THE DEVELOPMENT OF SUSTAINED FLUORIDE-RELEASING O-RINGS FOR THE PREVENTION OF WHITE SPOT LESIONS: AN IN VITRO STUDY

A Thesis

by

KATIVA JASMINE STRICKLAND

Submitted to the Graduate and Professional School of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Peter H. Buschang
Committee Members,	Yan Jing
	Amal Noureldin
	Reginald W. Taylor
Head of Department,	Madhu K. Nair

May 2022

Major Subject: Oral Biology

ABSTRACT

Background and Purpose

There is no ideal way to prevent white spot lesions. Current fluoride-releasing products exhibit an initial burst, which severely limits the duration of the effects. O-rings incorporated with fluoride have been previously studied for a more long-term continuous release of fluoride, however, have been unsuccessful in attaining therapeutic levels to prevent white spot lesions. The present study will utilize a novel method of dip coating O-rings to achieve sustained fluoride release.

Materials and Methods

To solve the challenge, we developed a novel strategy to incorporate calcium fluoride (400mg/ml) into 2.5%, 5% or 10% Polycaprolactone, and coated O-rings (n=16). Control and calcium fluoride O-rings were placed on upper lateral incisor brackets and soaked in distilled water. The amount of released fluoride was tested weekly for 7 weeks. Elastic properties were assessed with the Instron Mechanical test. Scanning electron microscopy investigated the integration of fluoride onto the O-rings.

Results

The average release rates of the 2.5%, 5% and 10% groups were 5.3, 10.3 and 10.0 (μg F^- /ring/day, respectively. These were all higher than the therapeutic range (1.2-2.8 (μg F^-/ring/day) and much lower than the toxic level (51 μg F^-/ring/day). At the 7^{th} week, the average daily release rates for 2.5%, 5% and 10% groups were 0.7, 6.5 and 7.0 (μg F^-/ring/day, respectively, indicating a continuous effective fluoride release in 5% and 10% groups. Scanning electron

microscopy showed successful integration of calcium fluoride onto the outer layer of the O-ring. Instron test revealed no significant change in elastic properties of the O-rings after coating.

Conclusions

The 5% and 10% groups of calcium fluoride O-rings exhibited long-term sustained and therapeutically effective fluoride release. This novel method can effectively and reliably coat PCL incorporated with calcium fluoride on the surface of O-rings. The calcium fluoride O-rings maintain elastic properties even after this dip coating modification. This study is promising for the possibility of calcium fluoride O-rings as a product for the prevention of white spot lesions in orthodontic patients.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Yan Jing, for providing foundation for the entire project and guidance through this process. Thank you to my committee co-chair, Dr. Peter Buschang for your support and assistance with statistics of this project. Thank you to Dr. Chi Ma for helping tremendously with the research design and technical lab support. Thank you to Dr. Amal Noureldin and Dr. Reginald Taylor for helping advise and provide guidance with reviewing the literature.

I would also like to thank my co-residents and the Class of 2022 for providing friendship and comradery throughout residency. I cannot wait for what our futures hold and to continue sharing clinical advice and knowledge as colleagues.

To my parents, Bill and Carista, and my sisters, Natasha and Sarina – thank you for always being my foundation throughout this long academic journey. And thank you to my fiancé, Jimmy, for your endless support and love throughout dental school and residency.

CONTRIBUTORS AND FUNDING

Contributors

This work was supported by a thesis committee including Dr. Yan Jing, Dr. Peter Buschang, and Dr. Reginald Taylor of the Department of Graduate Orthodontics, Dr. Chi Ma of the Department of Biomedical Sciences, and Dr. Amal Noureldin of the Department of Public Health.

Thesis work was completed by the resident, under the advisement of Dr. Yan Jing of the Department of Graduate Orthodontics.

Funding

Funding was provided by a research budget from the Texas A&M College of Dentistry Graduate Studies and the Robert E. Gaylord Endowed Chair in Orthodontics. Funding also provided by the American Association of Orthodontists Foundation (AAOF) Biomedical Research Award to Yan Jing (NIDCR K08 DE028593).

NOMENCLATURE

Ca-F	Calcium fluoride
EDX	Energy dispersive X-ray
ISE	Ion selective electrode
N	Newtons
O-ring(s)	Elastomeric O-ring(s)
PCL	Polycaprolactone
PEVA	Polyethylene co-vinyl acetate
ppm	Parts per million
SEM	Scanning electron microscopy
TISAB	Total ionic strength adjustment buffer
WSL(s)	White spot lesion(s)

TABLE OF CONTENTS

Page

ABSTRACT…… ii

ACKNOWLEDGEMENTS…… iv

CONTRIBUTORS AND FUNDING……..... v

NOMENCLATAURE……..... vi

TABLE OF CONTENTS…… vii

LIST OF FIGURES……..... ix

LIST OF TABLES……... xi

1. LITERATURE REVIEW……... 1

Introduction to White Spot Lesions…… 1
Treatment of White Spot Lesions……... 3
Prevention of White Spot Lesions……... 4
Fluoride Use in Prevention of White Spot Lesions…… 5
Introduction to O-Rings and Purpose in Orthodontics…… 7
O-Rings Materials and Properties…… 8
Fluoride Integration of O-Rings…….. 9
Burst Effect of Fluoride…….. 10
Previous Fluoridated O-Rings Studies…… 11
Calcium Fluoride for O-Rings…… 13
PCL and Materials Chemistry Overview…… 14
Introduction to Present Study…… 14

2. MATERIALS AND METHODS……... 16

Preparing Fluoridated O-Rings……..…….. 16

Scanning Electron Microscopy…… 19
Fluoride Release Test…… 19
Instron Mechanical Test…… 20
Statistical Analysis…… 21

3. RESULTS…… 23

Fluoride Integration…… 23
Fluoride Release…… 23
Elastic Properties…… 25

4. DISCUSSION…… 27

Improved Release Profile of Fluoride…… 27
Efficient Coating Method …… 31
Maintained Elastic Properties …… 33
Limitations and Future Studies …… 35

5. CONCLUSIONS…… 37

REFERENCES…… 38

APPENDIX A FIGURES…… 45

APPENDIX B TABLES…… 55

LIST OF FIGURES

Page

Figure 1	Workflow for Solution A, B, and C..……	45
Figure 2	Half-Branch of O-Rings……	45
Figure 3	O-Rings set on Brackets……	46
Figure 4	Fluoride Release Test Tubes……..…	46
Figure 5	O-Rings for Instron Mechanical Test……	47
Figure 6	O-Ring on Stainless Steel Hooks…...……	47
Figure 7	O-Ring Stretched…….……..	48
Figure 8	Cross-Section of O-Ring……	48
Figure 9	Thickness of PCL Layer..……	49
Figure 10	Percentage of Ca and F ions in PCL Matrix and O-Ring Matrix..…...……..	49
Figure 11	Percentage mass of F and Ca..…….……	50
Figure 12	Daily Fluoride Release Rate in 1st Week……...…….……..	51
Figure 13	Daily Fluoride Release Rate by Week..…..…….……..	51
Figure 14	Total Fluoride Accumulated...…….……	52
Figure 15	Loading Amount of O-Rings at T1..…….……	52
Figure 16	Change in Loading Amount of O-Rings from T1 and T2..……...……	53
Figure 17	Loading Amount of O-Rings at T2..…….……	53

Figure 18 Instron Test T1 Curve……..……..…… 54

Figure 19 Instron Test T2 Curve..……..…… 54

LIST OF TABLES

		Page
Table 1	Daily Fluoride Release Rate in 1st Week..……	55
Table 2	Daily Fluoride Release Rate by Week..……	56
Table 3	Total Fluoride Accumulated..……...……	57
Table 4	Instron Mechanical Test at T1, T2, and Change……..	57
Table 5	Instron Mechanical Test comparing T1 and T2 ……..	58

1. LITERATURE REVIEW

Introduction to White Spot Lesions

White spot lesions are characterized as the first sign of caries lesion on enamel that can be detected with the naked eye.[1] This decalcified enamel has an opaque and chalky appearance, refracting light differently than sound enamel with increased susceptibility to staining.[2] These lesions are more abundant in patients with fixed orthodontic appliances compared to those without appliances.[3] Thus, white spot lesions pose an esthetic problem after orthodontic treatment.[4]

As the environment of the oral cavity filled with saliva wavers in pH and undergoes the balance of the remineralization and demineralization processes of enamel, the decalcification process can occur when enamel is exposed to extended acidity. Decalcification, or the loss of calcified tooth substance, occurs during this change in pH environment in the presence of oral microbes and fermentable carbohydrates. This leads to the dissolution of calcium and phosphate ions out of enamel. Thus, an opaque, chalky, white spot develops, and cavitations may occur.[5]

To reverse the process of demineralization, there must be a reintroduction of calcium and phosphate ions into the enamel, thus a progression to remineralization. The remineralization process involves helpful components of saliva and therapeutics, involving reintroduction of calcium and phosphate ions to enamel structure concurrently with resolution in pH. Therapeutics, such as fluoride, can aid in this remineralization process. However, when the demineralization process takes hold, the enamel is susceptible to white spot lesions. The accumulation of plaque and poor oral hygiene pose as the main risk factors to the development of white spot lesions, as bacteria such as Streptococcus mutans and Lactobacillus incite the disease process of increasing acidity.[6]

According to the literature, there are 2 stages of white spot lesion development. First, surface softening occurs in that there is removal of the interprismatic substances and mineral loss on the enamel surface. Second, the mineral dissolution continues to invade deeper parts of the enamel, leading to a porous layer on the surface that is still mineral-rich, surrounding the inner mineral-poor area.[7, 8]

During orthodontic treatment, fixed appliances and brackets serve as harbors, attracting and instigating the accumulation of plaque.[9] Thus, white spot lesions begin to form as the enamel loses its sound structure, and now an optical difference in refraction of light from the tooth presents with a chalky, opaque appearance. From the start of orthodontic treatment, white spot lesions typically form within the first 6 months of treatment. White spot lesions can even arise after 4 weeks of plaque accumulation and retention around the fixed appliances.[10, 11] This poses a significant esthetic and pathogenic issue in patients undergoing orthodontic treatment, 25% of whom will experience these decalcifications.[2]

The prevalence of white spot lesions in orthodontic patients varies throughout the literature. A meta-analysis summarizes the literature to show the varying prevalence of WSLs in orthodontic patients as well as incidence of new WSLs. This revealed a range from 23-75% incidence and 33-97% prevalence, displaying an inconsistency in the literature.[12]

The most common teeth presenting with white spot lesions after orthodontic treatment are the maxillary lateral incisors.[13-15] Overall, the maxillary teeth more commonly develop WSLs than mandibular teeth, which is attributed to more saliva available in the mandibular regions to protect against WSLs. Furthermore, the smaller area of tooth surface of the maxillary laterals is more difficult to keep clean and can retain more plaque.

In terms of the most implicated surfaces of teeth, several studies determine that WSLs are most commonly found on the gingival margin or the gingival third of the facial surface of the teeth, most likely due to the area being more plaque retentive with fixed appliances and more difficult to access for proper hygiene.[13, 16-18] The gingival or cervical regions have greater aprismatic enamel, less density, and inferior etch quality for preventive sealants, explaining further the propensity for WSLs in this region.[19]

White spot lesions remain as one of the most common adverse effects of orthodontic treatment.[20] The prevalence of white spot lesions in orthodontic patients continues to be a pressing issue, as the risk of developing white spot lesions increases with poor oral hygiene and extended orthodontic treatment times.[21] In previous studies, it has been shown that orthodontic patients show higher incidence of white spot lesions than those who are untreated.[4] Furthermore, orthodontic treatment contributes to development of these enamel demineralization areas and severity of enamel opacities.[22] White spot lesions present an issue with esthetics for orthodontic patients, and as such, different modalities of prevention and treatment have been studied.

Treatment of White Spot Lesions

Despite these recommended prevention protocols, white spot lesions still develop in orthodontic patients and the need for treatment presents itself. Various treatment modalities include, fluoride applications, tooth whitening, casein derivates, laser, microabrasion, resin infiltration, and restorations such as direct composites or veneers.[20, 23] In the treatment of white spot lesions, it has been noted, however, that highly concentrated fluoride regimens can worsen the appearance of white spot lesions.[24] There are still downsides to treatment of WSLs. First, there is still a need for patient cooperation with some treatments that require applying a paste or

gel. Furthermore, there may be chair time required for clinical application of medicaments. These treatments can also present with expensive costs. With restorations specifically, there is invasiveness as there is a need to reduce and restore tooth structure. And lastly, there are lifetime replacement needs for restorations.[25] Overall, emphasizing preventive techniques mitigates the need for treatment of white spot lesions, and this may be solved by further investigating the use of fluoride.

Prevention of White Spot Lesions

This rampant issue resulting from orthodontic treatment with fixed appliances poses a demand for both prevention tactics and treatment modalities to solve this problem. First and foremost, good oral hygiene regimens and healthy diets with less fermentable carbohydrates help in prevention of white spot lesions. Currently, there is no known way to effectively prevent white spot lesions as shown adequately by evidence.[26]

Thus, there is still research to be done regarding the most efficient means of prevention of white spot lesions. Oral hygiene and effectiveness in removal of plaque are at the forefront of white spot lesion development.[15] This includes the patient having a regular hygiene recall appointment with their dentist. Since there is a relationship between the amount of salivary flow and the resistance to white spot lesion development, adequate salivary flow provides proper buffers to combat the demineralization cycle.[14]

In efforts to implement prevention tactics, various delivery systems of fluoride have been utilized. Prevention protocols typically include brushing teeth with fluoridated toothpaste 2-3 times a day, regular prophylaxis every 4 months, fluoride varnish application every four months,[3]

and daily sodium fluoride rinses.[20] One study concluded that daily .05% sodium fluoride rinses significantly reduced white spot lesion development in orthodontic patients.[27] Further studies have investigated the use of intraoral fluoride devices, however, their use in clinical practice have not yet been validated.[28, 29] Additional prevention techniques studied include chlorhexidine rinses, xylitol products, and MI Paste Plus, or CPP-ACP application.[30, 31] Sealants and orthodontic adhesives can also be used as a prevention method against WSL development.

Much like the downsides of WSL treatment modalities, the prevention tactics also have their cons. Oral hygiene and compliance are required. Furthermore, the adolescent patient population develops WSLs twice as often as adults. This age group presents difficulties in oral hygiene habits and motivation.[17] Further deficits of prevention methods include chair time required for clinical application of medicaments. Specifically with sealants and orthodontic adhesives, issues with retention and wear become problematic in preventing WSLs. Modalities used to prevent white spot lesions currently are still up to debate, as many of the methods are dependent upon patient compliance. Overall, no prevention methods have shown superior efficacy or long-term coverage in the literature.

Fluoride Use in Prevention of White Spot Lesions

Fluoride products used for prevention of white spot lesions work in the following manner. Fluoride works to disrupt the demineralization process caused by bacteria in plaque, thereby inciting the development of fluorapatite to remineralize enamel and stop the development of white spot lesions. Fluoride halts the production of harmful acids by inhibiting the bacteria at play.[32] Therefore, fluoride both protects the tooth surface and inhibits bacterial growth.[33]

Protection by fluoride will cease if fluoride intake is terminated, thus, a continuous supply of fluoride is needed to maintain protection. Reduction of white spot lesions was reported with even small concentrations of 0.02 ppm-1.0 ppm, if provided continuously.[34]

Fluoride can be effective, however, some delivery systems of fluoride, such as daily rinses and pastes, require patient compliance to be effective in prevention.[11] Fluoridated toothpastes require over 1000 ppm for effectiveness in preventing white spot lesions, however, higher concentrations of 5000 ppm are recommended for brushing with at night.[20] Fluoride toothpastes alone do not prevent white spots in most patients, thus, adjunctive fluoride methods must be used, such as mouthrinses. Daily use of 0.05% NaF mouthrinse has been shown to prevent these demineralizations during orthodontic treatment.[35, 36]

Other fluoride delivery systems avoid the roadblock of depending on patient cooperation and compliance. Although good oral hygiene will always be a critical factor in WSL prevention. Alternate methods of fluoride delivery in the luting of orthodontic brackets by way of the adhesives and sealants can be utilized. A study evaluating the use of fluoride-releasing adhesives for bonding orthodontic brackets showed a decrease of 58% in white spot lesion development.[37] On the other hand, another study showed that with the addition of fluoride to adhesive for fixed appliance bonding ("Rely-A-Bond"), there was no significant reduction in white spot lesion prevalence.[38]

Issues with glass ionomer cements incorporated with fluoride include the high release of fluoride on the first day of application, followed by a rapid decrease, also known as the "burst effect". Although patient compliance is eliminated with the use of fluoridated adhesives and cements for orthodontic brackets, this "burst effect" in the release of fluoride is problematic in providing long-term prevention of white spot lesions.[39]

Furthermore, regular fluoride varnishes can be applied by the clinician, and do not depend on patient compliance. According to a study, application of fluoride varnishes shows less demineralization for teeth with orthodontic appliances compared to those without fluoride varnish.[3] Fluoride varnish application led to a 44.3% decrease in white spot lesion development in orthodontic patients when applied every 6 months. However, the release of fluoride was greatest over the first 3 weeks, with a slower rate of delivery afterwards.[40] Overall, fluoride has been repeatedly studied as a mechanism for white spot lesion prevention, however, there is still no significantly effective means of white spot lesion prevention in orthodontic patients.

Despite all these prevention efforts, 46% of orthodontic patients still develop WSLs.[6] Ultimately, there is a problem with the release profile of these fluoride prevention modalities, with the concentrations of fluoride diminishing even within a few hours. There is a need for fluoride delivery that is slowly released to have a greater effect in preventing white spot lesions.[41] Currently, there are no modalities to effectively provide sustained fluoride applications. Thus, the implementation of fluoride with elastomeric O-rings may provide a solution for this sustained release during orthodontic treatment with fixed appliances.

Introduction to O-Rings and Purpose in Orthodontics

The role of the elastomeric ligatures, or "O-rings", in orthodontics is to engage the archwires into bracket slots. Their ease of use and speed of application have made them more popular in comparison to steel tie wire ligation. Furthermore, the variety of colors provides an esthetic and enjoyable experience for the patient at their orthodontic appointments. These O-rings are widely utilized by orthodontists in the initial level and align stages of orthodontic treatment.[42, 43] O-rings are changed at each adjustment appointment about every 4-6 weeks.[44]

O-Rings Materials and Properties

An elastomer is a material that rapidly returns to original dimensions after deformation.[45] Elastomeric ligatures, made of a polymer called polyurethane, have properties of tensile strength, extension to tensile strength, toughness, and modulus of elasticity all contributing to their ability to be stretched or ruptured upon excess stretching. One study found that in a simulated oral environment, elastomeric rings had a decrease in all these properties over a 28-day period, relaying the reason why they should be replaced at each orthodontic adjustment appointment.[46] Another study found that force decay decreased during the first 24 hours.[47, 48] and reached 75% by 8 weeks.[49] Further studies have shown that after 4 weeks elastomeric O-rings show a force decrease of 42%-66%.[46, 50] Thus, the elastomeric O-ring material properties are influenced by the moisture and heat of the oral cavity. Although the O-rings tend to deteriorate in terms of their elastic properties, this does not prevent their performance in terms of engaging archwires into bracket slots.[43]

Polyurethanes are influenced by heat, moisture, and enzyme exposure leading to aging, degradation, and force decay. One study explored the issue surrounding stretching and the environment of the oral cavity leading to aging of the elastomeric rings and alteration of its chemical and elastic properties. It was found that the elastomeric rings accumulate a protein coating that mineralizes on the material's surface, altering its structure. In previous studies, solutions of ethanol and water intended as *in vitro* simulations have failed to truly replicate the environment of the oral cavity and may not be accurately representing the change in elastic and chemical properties of polyurethanes *in vivo*.[47, 48]

Because these elastomeric rings are made up of polymer, they can be expanded when dipped in a good solvent, followed by contraction when placed in a poor solvent.[51] Furthermore, if this

dipping of polymer into solvent is extended over longer periods of time, then the polymer can expand and more deeply absorb contents into its structure.[52] Therefore, the polymer can be modified to integrate other substances via polymer swelling.[53]

Fluoride Integration of O-Rings

Integration of fluoride into elastomeric O-rings can make an impact on the prevention of white spot lesions. Because elastomeric O-rings are placed to activate orthodontic wires into bracket slots for about 4-6 weeks between adjustments, the O-ring may be used as a medium for harboring fluoride. Previous studies aimed to provide a long-term low dose release of fluoride since elastomers can be applied with ease and are replenished at each orthodontic visit.[35, 36, 39, 54] The problem lies in that current fluoride-infused O-rings do not have a long enough release profile to have a therapeutic impact on preventing white spot lesions.[55]

In combination with fluoride toothpastes and rinses, fluoridated elastomeric O-rings showed significantly increased amounts of residual fluoride in the elastic modules.[54] This is due to imbibition and absorption, allowing these fluoridated O-rings to recharge with fluoride from the oral cavity and from toothpastes and rinses used. In terms of elastic properties, one study concluded that stretching the fluoridated elastomeric O-rings on premolar brackets in distilled water solution shows an increase in fluoride release of 13% compared to the O-rings that were not stretched.[56] This is due to stretching causing deformation of the polyurethane structure and filament detachment to allow for more fluoride availability for release.[47, 48] Therefore, the stretching of O-rings necessary for them to be applied clinically can increase their release of fluoride.[56]

Burst Effect of Fluoride

Other studies have delved into the issue of a "burst effect" from fluoridated elastomeric ligatures, however, they still have disadvantages that will be targeted in the present study. First, the "burst effect" occurred because the experimental designs involved coating the fluoride on the elastomeric O-ring's outer surface only. Thus, only superficial layers were coated with fluoride leading to a quicker release of fluoride. There are two main ways to add fluoride to O-rings, either by coating an extra layer on superficially or by impregnating the fluoride into deeper layers.

One study discovered that a "dip-coating technique" helped to alleviate the burst effect issue. This dip-coating technique involves dipping the rings in a sodium fluoride solution and leaving them to dry overnight, to reach deeper levels of the elastomeric O-ring.[39] By penetrating deeper levels of the O-ring, more fluoride is incorporated, and release of fluoride is lengthened rather than occurring rapidly. This deeper impregnation of fluoride will be targeted in the present study.

The short-term efficacy of previously fluoridated O-rings has been exhibited with a "burst effect", limiting the amount of time that the fluoride is effective.[57] The "burst effect" involves a rapid release of fluoride, preventing continuous release over longer periods of time that is necessary to be therapeutic in preventing white spot lesions. Previously studied fluoridated elastomeric rings have shown loss of fluoride levels *in vivo* after just the first week of application.[56] Studies related to the amount of fluoride coated on the rings showed that only those with higher fluoride content were able to release a therapeutic level of fluoride for white spot lesion prevention.[39] If able to release fluoride over a longer period of time, infused elastomeric rings may help better prevent white spot lesions than other methods.

Previous Fluoridated O-Rings Studies

The integration of fluoride into elastomeric rings for the prevention of white spot lesions in orthodontic patients has been explored in previous studies. Previous studies primarily have utilized the commercialized "Fluor-I-Ties" (Ortho Arch Company, Inc.) comprised of tin fluoride; however, they have since been discontinued. An *in vitro* study using these O-rings in distilled water examined over 6 months showed that there was an initial burst of fluoride in the first 48 hours, followed by a decrease with 63% of the fluoride released by the first week and 88% released by the second week. By the end of the first month, swelling of the elastomers had occurred due to imbibition and absorption of water. There was still potential for adequate release of fluoride over one month with further investigation needed.[58]

Another study compared these Fluor-I-Ties *in vitro* and *in vivo* in a split mouth study with 6 patients. However, this study still showed an initial burst effect in the first 48 hours in both settings. The *in vivo* experiment showed 70 times more residual or leachable fluoride absorbed from the oral cavity compared to *in vitro*. These O-rings needed to be replaced every month due to the swelling and imbibition that occurred by this time point. Thus, these O-rings were able to absorb fluoride, creating a reservoir for fluoride release if able to investigate in further studies how to provide continual release of low dose fluoride.[59]

Furthermore, a study exploring the same Fluor-I-Ties in a clinical trial in 49 patients showed a 10% decrease in WSLs, however, still exhibiting a burst effect within the first week of application.[18] Another study performed a split mouth clinical trial and showed significantly fewer WSLs compared to controls, however, still displaying an initial burst effect of fluoride release.[60] A study utilizing an intraoral model for an in-situ study with the same Fluor-I-Ties showed no significant reduction in WSLs due to the burst effect.[55] Furthermore, a study investigating the

Fluor-I-Ties *in vitro* found a that when the O-rings were stretched over brackets, they released more fluoride as compared to the O-rings statically releasing fluoride on their own in distilled water.[56] Overall, the studies with the commercial Fluor-I-Ties utilized the method of coating the superficial layer of the O-ring, which can explain why the burst effect of fluoride release occurred.

Another downfall that was not delved into or further investigated, was the potential decrease in elasticity of the O-rings when infused with fluoride, leading to potential rupture or failure of the O-rings, making them unusable. This was noted with both sodium and tin solutions to integrate fluoride into the O-rings' surface.[58]

To improve upon previous studies, the present study will look to utilize calcium in its first dip solution, which has lower solubility, thereby slowing the release profile of fluoride. Overall, previous studies evaluating fluoridated elastomeric O-rings have been conflicting, due to this "burst effect" exhibiting a short-term release of fluoride, as well as changes in the elastic properties of the rings leading to their rupture. The present study will look to resolve these gaps in previous methodologies.

To counter the "burst effect" that has occurred in previous studies, the present study will take on a different method of fluoride delivery through elastomeric O-rings. The therapeutic daily level of fluoride intake to prevent white spot lesions is .024-.05ppm, so to provide continuous fluoride delivery, the initial "burst effect" must be overcome.[39] A release profile of 1.2-2.8 μg F^-/ring/day is necessary to be in therapeutic range, with a 51 μg F^-/ring/day toxicity level.[61] In the present study, fluoride will be impregnated in deeper layers to avoid the "burst effect" previously described. The goal will be to provide more long-term, continuous release of

fluoride, while also maintaining the elastomers' mechanical properties to carry out its main function of holding archwires in bracket slots.

Calcium Fluoride for O-Rings

The use of calcium fluoride, which can more slowly release fluoride than NaCl or SnCl2 solutions, can circumvent the previously mentioned "burst effect" and has been known to reduce demineralization.[41] Calcium serves as a mediator in formation of reservoirs of fluoride in oral fluids.[62] Calcium fluoride, having lower solubility, can more slowly release fluoride ions than the other solutions and is effective in low pH environments.[63] Calcium fluoride works to reduce the solubility of enamel against acid formation by bacteria in plaque. In terms of its mechanism of action, calcium fluoride releases fluoride from its built-up reservoirs whenever the pH level drops in acidic environments. Calcium fluoride remains adhered to the enamel surface and works to combat the caries development process.[64] During neutral pH environments in saliva, calcium fluoride builds up phosphate ions and incorporates them to form a fluorapatite layer on the surface of enamel. In contrast, when the pH drops to acidic levels, the previously adsorbed phosphate ions are lost followed by slow dissolution of calcium fluoride, leaching fluoride ions for incorporation in the hydroxyapatite lattice. Thus, calcium fluoride provides pH-controlled reservoirs of fluoride ions for release during caries formation.[63] Therefore, the present study will explore the use of calcium fluoride as a means of slowing the release of fluoride from elastomeric O-rings.

Thus, the questions posed by this study include: will infusion of elastomeric O-rings with calcium fluoride provide a slower release profile of fluoride? Will the elastic properties of the O-rings change with the infusion of this fluoride?

PCL and Materials Chemistry Overview

Polycaprolactone, or PCL, is a polymer that has favorable mechanical properties of capability of forming mixtures with other polymers and biodegradability.[65] PCL has a semi-crystalline structure, has good solubility, and a low melting point, making it biocompatible.[66] These properties make PCL a favorable medium for harboring calcium fluoride and integrating or coating with the polyurethane of the O-rings, while still maintaining elastic strength. PCL is biodegradable, bioresorbable, FDA approved, and can be excreted and not accumulated in the body. PCL is used in medical settings, for example, in sutures, in drug delivery systems, nerve regeneration, tissue engineering, and in material to fixate bone fractures.[66] Showing versatility and wide use clinically, PCL is favored for being safe, with low toxicity and biodegradability.[67] PCL has been targeted for long acting, sustained drug systems with controlled release delivery.[68]

In dentistry, PCL has been used in combination with the composite Resilon to form a root canal filling material that shows a successful seal. PCL has also been utilized as a scaffolding material in bone grafting associated with dental implants.[66] Thus, the present study will utilize PCL as a method of fluoride coating for the O-rings.

Introduction to Present Study

An initial pilot study was conducted utilizing O-rings dipped with a solution of calcium fluoride combined with polycaprolactone. To protect elastic properties of the fluoride-infused O-rings, preparation with polycaprolactone coating helped to preserve mechanical properties of the polyurethane O-rings, while still integrating and delivering fluoride. This new method involving

dip in solution of polycaprolactone (PCL) and acetone with calcium fluoride showed successful elastic property test results in the pilot study, with no ruptures upon stretching.

To date, there have not been studies that address fluoride infused O-rings having a longer release profile of fluoride. Expected findings of this research include targeting an effective means of integrating fluoride into O-rings. With this integration, it is not only important that fluoride is loaded, but also that fluoride can be released slowly while still maintaining the chemical composition and elastic integrity of the O-ring to be effective.[58, 59]

Controversy at hand may pose a question of whether fluoride will detriment the efficacy of elastomeric O-rings in maintaining its chemical and elastic properties to carry out its primary purpose of securing archwires within orthodontic brackets. This research will aim to achieve both long-term fluoride release and maintaining the elastic and chemical properties of the elastomeric O-rings.

If findings are successful, these fluoride-loaded O-rings can provide a more long-term means of fluoride release for patients in orthodontic treatment who are at risk for white spot lesions. Furthermore, patient compliance requirements with this prevention method are reduced. Thus, it may provide increased prevention against white spot lesions over the long term, as compared to the other methods of white spot lesion prevention that rely on patient cooperation.

Comparisons will be made with literature on previous fluoridated elastomeric O-ring products. The results of this study can help set the stage for future *in vitro* and *in vivo* studies of fluoridated elastomeric O-rings in a clinical setting as a method of preventing white spot lesions in orthodontic patients.

2. MATERIALS AND METHODS

The design of this study was comprised of three parts. First, scanning electron microscopy (SEM) was utilized to confirm the loading amount and impregnation depth of calcium fluoride into the O-rings. Second, the release of fluoride was measured from the fluoridated O-rings. Third, the elastic property of the fluoridated O-rings was measured via Instron mechanical test. Pilot studies were conducted to determine the basis of the dip concentrations, methods of measuring elastic properties and ion concentrations of fluoride, and the necessary effect size. With the original proposed sample size (n=8), 80% power can be attained with an effect size of 2.5. For fluoride release testing, the sample size was n=16. For Instron mechanical testing and SEM, the sample size was n=8.

O-rings were placed into 4 different groups based on varying concentrations of dip solution of polycaprolactone and acetone with calcium fluoride (control vs. 2.5% vs. 5% vs. 10%). The research design was carried out in these three parts, which ultimately address the long-term release of fluoride from the infused O-rings while determining if there were any changes to their elastic properties.

Preparing Fluoridated O-Rings

Elastomeric O-rings (American Orthodontics, Sheboygan, WI; 6 Sticks, .045" ID/.115" OD, color Red, item #: 854-668) were prepared with a solution of calcium fluoride (Ca-F) and acetone mixed with polycaprolactone (PCL) and acetone, as detailed below. All O-rings were prepared by one principal investigator.

To prepare the calcium fluoride and acetone solution with the polycaprolactone and acetone solution, the following instructions were followed. The solutions can be altered to make varying concentrations of PCL and acetone with calcium fluoride (i.e., 2.5% vs. 5% vs. 10%). This involves mixing a solution A (polycaprolactone and acetone) with solution B (calcium fluoride and acetone). The following prepares a 2.5%, 5%, or 10% solution of PCL and acetone with calcium fluoride solution.

First, solution A was made by adding polycaprolactone (PCL) to an empty 50 ml test tube by pouring measured PCL (1 g for 2.5% group, 2 g for 5% group, 4 g for 10% group) into the tube, first by taring the empty tube on the scale before measuring the PCL. Next, 20 ml of acetone was poured into the same test tube. A stirrer tab was used to mix the solution of PCL and acetone on a stirrer and hot plate. The tube was placed on a vortex-genie to prevent clumping beforehand. At this point, the mixture of solution A was thoroughly stirred with no clumps. This process can occur overnight on a stirrer with no heat or can proceed on a stirrer with heat for faster mixing. After this process, solution A was ready for use. Using a small green syringe, 0.5 ml of the solution A (PCL and acetone) was injected into a 1.5 ml microcentrifuge tube.

The calcium fluoride solution, solution B, was prepared from calcium fluoride powder (5 g vial of powdered calcium fluoride) with 12.5 ml of acetone to achieve a 400 mg/ml solution of calcium fluoride to acetone, mixed in a 50 ml test tube. A vortex mixer was first used, followed by an ultrasonic mixer to ensure proper consistency. The 50 ml test tube containing the mixture of 5 g calcium fluoride powder with 12.5 ml acetone was placed in a glass beaker filled with ice to maintain the temperature and prevent overheating. Aluminum foil was used to cover the test tube once the ultrasonic probe was placed in the tube. The probe did not touch the sides or bottom of the test tube to prevent melting. The ultrasonic settings were as follows: Pulse: 5

seconds/5 seconds, Amplitude: 90%, Time: 30 minutes. At 5-minute intervals of the cycle, the test tube containing the mixture was monitored and checked for proper consistency. After 30 minutes, the solution was properly mixed and ready to prepare solution B. This new solution B is now stored in the refrigerator before use.

Finally, solution A and solution B were combined to make **solution C**, which was used for dipping the O-rings. 0.5 ml of solution B was added using a small green syringe to the microcentrifuge tube already containing 0.5 ml of solution A (Figure 1). The combined solutions were mixed using the vortex for a few seconds immediately before dipping with O-rings. The solution C was re-mixed using the vortex if the O-rings were not ready for immediate dipping, as the solutions may settle and not be of proper consistency.

Each stick of 6 O-rings was divided in half vertically to include 3 O-rings for each dip (Figure 2). A half stick of O-rings was held with tweezers and carefully dipped into the microcentrifuge tube containing the combined solutions for 1 second. It was quickly removed, then placed in its own petri dish and covered to prevent evaporation or drying. About 4 half sticks of O-rings were prepared per microcentrifuge tube of solution C. The following diagram and photos show the preparation of O-rings process using PCL solution.

32 O-rings were prepared for each concentration of dip solution (2.5%, 5%, and 10%). They were used for the second two parts of the study: fluoride release and Instron mechanical test. 32 O-rings for each concentration, plus 32 O-rings for controls, makes for a total of 128 O-rings. Within each group, 16 O-rings were utilized for the fluoride release test and 16 were utilized for the Instron mechanical test. For SEM, there were 8 O-rings used for analysis prior to soaking in distilled water. Reliability for the standardization of the dipping procedure was established by

utilizing 4 test tubes for each group in the fluoride release test, which was established at 99.9% for intraclass correlation coefficient.

Scanning Electron Microscopy, n=8

Scanning electron microscopy (SEM) was utilized to verify that the PCL and calcium fluoride dip layer had successfully coated the O-rings. The morphology of the O-ring was characterized in cross section. A lower magnification image was used to investigate the PCL layer coating on the top of the O-ring matrix. A higher magnification image was utilized to display the embedding of the Ca-F particles in the PCL layer. The thickness of the coated PCL layers was measured using SEM.

To determine the amount of Ca-F in the PCL layers in different groups, energy-dispersive X-ray spectroscopy (EDX) associated with SEM (EDX/SEM) mapping was used to quantify the percentage of fluoride mass and atom ratio of calcium and fluoride in each group. This provided a means to determine if there was successful incorporation of fluoride in the PCL layer on the O-ring surface with a limited impact on the chemical composition of the O-ring.

Fluoride Release Test, n=16

For each experimental group, 16 O-rings were prepared and placed on brackets for the maxillary lateral incisor (American Orthodontics, Sheboygan, WI; Mini Masters Series, Low Profile, Maxillary Left Lateral, item #: 390-1604). 50 ml test tubes were prepared with 10 ml of distilled water. 4 O-rings placed on individual brackets were placed in each test tube (Figure 3).

Each experimental group had 4 test tubes, with 4 O-rings in each test tube, totaling 16 O-rings for each experimental group (Figure 4).

Each test tube containing 4 O-rings were incubated at 37°C. To assess the release profile of fluoride, measurements were taken at 10 time points, including Day 1, Day 3, Day 5, Week 1, Week 2, Week 3, Week 4, Week 5, Week 6, and Week 7. Fluoride concentrations were measured in ppm using the Thermo Scientific Orion Dual Star Ion Selective Electrode (ISE) Meter. Before the measurements were performed, calibration was conducted at 22°C per the Thermo Scientific Orion Dual Star ISE Meter instructions using the Fluoride with TISAB (total ionic strength adjustment buffer) II Standards for 1 ppm and 10 ppm.

At each subsequent time point, the O-rings were removed from the test tube, placed on a paper towel to dry, and transferred to a new test tube of 10 ml distilled water to incubate at 37°C. All O-rings were kept with their same group of 4 and labeled as such. Then, the water solution from the previous time point was used to measure the concentration of fluoride ions via electrodes with the Thermo Scientific Orion Dual Star ISE Meter (ASTM E815-17b). All fluoride testing was carried out by one principal investigator. Fluoride readings were each repeated three times to determine reliability. Reliability was established at 99.9% for intraclass correlation coefficient.

Instron Mechanical Test, n=8

8 O-rings from each of the four groups were tested using Instron to evaluate the force (N) of the O-rings (Figure 5). Testing was first conducted on the Instron prior to soaking in distilled water at T1. Each O-ring was placed on two stainless steel hooks attached to the Instron (Figure

6). The Instron software was opened, and the "O-rings" protocol pre-program was selected. The "balance" button was used to reset to zero (N) before starting. After the start button was pressed, the O-ring was stretched to 4 times its outer diameter at a rate of 100 mm/min, waiting for 5 seconds, then allowed to contract to 3 times its original outer diameter, followed by 30 seconds of holding (Figure 7). If the O-rings were unable to be stretched to 4 folds, then tensile failure was noted. Maximum force (N) at the time of breakage was recorded. If no breakage occurred, the final force (N) of the O-ring was recorded at the end of the test (ISO 21606:2007). All Instron testing was carried out by one principal investigator.

Since O-rings tested at T1 had been stretched, new O-rings were used for T2. 8 new O-rings for each group were soaked in distilled water for 7 weeks (T2). 4 O-rings were placed in a 50 ml test tube containing 10 ml distilled water. There were 2 test tubes per group containing 4 O-rings each, totaling 8 O-rings per group. At each time interval (Day 1, Day 3, Day 5, Week 1, Week 2, Week 3, Week 4, Week 5, Week 6, and Week 7), the distilled water was changed, and the O-rings were placed in a new test tube with fresh 10 ml distilled water, in the same fashion as depicted in the fluoride release study. For the purposes of the Instron mechanical test, however, fluoride readings were not carried out. At the end of the 7 weeks (T2), Instron testing was once again conducted, as previously described.

Statistical Analysis

Independent t-tests were used to compare groups for both the fluoride release and Instron experiments. ANOVA was used when comparing between more than two groups based on dip concentration.

All preparation of O-rings, fluoride release testing, and Instron mechanical testing was carried out by one principal investigator. Reliability was established for fluoride release testing by each fluoride measurement with the ISE Meter repeated 3 times. Intraclass correlation coefficient (ICC) was established at 99.9%. Further reliability between the 4 test tubes in each group was established at 99.9% for intraclass correlation coefficient.

Pilot studies were conducted to determine the basis of the dip concentrations, methods of measuring elastic properties and ion concentrations of fluoride, and the necessary effect size. With the original proposed sample size (n=8), 80% power can be attained with an effect size of 2.5. For fluoride release testing, the sample size was n=16. For Instron mechanical testing and SEM, the sample size was n=8.

3. RESULTS

Fluoride Integration

Scanning electron microscopy was utilized to characterize the morphology of the O-ring at T2. The lower magnification image showed that the coated PCL layer was on top of the O-ring matrix (Figure 8a). The higher magnification image further displayed the calcium fluoride particles (in white) embedded in the PCL layer (Figure 8b).

The thickness of the coated PCL layers between the 3 experimental groups showed a gradual increase of PCL layer thickness from the 2.5%, to 5%, to 10% group. The 2.5% group was significantly thinner than the 5% group ($p<.01$) and significantly thinner than the 10% group ($p<.001$) (Figure 9).

EDX images showed two absorbance peaks for fluoride and calcium ions in the PCL matrix from the 2.5%, 5%, and 10% groups, whereas there were no fluoride or calcium ions detected in the O-ring matrix (Figure 10).

Quantitative results revealed that the percentage of fluoride in the 2.5% group was less than half that of the 5% or 10% groups ($p<.05$). The 10% group was slightly higher than the 5% group (Figure 11). The percentage of calcium followed a similar trend, with significantly less percentage of calcium in the 2.5% group compared to the 5% and 10% groups ($p<.05$).

Fluoride Release

There were statistically significant differences within each group over the first week of fluoride release (Table 1a). For the 2.5% group, the fluoride release rate on the first day was 37.5 μg F^-/ring/day, then decreased from Day 3, 5, and 7. Statistically significant decrease occurred from Day 1 to Day 3 and from Day 5 to Day 7 for the 2.5% group ($p < .001$). In the 5% group,

fluoride release rate started at 43.9 μg F^-/ring/day on Day 1 and decreased to 25.0 μg F^-/ring/day, showing statistically significant decrease ($p < .001$). Slight increase occurred from Day 3 to Day 5 (25.2 μg F^-/ring/day), followed by decrease from Day 5 to Day 7 (24.1 μg F^-/ring/day). In the 10% group, fluoride release began on Day 1 at 34.1 μg F^-/ring/day with statistically significant decrease to Day 3 ($p<.001$). Further decrease in the 10% group occurred from Day 3 to 5 and from Day 5 to 7 with statistically significant decrease in the latter ($p<.01$). The control group started at a nominal .170 μg F^-/ring/day and showed statistically significant decrease from Day 1 to Day 3 ($p<.01$), followed by further decrease from Day 3 to 5, and slight increase from Day 5 to 7.

Between the groups there were statistically significant differences in the first week of fluoride release (Table 1b, Figure 12). For all group comparisons, there were statistically significant differences between all 4 groups at all 4 timepoints in the first week ($p<.001$). Significance value differed, however still significant, for the 2.5% compared to the 10% group at Day 1 ($p<.01$) and Day 3 ($p<.05$), and for the 5% compared to the 10% group at Day 3 ($p<.05$).

On a weekly basis, there were statistically significant decreases over the 7 weeks of fluoride release (Table 2a). For the 2.5% group, fluoride release began at 21.8 μg F^-/ring/day with statistically significant decrease that occurred from week to week ($p<.001$), however, not statistically significant from Week 6 to 7. For the 5% group, fluoride release began at 27.5 μg F^-/ring/day and incurred statistically significant decrease until the 7th week ($p<.001$), however, not statistically significant from Week 1 to 2. For the 10% group, fluoride release began at 24.5 μg F^-/ring/day and incurred decrease from week to week, with statistically significance from Week 1 to 2 and Week 3 to 4 ($p<.001$).

At each week, release of fluoride showed varying rates between the 4 groups (Table 2b, Figure 13). There were statistically significant differences between the 4 groups at each week ($p<.001$), except when comparing the 5% to 10% groups at Week 2, 3, 4, and 5. At Week 6, there was also statistically significant difference between the 5% and 10% groups, however, at different significance level ($p<.01$). The first week showed elevated fluoride release of 21.8 μg F^-/ring/day, 27.5 μg F^-/ring/day, and 24.5 μg F^-/ring/day for the 2.5%, 5%, and 10% groups, respectively. There was a decrease in fluoride release for all groups at week 2, however, release remained steady for the remainder of the timepoints for the 5% and 10% group. In the final 7th week, the fluoride release rates of the 2.5%, 5%, and 10% groups were 0.69 μg F^-/ring/day, 6.54 μg F^-/ring/day, and 6.97 μg F^-/ring/day, respectively.

The cumulative release curve shows fluoride release over the entire 7 weeks of the study. In the 5% and 10% groups, there is sustained fluoride release over the 7 weeks, while the rate of the 2.5% group plateaued after 3 weeks (Table 3, Figure 14). Total fluoride accumulated over 7 weeks was 260.8 μg, 504.9 μg and 489.5 μg for the 2.5%, 5%, and 10% groups, respectively.

Elastic Properties

Tensile failure did not occur for O-rings in any group at any time point. At T1 after the dip modification incorporating calcium fluoride and before soaking in water, the tensile force (N) of the loading amount of the 2.5% group was slightly decreased compared to controls, while the 5% group was moderately increased, and the 10% group had statistically significant increase ($p<.01$) compared to controls at T1 (Table 4, Table 5, Figure 15).

From T1 to T2, there were statistically significant decreases in tensile force (N) for the loading amount of the O-rings for the 2.5%, 5%, and 10% groups ($p<.001$) (Table 4, Table 5,

Figure 16). Within the groups from T1 to T2, percentage decreases in tensile force were -3.94%, -11.2%, -11.9%, and -26.6% for the control, 2.5%, 5%, and 10% groups, respectively. However, there were no tensile failures reported for any O-ring in any group.

At T2 after 7 weeks of soaking in distilled water, the tensile force of the Ca-F O-rings compared to the controls also showed a decrease in loading amount (N) (Table 4, Table 5, Figure 17). The tensile force of the 2.5%, 5%, and 10% groups were all lower compared to the controls at T2, with statistically significant decreases found in the 2.5% and 10% groups ($p<.001$) compared to controls.

At T1 and T2, the 4 groups showed similar curves throughout the entirety of the Instron mechanical test (Figure 18, 19).

4. DISCUSSION

Improved Release Profile of Fluoride

These novel calcium fluoride O-rings exhibited long-term sustained and effective fluoride release. In the first week of the present study, there was elevated fluoride release of 21.8 μg F^-/ring/day, 27.5 μg F^-/ring/day, and 24.5 μg F^-/ring/day for the 2.5%, 5%, and 10% groups, respectively. The recommended release rate in the therapeutic range for fluoridated O-rings is 1.2-2.8 μg F^-/ring/day.[39] Although an apparent burst effect of fluoride, the present study rates were above the therapeutic range and still below the toxic level established in previous literature of 51 μg F^-/ring/day.[39]

After the first week, the release rates for the remainder of the study into the 7th week were steady for the 5% and 10% groups. In the final 7th week, the fluoride release rates of the 2.5%, 5%, and 10% groups were 0.69 μg F^-/ring/day, 6.54 μg F^-/ring/day, and 6.97 μg F^-/ring/day, respectively. Therefore, the 2.5% group does not show therapeutic levels of fluoride release in the 7th week. The 2.5% group fell below the therapeutic range between the 5th and 6th weeks of the study, with fluoride release rates of 1.61 μg F^-/ring/day and .895 μg F^-/ring/day for the 5th and 6th weeks, respectively.

The 2.5% group had a diminished fluoride release rate, which may be due to less PCL medium loaded compared to the 5% and 10% groups. Thus, less fluoride was incorporated into the delivery system. There was more of an apparent burst effect, due to less PCL available to provide sustained fluoride release, leading to diminished fluoride release rates by the end of the study. However, in comparison to the previous studies, the present study shows improvement with the 5% and 10% groups in sustained, continuous fluoride release rates.

To show the present study's improvements, comparisons will be made to previous studies. An *in vitro* study using stretched Fluor-I-Tie O-rings in distilled water showed 9.68 μg F^-/ring/day on the first day of release. On the 7th day, this fluoride release dropped to .79 μg F^-/ring/day, and the overall average release rate over 196 days was .71 μg F^-/ring/day, falling out of the therapeutic range. This previous study does not show sustained fluoride release rates adequate for white spot lesion prevention.[56]

In a previous study using a polymer medium and sodium fluoride, fluoride release rates reached 115 μg F^-/ring in the first day, which far exceeds the toxic limit of 51 μg F^-/ring/day.[39] After this initial burst, the fluoride release rate averaged 6.7 μg F^-/ring/day for the first 10 days, and later dropped to .88 μg F^-/ring/day for the remainder of the study for 40 days, falling below the therapeutic range of 1.2-2.8 μg F^-/ring/day.[39]

Previous fluoridated O-ring studies show a burst effect of fluoride, releasing a rapid amount initially, followed by diminished release that was not in the therapeutic range.[58, 59] Most of the previous fluoridated O-ring studies integrated fluoride with highly soluble solutions, such as tin fluoride and sodium fluoride. This led to the rapid initial release rate once placed in distilled water or a saliva medium.[18, 60]

In order explore the deficits of previous studies with this burst effect, percentages of overall fluoride released can be compared to the present study. An *in vitro* study using Fluor-I-Ties found that 35% of total fluoride was released in the first 24 hours, followed by 63% released by the end of the 1st week.[58] In the present study, there was a burst effect only for the 2.5% group, with 14% released in the first day and 74% released by the end of the first week. However, the 5% and 10% groups are promising and show improvement. For the 5% group, there was 8.7% of fluoride released in the first day followed by 38% by the end of the 1st week.

The 10% group showed 6.9% of total fluoride released in the first day followed by 35% released by the end of the 1st week. Overall, the 5% and 10% groups in the present study shows much improvement in resolving the burst effect issue.

The mitigation of the burst effect of fluoride release in the present study may be attributed to the improvement in this novel dip solution. Previous studies did not utilize a polymer medium suited for a sustained delivery system of fluoride. The only study that did use a polymer medium did not use calcium fluoride, and instead used a more highly soluble sodium fluoride instead. Again, this study that used a polymer medium had an initial burst effect of fluoride exceeding the toxic limits in the first day.[39]

As reviewed in the literature, calcium fluoride is important as a fluoride reservoir that remains on the enamel surface. Calcium fluoride has lower solubility than solutions like tin fluoride and sodium fluoride. Calcium fluoride is stable in the oral cavity and activates in low pH environments, having cariostatic effects.[69] Calcium fluoride attaches to the enamel surface to later release fluoride ions to fight against caries and white spot lesion formation.[63, 64] Because white spot lesion prevention requires a consistent, low dose fluoride release,[41] calcium fluoride's role in providing constant fluoride reservoirs targets this problem.

Polycaprolactone is utilized as a medium for incorporating this fluoride on the outer layer of the O-ring. PCL is known as a polymer that is versatile, biodegradable, safe and has low toxicity.[65] It is commonly used in medical settings in drug delivery systems, sutures, contraceptive implants, and bone fixation materials.[67] PCL is biocompatible and has a semi-crystalline structure allowing for slower degradation, appropriate for controlled and sustained drug release via diffusion.[70] PCL has been used as a medium to deliver drugs including peptides, proteins, and vaccines via sustained drug release while capable of being fully excreted from the

body after use.[68] Coatings using PCL are known to have good wear resistance, chemical resistance, and can provide long-term drug delivery even up to 1 year, although can be tapered to the desired time for the drug release.[66] Thus, PCL serves as a successful medium for incorporating this fluoride into the outer layer of the O-rings while still maintaining the integrity of the original polymer. This method uses PCL as a carrier for fluoride release to prevent white spot lesions for the first time in orthodontics.

The advantages of calcium fluoride, having low solubility and serving as a fluoride reservoir, as well as PCL, acting as a polymer medium, allows for more long-term, sustained fluoride release. The present study has these advantages over previous studies with this novel solution for dip coating the O-rings. This method uses PCL as a carrier for fluoride release to prevent white spot lesions for the first time in orthodontics.

The 5% and 10% groups showed sustained fluoride release over 7 weeks. There was no statistically significant difference between the 5% and 10% groups in terms of fluoride release profile. This may be due to a limit on the amount of fluoride that can be released from the PCL medium surrounding the O-rings. Another possibility is that there is a maximum amount of PCL to be effective for sustained fluoride release, with no difference if more PCL were to be added. Thus, if 5% was hypothesized as the maximum amount of PCL that can be loaded into the outer layer of the O-ring to allow for fluoride release, there would be no difference in fluoride release with mediums of higher concentrations, such as the 10% group. The 5% group may be reaching a plateau in the level of efficacy for PCL, and the 10% group may not be providing any additional benefits for fluoride release. Overall, the 5% and 10% groups show sustained fluoride release that is in the therapeutic range for preventing white spot lesion development with this novel method.

Future studies will be needed *in vivo* to determine if fluoride release remains sustained in the oral cavity. A study comparing the first 24 hours of fluoride release from Fluor-I-Ties exhibited increased fluoride release *in vivo*, with 1.43 ug F^-/ring *in vivo* versus .37 ug F^-/ring *in vitro*.[59] In the first week of a previous study using Fluor-I-Ties, there was 90% fluoride release *in vivo* compared to 13% fluoride release *in vitro*. The increase in fluoride release *in vivo* was possibly due to additional fluoride absorption by the elastomer due to fluoride supplementation in the oral cavity via toothpastes and rinses.[54] A previous study using Fluor-I-Ties determined that stretching the O-rings over brackets released 13% more fluoride than unstretched O-rings due to more fluoride available for release from the surface area of the deformed polyurethane structure.[48, 56] Pilot investigations for the present study also determined a similar conclusion, however, future studies *in vivo* will require further investigation.

Previous Fluor-I-Tie clinical trials showed promising results for white spot lesion prevention, with one study showing a 49% reduction in decalcification scores.[18] Another Fluor-I-Tie clinical trial showed significantly fewer decalcifications compared to controls, even up to 50% fewer.[60] Even though these previous studies exhibited the burst effect, they still had promising clinical outcomes for white spot lesion prevention. Therefore, with the present study's improvements and advantages, these clinical results can be expanded upon and improved to prevent white spot lesions. To summarize, the present study design is promising for clinical application for sustained fluoride release in the prevention of white spot lesions.

Efficient Coating Method

The novel method of dip coating elastomeric O-rings with calcium fluoride and PCL solution shows successful integration into the outer polymer layer of the polyurethane O-ring.

Scanning electron microscopy results showed the PCL coating on the top layer of the O-ring and calcium and fluoride particles embedded into this PCL layer. The thickness of the outer layer of PCL was thickest for the 10% group, followed by the 5% group, and thinnest for the 2.5% group. Furthermore, the amount of calcium and fluoride ions embedded in the PCL layer followed a similar pattern, with the most in the 10% group, followed by the 5%, then 2.5% groups. This can be explained by the increased concentration of PCL in the 10% group, making a solution of thicker consistency, therefore, a thicker outer layer with more calcium and fluoride ions embedded.

To date, there have not been previous studies in the literature utilizing calcium fluoride and PCL incorporated with O-rings. No previous fluoridated O-ring studies mention the use of calcium fluoride. As mentioned before, the only study in the literature that utilizes a polymer medium as a controlled delivery system to incorporate fluoride into O-rings used a polymer called polyethylene co-vinyl acetate (PEVA) impregnated with sodium fluoride.[39] Although incorporation was successful, there were deficits in fluoride release, the sample size was small (n=3), and scanning electron microscopy was not utilized to further look at the layers of the O-ring and determine fluoride incorporation. Thus, the present study's novel method of utilizing PCL and calcium fluoride was successful in integrating with the elastomeric O-rings.

The incorporation of calcium fluoride via the PCL layer was integrated with the outer layer of the O-ring. Even with this fluoride incorporation, the O-ring was still able to carry out its original function of remaining on orthodontic brackets. EDX images displayed the absorbance peaks of calcium and fluoride ions in the PCL matrix, with no apparent effects on the elastic properties of the O-rings. Previous studies did not investigate the ionic composition of the

integrated fluoride O-rings. The new method further determines that fluoride was successfully incorporated and integrated into the outer layer of the O-ring via scanning electron microscopy.

This new process of dip coating the O-rings with a PCL and calcium fluoride solution is an easy and straightforward process. Advantages of this technique lie in that the solution and O-rings are easy to manipulate and do not take extensive time to produce. This technique also maintains the O-ring's polyurethane elastic features. Furthermore, the dipping procedure was shown to be consistent with reliability established at 99.9% for intra-class correlation coefficient when comparing the amount of fluoride released from different tubes of O-rings within the same experimental group. This novel method of dipping O-rings serves as a platform for future studies to further improve this system, whether it be by altering the calcium fluoride or PCL concentrations.

Maintained Elastic Properties

The O-rings maintain their elastic strength after this novel calcium fluoride modification. At T1, which was after dip modification and before 7 weeks of soaking in distilled water, no tensile failures were reported for any group. The 10% group showed increased tensile strength upon stretching after dip modification. This may be due to the increased thickness of the PCL layer compared to other groups, thereby increasing the overall thickness of the outer polymer layer. Thus, a thicker layer of polymer may be stronger with increased surface area, thereby increasing the tensile load (N) upon stretching.

At T2, which was after 7 weeks of soaking in distilled water, there again were no tensile failures reported for any group. There was minimal force decrease after 7 weeks of -3.94%, -11.2%, -11.9%, and -26.6% for the control, 2.5%, 5%, and 10% groups, respectively. Although

the experimental groups show statistically significant decrease in tensile strength from T1 to T2 compared to controls, these were not clinically significant changes since there were no failures and all O-rings remained on brackets in the 7-week fluoride release study. The 10% group exhibited the most change due to the increased tensile strength at T1 after dip modification before soaking, compared to other groups.

Previous fluoridated O-rings studies did not investigate or quantify the elastic changes of fluoridated O-rings on an Instron machine. The most comparable previous study utilizing a polymer medium for fluoridated O-rings did not inspect elastic properties at all.[39] In one previous study, there was mention of decrease in elasticity leading to potential rupture or failure of the O-rings due to alteration of the O-ring's polymer structure with their fluoride integration method.[56] Another *in vivo* study mentioned a rapid deterioration in the oral cavity resulting in loss of elastic properties and swelling of the O-rings due to imbibition, however, no measurements were conducted to support this apparent finding.[18] Overall, there are no quantitative results inspecting elastic properties in these previous studies.[39, 56]

After 7 weeks of soaking in the present study, the 2.5% and 10% groups had lower tensile force (N) compared to controls and the 5% group. This may indicate that the 5% group has the ideal amount of PCL, not too much or too little, to incorporate fluoride and remain integrated with the outer layer of the O-ring when soaked over time, which will require further investigation.

Based on the results, the 5% group shows the best elastic properties, although no clinical difference compared to the other groups. Overall, these novel fluoridated O-rings maintain elastic strength both after modification and after 7 weeks of soaking in distilled water medium. The same cannot be said for previous fluoridated O-ring studies. In addition to being maximally

stretched without failure, these novel calcium fluoride O-rings were able to fulfill their duty of remaining on brackets, which is important for clinical application.

Limitations and Future Studies

A limitation of the study is that the O-rings were tested in a distilled water medium. The oral environment is more acidic, contains bacteria, has susceptibility to pH cycles, and is subject to salivary flow. It will be important to investigate how the release profile and elastic performance of the O-rings are impacted *in vivo*, although it has been shown that calcium fluoride is stable in the oral cavity acting as a fluoride reservoir when placed in acidic environments.[64, 69] Calcium fluoride provides pH-controlled reservoirs of fluoride ions to combat white spot lesion formation.[63] Further investigation is needed to determine the viability of these calcium fluoride O-rings under the conditions of the oral cavity.

Previous fluoridated O-ring *in vivo* studies have shown that there is increased fluoride release and burst effect when placed in the oral cavity.[18, 60] In the first week of a previous study using Fluor-I-Ties, there was 90% and 13% fluoride release for the *in vivo* and *in vitro* settings, respectively.[54] Thus, it will be important to determine if the burst effect can still be overcome when this novel method is applied *in vivo*.

Another potential disadvantage of this dip coating technique is that there is an extra layer integrated on the outer portion of the O-ring that may be vulnerable to the mechanical forces of the oral cavity, including chewing and brushing. Previous clinical trials using fluoride-impregnated Fluor-I-Ties (Ortho Arch Company) did not discuss the wear of the O-ring and only mentioned that the fluoride O-rings tend to swell compared to controls.[60] The present study utilizes PCL to combat potential wear and rupture that may present with mastication and

brushing. As aforementioned, PCL has been used in drug delivery systems and has good wear resistance to withstand long-term drug delivery in the body, as with bone fixation materials, sutures, and contraceptive implants.[66] Thus, future studies should investigate the durability of the O-rings when put to the test in the oral cavity, which has not been investigated in previous fluoridated O-ring studies.

Despite these potential limitations, the present study results are promising for the possibility of calcium fluoride O-rings as a means of white spot lesion prevention in orthodontic patients. These O-rings were able to provide sustained fluoride release and have the potential when clinically applied to eliminate the issue of patient compliance, which is a major issue in white spot lesion prevention. The present study sets the stage for these novel O-rings to be tested *in vivo*, exposed to saliva and elements of the oral cavity, and applied in future clinical studies.

5. CONCLUSIONS

1. The 5% and 10% groups of calcium fluoride O-rings exhibited long-term sustained and therapeutically effective fluoride release.
2. This novel method can effectively and reliably coat PCL incorporated with calcium fluoride on the surface of O-rings.
3. The calcium fluoride O-rings maintain elastic properties even after this dip coating modification, with no tensile failures reported. All O-rings were able to fulfill their clinical application of remaining on brackets over 7 weeks.
4. This study is promising for the possibility of calcium fluoride O-rings as a product for the prevention of white spot lesions in orthodontic patients.

REFERENCES

1. Fejerskov, O., B. Nyvad, and E.A.M. Kidd, *Clinical and histological manifestations of dental caries.* Dental Caries: The Disease and Its Clinical Management, 2003: p. 71-99.

2. Julien, K.C., P.H. Buschang, and P.M. Campbell, *Prevalence of white spot lesion formation during orthodontic treatment.* Angle Orthod, 2013. **83**(4): p. 641-7.

3. Perrini, F., et al., *Caries prevention during orthodontic treatment: In-vivo assessment of high-fluoride varnish to prevent white spot lesions.* Am J Orthod Dentofacial Orthop, 2016. **149**(2): p. 238-43.

4. Ogaard, B., *Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment.* Am J Orthod Dentofacial Orthop, 1989. **96**(5): p. 423-7.

5. Mitchell, L., *Decalcification during orthodontic treatment with fixed appliances--an overview.* Br J Orthod, 1992. **19**(3): p. 199-205.

6. Tufekci, E., et al., *Prevalence of white spot lesions during orthodontic treatment with fixed appliances.* Angle Orthod, 2011. **81**(2): p. 206-10.

7. Ogaard, B., G. Rolla, and J. Arends, *Orthodontic appliances and enamel demineralization. Part 1. Lesion development.* Am J Orthod Dentofacial Orthop, 1988. **94**(1): p. 68-73.

8. Ogaard, B., et al., *Orthodontic appliances and enamel demineralization. Part 2. Prevention and treatment of lesions.* Am J Orthod Dentofacial Orthop, 1988. **94**(2): p. 123-8.

9. Morrier, J.J., *[White spot lesions and orthodontic treatment. Prevention and treatment].* Orthod Fr, 2014. **85**(3): p. 235-44.

10. Chow, C.K., C.D. Wu, and C.A. Evans, *In vitro properties of orthodontic adhesives with fluoride or amorphous calcium phosphate.* Int J Dent, 2011. **2011**: p. 583521.

11. O'Reilly, M.M. and J.D. Featherstone, *Demineralization and remineralization around orthodontic appliances: an in vivo study.* Am J Orthod Dentofacial Orthop, 1987. **92**(1): p. 33-40.

12. Sundararaj, D., et al., *Critical evaluation of incidence and prevalence of white spot lesions during fixed orthodontic appliance treatment: A meta-analysis.* J Int Soc Prev Community Dent, 2015. **5**(6): p. 433-9.

13. Artun, J. and B.O. Brobakken, *Prevalence of carious white spots after orthodontic treatment with multibonded appliances.* Eur J Orthod, 1986. **8**(4): p. 229-34.

14. Gorelick, L., A.M. Geiger, and A.J. Gwinnett, *Incidence of white spot formation after bonding and banding.* Am J Orthod, 1982. **81**(2): p. 93-8.

15. Geiger, A.M., et al., *The effect of a fluoride program on white spot formation during orthodontic treatment.* Am J Orthod Dentofacial Orthop, 1988. **93**(1): p. 29-37.

16. Mizrahi, E., *Surface distribution of enamel opacities following orthodontic treatment.* Am J Orthod, 1983. **84**(4): p. 323-31.

17. Khalaf, K., *Factors Affecting the Formation, Severity and Location of White Spot Lesions during Orthodontic Treatment with Fixed Appliances.* J Oral Maxillofac Res, 2014. **5**(1): p. e4.

18. Banks, P.A., et al., *Fluoride-releasing elastomerics--a prospective controlled clinical trial.* Eur J Orthod, 2000. **22**(4): p. 401-7.

19. Barnhart, E.C., et al., *The quality of etched enamel in different regions and tooth types and its significance in bonding and the development of white spot lesions.* Angle Orthod, 2021. **91**(5): p. 576-582.

20. Heymann, G.C. and D. Grauer, *A contemporary review of white spot lesions in orthodontics.* J Esthet Restor Dent, 2013. **25**(2): p. 85-95.

21. Brown, M.D., et al., *A practice-based evaluation of the prevalence and predisposing etiology of white spot lesions.* Angle Orthod, 2016. **86**(2): p. 181-6.

22. Mizrahi, E., *Enamel demineralization following orthodontic treatment.* Am J Orthod, 1982. **82**(1): p. 62-7.

23. Mellberg, J.R. and D.E. Mallon, *Acceleration of remineralization in vitro by sodium monofluorophosphate and sodium fluoride.* J Dent Res, 1984. **63**(9): p. 1130-5.

24. Lapenaite, E., K. Lopatiene, and A. Ragauskaite, *Prevention and treatment of white spot lesions during and after fixed orthodontic treatment: A systematic literature review.* Stomatologija, 2016. **18**(1): p. 3-8.

25. Bishara, S.E. and A.W. Ostby, *White Spot Lesions: Formation, Prevention, and Treatment.* Seminars in Orthodontics, 2008. **14**(3): p. 174-182.

26. Hochli, D., et al., *Interventions for orthodontically induced white spot lesions: a systematic review and meta-analysis.* Eur J Orthod, 2017. **39**(2): p. 122-133.

27. Geiger, A.M., et al., *Reducing white spot lesions in orthodontic populations with fluoride rinsing.* Am J Orthod Dentofacial Orthop, 1992. **101**(5): p. 403-7.

28. Marini, I., et al., *A retentive system for intra-oral fluoride release during orthodontic treatment.* Eur J Orthod, 1999. **21**(6): p. 695-701.

29. Pessan, J.P., et al., *Slow-release fluoride devices: a literature review.* J Appl Oral Sci, 2008. **16**(4): p. 238-46.

30. Abdullah, Z. and J. John, *Minimally Invasive Treatment of White Spot Lesions--A Systematic Review.* Oral Health Prev Dent, 2016. **14**(3): p. 197-205.

31. Reynolds, E.C., et al., *Fluoride and casein phosphopeptide-amorphous calcium phosphate.* J Dent Res, 2008. **87**(4): p. 344-8.

32. Khoroushi, M. and M. Kachuie, *Prevention and Treatment of White Spot Lesions in Orthodontic Patients.* Contemp Clin Dent, 2017. **8**(1): p. 11-19.

33. Liao, Y., et al., *Fluoride resistance in Streptococcus mutans: a mini review.* J Oral Microbiol, 2017. **9**(1): p. 1344509.

34. Margolis, H.C., E.C. Moreno, and B.J. Murphy, *Effect of low levels of fluoride in solution on enamel demineralization in vitro.* J Dent Res, 1986. **65**(1): p. 23-9.

35. Benson, P.E., N. Pender, and S.M. Higham, *Quantifying enamel demineralization from teeth with orthodontic brackets--a comparison of two methods. Part 1: repeatability and agreement.* Eur J Orthod, 2003. **25**(2): p. 149-58.

36. Benson, P.E., N. Pender, and S.M. Higham, *Quantifying enamel demineralization from teeth with orthodontic brackets--a comparison of two methods. Part 2: validity.* Eur J Orthod, 2003. **25**(2): p. 159-65.

37. Nascimento, P.L., et al., *Fluoride-Releasing Materials to Prevent White Spot Lesions around Orthodontic Brackets: A Systematic Review.* Braz Dent J, 2016. **27**(1): p. 101-7.

38. Banks, P.A., A. Burn, and K. O'Brien, *A clinical evaluation of the effectiveness of including fluoride into an orthodontic bonding adhesive.* Eur J Orthod, 1997. **19**(4): p. 391-5.

39. Baturina, O., et al., *Development of a sustained fluoride delivery system.* Angle Orthod, 2010. **80**(6): p. 1129-35.

40. Azarpazhooh, A. and H. Limeback, *Clinical efficacy of casein derivatives: a systematic review of the literature.* J Am Dent Assoc, 2008. **139**(7): p. 915-24; quiz 994-5.

41. ten Cate, J.M., *Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention.* Eur J Oral Sci, 1997. **105**(5 Pt 2): p. 461-5.

42. Graber, T.M., *Orthodontics; Principles and Practice*. 1972: Saunders.

43. Proffit, W.R., et al., *Contemporary Orthodontics - E-Book*. 2018: Elsevier Health Sciences.

44. Condo, R., et al., *Traditional elastic ligatures versus slide ligation system. A morphological evaluation.* Oral Implantol (Rome), 2013. **6**(1): p. 15-24.

45. Baty, D.L., D.J. Storie, and J.A. von Fraunhofer, *Synthetic elastomeric chains: a literature review.* Am J Orthod Dentofacial Orthop, 1994. **105**(6): p. 536-42.

46. Ahrari, F., T. Jalaly, and M. Zebarjad, *Tensile properties of orthodontic elastomeric ligatures.* Indian J Dent Res, 2010. **21**(1): p. 23-9.

47. Eliades, T. and C. Bourauel, *Intraoral aging of orthodontic materials: the picture we miss and its clinical relevance.* Am J Orthod Dentofacial Orthop, 2005. **127**(4): p. 403-12.

48. Eliades, T., et al., *In vitro degradation of polyurethane orthodontic elastomeric modules.* J Oral Rehabil, 2005. **32**(1): p. 72-7.

49. Aminian, A., et al., *Evaluation of the effect of different stretching patterns on force decay and tensile properties of elastomeric ligatures.* Dent Res J (Isfahan), 2015. **12**(6): p. 589-95.

50. Petersen, A., et al., *Force decay of elastomeric ligatures: influence on unloading force compared to self-ligation.* Angle Orthod, 2009. **79**(5): p. 934-8.

51. Desai, N.P. and J.A. Hubbell, *Solution technique to incorporate polyethylene oxide and other water-soluble polymers into surfaces of polymeric biomaterials.* Biomaterials, 1991. **12**(2): p. 144-53.

52. Schott, H., *Kinetics of swelling of polymers and their gels.* J Pharm Sci, 1992. **81**(5): p. 467-70.

53. Wang, Z.J., et al., *Site-Specific Pre-Swelling-Directed Morphing Structures of Patterned Hydrogels.* Angew Chem Int Ed Engl, 2017. **56**(50): p. 15974-15978.

54. Tinsley, D., J.J. O'Dwyer, and P.E. Benson, *Fluoridated elastomers: in vivo versus in vitro fluoride release.* J Orthod, 2003. **30**(4): p. 317-22; discussion 298.

55. Doherty, U.B., P.E. Benson, and S.M. Higham, *Fluoride-releasing elastomeric ligatures assessed with the in situ caries model.* Eur J Orthod, 2002. **24**(4): p. 371-8.

56. O'Dwyer, J.J., D. Tinsley, and P.E. Benson, *The effect of stretching on the release of fluoride from fluoridated elastomeric ligatures.* Am J Orthod Dentofacial Orthop, 2005. **128**(4): p. 471-6.

57. Robertson, M.A., et al., *MI Paste Plus to prevent demineralization in orthodontic patients: a prospective randomized controlled trial.* Am J Orthod Dentofacial Orthop, 2011. **140**(5): p. 660-8.

58. Wiltshire, W.A., *Determination of fluoride from fluoride-releasing elastomeric ligature ties.* Am J Orthod Dentofacial Orthop, 1996. **110**(4): p. 383-7.

59. Wiltshire, W.A., *In vitro and in vivo fluoride release from orthodontic elastomeric ligature ties.* Am J Orthod Dentofacial Orthop, 1999. **115**(3): p. 288-92.

60. Mattick, C.R., et al., *Fluoride-releasing elastomeric modules reduce decalcification: a randomized controlled trial.* J Orthod, 2001. **28**(3): p. 217-9.

61. Storie, D.J., F. Regennitter, and J.A. von Fraunhofer, *Characteristics of a fluoride-releasing elastomeric chain.* Angle Orthod, 1994. **64**(3): p. 199-209; discussion 210.

62. Vogel, G.L., *Oral fluoride reservoirs and the prevention of dental caries.* Monogr Oral Sci, 2011. **22**: p. 146-157.

63. Rolla, G., *On the role of calcium fluoride in the cariostatic mechanism of fluoride.* Acta Odontol Scand, 1988. **46**(6): p. 341-5.

64. Navarro, M., et al., *Calcium fluoride uptake by human enamel after use of fluoridated mouthrinses.* Braz Dent J, 2001. **12**(3): p. 178-82.

65. Labet, M. and W. Thielemans, *Synthesis of polycaprolactone: a review.* Chem Soc Rev, 2009. **38**(12): p. 3484-504.

66. Woodruff, M.A. and D.W. Hutmacher, *The return of a forgotten polymer—Polycaprolactone in the 21st century.* Progress in Polymer Science, 2010. **35**(10): p. 1217-1256.

67. Zhang, N. and S. Guo, *[Studies on a kind of new biodegradable material--polycaprolactone and developments in medical area].* Sheng Wu Yi Xue Gong Cheng Xue Za Zhi, 2003. **20**(4): p. 746-9.

68. Schlesinger, E., N. Ciaccio, and T.A. Desai, *Polycaprolactone thin-film drug delivery systems: Empirical and predictive models for device design.* Mater Sci Eng C Mater Biol Appl, 2015. **57**: p. 232-9.

69. Rolla, G. and E. Saxegaard, *Critical evaluation of the composition and use of topical fluorides, with emphasis on the role of calcium fluoride in caries inhibition.* J Dent Res, 1990. **69 Spec No**: p. 780-5; discussion 820-3.

70. Petersen, R.S., et al., *Controlled Drug Release from Biodegradable Polymer Matrix Loaded in Microcontainers Using Hot Punching.* Pharmaceutics, 2020. **12**(11).

APPENDIX A FIGURES

Figure 1. Workflow for Solution A, B, and C

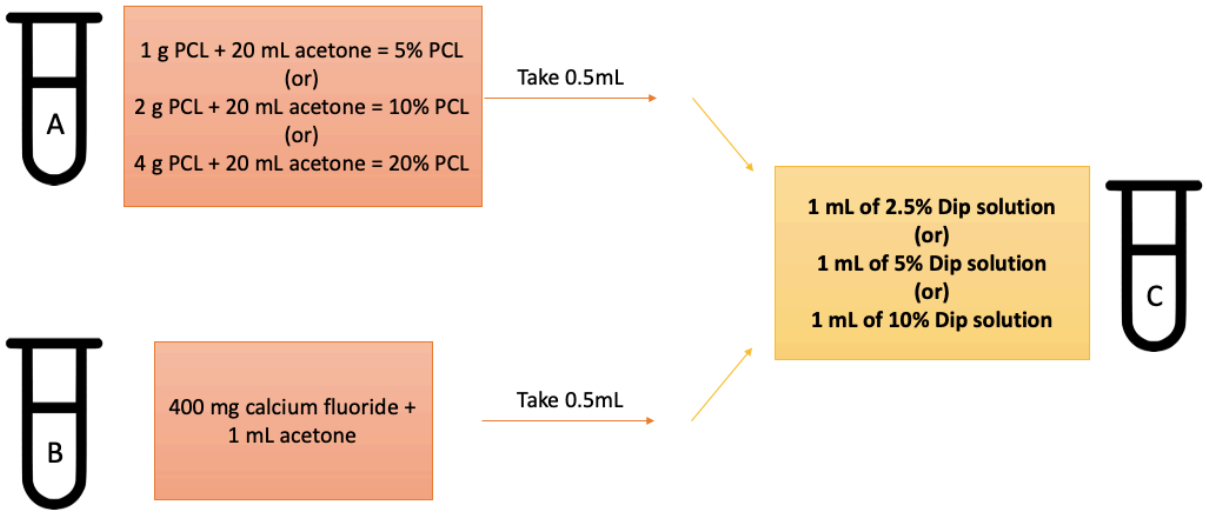


Figure 2. Half branch of O-rings in petri dish after dipping

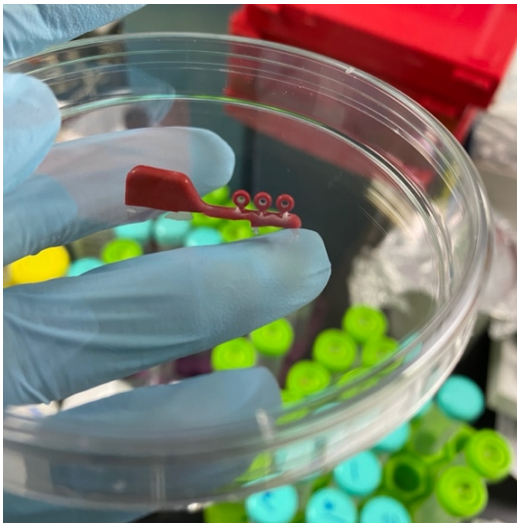


Figure 3. O-rings set on maxillary lateral incisor brackets

Figure 4. Diagram showing number of O-rings set on brackets in test tubes for fluoride release test

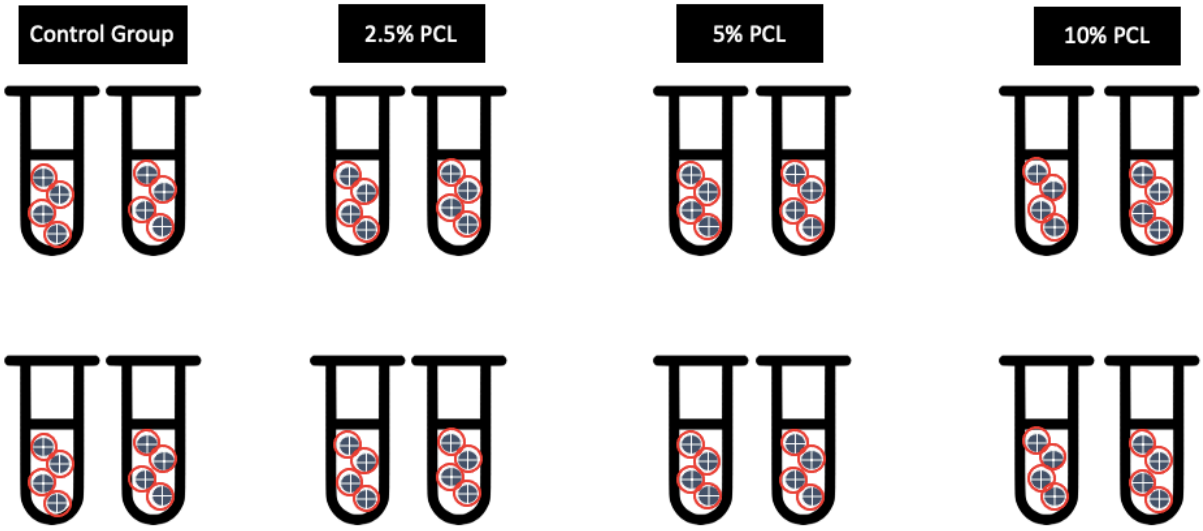


Figure 5. O-rings for Instron Mechanical Test

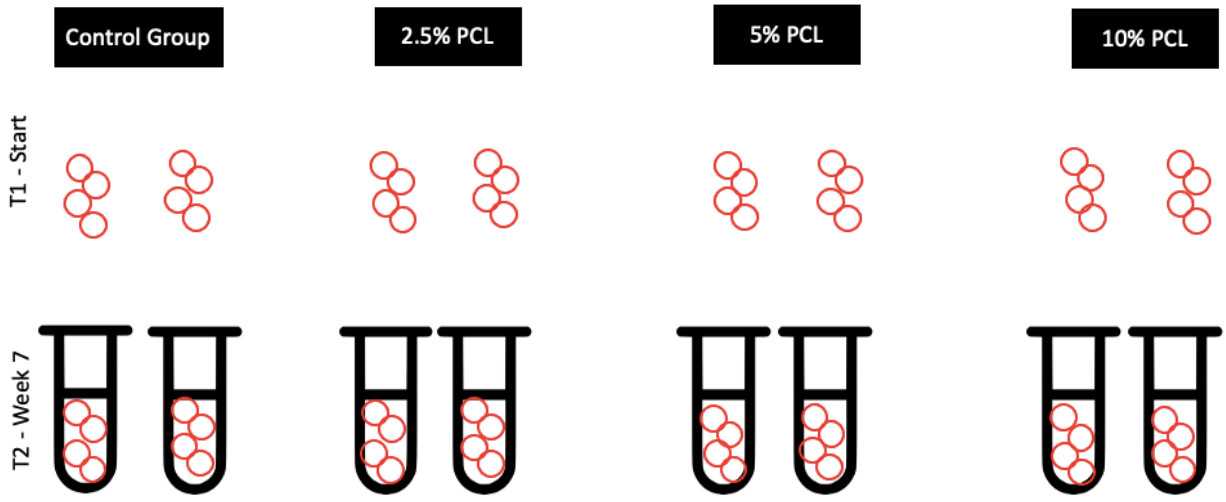


Figure 6. O-Ring on stainless steel hooks before stretching

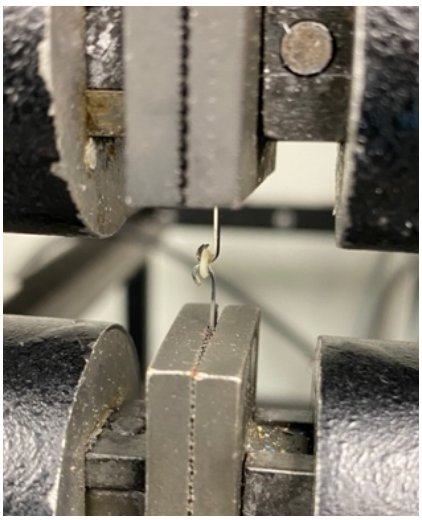


Figure 7. O-Ring on Instron stretched to 4 times original outer diameter

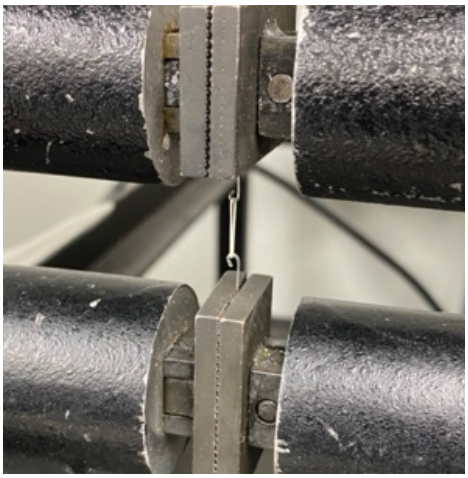


Figure 8. Cross-Section of O-Ring and Ca-F particles

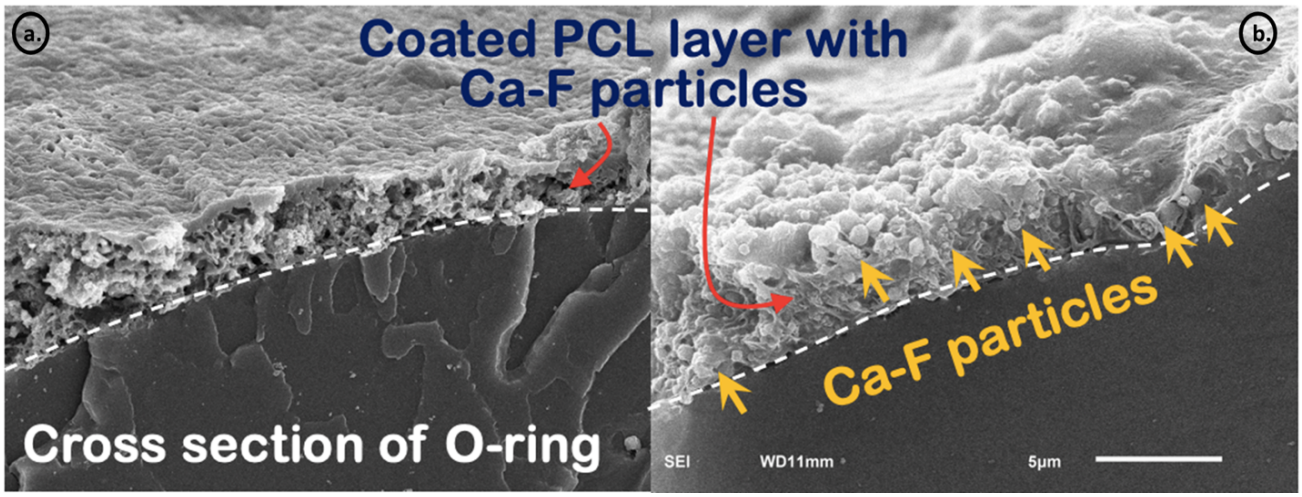


Figure 9. Thickness of the PCL Layer

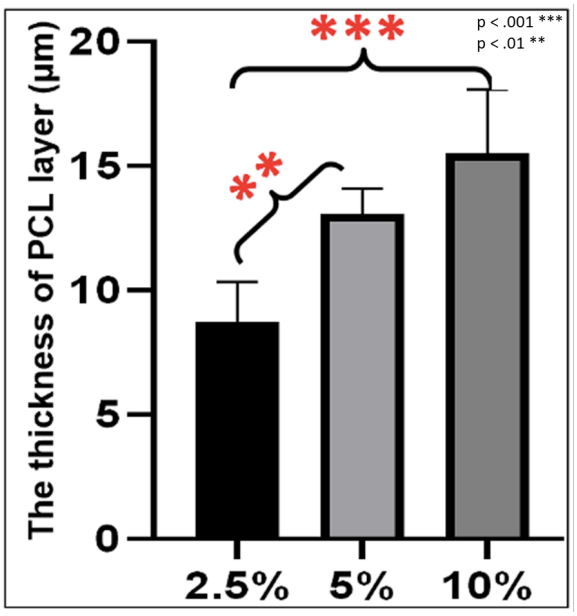


Figure 10. Percentage of Ca and F ions in PCL Matrix and O-Ring Matrix

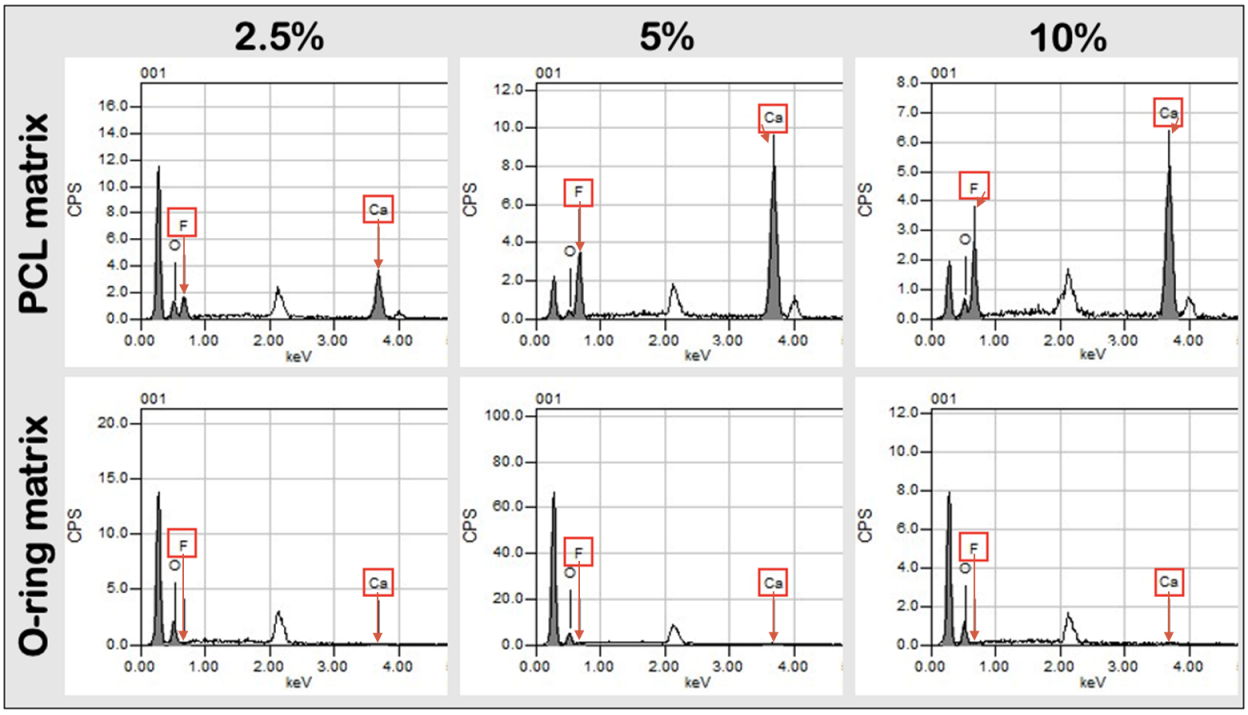


Figure 11. Percentage mass of F and Ca

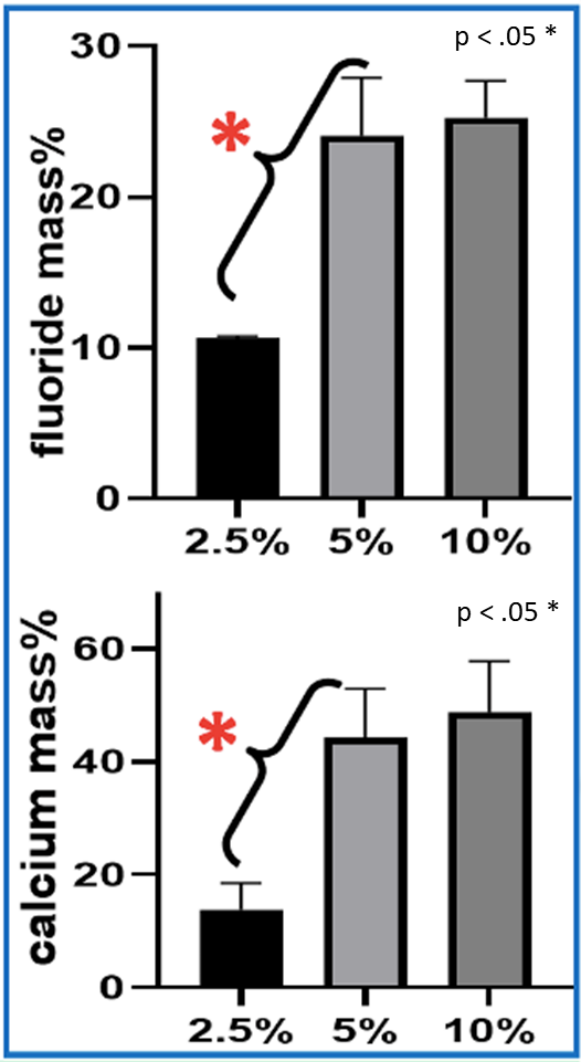


Figure 12. Daily Fluoride Release Rate in 1st Week (µg F-/ring/day)

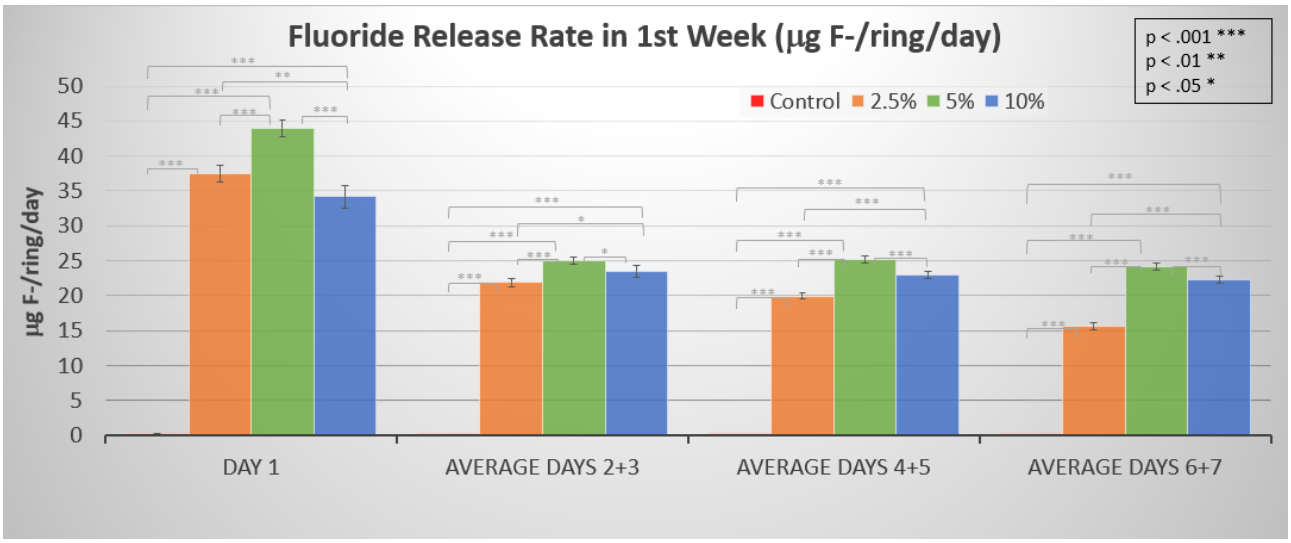


Figure 13. Daily Fluoride Release Rate by Week (µg F-/ring/day)

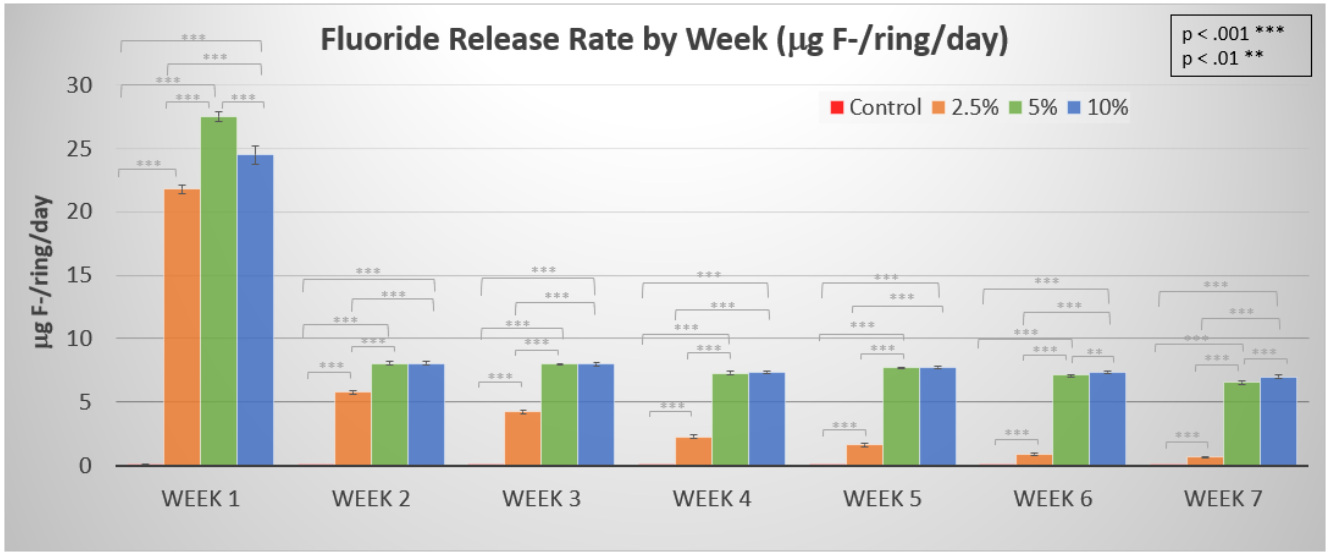


Figure 14. Total Fluoride Accumulated Over 7 Weeks (µg F-/ring)

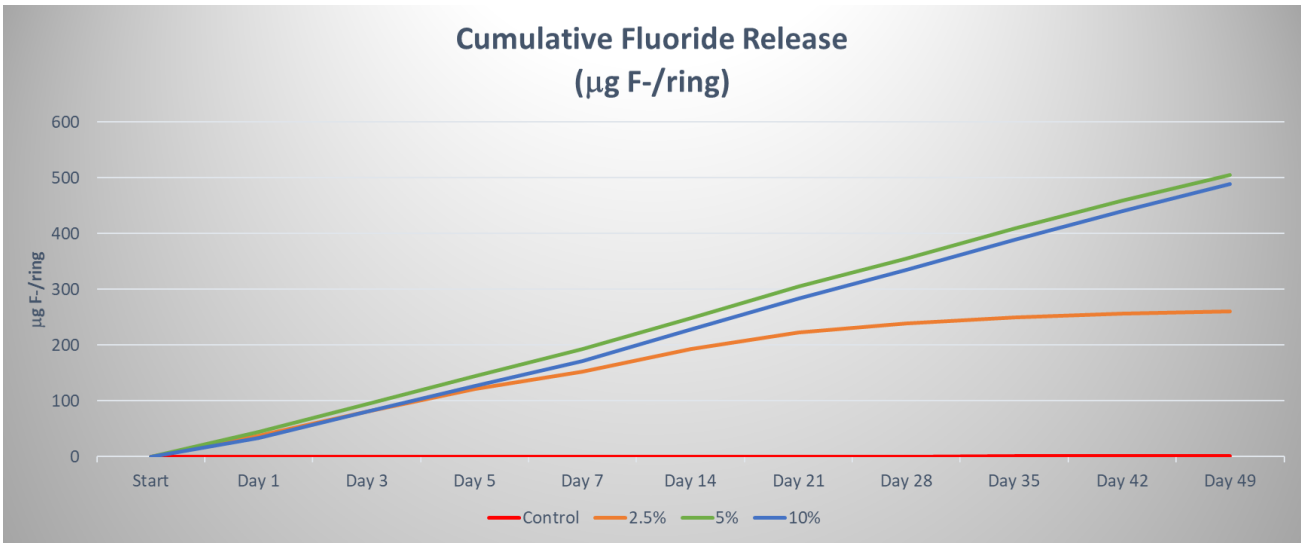


Figure 15. Loading Amount (N) of O-Rings at T1

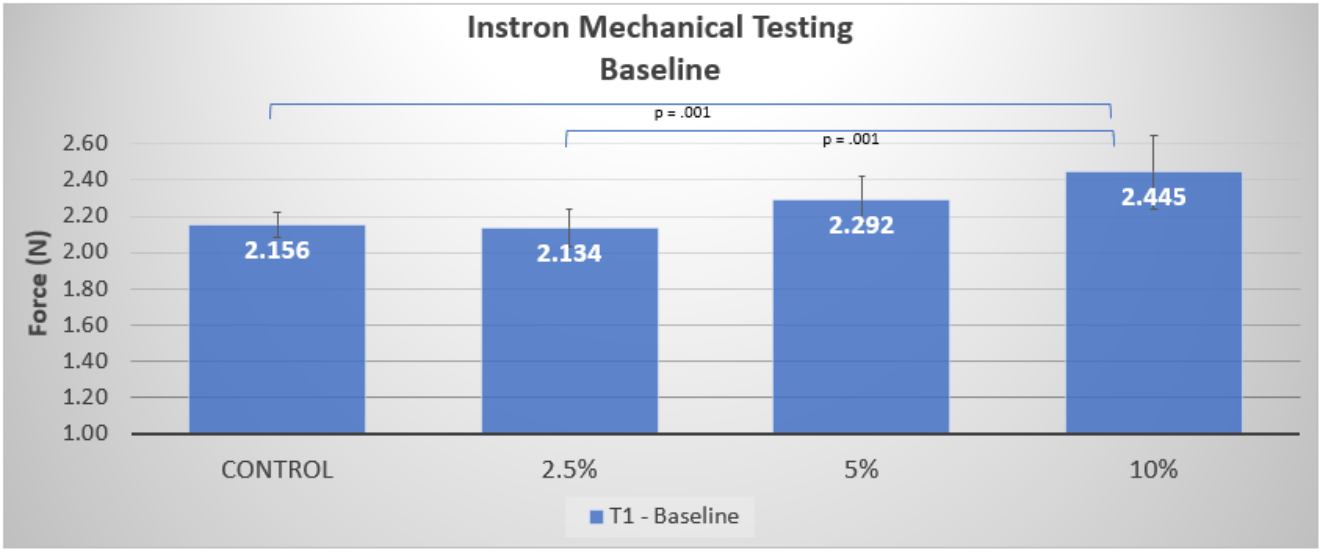


Figure 16. Change in Loading Amount (N) of O-Rings from T1 and T2

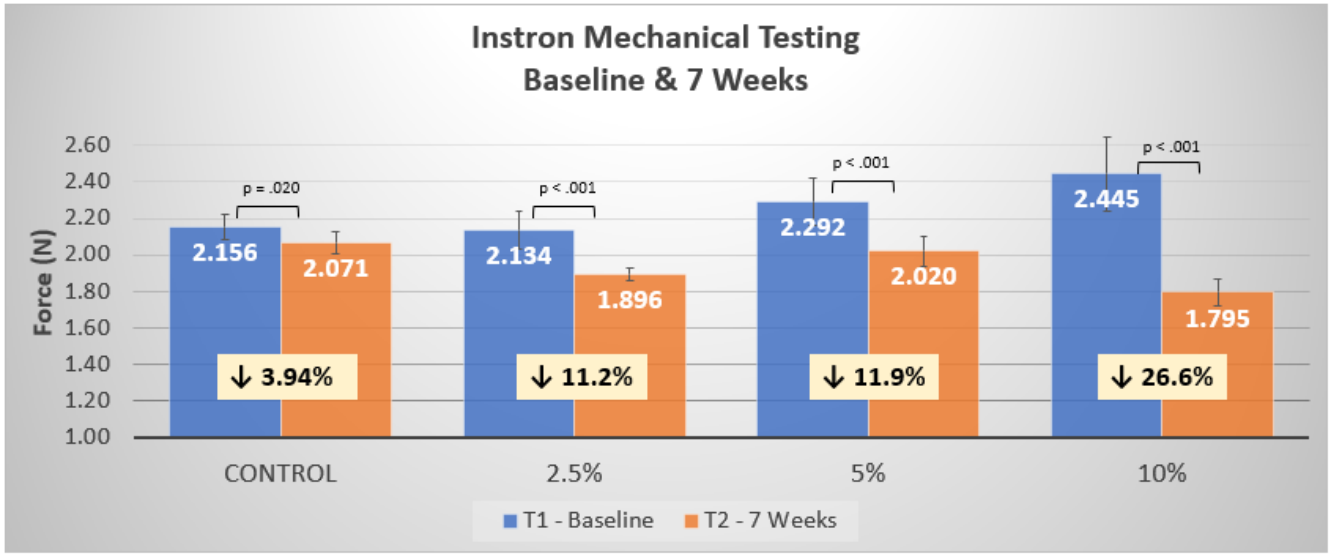


Figure 17. Loading Amount (N) of O-Rings at T2

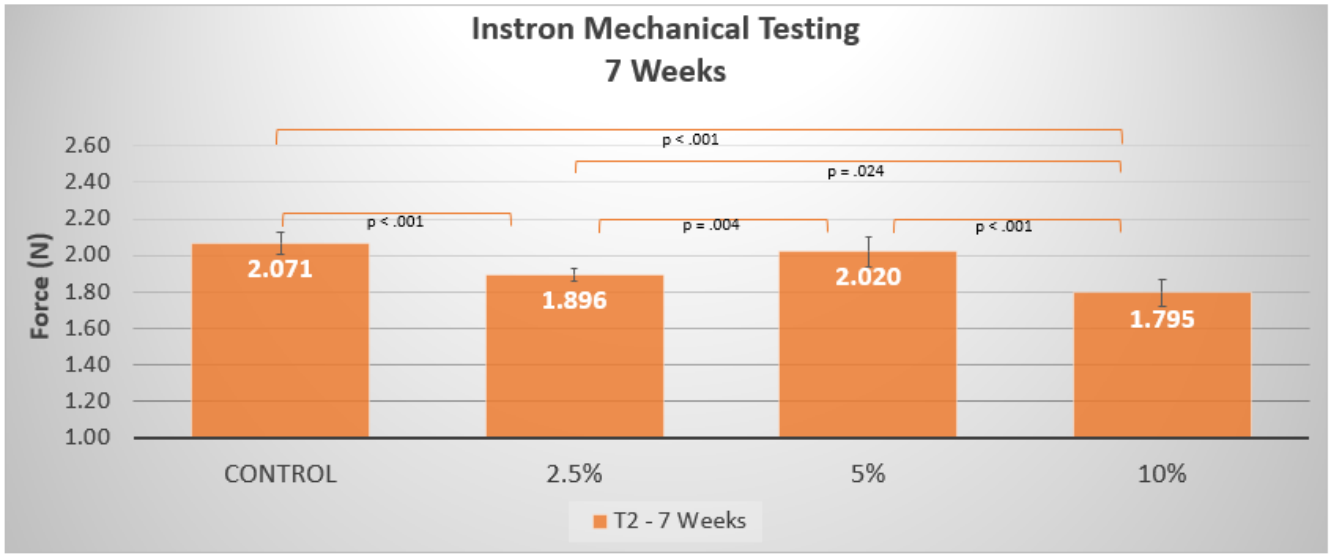


Figure 18. Instron Test T1 Curve

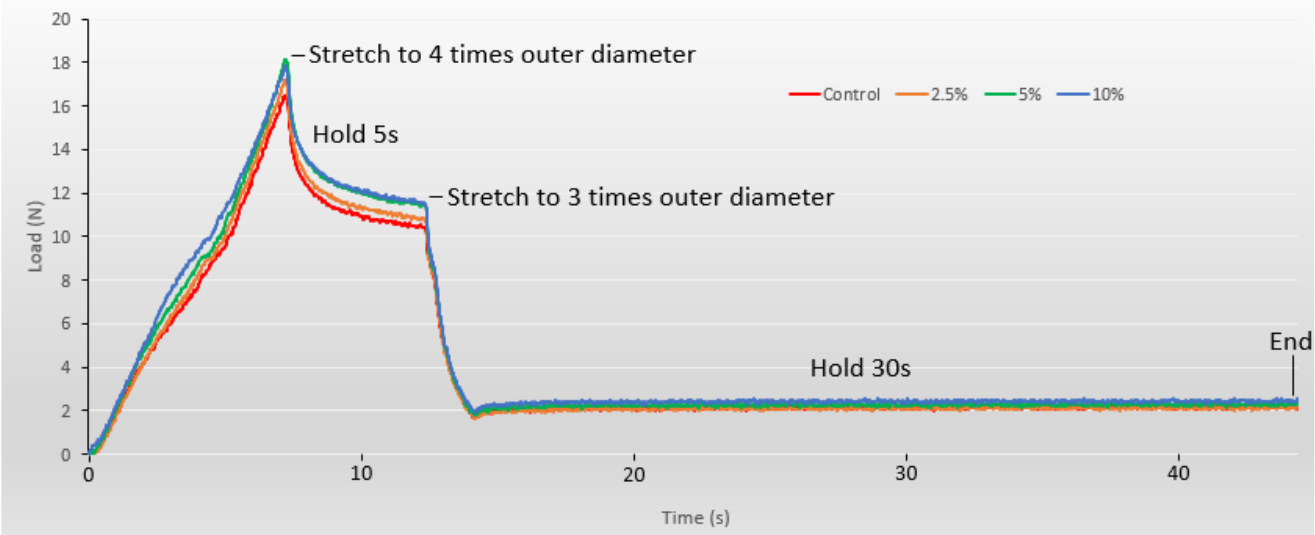
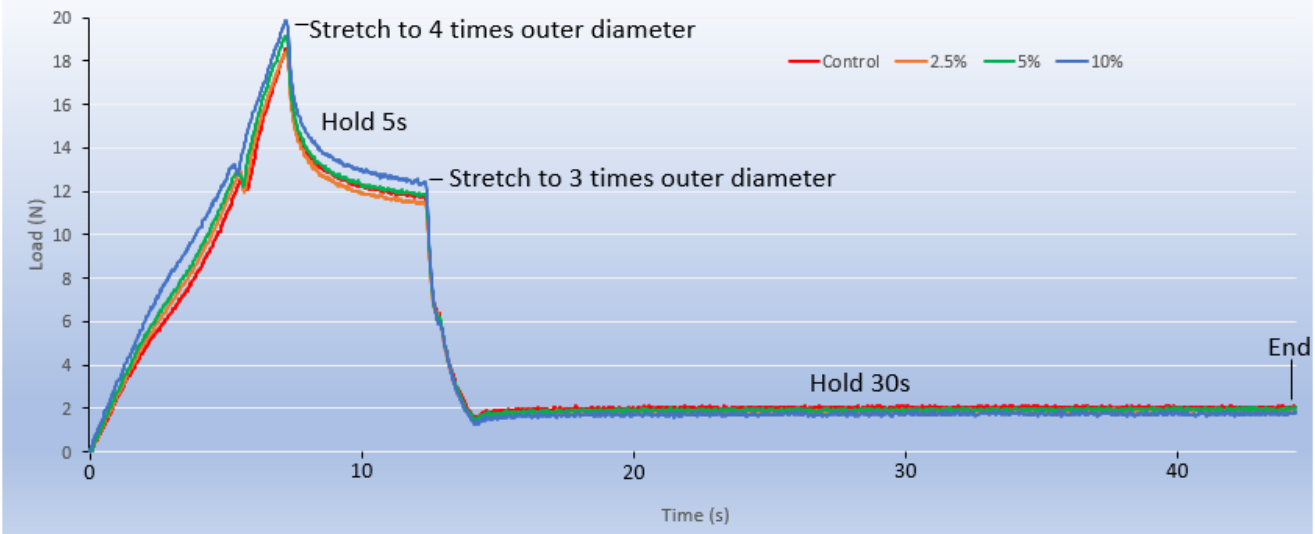


Figure 19. Instron Test T2 Curve



APPENDIX B TABLES

Table 1a. Daily Fluoride Release Rate in 1st Week (µg F^-/ring/day)

	Control			2.5%			5%			10%		
	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value
Day 1 (µg F-/ ring/day)	.170	.028	.005	37.521	1.220	<.001	43.958	1.228	<.001	34.146	1.577	<.001
Day 2+3 2 (µg F-/ ring/day)	.035	.022	.120	21.896	.580	.026	25.000	.458	.026	23.458	.817	.172
Day 4+5 2 (µg F-/ ring/day)	.019	.008	.273	19.969	.472	<.001	25.177	.512	.025	22.969	.515	.001
Day 6+7 2 (µg F-/ ring/day)	.025	.009		15.646	.498		24.135	.485		22.240	.504	

Table 1b. Daily Fluoride Release Rate in 1st Week (µg F^-/ring/day)

	Control		2.5%		5%		10%		2.5% vs. Control	5% vs. Control	10% vs. Control	2.5% vs. 5%	2.5% vs. 10%	5% vs. 10%
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	P-value	P-value	P-value	P-value	P-value	P-value
Day 1 (µg F-/ ring/day)	.170	.028	37.521	1.220	43.958	1.228	34.146	1.577	<.001	<.001	<.001	<.001	.009	<.001
Day 2+3 2 (µg F-/ ring/day)	.035	.022	21.896	.580	25.000	.458	23.458	.817	<.001	<.001	<.001	<.001	.010	.011
Day 4+5 2 (µg F-/ ring/day)	.019	.008	19.969	.472	25.177	.512	22.969	.515	<.001	<.001	<.001	<.001	<.001	<.001
Day 6+7 2 (µg F-/ ring/day)	.025	.009	15.646	.498	24.135	.485	22.240	.504	<.001	<.001	<.001	<.001	<.001	<.001

Table 2a. Daily Fluoride Release Rate by Week (µg F^-/ring/day)

	Control			2.5%			5%			10%		
	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value
Week 1 (µg F-/ ring/day)	.046	.011	.019	21.792	.334	<.001	27.512	.384	<.001	24.497	.704	<.001
Week 2 (µg F-/ ring/day)	.007	.002		5.795	.144		8.042	.139	.256	8.063	.157	.320
Week 3 (µg F-/ ring/day)	.005	.001	.044	4.211	.106	<.001	7.976	.063		7.982	.132	
Week 4 (µg F-/ ring/day)	.005	.001	.232	2.266	.129	<.001	7.280	.136	<.001	7.354	.114	<.001
Week 5 (µg F-/ ring/day)	.005	.001	.370	1.607	.126	.001	7.682	.074	.001	7.714	.129	.001
Week 6 (µg F-/ ring/day)	.005	.001	.151	.895	.110	<.001	7.098	.096	<.001	7.342	.086	.002
Week 7 (µg F-/ ring/day)	.005	.001	.436	.686	.016	.037	6.539	.145	<.001	6.970	.140	.003

Table 2b. Daily Fluoride Release Rate by Week (µg F^-/ring/day)

	Control		2.5%		5%		10%		2.5% vs. Control	5% vs. Control	10% vs. Control	2.5% vs. 5%	2.5% vs. 10%	5% vs. 10%
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	P-value	P-value	P-value	P-value	P-value	P-value
Week 1 (µg F-/ ring/day)	.046	.011	21.792	.334	27.512	.384	24.497	.704	<.001	<.001	<.001	<.001	<.001	<.001
Week 2 (µg F-/ ring/day)	.007	.002	5.795	.144	8.042	.139	8.063	.157	<.001	<.001	<.001	<.001	<.001	1.000
Week 3 (µg F-/ ring/day)	.005	.001	4.211	.106	7.976	.063	7.982	.132	<.001	<.001	<.001	<.001	<.001	1.000
Week 4 (µg F-/ ring/day)	.005	.001	2.266	.129	7.280	.136	7.354	.114	<.001	<.001	<.001	<.001	<.001	1.000
Week 5 (µg F-/ ring/day)	.005	.001	1.607	.126	7.682	.074	7.714	.129	<.001	<.001	<.001	<.001	<.001	1.000
Week 6 (µg F-/ ring/day)	.005	.001	.895	.110	7.098	.096	7.342	.086	<.001	<.001	<.001	<.001	<.001	.009
Week 7 (µg F-/ ring/day)	.005	.001	.686	.016	6.539	.145	6.970	.140	<.001	<.001	<.001	<.001	<.001	<.001

Table 3. Total fluoride accumulated over 7 weeks (µg F^-/ring)

	Control		2.5%		5%		10%	
µg F-/ ring	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Day 1	.170	.028	37.521	1.220	43.958	1.228	34.146	1.577
Day 3	.241	.050	81.313	1.981	93.958	1.504	81.063	3.155
Day 5	.280	.065	121.25	1.789	144.312	2.323	127.000	3.984
Day 7	.329	.077	152.542	2.336	192.583	2.690	171.479	4.931
Day 14	.375	.089	193.104	3.205	248.875	2.957	227.917	5.944
Day 21	.412	.097	222.583	3.470	304.708	3.234	283.792	6.635
Day 28	.445	.102	238.446	3.867	355.667	3.545	335.271	7.407
Day 35	.477	.105	249.698	4.735	409.437	3.871	389.271	8.282
Day 42	.514	.106	255.962	5.382	459.125	4.283	440.667	8.849
Day 49	.547	.114	260.762	5.365	504.896	4.867	489.459	9.766

Table 4. Instron Mechanical Test – T1, T2, and Change

	Control		2.5%		5%		10%		2.5% vs. Control	5% vs. Control	10% vs. Control	2.5% vs. 5%	2.5% vs. 10%	5% vs. 10%
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	P-value	P-value	P-value	P-value	P-value	P-value
T1 Force (N)	2.156	.069	2.134	.106	2.292	.128	2.445	.204	1.000	.328	.001	.173	.001	.193
T2 Force (N)	2.071	.060	1.896	.033	2.020	.082	1.795	.072	<.001	.770	<.001	.004	.024	<.001
Change T2-T1 Force (N)	-.085	.080	-.239	.111	-.272	.122	-.651	.186	.152	.047	<.001	1.000	<.001	<.001
% Change	- 3.94%		- 11.2%		- 11.9%		- 26.6%							

Table 5. Instron Mechanical Test – T1 versus T2

	Control			2.5%			5%			10%		
	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value	Mean	Standard Deviation	P-value
T1 Force (N)	2.156	.069	.020	2.135	.106	<.001	2.292	.128	<.001	2.445	.204	<.001
T2 Force (N)	2.071	.060		1.896	.033		2.020	.082		1.795	.072	