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Date

Stephen M. Gross, Ph.D., Chair

Mark A. Latta, D.M.D., M.S.

Barbara J. O'Kane, Ph.D., M.S.

Thomas P. Berry, D.D.S

Gail M. Jensen, Ph.D., Dean

METHODS FOR ZINC ION RELEASE FROM ORTHODONTIC CEMENT PASTES

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By

GRADY GORES

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A THESIS

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ABSTRACT

The prevention of plaque and calculus build up around orthodontic appliances is a difficult task for many patients. Current literature shows free zinc ions have the ability to hinder the progression of plaque and diminish the subsequent consequences. This study demonstrates the ability of two technologies to release zinc from orthodontic cement pastes. Microencapsulation and bioactive zinc glass were the two technologies studied. The cements were tested in neutral and acidic conditions as well as in the presence of competing ions. Results demonstrated competing ions cause zinc release profiles to fluctuate, likely due to changes in solubility resulting from different complexations. Bioactive zinc glass releases more zinc under acidic conditions, and microencapsulation releases about four times more zinc than bioactive zinc glass when loaded at the same weight percent. This study confirms the ability of microencapsulation and bioactive zinc glass to release zinc when incorporated into an orthodontic cement and demonstrates their potential to fight the accumulation of plaque around orthodontic appliances.

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Introduction

1.1 Plaque and Orthodontic Brackets

Many patients experience difficulty maintaining good oral hygiene while fitted with orthodontic appliances. Poor oral hygiene in combination with the placement of orthodontic brackets is a major contributor to the accumulation of plaque and the successive response of gingival inflammation [10]. Gingivitis and enamel demineralization may arise in response to plaque and calculus build up on orthodontic appliances. Persistence of these consequences has been found to be common [18]. Furthermore, plaque buildup around brackets increases treatment duration by an average of 1.2 months, causing a compounding effect on these negative results [68]. It would seem obvious regimes to control plaque and calculus should be explored in effort to maintain excellent oral and gingival health during orthodontic treatment.

1.2 Plaque and Calculus in the Oral Cavity

Plaque is a biofilm that adheres to the surface of the tooth. It is formed by soft deposits and composed of inorganic and organic materials derived from saliva, gingival crevicular fluid, and bacterial products, that adhere to the surface of the tooth. This biofilm serves as an organic matrix for mineralization of the deposit. Calcification of dental plaque results from mineral ions provided by bathing saliva or crevicular fluids. This calcification is called dental calculus, manifesting in a hard concretion forming on teeth and dental appliances. Dental calculus is composed of calcium phosphate mineral salts deposited in the presence of formerly viable microbes [108].

A layer of non-mineralized plaque covers calculus, making it difficult to separate their individual effect on gingival health. The correlation between calculus and gingivitis

is not as great as that between plaque and gingivitis, but the correlation is present and statistically significant nonetheless. In periodontal disease, the initial damage to the periodontal tissue is due to immunologic and enzymatic effects of the microbes in plaque. Supragingival calculus worsens this condition by promoting new plaque accumulations on its surface [108]. Similarly, plaque accumulation around the gingival margin leads to an inflammatory host response and increased flow of gingival crevicular fluid, which promotes the formation of calculus [84]. It is still not clear however, whether calculus and plaque together evoke a greater reaction than plaque alone, although there is some suggestive evidence for the former. The mineralized deposit does bring plaque closer to the supporting tissues, interfere with local self-cleansing mechanisms, and make plaque removal more difficult for the patient. Although efforts to determine whether the relationship between calculus and gingival health is primary or secondary may be futile, calculus is considered a pathogenic factor in periodontal disease [108]. Therefore, plaque and calculus must be controlled in order to maintain ideal oral health.

1.3 Potential Ways of Controlling Plaque and Calculus

An essential set of enzymes that contribute to the formation of the plaque biofilm are glucosyltransferases, which allow organisms like *S. mutans* to colonize the tooth surface [138]. These glucosyltransferases also play a significant part in the growth of dental plaque [52, 72]. Glucosyltransferases synthesize most of the polysaccharides in plaque, which make up 30-40% (dry wt) of the plaque matrix [21, 61, 77, 107]. Therefore, inhibiting the function of these plaque-building enzymes is of great interest as

a primary means of controlling bacterial colonization and the accumulation of plaque [137].

Periodontal disease is initiated by bacterial plaque and characterized by inflammation, which leads to the breakdown of collagen, loss of periodontal attachment, and bone destruction. It has been demonstrated that degradation of gingival tissue during active periodontitis is at least partly due to matrix metalloproteinases (MMPs). The inflammatory soft tissue breakdown has four recognized pathways: plasminogen-dependent, phagocytic, osteoclastic, and the MMP-dependent pathway [12]. MMPs have also been identified in both pulpal and periapical inflammation [49, 129]. They even more strongly correlate to periodontal diseases, since MMPs are the major players in collagen breakdown during periodontal tissue destruction [12, 74, 82]. Furthermore, MMPs are essential components in the growth and invasion of oral tumors [113], and may be important in the time-dependent loss of composite restoration adhesion [102, 123]. In the oral environment, MMPs are involved in many processes including developmental events involving teeth and salivary glands and in collagen turnover [46, 62], besides playing an important role in pathologic processes such as periodontal tissue destruction, oral lichen planus, dysplasia, squamous cell carcinoma [121], bone and cartilage degradation [47] and root caries [28, 69, 125].

There are several evidences indicating that MMP-2, -9, -13, and -14 play an important role in tissue destruction during periodontal disease. Periodontitis patients have significantly higher levels of MMP-2 and MMP-9 than healthy subjects, and the number of gelatinases decreases after periodontal treatment [82]. The activation of MMP-2 and MMP-9 was also shown to have a crucial role in the destruction of dentin by caries [125].

Additionally, these enzymes can potentiate the degradation of extracellular matrix by activating collagenase-3 (MMP-13) and neutrophil collagenase [92]. Many researchers have demonstrated that zinc can directly affect host response by inhibiting MMP-2 and MMP-9 [12, 19, 82], and other evidence suggests zinc may be capable of inhibiting the entire MMP family [126].

The control of supragingival dental plaque and calculus on the surface of teeth and around dental appliances is critical to gingival health. The mechanical action of tooth brushing can remove plaque most effectively. However, persistently effective brushing is uncommon suggesting a chemotherapeutic tactic might be beneficial [119]. Therefore, one of the most applicable methods may be using dental materials that prevent the accumulation of bacteria.

1.4 Zinc's Effect on the Oral Cavity

Our concern is with the release of zinc from an orthodontic cement. Current knowledge on zinc and its effect on the oral cavity has primarily been in two areas: its effect on bacterial growth and sugar metabolism [53]. However, more recently other functions have been explored, such as its role in calculus control, plaque acid production, and reduction of hydroxyapatite and enamel solubility [57, 81]. Zinc has been used in many dental materials, most often toothpastes and mouth rinses as an agent to control plaque and calculus through modification/inhibition of crystal growth. Further research suggests zinc ions may inhibit the adsorption of bacteria to the tooth surface as well as the growth of existing plaque [108]. The mechanisms remain unclear, but studies suggest

that free zinc ions are responsible [130] and that the inhibition of acid production is correlated with adsorption of zinc on the bacterial cell wall [23, 57].

It has been demonstrated that zinc oxide nanoparticles (ZnO-NPs) blended at 10 w/w% into dental composites display antimicrobial activity and reduce growth of bacterial biofilms by roughly 80% for a single-species model dental biofilm. Antibacterial effectiveness of ZnO-NPs was assessed against *S. sobrinus* ATCC 27352 grown both planktonically and as biofilms on composites [139]. Another investigation demonstrated that a dentifrice containing zinc citrate was able to prevent both the accumulation of plaque and the development of gingivitis [122]. Clinical studies have shown that mouth rinses and dentifrices containing zinc salts can reduce plaque accumulation and calculus formation [2, 55, 120]. This evidence indicates that releasing zinc from an orthodontic cement could be a very effective way of controlling plaque and calculus buildup.

Zinc may have other applications beyond plaque and calculus control, in adhesive dentistry. There is great concern about long-term stability of the adhesive interface, since it has been demonstrated that hydrophilic dentin adhesives deteriorate over time [29]. Exposed collagen matrices from acid-etched dentin, which have incompletely infiltrated collagen fibrils, are also susceptible to degradation [56]. The net effect of the deterioration of these structures is the loss of adhesion. The hydrolytic role of degradation was evident in an in vitro study [16]. Pashley et al. investigated the effect of proteolytic enzymes in the demineralized dentin matrix stored in water, artificial saliva, and oil [102]. They also analyzed whether proteolytic enzyme inhibitors prevented the demineralized collagen matrix from degrading. The results from this study demonstrated

that hydrolytic degradation of collagen fibrils can occur in the absence of bacterial colonization. The authors speculated that MMPs from the mineralized dentin matrix might have been activated during dentin acid etching and were probably responsible for collagen matrix degradation. Therefore, Pashley et al. considered prevention of the degradation of incompletely resin infiltrated collagen fibrils by MMPs in the hybrid layers would be an important procedure. Moreover, Tjaderhane found this function of MMPs to be upregulated in the presence of falling pH [125]. Later on, another study corroborated this approach, showing that self-destruction of collagen matrices occurred rapidly in resin-infiltrated dentin in vivo but it is arrested with the use of chlorhexidine as an MMP inhibitor [58]. Therefore, the release of zinc would likely improve adhesion by minimizing the effect of MMPs through direct inhibition and maintenance of a more neutral pH via plaque and calculus inhibition.

1.5 Naturally Occurring Zinc in the Oral Cavity

Zinc is found in all biological tissues and fluids [25]. Thus, it is worthwhile to consider the effect of physiological concentration on the oral cavity. Permanent teeth and bone contain 130 ppm zinc whereas 90 to 500 ppm has been measured in surface enamel [89]. Some studies have reported zinc levels to be as high as 1,500 ppm on surface enamel [37], but most is probably adsorbed to enamel apatite [63] and, therefore, not mobile. Plaque contains about 103 ppm zinc [54, 109], and almost all of this would be expected to be adsorbed to plaque organisms and not free in plaque fluid [106]. Saliva contains about 0.1 ppm zinc [38], a concentration lower than has been found inhibitory in

vitro. Thus, naturally occurring zinc in oral tissues and fluids is unlikely to have an inhibitory effect on plaque [57].

Zinc's natural presence in the oral cavity may explain its lack of unpleasant side-effects, which has led to its incorporation in many dental materials. However, clinically inhibitory concentrations appear to vary widely. For example, reduction of plaque formation by toothpaste containing 0.5% zinc citrate has been reported to range from 0 [66] to 42% [67]. Similarly, the zinc concentration responsible for halving the plaque pH drop following glucose exposure in vivo ranges from 5mM [4] to 100mM [42]. Factors other than concentration may account for such variation: a difference in zinc salts applied [130], methods of application (e.g. time between last rinse and sugar challenge), and different methods used to score plaque development or measure acidogenicity [57].

On the other hand, oral retention measurements of zinc are well agreed upon. Typically reported values of retained zinc are between 15-40% of the total dose delivered. These values have been confirmed in vivo [4]. The salivary decay curve demonstrates classic biphasic clearance (over a 5-120-minute period), the half-life for the bound phase of zinc being between 40 and 65 min. Zinc is a component or activator of several enzymes and is one of the substances which are needed in the metabolism of various inflammatory cells and, as a consequence, is mobilized from the tissues into the serum in the case of inflammation [51].

The oral mucosa is the bulk retention site for all clinically proven antiplaque agents. Supragingival plaque, tooth pellicle, and saliva may all be primary sites of action for these agents. Charged antibacterials are retained by electrostatic binding, most likely to carboxylic acid, sulfate, and phosphate residues of proteins and glycoproteins within

the surface layers of the oral mucosa, mucosal and tooth pellicle, and plaque [110, 111, 112]. Non-ionic antibacterials are retained by adsorption to lipophilic/hydrophobic regions within these receptor sites. Detailed understanding of these interactions is limited [22].

The retention sites for metal ions are, like chlorhexidine, proposed to be the oral mucosa, tooth pellicle, and supragingival plaque. Gingival tissues [44] and pellicle-coated teeth [43] have been shown to act as potential reservoirs for zinc in vitro. The adsorption of zinc and other metal ions to plaque bacteria may initially involve electrostatic interactions with cell surface proteins. However, they may be subsequently transported into the cell, where they are believed to inhibit uptake and metabolic processes, through interaction with sulfhydryl enzymes [110, 111, 112]. Although the inhibitory effect of zinc can be partially reversed when metals are washed away, it has been shown that zinc is retained for at least two hours in the mouth after tooth brushing with a 0.5% zinc-containing dentifrice [45]. The large amounts of zinc retained in plaque and on the pellicle covered tooth surface prolong the exposure to zinc.

1.6 Mechanisms of Action

Toxicological mechanisms of zinc ions play an important role in biofilm inhibition by inhibiting the active transport and metabolism of sugars as well as disrupting enzyme systems of dental biofilms [91, 116]. Zinc can also inhibit glucosyltransferase activity *in vitro* [13, 118] and *in vivo* [117]. The ions were found to inhibit growth at a minimum 8 mg/mL concentration against *S. sobrinus* [89]. The inhibition of glucosyltransferases by zinc is unlikely to be due to either covalently or

oxidatively induced damage. Kinetic studies of the glucosyltransferases of *S. sobrinus* by Devulapalle and Mooser showed that the zinc ions act as a reversible, competitive inhibitor at the fructose subsite within the active site of the glucosyltransferase [30].

The mechanism of enzyme inactivation by metals is not completely understood. It is assumed that metal ions bind to specific sites, causing conformational changes that inactivate the catalytic function of enzymes. Larsen and Auld have shown that the mechanism of zinc inhibition of carboxypeptidase A, a zinc metalloproteinase, is due to the formation of zinc monohydroxide that bridges the catalytic zinc ion to a cysteine side chain in the active site of the enzyme [73]. The non-competitive inhibition by other heavy metal ions is attributed to binding of the ion to a site distinct from the active site [83]. The fact that zinc inhibition of MMP-2 and MMP-9 can only be partially reversed after removal of soluble salt suggests the existence of more than one zinc-binding site within these enzymes, where the low affinity interactions of zinc and MMPs can be disrupted by simply decreasing the amount of zinc. $ZnSO_4$ is a strong inhibitor of MMP-2 and MMP-9. It has been shown that these MMPs can be fully inhibited by 3mM $ZnSO_4$. This inhibition was only partially reversed after removal of soluble $ZnSO_4$ by extensive washing of the gels [115], demonstrating the persistent efficacy of zinc. The effect of zinc on MMPs, glycosyltransferases, plaque, and calculus ultimately depends on concentration, time exposure, and contact surface area.

1.7 Delivery Methods

Currently, one of the best methods for zinc delivery is zinc oxide (ZnO). A certain amount of solid, inactive, ZnO provides a "depot" that accounts for prompt

repletion and rapid mobilization of zinc ions that diffuse to the site of any zinc-consuming reaction or to some receptor site on a microbe. A ZnO depot that consists of many small ZnO particles in a watery suspension acts as an immediate source of mobile zinc ions. In fact, in vivo mobilization by solution and dissociation of zinc from zinc oxide was found to proceed at a rate equivalent to that of zinc ions from an applied ZnCl solution. Thus, zinc oxide was considered an active compound. Zinc oxide is well tolerated by the body, which is assigned to the relatively insoluble nature of this compound [89].

Zinc oxide has traditionally been delivered in the form of zinc oxide eugenol (ZOE). The eugenol component is slightly soluble and is eluted when immersed in water. Zinc hydroxide is deposited through the hydrolysis of zinc eugonolate, which is driven by the equilibrium between zinc eugenolate and zinc and eugenol. Therefore, the zinc eugenolate matrix is decomposed by the continuous removal of eugenol from the cement [134]. Or, the cement forming reaction is effectively reversed [87]. The ease with which this occurs suggests that other zinc delivery methods should be explored.

Some other zinc releasing cements are zinc polycarboxylate and zinc phosphate. Zinc polycarboxylate cements have been widely used clinically as cavity liners, adhesives for the placement of crowns and for the adhesion of orthodontic appliances. These cements are composed of polycarboxylic acid, usually poly(acrylic acid), and a modified zinc oxide powder [132]. While the mechanical properties of this cement are acceptable, Cook showed that the reaction between zinc oxide and poly(acrylic acid) was almost quantitative and that the rate of diffusion of Zn^{2+} ions within the set matrix is low [18]. This was consistent with the conclusion of Crisp et al. that the loss of these ions from the

cement ceased upon aging [20]. The lack of zinc ion mobility was also consistent with other findings [95, 97]. Again, suggesting the need for a different method for zinc release.

One of the most widely used and studied zinc releasing cements is zinc phosphate, which typically contains about 10 w/w% zinc oxide in its deactivated form [135]. These cements have also shown to increase the pH of acidic storage solutions such as lactic acid, sodium lactate/lactic acid buffer solution [75, 94]. However, these solutions proved to be highly erosive to these cements, causing cements to erode 14.2% in moderate cases [26, 98]. Therefore, zinc phosphate too has left room for improvement in the field of zinc releasing dental cements.

Resin-Modified Glass Ionomer Cement (RMGIC) presents a similar level of fluoride release to traditional glass ionomer cement, but this release is known to drop off after a few days in both cements [39]. Currently, scholars disagree on whether it's anticariogenic effect is due to the fluoride or zinc present [86]. There are also those that believe it is the interaction between the two, zinc and fluoride, that influence the antibacterial activity [64, 78, 86, 103]. The addition of 10 w/w% $ZnSO_4$ to the RMGIC cement powder increased its solubility nearly five times. However, the highest value remained below the maximum value acceptable by ISO 7489 specification 14 [71]. The presence of zinc increased the growth inhibition halos, but only during the first fifteen-day period [78]. After this period, the inhibitory effect decreased abruptly [100]. Additionally, this cement has shown to not be rechargeable, which suggests a better method could exist.

The mentioned materials are bioactive via dissolution mechanisms. Dissolution mechanisms lead to reduced mechanical properties over time and therefore, reduced

longevity. Recently, a novel delivery system for calcium, phosphate, and fluoride ions has been developed. This system has the potential to allow ions to efficiently diffuse from a dental material to the oral environment [27]. This is done by encapsulation of aqueous solutions of ions in permeable microcapsules of polyurethane. The rate of ion release was shown to be controlled by varying the chemical structure of the microcapsules, the initial concentration of the salt, and the counter ion of the salt used in solution. These microcapsules have also been shown to be rechargeable [14]. Zinc release, using this novel delivery system, is explored in the current study.

Bioactive glasses have been studied for years, beginning with a melt-derived glass called Bioglass that generated a group of both melt-derived and sol–gel-derived glasses, collectively known as bioactive glasses [6, 59 65, 104]. In this study, we examine the release profiles on orthodontic cements containing a bioactive zinc glass. The silicate glass used in this study is manufactured by Schott, and contains 10 w/w% zinc oxide. Based on the literature, it is most likely prepared as a melt-derived glass, although we were unable to confirm this with the company [32, 79].

The release of zinc from a dental material, specifically an orthodontic cement would be beneficial for many reasons. Patients often have difficulty maintaining good oral hygiene while fitted with orthodontic appliances; this manifests in the buildup of plaque and calculus around said appliances and periodontitis. Studies have shown that zinc has the potential to act as an antimicrobial, antiplaque, and anticalculus agent through the aforementioned mechanisms. In effort to continuously strive for improvement in the field of dental materials, new delivery methods of zinc to the oral cavity should be explored. In the current study, we evaluate two different zinc delivery

methods. First, the release of zinc from the microcapsules described in the novel delivery system above when incorporated into an orthodontic cement. Second, the release of zinc from a bioactive zinc glass incorporated into an orthodontic cement at increasing weight percents.

Chapter 2

Materials and Methods

2.1 Pre-polymer Synthesis

A polyurethane pre-polymer was synthesized by a solution polymerization in cyclohexanone (Sigma Aldrich). For this study, polyurethane was synthesized using isophorone-diisocyanate (Fluka) and ethylene glycol (Sigma Aldrich). First, a flask was evacuated and flame dried. Next, nitrogen gas was gradually introduced to the flask. While the inert gas continued to flow through the flow adapter, cyclohexanone was added. The isophorone-diisocyanate was added next, using a syringe. The septum was replaced and secured. A syringe needle was inserted through the septum, to relieve pressure inside the flask, while the solution stirred for 30 minutes. After, ethylene glycol and an initiator were added. The septum was replaced a final time and secured. Next, the flask was carefully lowered into an oil bath. A degassing needle, connected to the manifold, was inserted until it rested at the bottom of the flask. Then the adjacent inert gas port on the manifold (connecting to the hose of the degassing needle) was opened slowly until the reaction mixture began to bubble. Once complete, the solution degassed at room temperature for 30 minutes. After, the degassing needle was removed and the reaction mixture was set to 70°C. Once the temperature was set, the reaction ran overnight.

On day two, the flask was removed from the oil bath and cooled for one hour. After, the solution was transferred, fitted to a flow adapter, and secured to a vacuum line from a cold trap. The dewar was filled approximately one third of the way with isopropanol (Fisher Scientific). Dry ice was added to the dewar in small pieces while the cold trap was in place. Once complete, the round bottom flask was lowered into the oil

bath, and set to 100°C. All exposed glassware including the cold trap, flow adapter, and round bottom flask were insulated.

The flask was then evacuated, so that the evaporating solvent could travel into the trap and liquefy in a cold environment. The vacuum was pulled until the pre-polymer appeared dry and all of the solvent was recovered from the product. The pre-polymer was then collected and stored in vials in a cabinet at room temperature until microcapsule synthesis.

2.2 Preparation of Oil Solution and Salt Solution

The preparatory solutions were made at least 24 hours prior to microcapsule synthesis. The oil solution consisted of methyl benzoate (Acros), an emulsifying agent, and the polyurethane pre-polymer. The three components were combined in an Erlenmeyer flask and stirred until the polyurethane was dispersed.

The salt solutions were made to accommodate our desired molarity for ion release. For this experiment, we used 2.5 M zinc sulfate heptahydrate (Fisher Scientific). 3.0 M potassium phosphate dibasic dibasic (Fisher Scientific), 5.0 M calcium nitrate tetrahydrate (Alfa Aesar) and 0.8 M sodium fluoride (MP Biomedicals) solutions were also made. It is important to note that the higher concentrations were given a significant amount of time to dissolve, in some cases, overnight. Solutions were stored at room temperature until use.

2.3 Microcapsule Synthesis

The prepared pre-polymer oil solution was agitated to create a reverse emulsion and the aqueous salt solution was then added incrementally over a period of fifteen minutes while the oil solution was agitated in a reactor at 70 ˚C. The polyurethane was then chain extended using ethylene glycol to create the desired product. The microcapsules were then centrifuged and stored for use in orthodontic cement formulation.

2.4 Orthodontic Cement Formulations

The formulations in this study were prepared with the same rheological properties as some of the industry leaders in single syringe, light initiated orthodontic bracket and band cements, but with the addition of either microcapsules or bioactive zinc glass (Schott). The formulations consisted of w/w% loadings of monomers BisGMA and TEGMA (Esstech), barium boroaluminosilicate glass, bioactive zinc glass, fumed silica (Evonik), photoinitiator, and microcapsules. The orthodontic cements were prepared with loadings of 0, 5, or 7 w/w% microcapsules containing various mixtures of 0.8 M sodium fluoride, 5.0 M calcium nitrate tetrahydrate, 3.0 M potassium phosphate dibasic, and 2.5 M zinc sulfate heptahydrate aqueous salt solutions. Cements were also prepared with loadings of 0, 5, 10, or 15 w/w% bioactive glass as seen in Table 1.

ID	Filler Glass	Zinc Glass	Total MCs	Zn^{2+} MCs	Ca^{2+} MCs	F^- MCs	PO_4^{3-} MCs
1	68	0	7	7	0	0	0
2	70	0	5	5	0	0	0
3	68	0	7	2.5	1	3	0.5
4	68	0	7	0	2	4	1
5	70	0	5	1	1.8	2	0.2
6	65	5	0	0	0	0	0
7	60	10	0	0	0	0	0
8	55	15	0	0	0	0	0
9	65	5	5	0	2	2	1
10	60	10	5	0	2	2	1
11	55	15	5	0	2	2	1

Table 1. Formulations of orthodontic cements containing varying loadings of bioactive glass and microcapsules. All values are given in w/w%.

2.5 Preparation for Zinc Ion Measurements

4.485g of microcapsules were loaded into dialysis tubing and places in a bath of 900 mL nanopure water. Three, one mL aliquots were taken immediately the first day at time zero, one hour, four hours, eight hours, sixteen hours, one day, four days, seven days, and weekly thereafter. It is important to note that the volume taken from the bath during each aliquot was refreshed afterwards with the same volume of nanopure water. Samples were stored in microcentrifuge tubes (Fisherbrand) until ion concentration measurements were performed. Each measurement was performed in triplicate.

The cement formulations were transferred into washers (Washers USA) which were fixed to glass microscope slides (Fisherfinest) using a standard water-resistant adhesive (AmazingGoop). The washers had an inner diameter of 0.375” and thickness of 0.032”. Cement was added until a smooth, uniform surface was achieved. Three washers were adhered to each slide, and each formulation contained 20 slides (for a total of 60 washers per formulation). Each slide was weighed before and after the cement was

placed. Once the cement transfer was complete, the slides were cured using a Spetrum 800 curing light at 600 mW/cm^2 for twenty seconds on each side, and then cured for an additional five minutes in a DENTSPLY Triad Visible Light Cure System. The following day, slides were weighed a final time, rinsed with nanopure water, and dried. Next, the slide dishes and stir bars were disinfected using a bleach solution (Clorox) then 70% ethyl alcohol solution (Fisher Scientific). The glassware was then flame dried and placed on a clean surface near an active Bunsen burner. Finally, our slide dishes were loaded. Slides were placed back to back in the slot holders so the washers faced outward into surrounding nanopure water or 0.02 M acetic acid, pH = 4.5 (HAc) buffer solution. Once all twenty slides were loaded, 200 mL of nanopure water or HAc buffer solution were added. Again, one mL aliquots were taken at the aforementioned times. The volume taken from the bath was replaced afterwards with the same volume of nanopure water or HAc buffer solution. Samples were stored in microcentrifuge tubes (Fisherbrand) until ion concentration measurements were performed. Each measurement was performed in triplicate.

2.6 Zinc Ion Measurements

Zinc ion concentrations were measured for samples with microcapsules containing zinc sulfate heptahydrate or bioactive zinc glass by Atomic Absorption Spectroscopy (AAS) using a Varian SpectrAA 200 Spectrometer. In this technique, a zinc hollow cathode lamp with a monochromator generates light with a wavelength of 213.9 nm that passes through a chamber containing the analyte. The greater the concentration of the analyte, the more light is absorbed. Concentration can then be determined through

Beer's Law: $A = \varepsilon lc$ (where A=absorbance, ε=absorptivity, l=pathlength, and c=concentration). Due to the linearity of Beer's law, were able use a set of standards (0.2 ppm, 0.5 ppm, 1.0 ppm, and 1.5 ppm zinc sulfate heptahydrate) and regression analysis (R^2>0.999) to generate a curve, which in turn was used to determine the concentrations of our samples.

Chapter 3

Results

3.1 Introduction

Zinc ions contribute to the disruption of plaque and calculus. Furthermore, scholars suggest zinc ions may play an important role in fighting the progression of gingivitis. Therefore, we chose to study two methods, microencapsulation and bioactive glass, of releasing zinc from an orthodontic cement paste. Aqueous solutions of sodium fluoride, zinc sulfate heptahydrate, calcium nitrate tetrahydrate, and potassium phosphate dibasic were encapsulated in ethylene glycol-polyurethane microcapsules and loaded into orthodontic cement paste formulations. Their release profiles were then studied. Moreover, the release profiles of cement formulations loaded with increasing amounts of bioactive zinc glass were assessed. The combined effects of these two release methods were also studied in both neutral and acidic environments. The figures and tables in this section demonstrate the release profiles and release rates of our experiments. All facts and figures are reported with normalized data, data adjusted to represent ion release per gram of orthodontic cement paste formulation.

3.2 Microencapsulation

3.2.1 Zinc release from orthodontic cement paste

The ability of zinc ions to diffuse out of the ethylene glycol-polyurethane microcapsules and into an aqueous environment of nanopure water was determined. Fig. 1 shows the part per million (ppm) of zinc being released per gram (g) of microcapsules

as a function of time in hours. These microcapsules encapsulated a 2.5 M zinc sulfate heptahydrate solution.

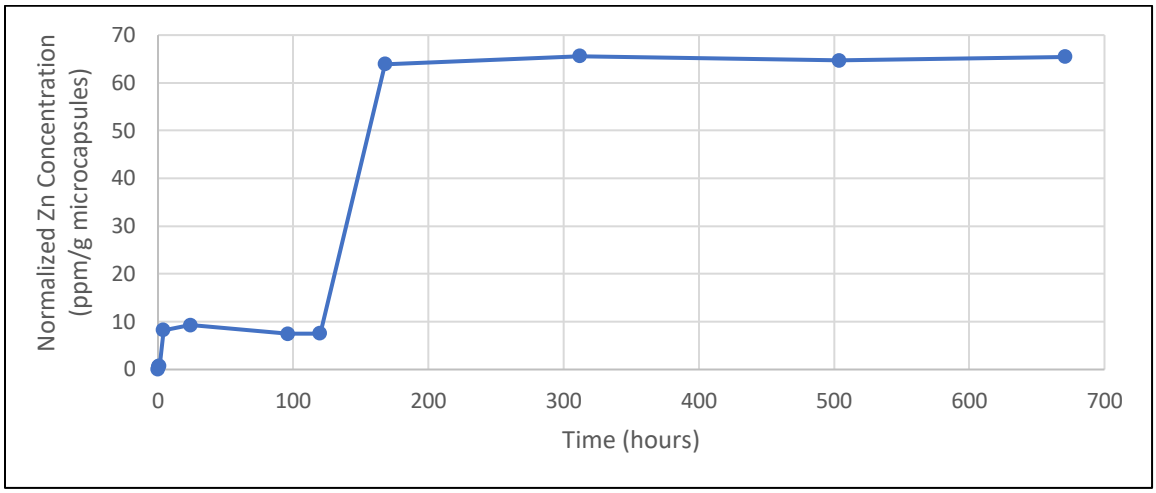

Figure 1, Normalized concentration of zinc ions released from microcapsules containing 2.5 M zinc sulfate heptahydrate. The plot is the concentration of zinc ions in ppm released per gram of microcapsules as a function of time in hours.

The zinc-loaded microcapsules showed an initial 8.228 ppm zinc/g microcapsules release at four hours. This level remained constant until 168 hours. As seen in Fig. 1, after 168 hours, the microcapsules reached equilibrium at 63.939 ppm zinc/g. This level persisted for the rest of the study. With evidence that the microcapsules were releasing zinc ions, we moved forward with our investigation of incorporating the microcapsules into resin-based cements.

3.2.2 Multiple ion release

The ion release profiles of zinc ions from orthodontic cements loaded with 5 w/w% and 7 w/w% microcapsules that contained an aqueous solution of 2.5 M zinc

sulfate heptahydrate are displayed in Fig. 2. The plot shows the ppm of zinc ion released per gram of orthodontic cement formulation as a function of time in hours.

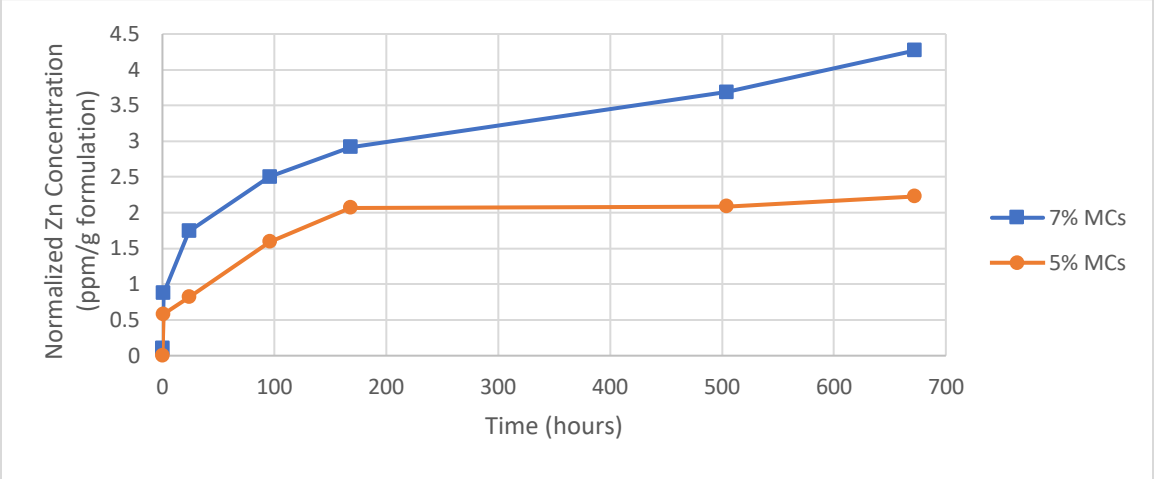

Figure 2, Normalized concentration of zinc ions released in ppm from microcapsules containing 2.5 M zinc sulfate heptahydrate loaded in an orthodontic cement paste formulation containing 5 and 7 w/w%. The plot shows ppm zinc released per gram of formulation as a function of time in hours.

The release profile in Fig. 2 illustrates an initial release of 0.066 ppm zinc/g formulation/hour for the first 24 hours for the formulation containing 5 w/w% zinc microcapsules. Zinc ion levels continued to increase for this formulation, at a slower rate, until leveling out at 2.070 ppm/g at 336 hours, where it remained constant for the remainder of the study. The 7 w/w% release profile had an initial rate of 0.0726 ppm/g/hour for the first 24 hours, and continued to release zinc throughout this study. This formulation released 2.040 ppm more zinc than the 5 w/w% formulation at 672 hours. All values can be found in Table 2.

Zinc Released from Microcapsules Loaded into an Orthodontic Cement (ppm/g formulation)		
Time (hours)	5 w/w% MCs	7 w/w% MCs
0	0.000	0.105
1	0.579	0.875
24	0.819	1.743
96	1.593	2.500
168	2.071	2.918
504	2.088	3.688
672	2.227	4.267

Table 2, Normalized concentration of zinc ions released in ppm from an orthodontic cement paste formulation containing 5 and 7 w/w% zinc sulfate heptahydrate microcapsules.

3.2.3 Effect of multiple ion release on zinc release profiles

We also studied the effect that fluoride, calcium, and phosphate ion release has on the release of zinc ions. Fig. 3 depicts the zinc ion release profiles of three different orthodontic cement formulations loaded with different types of microcapsules at totals of 5 and 7 w/w%. The specific w/w% breakdown of the various microcapsules in each formulation is as follows: 3% 0.8 M sodium fluoride, 2.5% 2.5 M zinc sulfate heptahydrate, 1% 5.0 M calcium nitrate tetrahydrate, and 0.5% 3.0 M potassium phosphate (2.5Z/1C/3F/0.5P); 2% 5.0 M calcium nitrate tetrahydrate, 4% 0.8 M sodium fluoride, and 1% 3.0 M potassium phosphate dibasic (2C/4F/1P); 2% 0.8 M sodium fluoride, 1% 2.5 M zinc sulfate heptahydrate, 1.8% 5.0 M calcium nitrate tetrahydrate, and 0.2% 3.0 M potassium phosphate (1Z/1.8C/2F/0.2P). Please refer to Table 1 for further breakdown of each formulation, under IDs 3, 4, and 5, respectively. The plot

shows the ppm of zinc ions released per gram of orthodontic cement formulation as a function of time in hours.

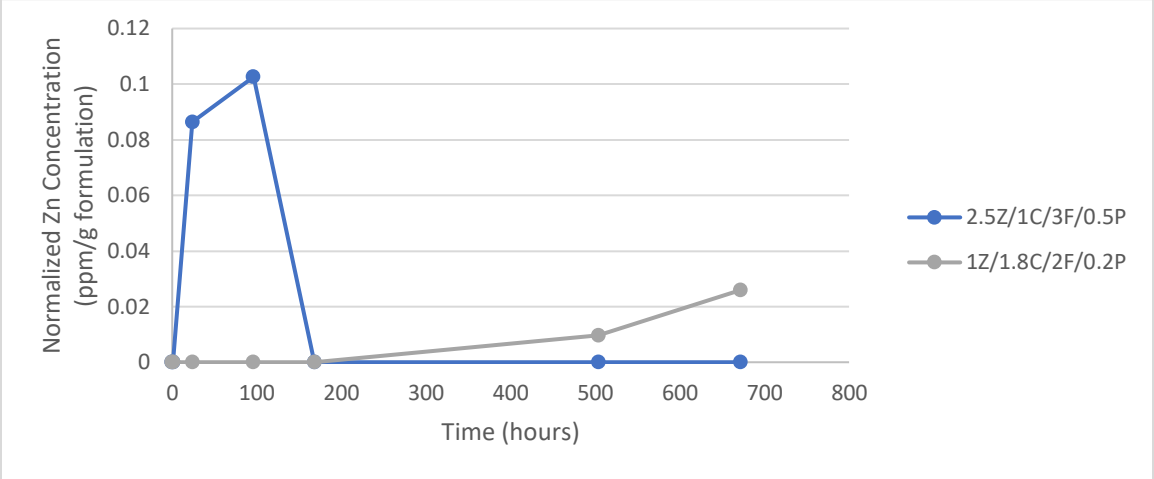

Figure 3, Normalized concentration of zinc ions released from an orthodontic cement paste formulation containing a total of 5 or 7 w/w% of microcapsules of different ions. Specifically, 3% 0.8 M sodium fluoride, 2.5% 2.5 M zinc sulfate heptahydrate, 1% 5.0 M calcium nitrate tetrahydrate, and 0.5% 3.0 M potassium phosphate (2.5Z/1C/3F/0.5P); 2% 0.8 M sodium fluoride, 1% 2.5 M zinc sulfate heptahydrate, 1.8% 5.0 M calcium nitrate tetrahydrate, and 0.2% 3.0 M potassium phosphate (1Z/1.8C/2F/0.2P) The plot shows zinc ion concentration released in ppm per gram of formulation as a function of time in hours.

As seen in Fig. 3, the release profile for formulation 2.5Z/1C/3F/0.5P reached a peak of 0.103 ppm zinc/g formulation at 96 hours, before falling to zero upon the next reading. That is two times lower than the maximum amount of zinc (2.227 ppm) released from the formulation that contained 5 w/w% of exclusively zinc microcapsules. However, this level was over three times greater than the other formulations in this figure. The other two formulations showed much slower zinc mobilization. Formulation 1Z/1.8C/2F/0.2P displayed minimal zinc release, with a small peak of 0.028 ppm zinc/g at 504 hours. All values can be seen in Table 3.

Zn Released per Gram of Formulation Containing Various Microcapsules

Time (hours)	Formulation .8F/2.5Z/1.8C/.2P	Formulation .8F/1Z/1.8C/.2P
0	0	0
1	0	0
24	0.086	0
96	0.103	0
336	0	0
504	0	0.009
936	0	0.026

Table 3, Normalized total concentration of zinc sulfate heptahydrate ions released from an orthodontic cement paste formulation containing a total of 5 and 7 w/w% microcapsules of different ions. Specifically, 3% 0.8 M sodium fluoride, 2.5% 2.5 M zinc sulfate heptahydrate, 1% 5.0 M calcium nitrate tetrahydrate, and 0.5% 3.0 M potassium phosphate (2.5Z/1C/3F/0.5P); 2% 0.8 M sodium fluoride, 1% 2.5 M zinc sulfate heptahydrate, 1.8% 5.0 M calcium nitrate tetrahydrate, and 0.2% 3.0 M potassium phosphate (1Z/1.8C/2F/0.2P). Data is reported in ppm zinc/g formulation.

3.3 Bioactive Zinc Glass

3.3.1 Weight percent loading

The ability of a bioactive zinc glass to release free zinc ions into an aqueous environment of nanopure water or an acidic environment was also assessed. Fig. 4 shows the part per million of zinc being released per gram of formulation as a function of percent loading of bioactive zinc glass in a neutral pH, aqueous environment. These

formulations contained 5, 10, and 15 w/w% loadings of bioactive zinc glass. Each data series represents a given time in hours.

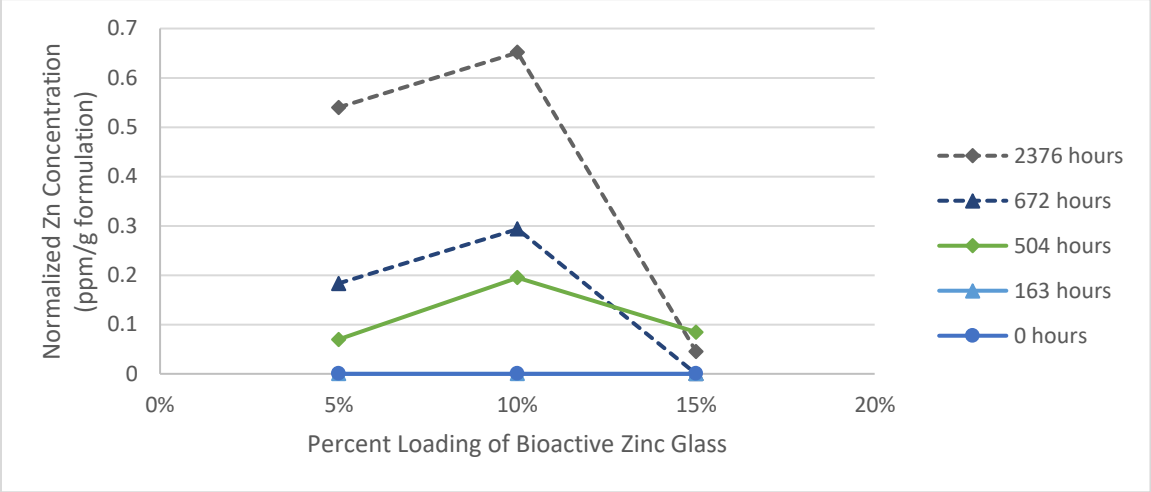

Figure 4a, Normalized concentration of zinc ions released from cements containing 5, 10, and 15 w/w% bioactive zinc glass in neutral pH. The plot is the concentration of zinc ions in ppm released per gram of orthodontic cement paste as a function of percent loading of bioactive zinc glass. Each series in given in hours.

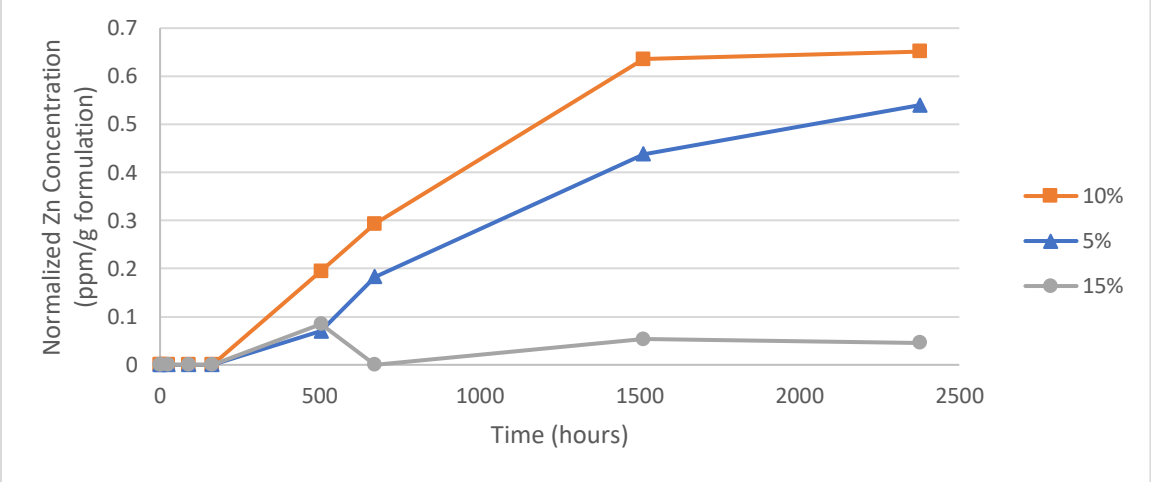

Figure 4b, Normalized concentration of zinc ions released from cements containing 5, 10, and 15 w/w% bioactive zinc glass in neutral pH. The plot is the concentration of zinc ions in ppm released per gram of orthodontic cement paste as a function of time in hours.

As seen in Fig. 4a, the release profiles for all three formulations were near zero for all readings within the first 168 hours. After 168 hours, all formulations began to show ion release. The orthodontic cement paste formulation loaded with 10 w/w%

bioactive zinc glass displayed the greatest release of 0.293 ppm zinc/g formulation. The cements loaded with 15 and 5 w/w% bioactive zinc glass released a maximum of 0.084 and 0.128 ppm zinc/g, respectively.

3.3.2 Acidic conditions

Due to the fact that most harmful bacteria in the oral cavity, plaque, and calculus thrive under acidic conditions, we studied the effect an acidic environment (pH = 4.5) had on the release profile of an orthodontic cement loaded with bioactive zinc glass.

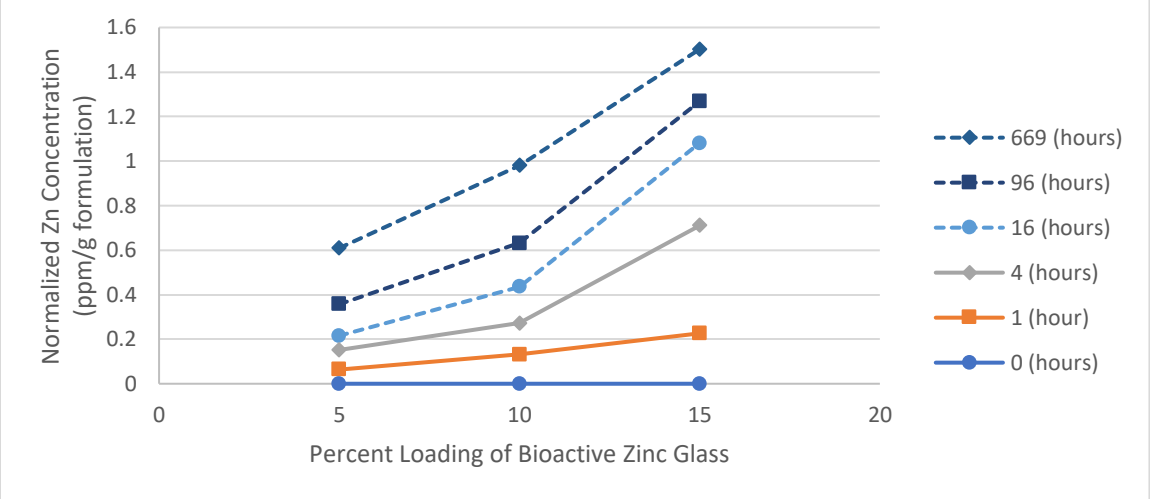

Figure 5a, Normalized concentration of zinc ions released from 5, 10, and 15 w/w% bioactive zinc glass. The plot is the concentration of zinc ions in ppm released from bioactive zinc glass loaded in an orthodontic cement paste, in an acidic (pH = 4.5) environment of acetic acid buffer solution, as a function of percent loading of bioactive zinc glass. Each data series represents time in hours.

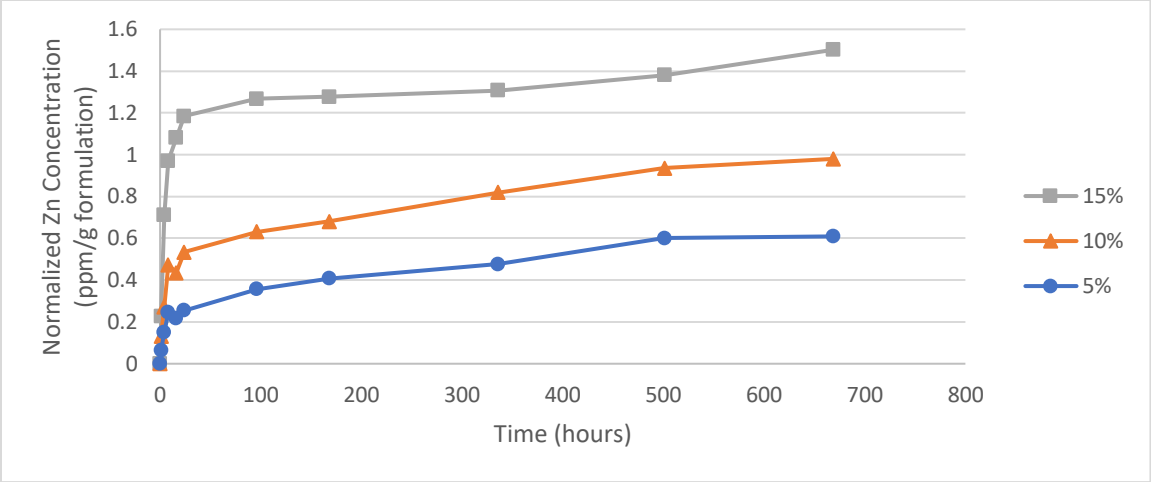

Figure 5b, Normalized concentration of zinc ions released from 5, 10, and 15 w/w% bioactive zinc glass. The plot is the concentration of zinc ions in ppm released from bioactive zinc glass loaded in an orthodontic cement paste, in an acidic (pH = 4.5) environment of acetic acid buffer solution, as a function of time in hours.

As seen in Fig. 5a and 5b, the three release profiles showed an almost linear increase in the equilibrium they reached when immersed in the acidic environment. At 669 hours, the 5, 10, and 15 w/w% cements released 0.609, 0.980, and 1.502 ppm zinc/g formulation, respectively. A brief dip in the 5 and 10 w/w% bioactive zinc glass formulations can be seen between 8 and 24 hours. In both cases, there is about a 0.05 ppm zinc/g decrease observed at 16 hours. The decrease is recovered in the 24 hours reading and the release profiles continue to increase thereafter. This small decrease was not observed for the 15 w/w% bioactive zinc glass formulation. The release profile for this formulation continued to increase, at a decreasing rate, for the entirety of our study. Rates varied from 0.226 ppm zinc/g/hour during the first hour to nearly zero at 336 hours. All values can be seen in Table 4.

Effect of an Acidic Environment on Release Profiles Without Microcapsules			
Time (hours)	5% Zn Glass	10% Zn Glass	15% Zn Glass
0	0	0	0
1	0.064	0.131	0.226
4	0.152	0.272	0.710
8	0.245	0.474	0.971
16	0.216	0.436	1.081
24	0.255	0.533	1.184
96	0.357	0.631	1.268
168	0.408	0.681	1.277
336	0.477	0.819	1.307
501	0.600	0.935	1.381
669	0.609	0.980	1.502

Table 4, Normalized concentration of zinc ions released in ppm per gram of orthodontic cement paste formulation containing 5, 10, and 15 w/w% bioactive zinc glass without microcapsules in an acidic (pH = 4.5) environment of acetic acid buffer solution.

3.4 Microencapsulation and Bioactive Glass

3.4.1 Neutral conditions

The effect of the addition of microcapsules to the orthodontic cement formulation containing bioactive zinc glass was assessed. For this assay, all formulations were loaded with a total of 5 w/w% microcapsules. The specific breakdown was the same for all three and is as follows: 2% 5.0 M calcium nitrate tetrahydrate, 2% 0.8 M sodium fluoride, and 1% 3.0 M potassium phosphate dibasic. Fig. 7 shows the release profiles in ppm zinc/g

formulation as a function of percent loading of bioactive zinc glass. Each data series represents time in hours.

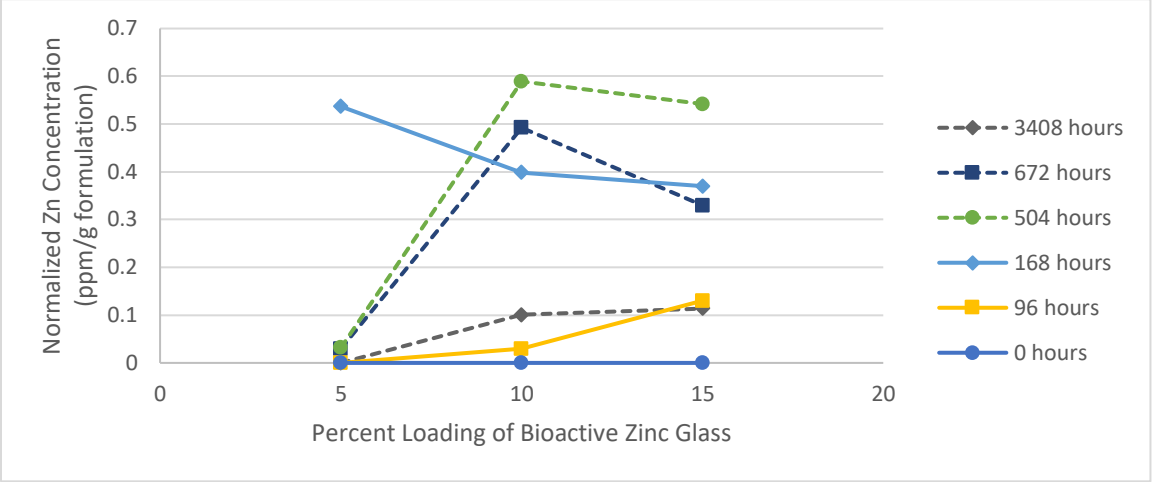

Figure 6a. The plot is the concentration of zinc ions in ppm per gram formulation released from an orthodontic cement paste as a function of percent loading of bioactive zinc glass. Each series is given in hours.

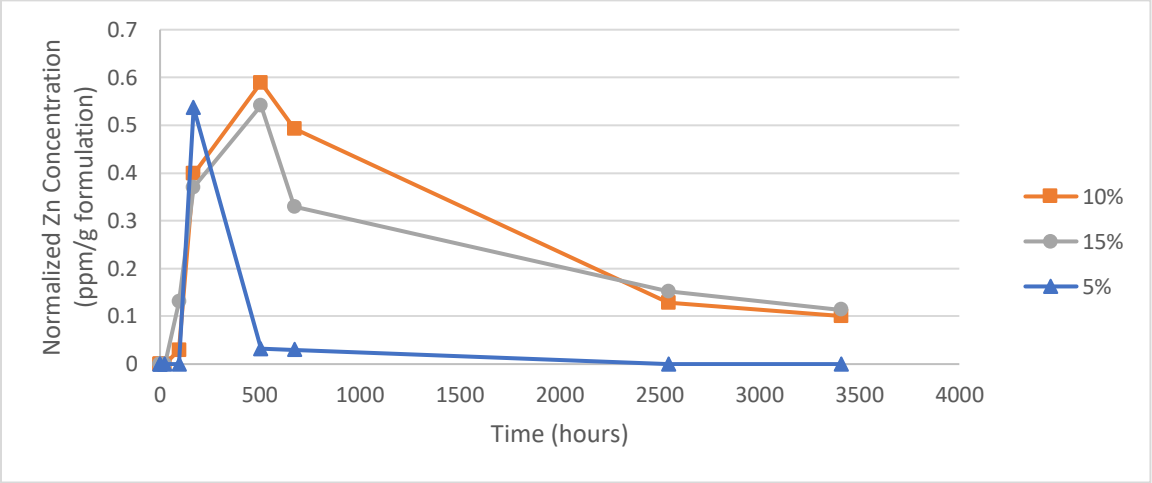

Figure 6b. The plot is the concentration of zinc ions in ppm per gram formulation released from an orthodontic cement paste as a function time in hours.

At 168 hours, the formulation loaded with 5 w/w% bioactive zinc glass displayed an ion release of 0.537 ppm zinc/g formulation. However, both the 10 and 15 w/w% bioactive zinc glass formulations released more zinc over time, each showing 0.589 and 0.541 zinc/g at 504 hours, respectively.

3.4.2 Acidic conditions

Finally, the combined effects of an acidic (pH = 4.5) environment and the presence of microcapsules in a cement containing bioactive zinc glass were assessed. These formulations were the same as seen in section 3.4.1, loaded with a total of 5 w/w% microcapsules and increasing weight percents of bioactive zinc glass. Further breakdown of the 5, 10 and 15 w/w% formulations can be found in Table 1 **under ID's 1, 2, and 3.**

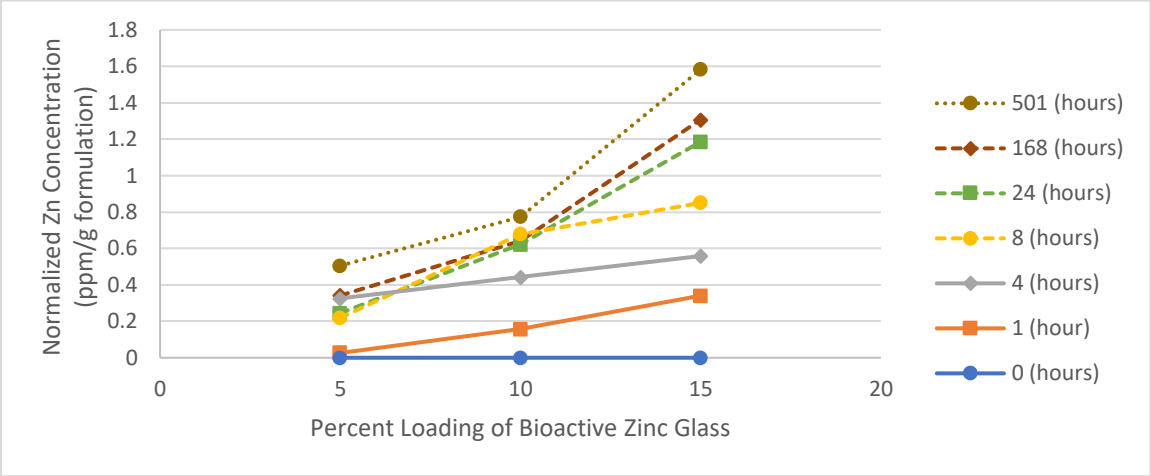

Figure 7a, The plot is the concentration of zinc ions in ppm released from bioactive zinc glass loaded at increasing weight percents in an orthodontic cement paste as a function of percent loading of bioactive zinc glass. Each data series represents a given time in hours.

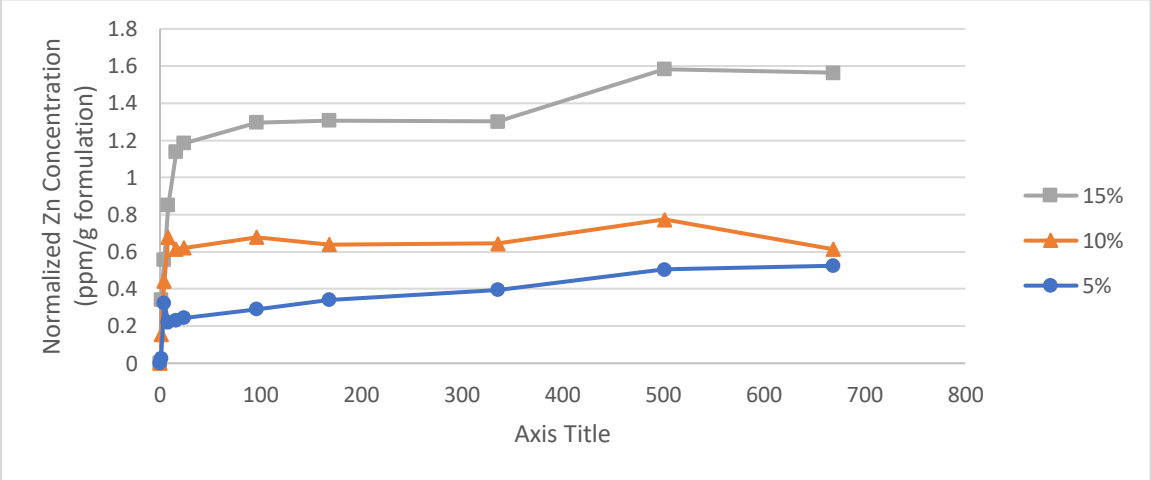

Figure 7b, The plot is the concentration of zinc ions in ppm released from bioactive zinc glass loaded at increasing weight percents in an orthodontic cement paste as a function of time in hours.

These formulations showed quick mobilization of zinc, as all release profiles hit about 50% of their maximum zinc release levels in the first 24 hours. Fig. 7 demonstrates the formulation loaded with 10 w/w% bioactive zinc glass released 0.250 ppm zinc/g formulation more than the formulation loaded with 5 w/w% zinc glass at both of their maximums. The cement that contained 15 w/w% bioactive zinc glass released 1.582 ppm zinc/g formulation at its maximum, over triple the amount displayed by the 5 w/w% release profile.

Rapid zinc release is seen across all three formulations in the first 24 hours. The 5 w/w% zinc glass formulation displays an early peak at four hours. Its release rate was 0.0814 ppm zinc/g/hour for the first four hours. Total zinc decreased to 0.219 ppm zinc/g. The peak observed at four hours of 0.325 ppm zinc/g wasn't surpassed until the formulation had been exposed to the acidic environment for 168 hours, when 0.341 ppm zinc/g was observed. The initial release rate for the 10 w/w% zinc glass formulation was 0.110 ppm/g/hour for the first four hours and 0.139 ppm/g/hour for the 15 w/w% cement. The 15 w/w% zinc glass cement displayed the fastest release rate and consistent increases in total zinc released throughout the study. However, 10 w/w% zinc glass cement displayed a similar trend shown by the 5 w/w% formulation. The 10 w/w% zinc glass cement release profile displays a peak of 0.679 ppm/g at eight hours. This peak was recovered in the first one hundred hours (0.679 ppm/g at 96 hours), unlike the 5 w/w% formulation. All values can be observed in Table 5.

Effect of an Acidic Environment on Release Profiles With Microcapsules			
Time (hours)	5% Zn Glass	10% Zn Glass	15% Zn Glass
0	0	0	0
1	0.026	0.157	0.341
4	0.326	0.442	0.558
8	0.219	0.679	0.850
16	0.232	0.616	1.137
24	0.245	0.621	1.185
96	0.292	0.679	1.297
168	0.342	0.639	1.307
336	0.396	0.645	1.302
501	0.505	0.775	1.583
669	0.525	0.614	1.564

Table 5 Normalized total concentration of ions in ppm per gram orthodontic cement paste formulation containing 5, 10, and 15 w/w% bioactive zinc glass and 5 w/w% microcapsules.

3.5 Effect of pH and multiple ions at constant bioactive zinc glass loading

In this section, we compare the release profiles of formulations loaded with the same amount of bioactive zinc glass when exposed to a neutral environment of nanopure water verses an acidic environment of acetic acid buffer solution (pH=4.5). We also demonstrate how the presence of microcapsules effects these release profiles. Each graph has four data series. An orthodontic cement paste containing a given loading of bioactive zinc glass (5, 10, or 15 w/w%) without microcapsules, accounts for two of the series- one in the neutral environment and one in the acidic environment. The other two series represent an orthodontic cement formulation with the same amount of bioactive zinc glass, but these cements contain a total of 5 w/w% microcapsules (2% 5.0 M calcium nitrate tetrahydrate, 2% 0.8 M sodium fluoride, and 1% 3.0 M potassium phosphate

dibasic). Again, this formulation was exposed to both the neutral and acidic environments.

3.5.1 5% zinc glass

A comparison of release profiles for formulations containing 5 w/w% bioactive zinc glass is illustrated in Fig. 8. There were two formulations used, one without microcapsules and the other with a total of 5 w/w% microcapsules; 5.0 M calcium nitrate tetrahydrate (2 w/w%), 0.8 M sodium fluoride (2 w/w%), and 3.0 M potassium phosphate dibasic (1 w/w%). These microcapsules were used for all assays in this section. Both of the formulations were exposed to neutral and acidic environments, creating four series of data. The plot shows the normalized concentration of zinc released per gram of formulation at a function of time in hours.

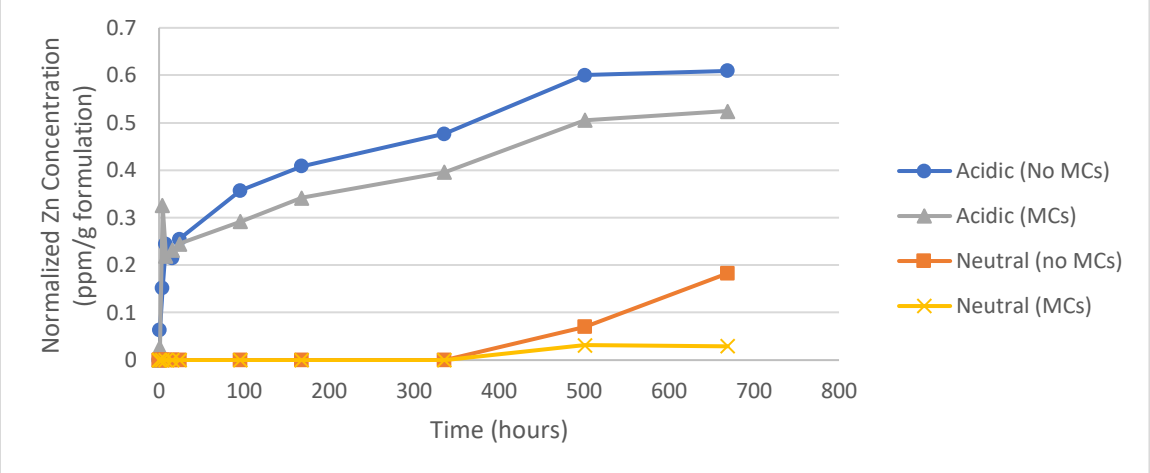

Figure 8, The graph shows the normalized concentration of zinc in ppm per gram of formulation as a function of time in hours. Two formulations were each exposed to a neutral environment of nanopure water and an acidic environment of acetic acid buffer solution (pH = 4.5), creating four series of data. Both formulations were loaded with 5 w/w% bioactive zinc glass. One was also loaded with 5 w/w% of microcapsules (2% 5.0 M calcium nitrate tetrahydrate, 2% 0.8 M sodium fluoride, and 1% 3.0 M potassium phosphate dibasic), while the other had no microcapsules.

The acidic environment had a large effect on the amount of zinc released from these formulations. The microcapsule-containing cement displays the most rapid release

of zinc (0.326 ppm/g formulation at four hours) when exposed to the acidic environment. However, the same cement released virtually no zinc in the neutral bath of nanopure water. The orthodontic cement paste absent of microcapsules released the most zinc when bathed in the acidic environment. At its maximum, the formulation without microcapsules released 0.600 ppm/g formulation, about 0.100 ppm/g formulation more than the formulation with microcapsules in the same environment. The formulation absent of microcapsules released much less zinc in the neutral environment, exhibiting a maximum release of 0.182 ppm/g at 669 hours.

3.5.2 10% zinc glass

Release profiles for 10 w/w% bioactive zinc glass loaded formulations were also analyzed in neutral and acidic environments. Two formulations were used, both containing 10 w/w% bioactive zinc glass, one with 5 w/w% microcapsules and the other with no microcapsules. Each formulation was exposed to both neutral and acidic environments, creating four data series. Fig. 9 demonstrates the normalized concentration of zinc released per gram of formulation as a function of time in hours.

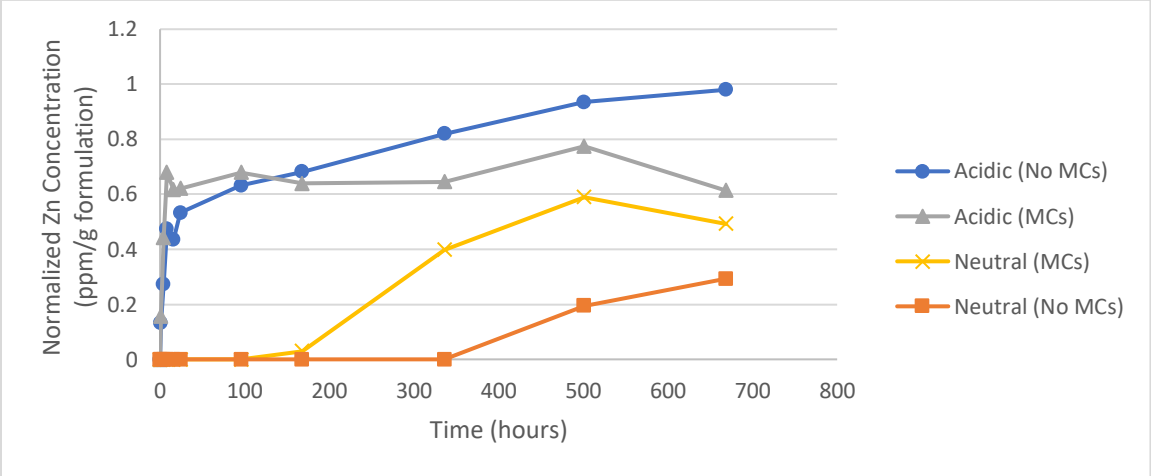

Figure 9, The graph shows the normalized concentration of zinc in ppm per gram of formulation as a function of time in hours. Two formulations were exposed to a neutral

environment of nanopure water and an acidic environment of acetic acid buffer solution (pH = 4.5), creating four series of data. Both formulations were loaded with 10 w/w% bioactive zinc glass. One was also loaded with 5 w/w% of microcapsules (2% 5.0 M calcium nitrate tetrahydrate, 2% 0.8 M sodium fluoride, and 1% 3.0 M potassium phosphate dibasic), while the other had no microcapsules

The cement loaded with microcapsules in the acidic environment exhibited the fastest zinc release (0.679 ppm/g formulation at eight hours). At 168 hours, the non-microcapsule containing formulation surpassed the cement containing microcapsules, both in the acidic environment. The non-microcapsule loaded cement released the most zinc over time (0.980 ppm/g formulation at 669 hours), almost 0.4 ppm/g formulation more than the microcapsule containing cement at the same time (0.614 ppm/g).

In the neutral environment, the cement with microcapsules released more zinc than the cement without microcapsules at all time points. The microcapsule containing cement released 0.589 ppm/g formulation at 501 hours, compared to 0.195 ppm/g for the cement absent of microcapsules. However, at 669 hours, the cement with microcapsules only released about 0.2 ppm/g more than the cement without microcapsules. It is important to note that the formulations exposed to the acidic environment released more zinc at all time points.

3.5.3 15% zinc glass

Release profiles for cements loaded with 15 w/w% bioactive zinc glass were compared. The two formulations of 15 w/w% bioactive zinc glass (one with 5 w/w% microcapsules, one without) were exposed to both neutral and acidic environments. Fig. 10 displays the normalized zinc concentration in ppm/g formulation as a function of time in hours.

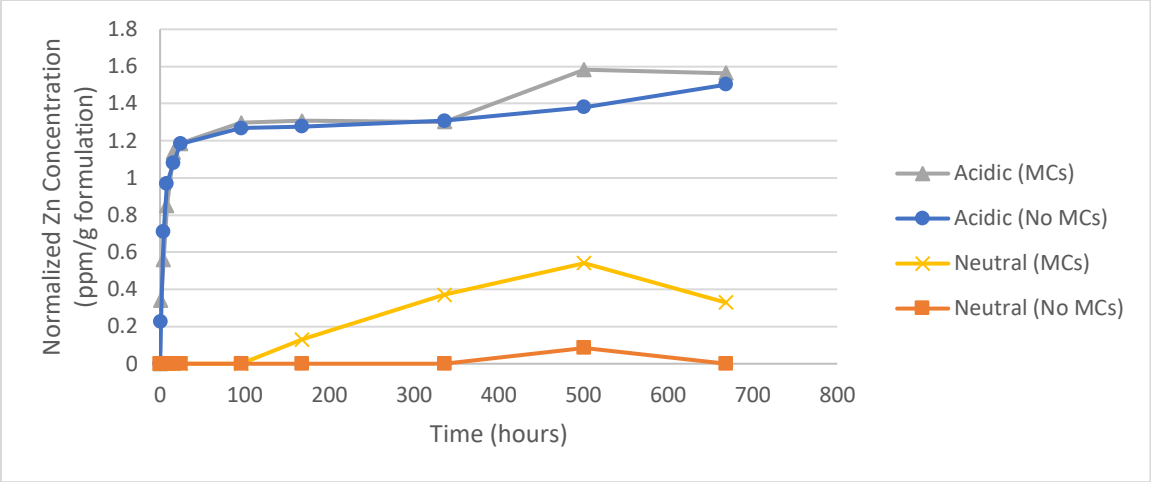

Figure 10, The graph shows the normalized concentration of zinc in ppm per gram of formulation as a function of time in hours. Two formulations were each exposed to a neutral environment of nanopure water and an acidic environment of acetic acid buffer solution (pH = 4.5), creating four series of data. Both formulations were loaded with 15 w/w% bioactive zinc glass. One was also loaded with 5 w/w% of microcapsules (2% 5.0 M calcium nitrate tetrahydrate, 2% 0.8 M sodium fluoride, and 1% 3.0 M potassium phosphate dibasic), while the other had no microcapsules

The release profiles for both formulations in the acidic environment displayed in Fig. 10 are very similar. Rapid zinc release was seen, as both formulations passed the 1 ppm/g formulation mark in the first 16 hours. They separate briefly at 501 hours, when the microcapsule containing cement release about 0.202 ppm/g more than the cement without microcapsules. However, the release profiles came together again at 669 hours, when both cements released about 1.5 ppm/g formulation.

In the neutral environment, again less zinc of released. The microcapsule containing cement released more zinc than the cement without microcapsules at all times. The largest difference was at 501 hours, when the cement with microcapsules released almost 0.5 ppm/g formulation more than the cement without microcapsules. It is important to reiterate that the acidic environment caused much more zinc to be released from these formulations. All data can be found in Table 6.

Comparison of Release Profiles in Neutral and Acidic Environments												
Time (hrs)	5%				10%				15%			
	MCs	No MCs	MCs	No MCs	MCs	No MCs	MCs	No MCs	MCs	No MCs	MCs	No MCs
	Acidic		Neutral		Acidic		Neutral		Acidic		Neutral	
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.026	0.064	0	0	0.157	0.131	0	0	0.341	0.226	0	0
4	0.326	0.152	0	0	0.442	0.272	0	0	0.558	0.710	0	0
8	0.220	0.245	0	0	0.679	0.474	0	0	0.850	0.971	0	0
16	0.232	0.216	0	0	0.616	0.436	0	0	1.137	1.081	0	0
24	0.245	0.255	0	0	0.621	0.533	0	0	1.185	1.184	0	0
96	0.292	0.357	0	0	0.679	0.631	0.000	0	1.297	1.268	0	0
168	0.342	0.408	0	0	0.640	0.681	0.029	0	1.307	1.277	0.130	0
336	0.396	0.477	0.537	0	0.645	0.820	0.398	0	1.302	1.307	0.370	0
501	0.505	0.600	0.032	0.070	0.775	0.935	0.589	0.195	1.583	1.381	0.541	0.085
669	0.525	0.609	0.030	0.183	0.614	0.980	0.492	0.293	1.564	1.502	0.329	0

Table 6 displays the values of all release profiles in section 3.5. The release profiles represent six formulations in total, all exposed to a neutral environment of nanopure water and an acidic environment of acetic acid (pH=4.5). Two formulations were manufactured with 5 w/w% loadings of bioactive zinc glass. One of these formulations was then loaded with 5 w/w% of microcapsules (2% 5.0 M calcium nitrate tetrahydrate, 2% 0.8 M sodium fluoride, and 1% 3.0 M potassium phosphate dibasic), and the other was left absent of microcapsules. The same of done for cements containing 10 and 15 w/w% bioactive zinc glass. All values are given in ppm/gram formulation.

3.6 Comparison of Microcapsules and Bioactive Zinc Glass

A comparison of the two release methods in this study, microencapsulation and bioactive zinc glass, was made when loaded at 5 w/w% into an orthodontic cement paste formulation. The first formulation with loaded with 2.5 M zinc sulfate heptahydrate microcapsules and exposed to a neutral environment. The second cement was manufactured with bioactive zinc glass and exposed to both neutral and acidic environments. Fig. 11 shows normalized zinc concentration in ppm/g formulation as a function of time in hours.

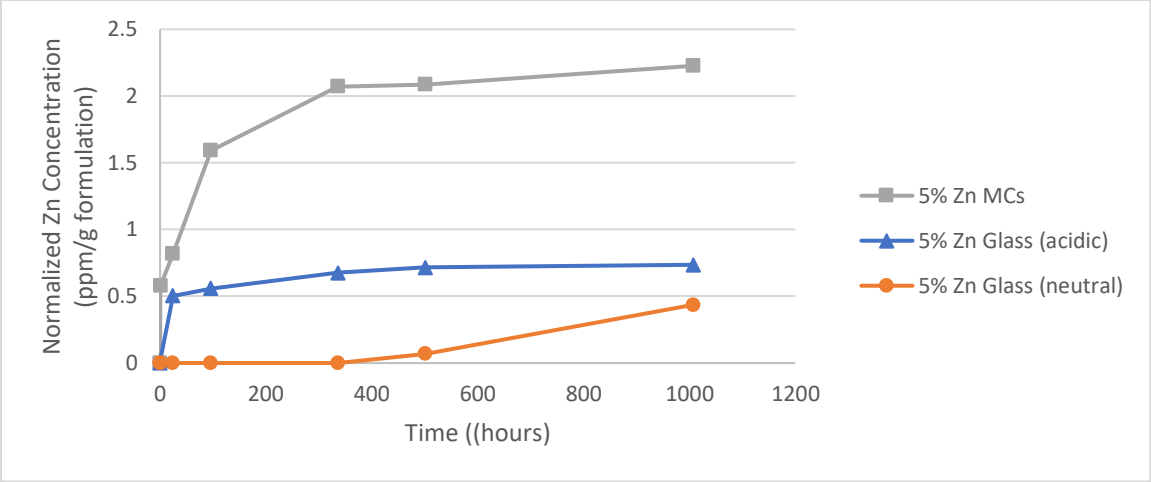

Figure 11, The plot shows normalized zinc concentration in ppm per gram of orthodontic cement formulation as a function of time in hours. Two cements were used in this assay. The first was loaded with 5 w/w% 2.5 M zinc sulfate heptahydrate microcapsules and exposed to a neutral environment. The second was loaded with 5 w/w% bioactive zinc glass and exposed to both neutral and acidic environments.

As illustrated in Fig. 11, the 2.5 M zinc sulfate heptahydrate microcapsules released 2.2 ppm zinc/g formulation. The bioactive zinc glass formulations released 0.6 ppm/g in acidic conditions and 0.4 ppm/g in neutral conditions. The microcapsule-loaded cement released the same amount of zinc in the first hour as the bioactive zinc glass cement did in the entirety of this study (in acetic acid).

Chapter 4

Discussion

It has been demonstrated that microencapsulation and bioactive glass are capable of releasing zinc when incorporated into an orthodontic cement. Previous research has shown that zinc ions can be useful in fighting the progression of plaque, calculus, and gingivitis [7, 11, 50, 100, 127]. The current study analyzed the efficacy of the mentioned zinc release mechanisms when incorporated into an orthodontic cement paste formulation in neutral and acidic conditions.

A novel, retentive polymer has been developed for the delivery of ions. As seen in Fig. 1, the ethylene glycol-polyurethane microcapsules released over 60 ppm zinc/g of microcapsules. This is in agreement with previous work, which has demonstrated that these microcapsules are capable of releasing calcium, fluoride, and phosphate ions [33, 14]. When incorporated into cement formulations, the higher percent loading of 2.5 M zinc sulfate heptahydrate microcapsules (7 w/w%) released 2.1 ppm zinc/g formulation more than the 5 w/w% loading, as expected (Fig. 2). The 7 w/w% formulation released a maximum of 4.3 ppm zinc/g formulation. These weight percent loadings were chosen because Gubrud and Adler showed that the mechanical properties of the cement are not compromised at these levels [48, 3].

Release profiles are erratic when microcapsules containing different ions are loaded into a cement. Fig. 3, illustrates fluctuating zinc release when sodium fluoride, calcium nitrate tetrahydrate, and potassium phosphate dibasic microcapsules are also incorporated into the formulations. This is likely due to complexation with other ions leading to either increased solubility in the case of zinc nitrate (k_{sp} = 206, compared to 35.7 for zinc sulfate) or precipitation in the case of zinc fluoride or zinc phosphate, which are insoluble in water.

Melt-derived bioactive zinc glass containing 10 w/w% ZnO was also analyzed in this study. Release profiles are displayed from cements loaded with 5, 10, and 15 w/w% of this bioactive zinc glass in neutral pH (Fig. 4) and in low pH (Fig. 5). Interestingly, in a neutral environment of pH 7, the 10 w/w% loading released more zinc than the 15 w/w% loading, as did the 5 w/w%. This can be seen in Fig. 4a and Fig. 4b. These results may be explained by increased chemical durability of the glass due to the incorporation of zinc, as suggested by previous research [76, 79]

Although this previous research was not motivated specifically by the zinc releasing potential of bioactive glass, the findings can still be applied to the current study. Linati showed that varying the chemical nature and the concentration of the glass constituents can create new properties that can be created for specific applications [76]. Specifically, the addition of zinc improves the chemical durability of silicate, borosilicate, and phosphate glasses in water [76, 79]. Other scholars agree that zinc slows down the degradation and overall leaching activity of glass [50, 6]. This phenomenon is explained by Lusvardi and Linati, through molecular dynamics simulations. These simulations suggest that zinc adopts a tetrahedral coordination irrespective of its concentration and copolymerizes with the silicon tetrahedral, which causes an overall complexation of the network and increase of reticulation [76, 79]. Thus increasing the chemical durability in water. In another study, Lusvardi suggested that zinc enhances the chemical durability of glasses by acting as a chaperone for the insertion of phosphorus into the glass network [79]. This may explain the slow dissolution rate of zinc glass in water.

Moreover, the initial step of glass degradation involves the exchange of sodium with H_3O^+ ions in the solution. This is blocked by the progressive obstruction of the percolation channels used for the diffusion of Na ions as a function of Zn concentration [76]. Furthermore, the excess of negative charge on $(ZnO_4)_2$ tetrahedrals restrains the sodium ions mobility even more [6]. Therefore, high concentrations of zinc seem to be responsible for the drastic reduction in the overall leaching activity of the zinc.

We propose that the incorporation of bioactive zinc glass had an insignificant effect of the chemical durability of the cement as a whole at the 5 w/w% and 10 w/w% levels, resulting in higher zinc release from these formulations. We hypothesize that it isn't until the bioactive zinc glass is loaded at 15 w/w% does it improve the chemical durability of the orthodontic cement in water, which manifests in very low levels of zinc release for this formulation. Fig. 4a illustrates that the amount of zinc released from the 15 w/w% loading was near the detection limits of atomic absorption spectroscopy, while the 5 and 10 w/w% loadings both released over 0.5 ppm/g formulation.

Considering that during a cariogenic challenge the pH value drops as a result of the metabolic activity of the plaque microbes [40]. Featherstone and others as well as Ten Cate explained that laboratory models should simulate this [34, 124]. Therefore, the effect of an acidic environment on the release of zinc from these cements was analyzed.

As seen in Fig. 5a and 5b, the orthodontic cement formulations released more zinc in acid as more bioactive zinc glass was incorporated. Table 4 shows the 5, 10, and 15 w/w% bioactive zinc glass cements released 0.6, 1.0, and 1.5 ppm zinc/g formulation at their maximums, respectively. Previous research has found that dental materials generally release more ions in acidic conditions [10, 60]. It is noteworthy that most of these

materials release ions via dissolution mechanisms. This is likely due to the degradation of both the polymer and glass in acidic conditions, as it is in many of the studies previously referenced. Mueller showed that BIS-GMA-based polymers are highly susceptible to chemical softening [90]. Asmussen and Wu demonstrated a softening of composites stored in solvents, such as ethanol and organic acids, that are present in the plaque [8, 85, 90, 136]. Moreover, Watts showed that the surface integrity of a compomer remained intact in neutral environments but softened under acidic conditions, due to the loss of structural ions from the glass phase [1, 131]. In low-pH environments, compomers demonstrate increased solubility and selective dissolution of matrix-forming elements, calcium, strontium, and aluminum [35, 131]. The dissolution of the matrix-forming elements by a low pH may result in the extraction of zinc from the depths the matrix [1, 31]. Also, acid etching increases surface, which in turn increases ion release [6, 36, 133]

Moreover, zinc oxide, the zinc compound found in the bioactive zinc glass used, is an amphoteric species. Yoshida confirmed its reactivity in acid, reporting that leaching rates of zinc oxide increase as pH values decrease. We propose that the degradation of the polymer due to acidic conditions overwhelms the increased chemical durability of the 15 w/w% bioactive zinc glass cement. This, along with the increased solubility of zinc oxide in acid, results in a directly proportional relationship between amount of zinc glass loaded in a given formulation and the amount of zinc released in acidic conditions.

Fig. 6a and b illustrate the release of zinc from formulations containing increasing loadings (5, 10, and 15 w/w%) of bioactive zinc glass along with microcapsules in a neutral environment. The ions encapsulated were 2 w/w% 5.0 M calcium nitrate tetrahydrate, 2 w/w% 0.8 M sodium fluoride, and 1 w/w% 3.0 M potassium phosphate

dibasic. This assortment of microcapsules will be referred to as the calcium, phosphate, and fluoride containing microcapsules for the remainder of this thesis. As seen in Fig 6b, zinc mobilizes from the cement to a maximum concentration of 0.6 ppm/g formulation after a short induction period before decreasing toward zero. Zinc oxide has low solubility in water. So, other factors are likely acting on the cement to invoke the release of zinc. A potential explanation could be nitrates from the calcium nitrate tetrahydrate microcapsules adsorbing onto the surface of the bioactive zinc glass and extracting zinc from the glass by forming zinc nitrate, which would then dissolve in solution. However, over time the release profile trends toward zero. This may be explained by zinc complexing with other ions in the microcapsules such as phosphate and fluoride, which are both essentially insoluble.

The same three formulations (5, 10, and 15 w/w% bioactive zinc glass with the calcium, phosphate, and fluoride containing microcapsules) were also tested in an acidic environment. Release profiles for this assay can be seen in Fig. 7a and b. Fig. 7b closely resembles Fig. 5b, which displays orthodontic cement formulations loaded with 5, 10, and 15 w/w% without microcapsules in an acidic environment. This suggests that the pH of the solution has a much greater effect on zinc release than the incorporation of the calcium, phosphate, and fluoride containing microcapsules. However, the microcapsules do have a small, highly nuanced effect, which will be discussed later. We believe the increased solubility of zinc oxide in acid compared to water and the increased degradation of the cement as a whole are the main reasons for the greater zinc release in low pH.

To take a different look at the data, zinc release profiles of orthodontic cements containing a constant loading of bioactive zinc glass, while changing other variables, namely the pH of solution and incorporation of the calcium, phosphate, and fluoride containing microcapsules are displayed. In Fig, 8 and 9, it is seen that the cement loaded with microcapsules bathed in an acidic environment released zinc at the fastest rate during the first one hundred hours. As previously mentioned, zinc oxide's increased solubility in acid and nitrates adsorbing onto the surface of the glass are possible explanations for this. Also, the microcapsule-containing cements are inherently more polar due the micron sized pockets of solution distributed throughout the material. This increase polarity could also be affecting the release of zinc. After the first one hundred hours the release profile of the microcapsule-containing cement in acid is surpassed by the formulation without microcapsules. Again, this is possibly explained by zinc complexing with phosphate and fluoride and precipitating out of solution.

The presence of the calcium, phosphate, and fluoride containing microcapsules has a much smaller effect of zinc release when the cement is loaded with 15 w/w% bioactive zinc glass and exposed to low pH, as seen in Fig. 10. We believe the acidic environment is causing the zinc oxide to release zinc ions at a large enough rate to overwhelm the effects of the microcapsules in this formulation. However, the calcium, phosphate, and fluoride containing microcapsules appears to play a role in the release of zinc from these formulations in neutral conditions. Fig. 9 and 10 show the microcapsule containing cements release more zinc in neutral conditions compared to the cements absent of microcapsules. We hypothesize the adsorption of nitrates onto the surface of the glass and the subsequent formation of zinc nitrates to effectively extract zinc from the

glass into solution, and the increased polarity of the materials as potential explanations of this.

Fig. 11 demonstrates that microencapsulation releases more zinc than bioactive glass (in acidic or neutral conditions) when loaded into an orthodontic cement at 5 w/w%. These microcapsules appear to have two notable differences compared to bioactive zinc glass. First, as seen in Fig. 11 the microcapsules release 1.6 ppm zinc/g formulation more than the bioactive zinc glass in acid, and 1.8 ppm zinc/g formulation more when the glass is in a neutral environment. Secondly, the microcapsules do not use a dissolution mechanism for release, like the bioactive zinc glass. Therefore, in theory, the mechanical properties are not compromised as more zinc is released as proposed by [88, 93].

Future research could take the direction of synthesis of nanoscale bioactive glasses. Studies suggest that bioreactivity can be enhanced or modified and controlled to a greater extent if nanoparticles are available, as opposed to conventional micron-sized powders seen in this study [105]. There is evidence in the literature that faster deposition or mineralization of tissues such as bone or teeth is possible when these tissues are in contact with nanoscale particles, [70, 101]. Additionally, bioactive silicate glass nanoparticles can produce a higher alkalinity, which could buffer to a greater extent the acidic degradation of polymers [128]. Perhaps glasses of this scale could be more effective. However, it is usually difficult to synthesize bioactive glasses in nano-size scale with addition of zinc ions [7] and glasses of this scale have extreme effects on the handling properties of composite resins. Therefore, application of nano-scale bioactive glasses may be limited to dentifrices and rinses. Finally, it is known that electrostatic binding is the means by which charged antibacterials are retained in the oral cavity, but

these interactions are not currently understood in detail. Further knowledge could provide valuable information as how to best manufacture materials for delivery of antibacterials.

Chapter 5

Conclusion

It has been demonstrated that microencapsulation and bioactive zinc glass are both viable methods of releasing zinc ions into the oral cavity. Bioactive zinc glass releases more zinc in acidic conditions when loaded into an orthodontic cement paste, and this release is via dissolution. When compared at an equal weight percent, microencapsulation releases over three times the amount of zinc as bioactive zinc glass. It is noteworthy that microencapsulation releases zinc by diffusion, as opposed to dissolution. Future research should explore the efficacy of zinc ions at the reported levels in fighting plaque and calculus accumulation around orthodontic bracket as well as the progression of gingivitis. Further understanding on how charged antimicrobials are retained in the oral cavity may provide useful knowledge for future release methods.

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