifrices often claim various beneficial effects such as plaque removal, anti-caries effects, remineralization effects, antibacterial effects, tooth whitening, reduction in oral malodor, anti-gingivitis effects, desensitization effects, periodontal healing among many other effects [172].

Dentifrices often contain remineralization agents such as various fluoride compounds, potassium, calcium and phosphate as well as Novamin [28], [171], [173]. Fluoride compounds, as discussed earlier, can reduce dissolution through forming compounds which prevent it. SnF_2 , an ingredient in many toothpastes, has been shown to form complexes which are more resistant to acid attack *in vitro* [174]–[176] and well as *in situ* using toothpastes and rinses [177] through the deposition of a tin-rich surface on the enamel [171], while ZnF_2 , another common ingredient in toothpaste,

can provide the antibacterial action of zinc in addition to the cariostatic effect of fluoride [175], [178], [179]. Potassium can provide relief from dentin hypersensitivity through occlusion of tubules [17], [28], [173], [180] . Calcium and phosphate compounds are valuable in remineralizing hydroxyapatite [181]. Novamin is a bioactive glass which provides a source of calcium and phosphate, as well as limited antiseptic capability [15].

It is commonly accepted that use of dentifrices have shown an ability to remove plaque buildup [182]. Plaque is a microbiological biofilm and is the chief cause of dental caries and periodontal disease [4]. However, Valkenburg *et al.* found that toothbrushing with a dentifrice does not provide a benefit for mechanical removal of dental plaque [172]. They suggested that the mechanical action provided by the toothbrush itself and the skill of the individual play a larger role in reducing plaque [182], [183]. While toothpastes often contain abrasives, these are not effective in removing plaque build-up [183], [184]. The abrasives in toothpastes are far more effective at removing stained pellicle, and preventing teeth from becoming stained [185], [186]. The pellicle is a thin film which is considerably tougher to remove than plaque and cannot be broken through the bristles on a toothbrush [186]. Abrasive particles penetrate and remove the stained pellicle films [186].

Sapra *et al.* tested an herbal extract, *Spilanthes calva*, an herb found in India commonly known as Akarkarah (toothache plant), along with an herbal dentifrice (Arodent) and a commercial dentifrice (Colgate) [187] against salivary amylase. They found that *s. calva* was effective in reducing plaque build-up through inhibiting salivary amylase [187]. Salivary amylase is a digestive enzyme produced in the salivary glands which plays a key role in metabolizing Streptococcus, leading to the formation of dental plaque and dental caries [188]. The results are summarized in Table 10.

Table 10: Effect of various dentifrices on salivary amylase activity. All results are statistically significant at $p < 0.05$ except for the control. [187]

Dentifrice	Pre-Application (mg maltose/ml)	Post-Application (mg maltose/ml)	Difference
<i>S. Calva</i>	8.08±0.60	4.11±0.33	3.97±0.46 (maximum)
Arodent	8.01±0.66	6.05±0.83	1.96±0.69
Colgate	8.22±0.68	6.73±0.95	1.49±0.41
Control	7.58±0.69	7.40±0.92	0.18±0.13 (minimum)

As shown above, *s. calva* was more effective than traditional dentifrices in inhibiting plaque build-up. The amylase activity was measured through how much maltose was produced from starch supplied to the assays [187].

Tooth whitening is another function of dentifrices. Whitening agents promote decomposition of pigmented organic materials through their oxidation [189]. They release free radicals, such as oxygen or hydroxyl, which have unpaired electrons in the outermost electron shell, giving them a strong tendency to interact with other electrons under the same conditions, stabilizing their incomplete orbits [190], [191]. The released free radicals initiate an oxidation process on the teeth in which dark-coloured, high-weight molecules break down into lighter-coloured low-weight molecules, whitening the teeth [191]. However, while they are effective at attacking organic pigment-generating tissues, they also produce adverse effects such as tooth demineralization and irritation of neighbouring tissues [189], [192]. When whitening agents are embedded into dentifrices, it is believed the mineral loss is greater from the mechanical action of brushing than from the whitening agents [193], [194]. It is believed that the duration of exposure on enamel of the whitening agent has a far greater effect than the concentration of the agent

[194], [195]. A study by de Araujo *et al.* tested three dentifrices containing various bleaching agents, as listed in Table 11 below [189].

Table 11: Dentifrices containing bleaching agents tested by de Araujo *et al.* [189]

Dentifrice	Manufacturer	Bleaching Agent
Mentadent	(Chesebrough Pond's USA Co., Oral Division, Greenwich, CT, USA)	Hydrogen Peroxide
Rembrandt	(Den-Mat Corporation, Santa Maria, CA, USA)	Carbamide peroxide
Colgate	(Colgate-Palmolive Company, S. Paulo, Brazil)	Sodium Bicarbonate
Colgate Total 12	(Colgate-Palmolive Company)	None

They found that while all were effective as whitening agents, the abrasive sodium bicarbonate treatment resulted in lesions of the greatest severity while treatments involving carbamide peroxide produced lesions of the least severity.

An *in vitro* study by Roopa *et al.* found that compomer and composite restorations treated with conventional toothpastes and whitening toothpastes showed no significant differences in microhardness after two and four weeks, while they did detect a significant colour difference with the compomer and composite restorations when using the whitening toothpaste as compared with the conventional toothpaste [196]. They also found that the whitening toothpaste produced a more significant change in surface roughness than conventional toothpastes on both compomer and composite restorations at both two and four weeks. The results are summarized in Table 12 below.

Table 12: Comparison of Conventional and Whitening Toothpaste on Compomer and Composite Restorations. Microhardness values are in VHN with 25g for 20s. Colour was measured as ΔE (total colour change) with a spectrophotometer with a 10mm aperture and D65 illuminant. Surface roughness was measured in Ra (μm) with a radius of $1.5\mu\text{m}$ at a constant speed of 0.1mm/s with a force of 0.7mm, with a cutoff at 0.25mm, measured with a surface roughness machine. [196]

Group	2 Weeks Mean Change	4 Weeks Mean Change
Compomer With Conventional Toothpaste	Microhardness 0.18 ± 0.47 Colour Stability 0.71 ± 0.47 Surface Roughness 0.09 ± 0.47	Microhardness 0.81 ± 1.13 Colour Stability 1.03 ± 0.62 Surface Roughness 0.16 ± 0.05
Composite With Conventional Toothpaste	Microhardness 0.39 ± 0.93 Colour Stability 1.02 ± 0.11 Surface Roughness 0.01 ± 0.03	Microhardness 0.21 ± 1.06 Colour Stability 0.97 ± 0.31 Surface Roughness 0.12 ± 0.04
Compomer With Whitening Toothpaste	Microhardness 0.35 ± 0.98 Colour Stability 2.12 ± 0.32 Surface Roughness 0.10 ± 0.04	Microhardness 1.17 ± 0.91 Colour Stability 2.19 ± 0.06 Surface Roughness 0.21 ± 0.01
Composite With Whitening Toothpaste	Microhardness 0.75 ± 1.37 Colour Stability 1.93 ± 0.29 Surface Roughness 0.05 ± 0.03	Microhardness 0.01 ± 0.52 Colour Stability 1.98 ± 0.22 Surface Roughness 0.20 ± 0.04

The whitening toothpaste contained an ingredient known as perlite, an amorphous mixed glossy silicate compound which is chemically inert and pH neutral [196]. It is often used in professional prophylactic pastes as it demonstrates an excellent ability to clean and polish; when combined with silica in toothpaste, it has also proven to be a potent stain removal and stain prevention agent [197].

Dentifrices normally contain an abrasive, which can be useful for reducing or eliminating stains [196], [198]. Dentifrices containing carbamide or hydrogen peroxide, along with alumina, silica and calcium carbonate are known to produce changes in surface roughness [198]. Tooth brushing can erode the soft polymer matrix of resin composite materials [198]. The difference in the

surface roughness reported by Roopa *et al.* in the composite and compomer materials can be attributed to the differences in the wear resistance between composite and compomer [196].

1.4 Innovations in Current Solutions

A major problem with implantable devices are infections due to biofilm growth near an implant. Implants tend to produce ideal substrates for biofilms to develop on [199]–[201] and often, implant sites require revision surgery to deal with biomaterial-centered infections [202], [203]. The use of invasive devices can allow microorganisms to bypass the natural immune system and create infections [201], [204]. Therefore, many biomaterials are doped with antimicrobial ions such as silver or gallium, which can provide an effective alternative to antibiotic treatments, especially with inhibiting biofilm growth [205]–[210].

Strontium has also garnered significant attention as it can be readily accepted into HA crystals through substitution for Ca and creates stronger bones [32]. Osteoporotic patients treated with strontium ranelate were found to have fewer fracture incidences [211]. Strontium ions (Sr^{2+}) have been found to enhance preosteoblastic cell replication and collagen synthesis while simultaneously inhibiting osteoclasts, resulting in increased bone mass [212]. Some studies have shown that Sr^{2+} may possess antibacterial properties through a synergistic action with fluorine ions, however this mechanism is not well understood [201], [213], [214]. Sr^{2+} have shown antibacterial properties when tested against periodontal bacteria such as *S. mutans*, *Lactobacillus*, *P. gingivalis* and *E. coli* [215], [216]. However, others still have challenged the antibacterial properties of Sr^{2+} as actually being very weak, and that the observed cariostatic effect is due to the enhanced remineralization effect of Sr^{2+} , rendering enamel stronger against caries attacks [217], [218].

Other ions that have been included in biomaterials include known antibacterial ions such as Zn^{2+} and Ag^+ [214]. Silver nitrate has been used to treat wounds and ulcers, while silver containers are used for disinfecting food and water [219]. The exact mechanism by which silver exerts its antimicrobial activity is not fully understood but the most commonly accepted explanation describes silver as bonding to sulfhydryl groups on the surface of cell membranes, blocking respiration and electron transfer inside the cell as well as blocking the proton motive force have been proposed [220], [221]. These actions can deenergize the cell membrane and ultimately lead to cell death.

1.4.1 Role of Strontium

Strontium has a similar atomic radius to calcium and is readily substituted for calcium in the apatite lattice. In a study by Curzon et al., strontium concentrations in drinking water were taken from Fort Recovery, Delphos and Portsmouth (all OH, USA) while children were surveyed for decayed, missing and filled surfaces (DMFS) scores [18]. Fort Recovery and Delphos boasted strontium concentrations of about 5.3 and 5.45 ppm respectively whilst Portsmouth only had 0.2 ppm. Children were found to have DMFS scores of 3.56 per child in the former two cities while Portsmouth had a score of 5.54. Ten years later, the concentrations and surveys were completed again and it was found that Fort Recovery and Delphos had an increased Strontium concentration; up to 10.98 and 7.25 ppm respectively. Portsmouth remained the same. However, DMFS scores for Portsmouth went up to 7.63 while Fort Recovery and Delphos went down to 2.4 on average. Increasing strontium concentrations in enamel and plaque were correlated with decreasing DMFS scores. This is a mathematical relationship and a cause-and-effect condition was not established [18].

Another study, also performed by Curzon et al., in Wisconsin, USA, corroborated these results [19]. Seven communities were surveyed for caries scores and strontium content in drinking water. Sr^{2+} concentrations in water varied from 0.022 ppm at their lowest to 33.9 ppm at their highest. The two communities with the lowest caries scores had strontium concentrations of 5.4 and 8.3 ppm respectively. Strontium ion concentrations higher or lower than these were associated with higher caries scores. This suggests that there is an optimum level of strontium required for the maximum remineralization effect. It is also worth noting that all communities had reported a similar fluoride concentrations in their water, ranging from 1.00 and 1.29 ppm. It is believed that strontium may end up in plaque and is available for remineralization [19]. However, a synergistic effect between fluoride and strontium is the most plausible explanation for the low-caries incidences in these communities.

1.4.2 Role of Strontium-Fluoride

Yassen performed a study to investigate the effects of strontium and strontium-fluoride combinations on caries and found that strontium alone did not increase remineralization [222]. However, when combined with fluoride, a synergistic effect was noticed and this provided the second strongest remineralizing effect, after the fluoride without strontium group. Specifically, the 10 ppm strontium combined with 0.05 ppm fluoride group repaired lesions the best with the exception of 0 ppm strontium and 0.05 ppm fluoride group. This claim is further supported by Thuy *et al.* who performed a similar study [20], where fluorine was added to a remineralization solution in varying concentrations. Strontium was then mixed in at 10 ppm. The study reported strontium providing more mineralization in terms of both quality and quantity. Figure 21 below shows microradiographs of enamel remineralization due to different concentrations of fluoride and strontium. The addition of strontium was found to produce approximately 10% greater average remineralization than fluoride alone.

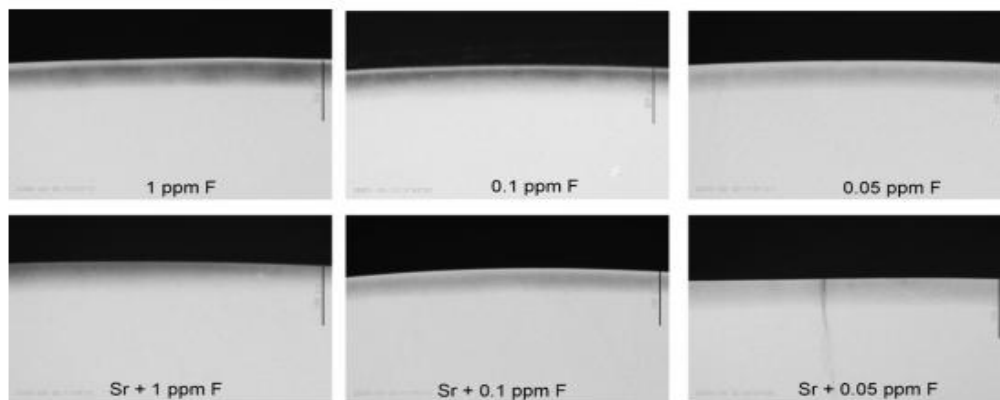


Figure 21: Remineralization Microradiographs. The faint grey areas near the top of the enamel specimens shows demineralization while the darker greys in the bulk of the specimen show mineralized enamel. [20]

Strontium regimens are also very effective in increasing acid resistance in enamel. In a study by Koletsi-Kounari *et al.*, strontium-fluorine regimens were identified as one of the strongest candidates in terms of remineralization and acid resistance properties as compared with the other regimens such as aluminum and fluoride, strontium with aluminum, and fluoride alone [223]. Fluorine ranked the highest at 15.42% mean surface hardness recovery while strontium-fluorine provided greater than 13% mean surface hardness recovery. However, strontium-fluorine had the highest acid resistance, with 75.6% relative loss, the lowest of all tested groups. This value was closely followed by the aluminum-fluorine which boasted an acid resistance of 75.8% relative loss. Unfortunately, the aluminum-fluorine group only supported 9.61% mean surface hardness during remineralization. Therefore, it can be surmised that Sr-F and Al-F treatments are more effective than F treatments alone in pre-softened enamel, showing enhanced remineralization of enamel and inhibited acid dissolution [223].

1.4.3 Role of Silver

Silver has long been used for its antimicrobial properties and low toxicity to human cells in topical burn creams, dental amalgams and even water system disinfectants [221]. The use of silver as an antimicrobial agent has increased due to concerns with antibiotic resistant

microorganisms. Due to the broad-spectrum nature of silver-based antiseptics, it is believed to be less likely to induce microbial resistance as compared to antibiotics [22]. While human toxicology to silver is not well documented, studies by Drake and Hazelwood (2005) and by Lansdown (2010) report low health risks from systemic absorption of silver ions [224], [225]. The most common consequence to silver exposure is argyria, an irreversible condition in which silver deposits into skin and eyes, leaving a bluish-grey hue [221]. It is not believed to be a life threatening condition and does not cause irreversible tissue damage [224]. The World Health Organization (WHO) recommends a maximum lifetime intake of 10g of silver as this has no observable adverse health effects on humans [221]. Drinking water concentrations are limited to 0.1 mg/m³ according to the National Institute for Occupational Safety and Health and the American Conference of on Governmental Industrial Hygienists. WHO has reported that it is unlikely for a person to consume enough silver over a lifetime in order to develop symptoms of argyria [226]. Risks may increase depending on geographical location however; for example, residents of the San Francisco Bay area may deal with silver residues from factory wastes entering the food supply and this may increase the risks of developing argyria.

Nano-silver particles have been investigated for their cariostatic properties on primary teeth. A study by Santos V.E. *et al.* (2014) found the effectiveness to be 66.7% when applied once a year [227]. Additionally, the treatment did not stain any teeth black as the nano-silver compounds did not oxidise. This is in contrast to 30% silver diamine fluoride solutions which had a caries arrest rate of 66.9% when applied once per year. However, the black staining of teeth is still encountered with silver diamine fluoride treatments [228]. Furthermore, silver nanoparticles have also demonstrated a significant antibacterial effect on *s. mutans*, a bacterial strain commonly associated with dental caries [23]. Bacteria exposed to silver nanoparticles had a

survival rate of about 2% whereas chlorhexidine, a common antiseptic agent yielded a survival rate of 60%.



Figure 22: A primary tooth treated by nano-silver solution after 12 months. Note the lack of black staining on the lesion.[227]

While the antibacterial action of silver has been well documented [22], [112], [204], [206], [221], the mechanism of action is not fully understood [229]. Several proposals to explain the antibacterial effect of Ag^+ ions have been developed. They have been reported to inactivate metabolism and inhibit bacterial growth [230], uncouple the respiratory chain from phosphorylation [231], interfere with membrane permeability to protons and phosphate [232, p.], and react with thiol groups of membrane-bound enzymes and proteins to inactivate bacteria [229]. It is observed that the most common site of action at low concentrations is the cytoplasmic membrane [233]. Many important proteins, such as enzymes for respiratory chains reside here [231], [233]. At higher concentrations, Ag^+ ions have been observed to interact with cytoplasmic components within the cell [229]. In a study by Holt and Bard, $\leq 10 \mu\text{M}$ AgNO_3 were added to immobilized *E. coli*. Measurement of Ag^+ uptake by the cell found that approximately 60% of the silver was transported into the cell while the remaining 40% was bound to the outside of the

cell [231]. The bacteria reacted to the addition of silver with stimulated respiration before death, suggesting the interaction of Ag^+ and enzymes of the respiratory chain.

1.4.4 Role of Zinc

An essential trace element to the body, zinc is naturally present in bone (0.6g), muscle tissue (1.2g) and even skin (0.1g) [234]. It is often added to toothpastes and mouth rinses as an antibacterial agent to fight against plaque, calculus formation and halitosis [24], [25]. Zinc ions react with volatile sulfur compounds such as hydrogen sulfide in the mouth, and it is in these compounds which are responsible for the malodour associated with halitosis [235]. In a study by Giertsen *et al*, caries incidences on partially desalivated rats were treated with zinc combined with either chlorhexidine or cetylpyridinium chloride [236]. Zinc was effective at significantly reducing smooth-surface caries incidences but showed no significant synergistic effect with either chlorhexidine or cetylpyridinium chloride, while sodium fluoride showed the greatest cariostatic effect in reducing smooth-surface incidences. However, in determining total viable populations of *streptococcus sobrinus*, zinc showed a great synergistic effect with cetylpyridinium chloride, as this combination was the most successful in suppressing the bacteria. Interestingly, zinc alone was not more effective than placebo. From the results, it can be suggested that perhaps a stronger concentration of zinc may be required to inhibit caries development in teeth when subjected to strong cariogenic challenges.

Zinc was found to be almost as effective as fluoride in equimolar concentrations due to its ability to form insoluble zinc-phosphate compounds [236], [237]. A study by Mohammed *et al*. confirmed that Zn^{2+} ions were effective at reducing enamel demineralization by acting on enamel surfaces to form zinc phosphate phases [26]. Additionally, zinc retards the ability of fluoride to arrest lesions, allowing more complete remineralization of the lesion at greater depths. Due to the

highly mineralized surface zone, which is characteristic of lesion arrests, diffusion deeper into the enamel and the hypomineralized lesions is reduced which ultimately hinders remineralization efforts [179]. Zinc maintains surface porosity through its ability to inhibit crystal growth, which allows ions such as calcium and fluoride to penetrate deeper into the enamel and repair lesions more effectively. However, a three-year study by Stephen *et al.* found that there was no noticeable difference between groups of children using zinc citrate and groups using zinc-free toothpastes in caries incidence [29]. 3000 12-year old children were evaluated using six custom toothpastes with three different concentrations of sodium monofluorophosphate, 1000 ppm 1500 ppm, and 2500 ppm while 0.5% zinc citrate was either included or not included in each of the toothpastes. The dentifrices also contained 50% alumina trihydrate abrasive, surfactant, flavouring and minor excipients. While differing concentrations of sodium monofluorophosphate provided different DMFS index scores, there were no significant differences between the dentifrices including and excluding zinc citrate. It was concluded that the insignificance of zinc citrate on dental caries was independent of the fluoride level. Turning then to the antiglycolytic activity of zinc, *in vivo* acid production by plaque can be reduced up to four hours after a minute long rinse of 10 mL of 20 mM ZnCl_2 [238]. While zinc may not reduce *s. sobrinus* counts, Giertsen still found a 30% reduction in oral flora [236]. It is suspected that zinc may either suppress other bacteria present in the oral environment, or it may interfere with the acid-production of bacteria, or perhaps a combination of these effects. Indeed, studies by Gallagher and Cutress suggested that *s. mutans* is sensitive to zinc [239]. These results are further backed by a study by He *et al.* in which different bacterial strains such as *streptococcus mutans*, *streptococcus salivarius*, *streptococcus sobrinus*, *actinomyces naeslundii* and *lactobacillus casei* were tested singly and in mixed cultures to determine the glycolysis-inhibiting effect of zinc

[240]. *S. mutans* was the most sensitive to zinc, followed by *s. salivarius* and *s. sobrinus*. Mixed cultures demonstrated similar effects to zinc as the *streptococci* tested but this may be due to the larger share of *streptococci* bacteria in the mixtures. Therefore, it is currently suspected that the zinc ion helps remineralization chiefly through its antibacterial ability and synergistic effects with other ions rather than its ability to remineralize and diffuse through enamel, although no clear link between antibacterial effects and cariostatic effects has been determined [234]. Greater mechanistic studies are required to better understand zinc-enamel interactions and the efficacy of zinc regarding its ability to fight dental caries.

Zinc has shown great oral substantivity. Typically, 15-40% of zinc doses are retained in the mouth [234] while up to one third of the dose from toothpaste can be retained for at least two hours [241]. Loosely bound zinc is removed rapidly while more firmly bound zinc clears at a slower rate [24]. Repeated applications have demonstrated a kind of build-up effect [242], [243].

1.4.5 Rapidly Dissolving Bioglass

Recent work by Hill and Wadke has resulted in a bioglass with an open network structure[244].

An open network structure enables alkali and alkali-earth cations (Na^+ , K^+ , Ca^{2+}) to be accommodated as network modifiers, disrupting the continuity of the network of Si-O-Si bonds, leading to the formation of non-bridging oxygen groups (Si-O-NBO) [245]. This allows the glass to dissolve quickly in the presence of saliva. Ions such as calcium and phosphate are released from the dissolution, which is pH dependent, and form an apatite-layer on the enamel surface[246]. Furthermore, the glass particles themselves can block exposed tubules, relieving patients of tooth pain [161]. Tooth pain and sensitivity is caused through stimulation in dentinal tubules. The stimulation may be due to hot or cold fluids, or even mechanical stimulation. These tubes are typically covered but, as the gums recede or tooth decay breaks down the enamel layer,

the tubes become exposed. Mechanical agitation through activities such as brushing one's teeth can also expose them [247]. However, there are some products which can physically block or occlude the tubules due to their structure and particle sizes such as NovaMin [28]. There is increasing evidence that bioglasses dissolve more rapidly during acidic conditions, such as those related to dental caries [17]. This makes them especially desirable as they can act as a 'smart' material which responds to caries incidences. In developing a rapidly dissolving bioglass treatment, Gillam proposed a list of properties an ideal material would possess. Even though a material meeting these criteria may not necessarily be suited for all purposes, the properties still serve as a good general design criteria. Note these properties are primarily tailored towards treating dentin sensitivity but can be just as effective at treating caries with minor adjustments.

Table 13: Gillam's recommendations for an ideal bioglass material to treat sensitivity and caries. [17]

Dissolve time	Rapid dissolution into apatite in less than six hours.
Apatite Formation	Forms fluoroapatite (FA) instead of hydroxycarboapatite (HCA).
Particle Size	A binary distribution of small particles for occlusion of tubules (<3 um) and large particles for longer-term dissolution
Ion Composition	Strontium and Fluoride are recommended for their caries inhibition effects as well as their remineralization capabilities. Potassium can also numb nerves to relieve pain from sensitivity.
Acid Neutralization	Be able to keep pH from rising above 8.0
Mechanical Properties	Should not be significantly harder than enamel at 3.5 GPa.
Cost	Should be inexpensive. Strontium compounds can be prohibitively expensive.

NovaMin is the commercial iteration of 45S5 bioglass that is designed to treat dentinal hypersensitivity [11], [158]. Table 14 lists some commonly available bioglass products and their

applications. NovaMin, a calcium sodium phosphosilicate bioactive glass, serves as the current solution in rapidly dissolving bioglasses but has several drawbacks[17].

- It is believed to form hydroxycarboxylic apatite, which is more soluble than hydroxyl apatite. It is more advantageous to form fluorapatite, a more durable mineral less likely to be susceptible to acid attacks.
- The 45S5 glass it employs has a higher hardness value than enamel, which can cause additional abrasion and enamel loss. A glass with a hardness closer to enamel would be ideal as it would minimize the enamel loss from glass-enamel abrasion. Changing the particle shape to be less sharp and angular and instead, be more rounded can also reduce the abrasive effect of the glass on enamel. This can be achieved through grinding. Ball milling tends to produce more rounded particles while vibratory milling yields more angular particles [17]. Additionally, the hardness of 45S5 is too high for softer exposed dentin surfaces and thinner enamel surfaces. Ideally, the bioglasses would have a hardness no greater than 3.7 GPa.
- NovaMin raises the pH in the environment upon dissolution by releasing alkali components such as Ca^{2+} and PO_4^{3-} ions. While the pH levels are deemed safe, it is still far more desirable to not cause pH spikes since local pH levels can exceed the $\text{pH} < 8.0$ due to concentration of dissolution products, even when global pH levels are considered acceptable.

NovaMin also lacks a biocidal or anti-caries agent, as well as a nerve numbing agent to couple with the occlusion in order to minimize patient discomfort. Apatite formation can take up to weeks with NovaMin while relief from pain may be needed more urgently. The inclusion of potassium instead of sodium can help offset the pain as it will not change key properties of the

bioglass but its dissolution can aid in blocking nerves. The replacement of sodium with potassium also has the additional advantage of reducing glass hardness slightly.

Table 14: Some commercially available bioglasses based on 45S5 with common applications [158]

Product Name (Manufacturer)	Applications
Perioglass (NovaBone)	<ul style="list-style-type: none"> • Restore bone loss from periodontal disease in infrabony defects • Alveolar ridge augmentation • Tooth extraction sites
NovaBone (NovaBone)	<ul style="list-style-type: none"> • Non-load bearing site orthopaedic bone grafting
NovaMin (GlaxoSmithKline)	<ul style="list-style-type: none"> • Dentinal hypersensitivity • Cariostatic agent

NovaMin was not specifically formulated for dental applications and so many of these drawbacks were simply not in the scope of design. This increases the need for a specifically tailored solution designed to deal with caries and hypersensitivity effectively and promptly [169]. Despite that, NovaMin has demonstrated limited effectiveness as a cariostatic agent; NovaMin-fluoride treatments were found to have the greatest remineralization effect on white-spot lesions in bovine enamel. Casein phosphopeptide-amorphous calcium phosphate, or Recaldent as its commercially known, combined with fluoride was found to be the least effective, as can be seen in

Table 15.

Table 15: Results of an in vitro enamel white-spot lesion microhardness study on bovine tooth crowns. [27]

Tested Specimen	Knoop Microhardness (gf/mm^2)
Prepared enamel	119.36 \pm 6.78
Artificial lesion	5.17 \pm 0.35
5000ppm F	14.40 \pm 1.30
Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) with 900ppm F	6.53 \pm 0.70
NovaMin with 5000ppm F	33.52 \pm 4.96

NovaMin remineralizes enamel through ion exchange with bone which results in the increase of hydroxyapatite phases around NovaMin particles [248]. Particle size influences the activity of bioglasses in oral applications. A study by Vollenweider *et al.* [249] found that nanoparticle bioglass treatments were more effective than conventional NovaMin treatments. After 10 minutes in solution, nanoparticle treatments released up to 20 times more calcium and silicon than NovaMin. Smaller particle sizes correlate to greater dissolution rates [250]. However, promoting remineralization is not enough; Vollenweider *et al.* (2007) also found that there was no increase in flexural strength or Young's Modulus after 30 days of remineralization [93]. This suggested that if the apatite does not form a composite with the collagen, a mechanically unstable mineral will form. Furthermore, 45S5 has been found to have significant antibacterial

effects in both supra and subgingival bacteria. A study by Allan *et al.* (2001) found that particulate 45S5 bioglass was effective against *Streptococcus mutans*, *Streptococcus sanguis*, and *Actinomyces viscosus*, with the greatest effect being noticed after three hours, regardless of the suspending solution [251]. The mean bacteria killing percentage was found to be 93% in nutrient broth and 85% in artificial saliva after one hour while the mean killing percentages were 99% in nutrient broth and 98% in artificial saliva after three hours. In subgingival bacterial species, bioglass particulate was found to kill 100% of *Prev. intermedia* in basal medium.

Table 16: Percentage of bacteria killed in nutrient broth and artificial saliva by 45S5. [251]

Bacteria and Medium	% supra-gingival bacteria killed		% sub-gingival bacteria killed after 1 hour
	After 1hour	After 3 hours	
<i>S. sanguis</i> + nutrient broth	98.0	99.5	n/a
<i>S. sanguis</i> + artificial saliva	65.8	94.4	n/a
<i>S. mutans</i> + nutrient broth	83.1	97.3	n/a
<i>S. mutans</i> + artificial saliva	94.4	98.4	n/a
<i>A.viscosus</i> + nutrient broth	97.3	99.5	n/a
<i>A.viscosus</i> + artificial saliva	96.2	99.6	n/a
<i>P. gingivalis</i> + basal medium	n/a	n/a	91.2
<i>F. nucleatum</i> + basal medium	n/a	n/a	95.0
<i>Prev. intermedia</i> + basal medium	n/a	n/a	100
<i>A.actintomycetemcomitans</i> + basal medium	n/a	n/a	98.6

Another study by Liu *et al.* (2014) created a novel calcium phosphate glass (CPG) with an intended Ca/P molar ratio of 0.6 [216]. The analyzed composition of CPG as determined by X Ray Fluorescence (XRF) is described in Table 17.

Table 17: Composition (wt%) of Liu *et al.* CPG doped with Zn and F as determined by XRF

Component (wt%)	Calcium Phosphate Glass (CPG)	CPG + Zn	CPG + F	CPG + Zn + F
CaO	36.4	36.0	37.7	36.5
MgO	1.2	1.1	1.3	1.1
Na ₂ O	-	-	1.3	1.0
P ₂ O ₅	62.3	61.7	59.7	60.2
ZnO	-	1.2	-	1.2
NaF	-	-	0.5	0.5
Cacluated Ca/P ratio	0.58	0.58	0.63	0.61

Antibacterial studies on this bioglass found it was effective against *S. mutans* (Figure 23). The greatest effect on viability was noticed at 0.01g of CPG doped with zinc after two hours. After 4 hours, both CPG with zinc and CPG with fluoride demonstrated complete biocidal activity for the length of the study. The increased concentration of the 0.05g study showed maximum biocidal activity at two hours instead of four, with the base CPG completely inhibiting bacterial growth at four hours. The effects of CPG with both zinc and fluoride were unaffected by the change in concentration.

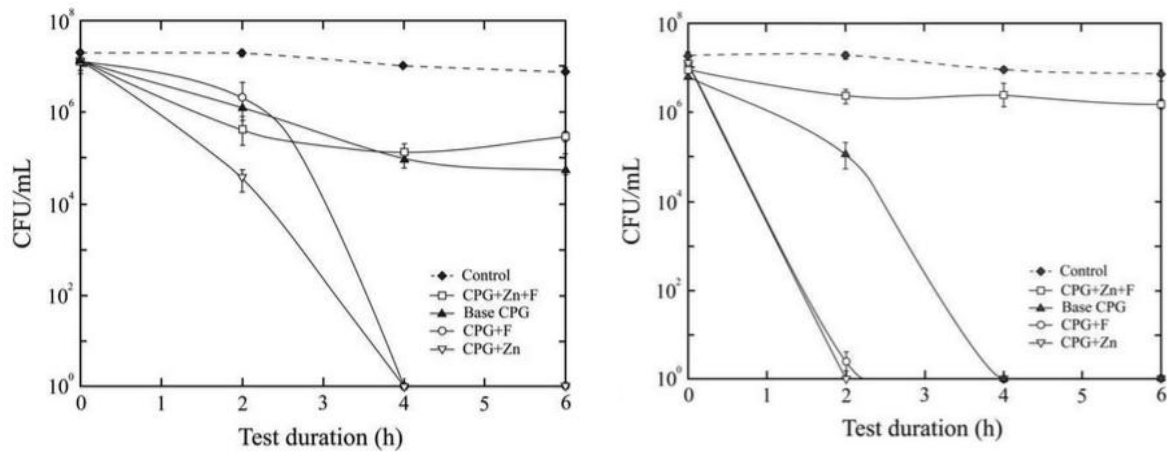


Figure 23: a) Antibacterial effect of 0.01g of calcium phosphate glass on *S. mutans*. b) Antibacterial effect of 0.05g of calcium phosphate glass on *S. mutans* [216]

1.4.6 Restorative Dentifrices

Fluorapatite is a more resistant mineral to acid attack than HA, which in turn is more resistant than carbonated apatite [181]. Gjorgievska *et al.* studied the remineralizing effects of several toothpastes with different additives in order to compare their effects [181]. The toothpastes and their respective additives are listed below in Table 18.

Table 18: Commercial toothpastes with additives studied by Gjorgievska *et al.* (2013). [181]

Toothpaste (Manufacturer)	Additive
Mirasensitive HA+ (Miradent, Hager & Werken GmbH & Co, KG, Germany)	30% HA, xylitol, 1450 ppm fluoride
Mirawhite tc (Miradent, Hager and Werken GmbH & Co, KG, Germany)	5.5% 45S5 (<90µm particle size, calcium-sodium-phospho-silicate glass)
Sensodyne rapid relief (GlaxoSmithKline, UK)	8% strontium acetate, 1040 ppm NaF

The study found that dentifrices containing HA and bioglass were the most effective at enamel remineralization on human permanent molars. Their use resulted in the formation of protective deposits of bioactive glass attached to the enamel surface. The use of the HA containing

toothpaste, Mirasensitive HAp+ (Miradent, Hager & Werken GmbH & Co, KG, Germany), resulted in an increase in calcium and phosphorous content in the enamel layer while the bioactive glass containing paste resulted in an increased concentration of fluorine, magnesium, aluminium, silica, phosphorous, chlorine, calcium, copper and zinc in the enamel layer. The strontium acetate toothpaste did not result in any significant remineralization [181]. SEM micrographs of the remineralization from the dentifrices can be seen in Figure 24 below.

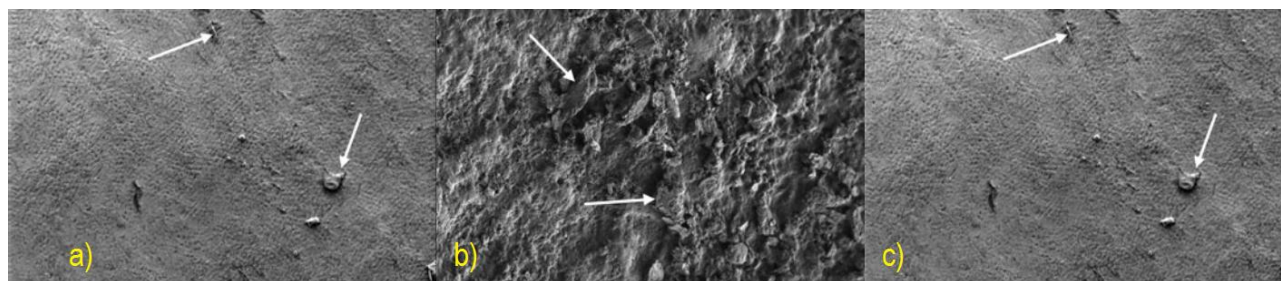


Figure 24: SEM micrographs of mineralization effects of three different toothpastes. Arrows point to some of the deposits. (a) Hydroxyapatite containing toothpaste left deposits on enamel surface which fill irregularities, leaving porous enamel. (b) Bioactive glass containing toothpaste has created significant remineralization across the surface, sealing irregularities and leaving residual glass particles firmly attached to enamel surface. (c) Strontium-acetate and fluoride containing toothpaste has not created any significant remineralization and only few small deposits.

A commercial toothpaste and oral rinse containing F and HA were also investigated by Hill [252]. Ultradex Recalcifying and Whitening is a commercial product which aims to combine the effects of fluorine remineralization with the calcium and phosphate present in order to repair teeth. The toothpastes contained 1000 ppm fluoride and nano-hydroxyapatite (7.5%). Using F magic-angle spinning nuclear magnetic resonance (MAS-NMR) which uses the ^{19}F isotope to identify fluorine containing compounds with greater accuracy than diffraction based tests when evaluating disordered systems [253], enamel slices were analyzed to determine the weight loss after demineralisation treatments. The toothpaste containing only HA corresponded to the largest enamel loss at just under 2% while the toothpaste with only fluorine corresponded to an enamel loss of about 0.5%. The oral rinse containing both HA (5% wt) and fluorine (600 ppm) performed between the two toothpastes at around 1.5%. The toothpaste with both HA and fluoride

corresponded to the smallest enamel loss (Figure 25). However, smaller concentrations of fluorine are recommended as they form the more valuable fluorapatite instead of the fluorite (CaF_2) that larger volumes tend to precipitate [252].

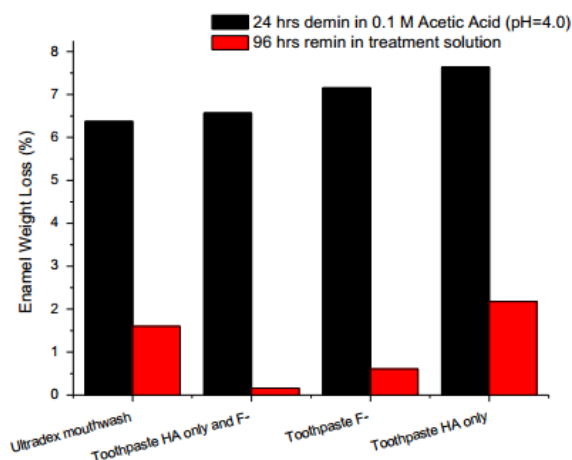


Figure 25: Enamel weight loss by different toothpaste and oral rinse treatments. Measurement error is estimated at 0.14%. [252]

Similar results were also found in a study by Porcelli *et al.* (2015) in which an ordinary fluoridated commercial toothpaste was found to be the most effective at remineralizing enamel erosive lesions, as indicated by their 9.91% mean Knoop hardness change [254]. The nano-HA containing dentifrice performed at 7.96% mean Knoop hardness change and was the second most effective tested treatment at remineralizing enamel. Interestingly, the high fluoride content dentifrice exhibited limited effectiveness, netting only 2.12% mean Knoop hardness change. The study determined that the nano-HA dentifrice and the ordinary fluoridated toothpaste were both able to arrest lesion growth and also remineralize teeth and decrease the erosions already present. This is in contrast to the high fluoride content toothpaste and the calcium carbonate containing dentifrices which only delayed the new erosive lesions formation. The results of the study are summarized in Figure 26.

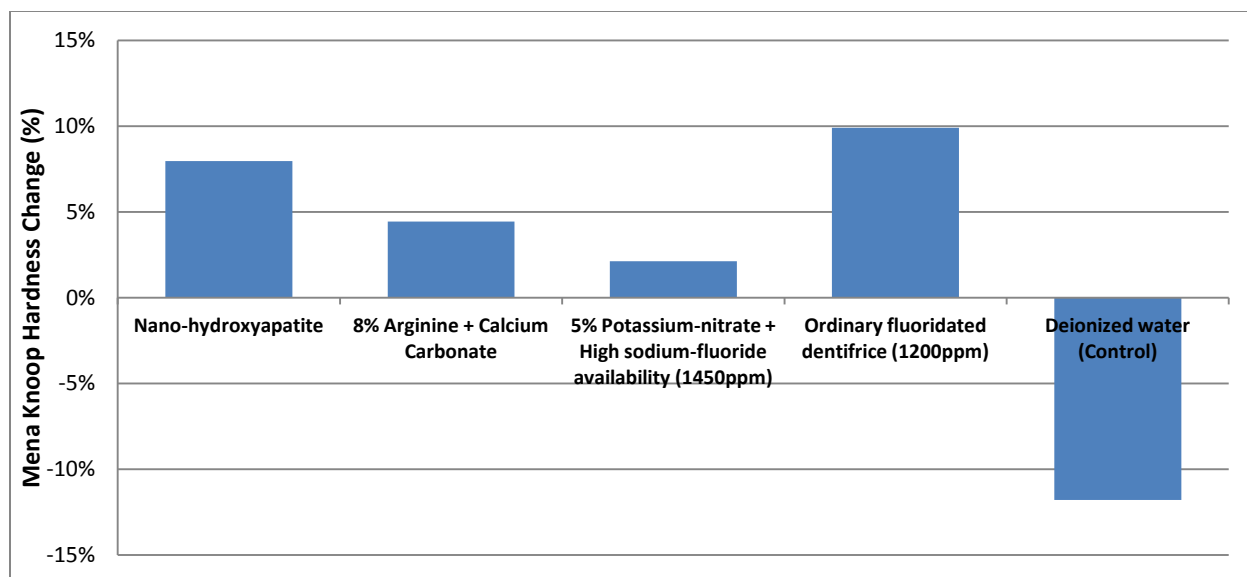


Figure 26: Mean Knoop Hardness change as a measure of mineralization for four different dentifrices. [254]

Danelon *et al.* (2015) studied the effects of trimetaphosphate (TMP) additive to fluoridated toothpastes and their efficacy against dental caries *in situ* [255]. A toothpaste containing 1100 ppm F, 1100 ppm F with 3% TMP and 1100 ppm F with 3% nano-sized TMP were tested against a control toothpaste with no F or TMP. The addition of nano-sized TMP particles were found to be the most effective, increasing mineral hardness by 20% compared to the 1100 ppm F and demonstrated 66% more fluorine uptake than the non-nano sized TMP. It is believed that the nano-sized particles yield a larger surface area to volume ratio which makes them more reactive.

1.5 Summary

Caries is the most common preventable disease in the world today [1]. It is characterized by cariogenic bacteria such as *s. mutans* and *lactobacillus*, which exist in plaque to produce acids which demineralize enamel and dentin by leeching out calcium and phosphorous from the HA that they are made of. If the disease reaches an advanced stage, it will cause lesions and cavities to form in the teeth, allowing the bacteria to infect the pulp. This can lead to cell death and further complications from infection. There are a variety of restorations which can fill these

cavities, ranging from amalgams to composites to cements. New restorations are being developed which involve the use of bioactive glasses. These materials can be used in conjunction with cements to give a therapeutic benefit to the filling such as antibacterial effects, remineralization effects and even relief from hypersensitivity. However, most of these restorations are solutions that are used after the disease has reached an advanced stage. There exists a need for better preventative dental treatments. Most dental procedures deal with repairing teeth after damage or decay; usually by filling in lesions or creating restorations. Many of these procedures have their own limitations, such as strength, toxicity, durability and aesthetics. New glasses are being developed which can be used proactively, preventing cariogenic bacteria from forming biofilms by killing them, as well as promoting remineralization of dental tissues. These glasses can be embedded in dentifrices which is used as a delivery vehicle, depositing the glass in the mouth until an acidic challenge dissolves them and releases their therapeutic ions.

1.6 Scientific Rationale

This research aims to create a novel, rapidly dissolving bioglass treatment which can be used regularly to minimize caries risks. By embedding it in a dentifrice such as toothpaste, the dissolution of the glasses will release therapeutic ions to teeth.

The glasses will be fired through the melt-quench method and tested to ensure they can provide the necessary effects a daily preventative dental treatment will need. Calcium and phosphorous are included in order to increase the remineralization effects, as both are key parts of HA. Silver is included as an antibacterial agent as its antibacterial properties have been well understood for centuries. By investigating a series of glasses with variable silver content, their effects on enamel remineralization and biofilm formation can be determined.

2. Materials and Methods

2.1 Glass Synthesis

2.1.1 Formulations

Three SiO_2 -CaO- P_2O_5 - Na_2O_5 - Ag_2O glasses were synthesized in a series with increasing silver content at the expense of silica. Appropriate amounts of analytical grade reagents were measured out and mixed together thoroughly. The mixture was then fired using the melt-quench method in which the mixture was placed in a platinum crucible. The crucible was then placed in a furnace and fired at 1600°C for one hour. After the allotted time, the melt was shock quenched in cool water. The resulting glass frit was retrieved and dried for 24 hours in an incubator at 37°C . The required reagents are listed in Table 19. The quantity of each reagent was selected through trial and error, as concentrations involving greater amounts of silver or phosphorous would not form glass or would not incorporate silver in to the network structure.

Table 19: Silica glass series composition (mol%)

Glass	SiO_2	CaO	P_2O_5	Na_2O	Ag_2O
Si-Control	70	12	3	15	0
Si-02	69.8	12	3	15	0.2
Si-05	69.5	12	3	15	0.5

2.1.2 Network Connectivity

Network connectivity can be calculated using the equation below. The network formers are assumed to be SiO_2 while the network modifiers are assumed to be CaO, Na_2O and P_2O_5 . Ag_2O generally behaves as a network modifier but can change its role to that of a network former [256]. This usually occurs with high concentrations, or if metallic silver particles form during glass synthesis. Both cases of silver behaving as a modifier or as a former will be examined.

$$\text{Network Connectivity} = \frac{\text{Bridging Oxygens} - \text{Nonbridging oxygens}}{\text{Total bridging species}} \quad (1)$$

2.2 Glass Characterization

2.2.1 Particle Size Analysis (PSA)

The dried glass was placed inside a 50 mL grinding jar for use in the Retsch PM100 Planetary Ball Mill (Retsch GmbH, Haan, Germany). 25mL of distilled water was added to the grinding jar along with eight 10 mmØ grinding balls. The remainder of the volume was filled with glass. The jar was placed inside of the mill and allowed to grind at 550 RPM for 15 minutes.

The solution was then removed and placed in a Buchner funnel with a #1 size 9 cmØ filter paper and a vacuum pressure of about 300-350 mmHg was maintained overnight until the powder was dry. The powder was then scraped off the filter paper and sieved through a 20 µm sieve using a sieving brush.

A Microtrac S3500 Particle Size Analyzer (Microtrac Inc., USA) was used in order to collect particle size information about the glass powders.

2.2.2 X-Ray Diffraction (XRD)

Ground glass samples were placed on a glass slide and inserted into an X'Pert PRO PANalytical XRD machine (PANalytic Inc., St Laurent, QC, Canada). Samples were adhered to glass slides and placed inside the machine. The scan was run from a start position of 10° to 80° 2θ with a step size of 0.0500° and a step time of 1.0000 s. The generator was set to 40 mA, 45 kV.

2.2.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed on a PerkinElmer Spectrum One IR (PerkinElmer, Waltham, MA, USA). Ground glass ($\leq 20\mu\text{m}$) was placed inside the machine measuring from 450 cm^{-1} to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} .

2.2.4 Differential Scanning Calorimetry (DSC)

DSC was performed on a NETZSCH STA 449F3 Jupiter (NETZSCH-Geratebau GmbH, Germany) using sapphire crucibles. The analysis was performed from 20°C to 1000°C with a step size of $20^{\circ}\text{C}/\text{min}$.

2.2.5 Scanning Electron Microscope-Energy Dispersive Xray Spectroscopy (SEM-EDX)

Ground glass samples were placed on a stage using two sided tape to hold the powder while excess powder was shaken off. The stage was then placed inside of a JEOL JSM-6380LV scanning electron microscope (JEOL Ltd., Tokyo, Japan) and examined using a beam energy of 20 kV. EDX spectra were also collected in order to determine the elements present in the glass. Three different sites were examined and averaged in order to limit the impact of localized testing. Multiple representative particles were measured in order to collect particle size information at different sites in a sample.

2.3 Solubility Studies

A buffer solution was prepared by mixing 15.09 g of tris (hydroxymethyl) aminomethane in approximately 800 mL of deionized water and adding 44.2 mL of 1M hydrochloric acid. The volume was then filled to 2L using deionized water and maintaining a pH of 7.30 by adding hydrochloric acid and testing with a pH meter. The solution was then left at 37°C overnight.

75 mg of each glass powder was added along with 50 mL of tris solution in Nalgene plastic bottles. The bottles were then placed in an incubator at 37°C for 6, 12 and 24 hours. The bottles were removed and the solutions were run through a filter paper to remove undissolved glass. The fluid meanwhile was collected and tested for pH and ion release.

The collected fluid from the tris buffer solubility test was run through an AAS machine, PinAAcle 500 Flame Atomic Absorption Spectrometer (PerkinElmer, Waltham, MA, USA). Calibration standard solutions were prepared for each ion by mixing their corresponding AAS standard in deionized water. 1000ppm AAS standards were diluted to 0.1ppm, 0.5ppm, 1ppm, 5ppm, 10ppm and 20ppm. Deionized water was also kept for testing as a blank. Each sample was tested three times.

2.4 Antibacterial Studies

Oral cavity bacteria were collected by swabbing the teeth, gums and tongues of three volunteers at Ryerson University (Toronto, ON, Canada). The swabs were then placed in nutrient broth and allowed to grow for 3 days, after which they were inoculated onto strep agar plates, and allowed to grow for another 3 days. Four colonies of interest, such as those exhibiting satellitism or micro colonies were then extracted and suspended in broth again for three days. These isolates were then streaked across agar plates with glass powder. The isolate streaked agar plates were divided into thirds and 0.5g of each glass composition measured out in Eppendorf tubes were placed in each third. After six hours, the inhibition zone was measured for each composition by comparing the dark, inoculated spaces against the lighter, inhibited spaces and measuring using a caliper. The difference in the diameters of the inhibited zone and the glass deposit was measured.

2.5 Toothpaste Remineralization Studies

Fifteen lamb molars were obtained from a local butcher in Toronto and their buccal surfaces were ground with 600 grit sandpaper on a water-cooled disc grinder. They were then mounted three per puck in cold-curing resin, with the buccal surfaces facing upward. The resin was then grinded down with 400, 600 and 1200 grit sandpaper on a water-cooled bench disc grinder (Buehler Ltd., IL, USA). The teeth were tested for their Knoop micro hardness and SEM images were taken of each. All five pucks were then placed in a container of 1.0M HCl acid until fully submerged overnight (12h) at 37°C. The pucks were then retested for their micro hardness and SEM images were captured after this period. Three of the pucks were selected for remineralization through brushing with a glass-embedded toothpaste while two pucks were selected for control. One control involved brushing with no glass embedded in the tooth paste while the other involved brushing with deionized water. After remineralization, micro hardness results and SEM images were taken again using a JSM-6380LV SEM (JEOL Ltd., Tokyo, Japan). The pucks are pictured below in Figure 27.



Figure 27: Dental Pucks mounted in resin and with their buccal surfaces exposed. Picture is taken after they were brushed

The degree of remineralization will be quantified by the percentage of surface hardness loss (%SHL), a standard metric commonly used to describe mineral loss during dissolution as a function of microhardness [13], [167], [170]. A lower %SHL is desirable as it indicates less mineral loss. %SHL can be calculated using **Error! Reference source not found.** below.

$$\%SHL = \frac{Baseline\ Hardness - Treated\ Hardness}{Baseline\ Hardness} * 100\% \quad (2)$$

3. Results

3.1 Network Connectivity

Network connectivity of the different glasses were calculated using Equation 1. The network former is SiO_2 contributing two bridging oxygens. The network modifiers are P_2O_5 , CaO , Ag_2O and Na_2O . Ca^{2+} provides two non-bridging oxygens and Na^{1+} provides one non-bridging oxygen per Na^{1+} ion. Recent work by Hill has shown that P_2O_5 functions as an orthophosphate (Q_0) modifier, contributing three non-bridging oxygens per PO_4^{3-} ion [257], [258, p. 5]. Each Ag^+ ion provides a non-bridging oxygen [256], [259]. However, since Ag^+ ions can function as network formers under certain circumstances, network connectivity has been calculated for the case of Ag^+ as a network modifier and as a network former.

Table 20: Network connectivity for each composition assuming silver is either a network modifier (NM) or network former (NF).

Glass	SiO_2	CaO	P_2O_5	Na_2O	Ag_2O	Network Connectivity	
						NM	NF
Si-Control	70	12	3	15	0	1.314	1.314
Si-02	69.8	12	3	15	0.2	1.309	1.315
Si-05	69.5	12	3	15	0.5	1.302	1.317

3.2 Glass Characterization

3.2.1 Particle Size Analysis (PSA)

This is close to the $3\mu\text{m}$ particle size prescribed by Mneimne *et al.* so that the glass may enter the dentinal tubules [261]. Precipitation of apatite inside the tubules would occlude them and decrease sensitivity.

Table 21 contains the PSA results. Particle sizes were all required to be comparable to each other and below $20\mu\text{m}$ as smaller particle sizes promote faster dissolution due to their greater specific

surface area [249]. This allows for the particles to degrade faster and thereby release therapeutic ions more quickly [260]. The average particle size was found to be approximately 4-5 μ m. This is close to the 3 μ m particle size prescribed by Mneimne *et al.* so that the glass may enter the dentinal tubules [261]. Precipitation of apatite inside the tubules would occlude them and decrease sensitivity.

Table 21: PSA Results. All measurements are in micrometers (μ m).

Glass	D10	D50	D90
Si-Control	2.29	4.22	9.31
Si-02	2.29	4.31	10.33
Si-05	2.38	5.11	12.73

3.2.2 X Ray Diffraction (XRD)

The XRD patterns were used to confirm whether the glasses were amorphous. From Figure 28, it was apparent that each of the glasses was amorphous. Despite the noise, each of the figures demonstrate the characteristic amorphous ‘hump’ near 25° 2 θ . The amorphous hump occurs due to the non-periodicity of the crystal structure of amorphous materials. For crystalline materials, sharp Bragg peaks were observed at high intensities where the x-rays encounter and are scattered by the formed lattice planes. However, amorphous materials do not have a formed lattice plane and so they scatter the X-rays in many directions leading to a large distributed bump.

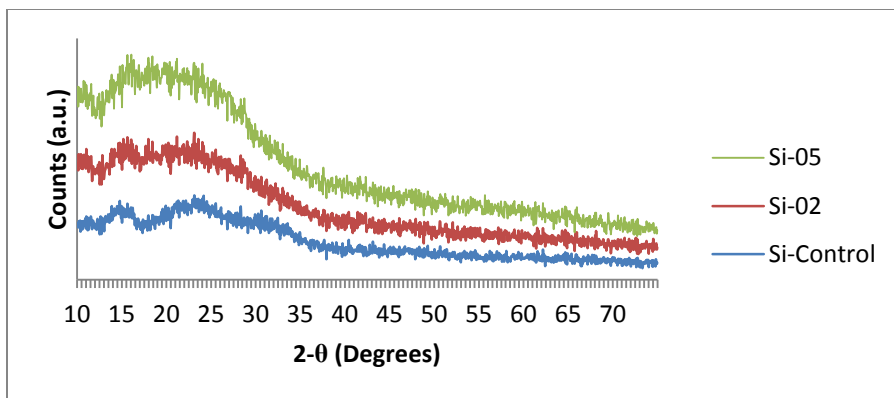


Figure 28: XRD patterns of glass compositions

3.2.3 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 29 shows the FTIR spectra of all three glasses. As can be seen, there is the characteristic Si-O-Si (stretching) bonding band between 1000 and 1300 cm^{-1} [262], with an intense peak centered around 1024 , 1022 , and 1031 cm^{-1} for Si-Control, Si-02 and Si-05, respectively. There is also an Si-O (bending) bonding band between 700 and 800 cm^{-1} [262], with peaks centered around 777 , 786 and 749 cm^{-1} . Bands associated with Si-O-NBO per SiO_4 tetrahedron were found at between 890 and 975 cm^{-1} [262], with peaks centered at 946 , 974 and 955 cm^{-1} . The results are summarized in **Error! Reference source not found.**

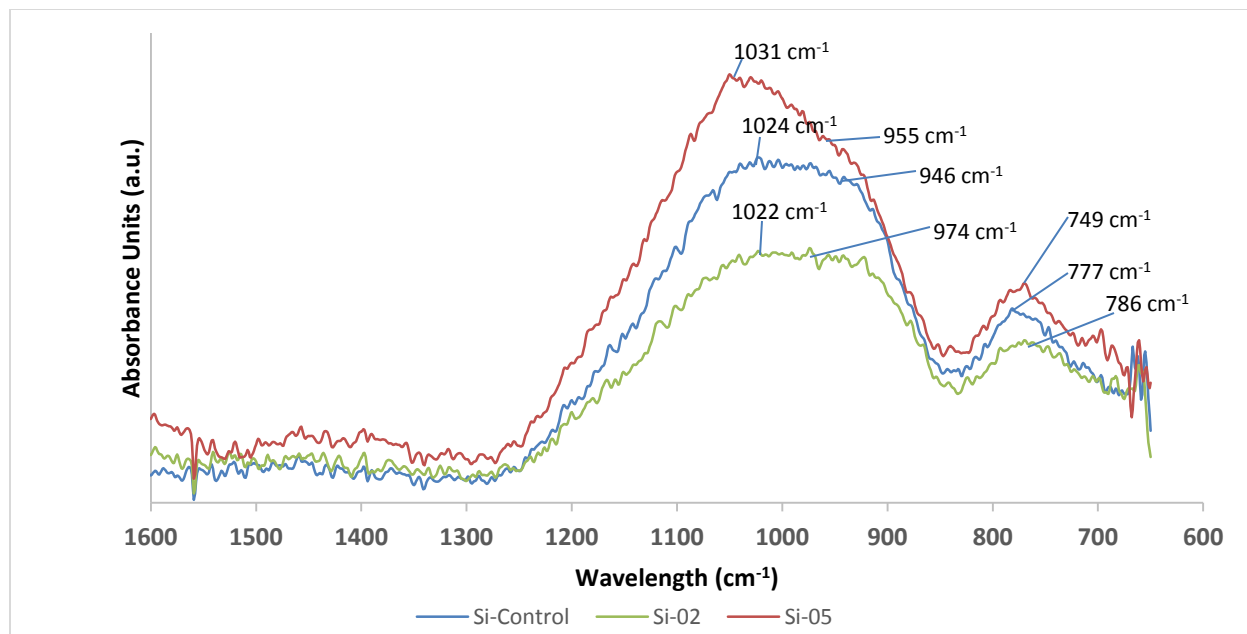


Figure 29: FTIR spectra of three glass compositions in absorbance units

As silver is added at the expense of silica, the intensity of each peak should decrease with increasing silver content as there is less network former available [262]. While this holds true from Si-Control to Si-02, Si-05 exhibits more intense peaks. Furthermore, peaks should be shifted towards lower wavelength numbers, and this trend holds the same with Si-Control to Si-02 but not with Si-05.

It is possible that this occurs due to the complex role of silver addition in the network. In Si-02, silver addition functions like a modifier, creating more NBO sites and introducing discontinuities in the network as the peaks are decreasing in intensity. However, in Si-05 spectra, the addition of silver behaves more like a network former, with peaks increasing in intensity as compared with Si-Control and Si-05. Generally, increasing intensity indicates a greater concentration of these types of bonds [263]. With the increasing intensity of peaks correlated with Si-O-Si bending and Si-O-Si stretching, it can be surmised that the quantity of bridging oxygen groups are increasing, indicating a reduction in NBO groups. Therefore, Si-05 is behaving like a network former.

There is a precedent for the change in role from network modifier to network modifier; Baltzer and Copponnex report that quantities of silver greater than 3 mol% transition their role from modifiers to formers due to the precipitation of spherical metallic silver nanoparticles [256]. Delben *et al.* found that bioactive glass samples containing 3% and 5% silver showed an increase of FTIR bands attributed to BO groups [264]. They found that increasing silver content was linked to higher Si-O-Si bond energies [264]. Furthermore, a study of Fe-doped borosilicate glasses by El-Damrawi *et al.* showed that quantities greater than 6 mol% transitioned the role of Fe from modifier to former due to the reduction of NBO bonding sites [265]. Additional Fe removes NBO from the silicate network and consumes part of the modifier to form FeO₄ groups. However, XRD traces of Si-05 showed amorphicity without the presence of metallic silver phases. The quantity of Ag ions is also significantly lower than the 3 mol% suggested in the transitional behaviour. However, since silver is added at the expense of silica, it is possible that some interactions are causing Ag addition to shield BO sites.

3.2.4 Differential Scanning Calorimetry (DSC)

Table 22 below summarizes the glass transition (T_g) and crystallization temperatures (T_x) of the three glasses. It is apparent that as more silver is added into the network structure, both the T_g and T_x increases.

Table 22: Crystallization and Glass Transition Temperatures of glass compositions

Glass	T_g (°C)	T_x (°C)
Si-Control	609.5	825.6
Si-02	617.4	837.9
Si-05	619.6	848.1

3.2.5 SEM-EDX

SEM photos are exhibited below in Figure 30, with (a) as Si-Control, (b) as Si-02 and (c) as Si-05. The photos confirm that the compositions are mostly homogenous in their particle sizes. However, Si-05 appears to be more granular and has sharper edges in its particles.

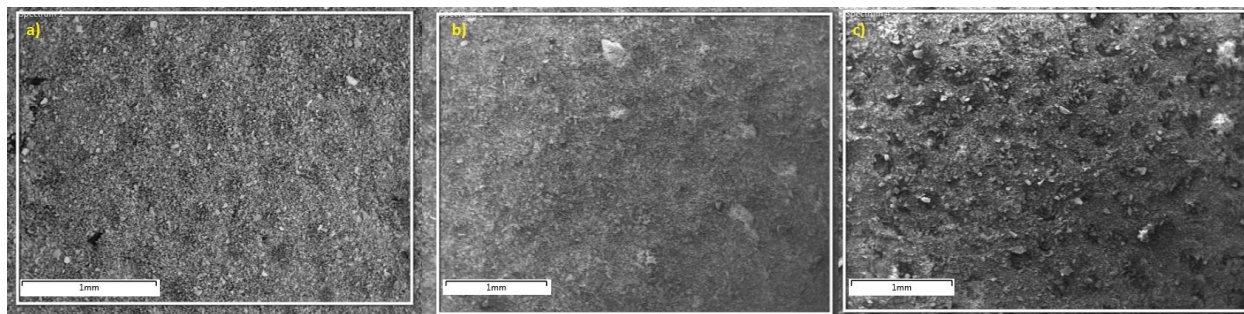


Figure 30: SEM photos of compositions: a) Si-Control b) Si-02 c) Si-05

EDX images were taken at multiple sites to determine the composition of the glasses and minimize the localization effects experienced. As can be seen in

Table 23, the glass compositions were close to the reagent amounts fired in the crucible. This confirms that the glass thoroughly melted during the firing process.

Table 23: Average of wt% in compositions for each composition

Element	Si-Control	Si-02	Si-05
O	50.6	54.8	54.7
Si	29.0	25.9	24.9
Na	10.3	10.3	10.6
Ca	7.60	6.60	6.77
P	2.50	2.03	2.03
Ag	0	0.3	0.97

3.3 Solubility Results

Figure 31 below summarizes the pH values found for each of the tests.

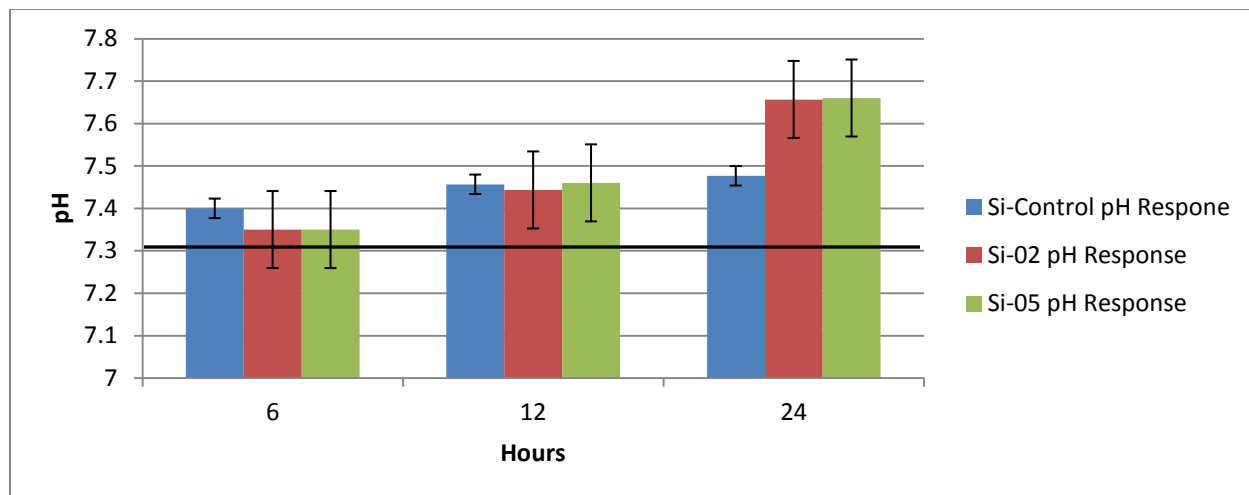


Figure 31: pH response of tris buffer solution upon glass dissolution after six, twelve and twenty-four hours. Black horizontal bar represents the initial pH value of the Tris buffer solution before dissolution.

It is apparent from the figure above that the pH of the tris buffer solution increases over time as the glass degrades. The pH of the tris solution is highest for Si-Control at six hours but does not reach the higher pH values of tris containing Si-02 and Si-05 over the longer time periods of twelve hours and twenty-four hours. It appears that the cations are not as readily exchanged during the early stages of Si-02 and Si-05 dissolution.

Figure 32 (a-d) below summarizes the ion release data.

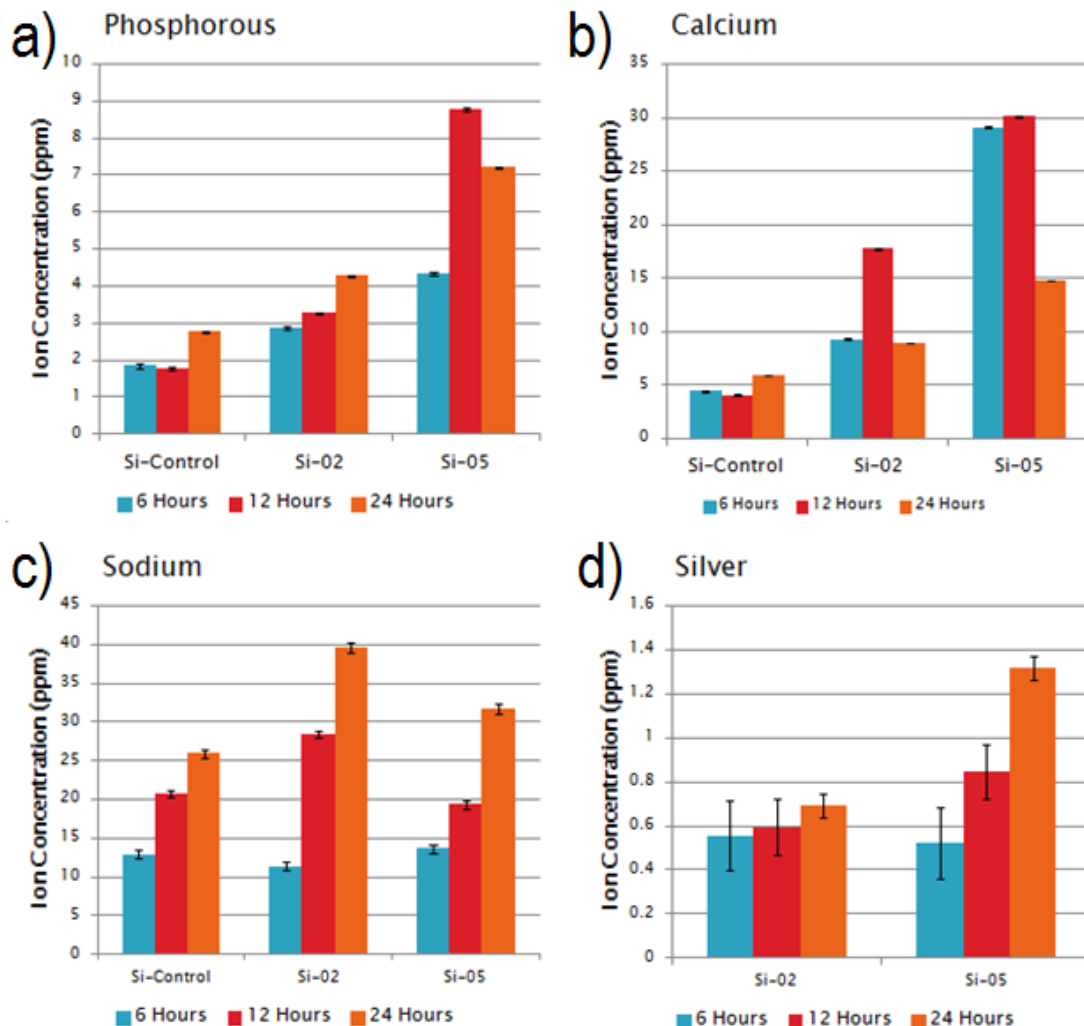


Figure 32 (a-d): Ion release profiles for (a) Phosphorous, (b) Calcium, (c) Sodium and (d) Silver. Si-Control silver release has been omitted from (d) as no silver was present in the composition.

While Si-05 releases more silver than Si-02, Si-02 releases a greater concentration of calcium, sodium and phosphorous. Si-05 releases a smaller concentration of calcium, sodium and phosphorous ions when compared with Si-02 and Si-Control after 24 hours. While all ion amounts increase over time, they do not increase linearly. Si-02, for example, exhibits a marked spike in concentration from 12 hours to 24 hours for its phosphorous release, overtaking the phosphorous ion release from Si-Control and Si-05. Si-02 also experiences an increase at 12 hours in its calcium release, while Si-Control undergoes a similar spike after 24 hours in calcium

release. Sodium ion release exhibits more constant ion release rates for all compositions across all time spans.

To measure the ion release kinetics of an alkali-silicate glass, the heterogenous model was used [121], [266]. To confirm dissolution behaves under the heterogenous model, the total silver ion concentration was graphed against the square root of the time and a linear regression was employed. The correlation coefficient (R^2) was above 0.95 for both Si-02 and Si-05 compositions, confirming the model. The network is predicted to be very complex, formed by a backbone of SiO_4 tetrahedra connected at the oxygen atoms to form a 3D network. However, the presence of alkali species breaks up the network continuity, as the oxygen atoms no longer bridge to the next tetrahedra but instead to an alkali ion. This should increase dissolution rates as these non-bridging oxygen bonds are easier to break than bridging oxygen bonds. However silver ions were added at the expense of silica ions, which should reduce the network connectivity as network formers were replaced with network modifiers. This leaves less silica ions available to form the backbone, as well as further disrupting the network. Ion release rates should be seen to be higher in Si-05 than Si-02, as well as being higher in Si-02 than Si-Control. While this is observed over short time periods, with the exception of silver release, longer dissolution time periods favour Si-02.

3.4 Antibacterial Studies

Error! Reference source not found.Figure 33 describes the inhibition zones. The inhibition zone is useful for measuring how antibacterial these glasses are. As expected, Si-05 consistently exhibited a larger inhibition zone against all tested bacteria and therefore a stronger antibacterial response. Additionally, the inhibition zone increases over time for all bacteria except strain D4, which experiences a dip in the inhibition zone before climbing again. However, one-way

ANOVA analysis showed this difference is not significant. Furthermore, since the control glasses did not experience any inhibition zone at any time period, the effects of pH rise can be eliminated as a potential bactericidal effect. As silver is the only ion species not present in Si-Control, it is the most likely bactericidal agent responsible here. Silver has a known wide-ranging antibacterial effect and the increase of silver release over time is consistent with the growing inhibition zone.

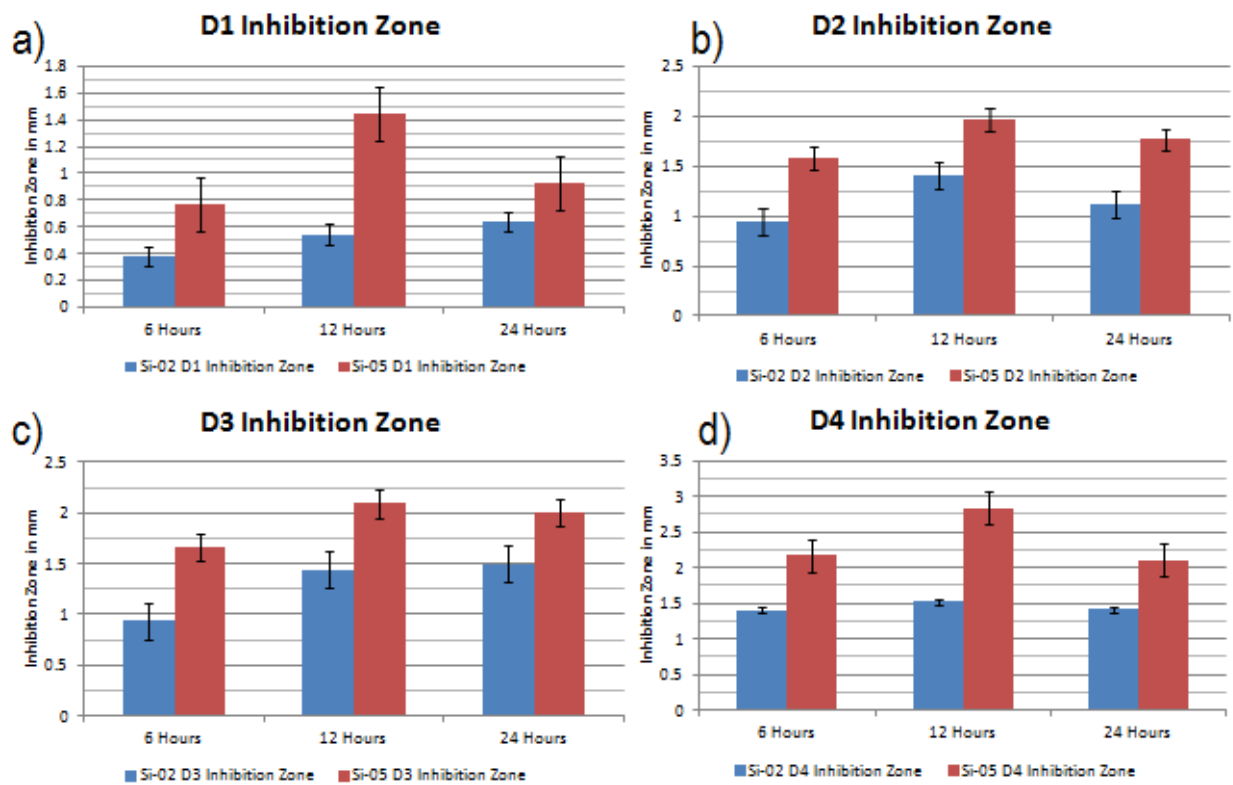


Figure 33: (a) Inhibition Zone against bacterial strain D1 (b) Inhibition Zone against bacterial strain D2 (c) Inhibition Zone against bacterial strain D3 (d) Inhibition Zone against bacterial strain D4. The inhibition zone produced by glass powders against four bacterial isolate strains. Si-Control was excluded as it produced no inhibition zone throughout the time period measured.

3.5 Toothpaste Remineralization Studies

Figure 34 shows the demineralization and remineralization of the dentin molars embedded in resin pucks. The degree of mineralization was quantified through Knoop microhardness and

control measurements were taken after the pucks were prepared and before they were subjected to an acid challenge, as well as before and after their dentifrice remineralization treatments.

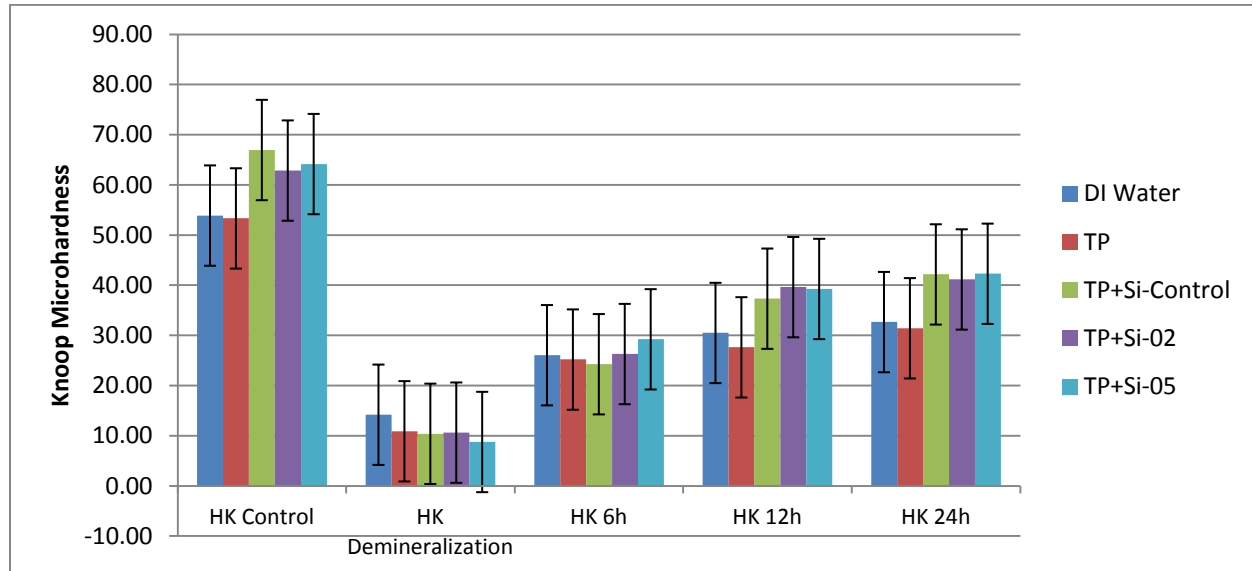


Figure 34: Knoop Microhardness Remineralization and Demineralization on lamb molar dentin samples. All measurements are Knoop Hardness (HK). DI is deionized water, TP is toothpaste.

The percentage of remineralization and demineralization are quantified through percentage hardness loss (%SHL) in Table 24.

Table 24: Percent Surface Hardness Loss (%SHL) for each stage of treatment as compared with baseline Knoop values

Treatment	%Demin	%Remin after 6 hours	%Remin after 12 hours	%Remin after 24 hours
DI Water	74%	52%	43%	39%
TP	80%	53%	48%	41%
TP+Si-Control	84%	64%	44%	37%
TP+Si-02	83%	58%	37%	35%
TP+Si-05	86%	54%	39%	34%

It is apparent through these results that the glass compositions were more effective than the controls, with the highest microhardness after twenty four hours belonging to TP+Si-02 followed by TP+Si-Control, TP, TP+Si-05 and finally DI water. However, when taking into account the initial demineralization effects, TP+Si-Control, TP+Si-02 and TP+Si-05 had similar remineralization percentages, as per Table 24. Additionally, the response was higher than just the

dentifrice treatment alone, indicating that the glass had a positive contribution to the mineralization treatments.

4.0 Discussion

Bioactive glasses cause pH rises upon dissolution in aqueous solutions, such as tris buffer. This facilitates apatite formation but can also cause a pH rise too great for the oral environment; an oral pH greater than 7.5 can irritate the oral mucous membranes [261], [267]. As per Figure 31, the tris buffers containing the Si-02 and Si-05 compositions exhibit a pH of 7.35 at six hours while the Si-Control solution shows a higher pH rise at 7.4. These values were not determined to be significantly different at $p < 0.05$. After twelve hours, the pH responses of the solutions are 7.46, 7.44 and 7.46 for Si-Control, Si-02 and Si-05 respectively indicating that the advantage gained by the control glass' early pH rise is lost over longer time frames. Indeed, by 24 hours, solutions containing Si-02 and Si-05 both show a pH of 7.66 while the solution containing Si-Control shows a pH of 7.48.

This compares favourably with other bioactive glasses in aqueous solution. Brauer *et al.* found the pH response of an SBF solution containing fluoridated bioactive glass can reach 7.6 after 24 hours but to plateau at 7.8 shortly afterwards from day 3 to the end of the experiment [267]. Tris buffer solutions containing Si-02 and Si-05 both reached the same pH level after 24 hours while the solution containing Si-Control did not show a significant pH rise after 12 hours. Time frames greater than 24 hours were not examined as they were not deemed relevant to the application of toothpaste in the oral environment; it is highly unlikely that the ions deposited from the degrading glass powders would remain in the mouth after a days consumption of liquids diluting the contents of the mouth [261].

These glasses were designed with rapid ion release in mind and it was observed that, after twelve hours, the release rate accelerates. As per Figure 32 (d), Si-02 releases silver, on average, 2.5 times faster after 12 hours while Si-05 releases silver on average 1.5 times faster after 12 hours. The incorporation of silver into the network structure changes the more linear response of the control composition into an accelerating one, albeit with a slower initial response time. This is due to the complex nature of the network structure when silver is incorporated, as shown by the apparent change of its role from network modifier in Si-02 to a network former in SI-05 in the FTIR spectra, as can be seen in Figure 29. As silver is added at the expense of silica, a network modifier is added at the expense of a network former, which should increase bioactivity and solubility of the glass as bridging oxygens are being lost and replaced with non-bridging oxygens, which should facilitate a greater release of cations, specifically sodium and calcium ions, which are basic [261], [268], and will produce a greater pH rise. The pH rise should disrupt the dissolution mechanism and promote remineralization. However, the initial lag before the 12-hour acceleration indicates that the cations are not exchanged as readily during the early stages of dissolution. It may be that the complex network structure takes longer to degrade and favors dissolution of its formers more than its modifiers. This trend holds up when compared with the ion release profiles. The larger silver content yields higher initial release volumes but smaller release rates over time, leading to lower cumulative final release volumes. Interestingly, the Si-Control composition generally displays ion release volumes either between the two silver-containing compositions or closer to Si-02 ion profiles, as seen in Figure 32 (a-c). Si-05 releases less silver ions initially but greater quantities of all other ions when compared with Si-02 after six hours, with the exception of phosphorous. However, over time, silver ion release rates increase rapidly with Si-05 while Si-02 releases silver ions at a constant rate. Si-02 also generally

exhibits higher ion release volumes than Si-05 after 24 hours for sodium, calcium and phosphorous. Generally, the more silver ions released, the less of every other ionic species is released. Silica release was not measured but it may be useful in further explaining how the network degradation functioned as silica was the backbone of the network.

Phosphorous ion release behaves differently to the sodium, calcium and silver release as it reaches a peak around 12 hours and then diminishes, as can be seen in Figure 32 (a). The decrease of phosphorous over longer time periods is associated with re-precipitation of Ca-phosphate species, such as apatite and octacalcium phosphate, a sort of precursor mineral for apatite formation [269], [270]. However, the deposits would have to be examined under FTIR and XRD analysis in order to determine if any apatite has in fact precipitated, or if a different mineral has formed. Conventionally, the bioactivity of glasses has been defined as the apatite-forming ability of glasses after immersion in SBF [261], but immersion in tris buffer represents a more conservative test as it does not supply Ca^{2+} or phosphate species. This is a better model of the oral environment as saliva can be diluted when taking in liquids and become depleted of Ca^{2+} and phosphate species [261]. Phosphorous ions are also known to diminish the pH rise from dissolution due to their acidity. This effect can help moderate the pH rise associated with the other cations released from the glass dissolution and prevent pH spikes from local concentrations of cations, as well as promote bioactivity since a pH of approximately 7.3 is considered ideal for apatite precipitation [268].

Cell culture studies were singularly affected by Ag^+ release as all agar discs were kept under similar conditions and Si-Control exhibited no antibacterial response. It is therefore possible to eliminate pH as a bactericidal factor, since all compositions are known to cause similar pH rises, as per Figure 31. Referring to Figure 33 (a-d), across all time frames and all bacterial strains, Si-

05 outperformed Si-02 in its bactericidal efficacy, which further suggests that silver is the lone bactericidal agent. However, at six hours, less silver was reported as having leached out in Si-05 as compared to Si-02, although the difference was not statistically significant at $p < 0.05$. Silver is known to have wide ranging antibacterial properties; it is believed that DNA stops being able to replicate and proteins become inactive after contact with Ag^+ [266]. It has also been suggested silver ions interfere with the metabolism of the bacteria, which ultimately inhibits their growth [22].

Referring to Figure 34, all three glasses significantly remineralized enamel after 24 hours. As Si-02 released the greatest amount of calcium and phosphorous after 12 hours (17.7ppm and 8.79ppm respectively), it should have exhibited the greatest degree of remineralization at that time frame, which is consistent with the results, and indeed does not remineralize much more; from 12 hours to 24 hours, the teeth treated with the toothpaste embedded with Si-02 only remineralize from 37% to 35%. There is an upper limit to the amount of restoration which is possible after demineralization, which is approximately 35% surface hardness loss (%SHL). To compare, a toothpaste containing 1100 ppm F exhibited a maximum of 38% SHL after four 7-day phases of demineralization/remineralization cycles [271]. Percentage hardness losses for enamel treated with over the counter bleaching agents and then stored in artificial saliva range from 12-40% according to Zantner *et al.* (2007), while a study by Chen *et al.* (2008) found an 18-32% SHL for enamel treated with fluoridated bleaching agents followed by buffer saline solution [272], [273]. The upper limit can be explained by calcium and phosphorous uptake into the enamel being limited by the concentration of the ions available. Therefore, there may simply not be enough calcium or phosphorous to repair the lesions and form HA. It is also possible that apatite crystals precipitated over the surface level of the pucks, preventing the penetration of

glass deeper into the lesions and remineralizing the bulk of the material. However, it is also possible that the reason the teeth did not fully remineralize is damage to the underlying dentin and cementum, as well as the resin holding the teeth. Since all compositions had the same amount of calcium and phosphorous, all pucks restored with the glass-loaded dentifrices are expected to reach a similar remineralizing point. While the dentifrice alone was effective at remineralizing the dentin (41%SHL), the glass loading did increase the remineralization slightly, with a percent difference of 15.4% between the average glass remineralization and the toothpaste surface hardness loss. However, the difference was not statistically significant at the $p < 0.05$ level.

5.0 Conclusion

The goal of this project was to develop a silver-doped bioactive glass that could be incorporated into a dentifrice to remineralize dental tissues. Three glass compositions were synthesized and examined to determine effectiveness as an anticaries treatment. The glasses were characterized and tested for their antibacterial properties as well as their enamel remineralization capability.

- The addition of 0.2% and 0.5% silver to a $\text{SiO}_2\text{-CaO-P}_2\text{O}_5\text{-Na}_2\text{O-Ag}_2\text{O}$ glass composition at the expense of silica did not cause the glasses to crystallize when fired through the melt-quench method.
- The glasses caused a pH response in tris buffer solution, raising the pH from 7.3 to approximately 7.6. This is invaluable as remineralization and demineralization are pH dependent processes. An acidic environment will promote demineralization while a more alkaline environment will be more conducive to remineralization. However too great a change in the oral pH can cause irritation.
- Ion release profiles were found to be dependent on time and silver content. The network structure of the glass is complex and glass dissolution follows the heterogenous model, meaning the release of silver occurs in two phases. The first phase involves alkali ions being replaced with hydronium ions which varies with the square root of time while the second phase is the dissolution of the network which varies linearly with time
- The Si-05 glass exhibits a stronger antibacterial response than the Si-02 glass, while Si-Control exhibits no antibacterial effect. This stronger antibacterial response is due to the silver content in each composition; while pH changes are caused by all glasses, Si-Control contains no silver. It can be said then that Ag^+ is the sole bactericidal factor in the glasses.

- All glasses remineralize lamb dental enamel when incorporated into a toothpaste. This remineralization effect is more potent than the toothpaste alone, which is more effective than deionized water. All compositions contain the same amount of calcium and phosphorous. As these are the constituent ingredients in HA, it is believed that Ca^{2+} and P^{3-} cause the remineralization effect.

6.0 Future Work

Further work on these glasses would involve tailoring their ion release profiles to release the majority of their ions during the early stages of dissolution, rather than accelerate after small releases. This is useful for the purposes of maximising the therapeutic effect of the glasses by allowing most of the ions to do their work before they can be leached out of the mouth through daily liquid intake. This can be accomplished by increasing the bioactivity further, by decreasing the network connectivity or by shock quenching faster when firing the glasses. This can also be accomplished by further reducing particle size, or introducing a bimodal distribution of 'coarse' and 'fine' particles. Fine, or smaller particles would degrade faster, providing quick initial relief from dentinal sensitivity while ions released would promote immediate remineralization. The pH response provided would facilitate the coarser, or larger particles to provide a sustained release over time and reduce the daily enamel and dentin demineralization.

Furthermore, human enamel and dentin samples should be tested against these glasses to further confirm the remineralization treatments on human subjects as well as pre-existing teeth restorations, both *in vivo* and *in vitro*. Microfluidic techniques can also be employed to model the effects of the glass on biofilm formation and the ability of the glass to penetrate into cavities and cracks, including microcracks which occur in teeth restorations.

Appendix

List of tested products from Kleverlaan 2005 study, as summarized in Table 6.

Product	Manufacturer
HelioMolar	Ivoclar Vivadent
Filtek A110	3M ESPE
Filtek Z250	3M ESPE
Filtek P60	3M ESPE
Filtek Supreme	3M ESPE
Filtek Z100	3M ESPE
Prodigy Condensable	Kerr
Tetric Cream	Ivoclar Vivadent
Herculite XRV	Kerr
Spectrum TPH	Densply deTrey
Point4	Kerr
Aelite Flo	Bisco
Revolution Formula 2	Kerr
Flow-It	Jeneric/Pentron
UltraSeal XT Plus	Ultradent

Products tested in Table 5, including their classification and manufacturer [91], [92].

Product Brand (Manufacturer)	Material Class	Source
Filtek Z250 (3M ESPE, USA)	Adhesive Composite	Aghazadeh <i>et al.</i> (2007) [92]
Tetric Ceram (Ivoclar Vivadent, Liechtenstein)	Adhesive Composite	
Heliomolar (Ivoclar Vivadent, Liechtenstein)	Adhesive Composite	
Gradia Indirect (GC Gradia, Japan)	Traditional Composite	
Gradia Direct (GC Gradia, Japan)	Traditional Composite	
Geristore (DenMat, USA)	Adhesive Composite	Berry and Powers (1994) [91]
Tenure (DenMat, USA)	Bonding Agent	
Marathon (DenMat, USA)	Traditional Composite	

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