Type of Award: Biomedical Research Award

Name(s) of Principal Investigator: Yan Jing

Title of Project: Development of sustained fluoride-releasing O-rings for the prevention of white spot lesions

Period of AAOF Support (e.g. 07-01-20 to 06-30-22):

Amount of Funding: 30k

Summary/Abstract (250 word maximum)

There is no ideal way to prevent white spot lesions. O-rings incorporated with fluoride have been previously studied for a long-term continuous release of fluoride, however, have been unsuccessful in attaining therapeutic levels to prevent white spot lesions. The present utilized a novel method of dip coating with calcium fluoride (400mg/ml) into 2.5%, 5% or 10% Polycaprolactone O-rings to achieve sustained fluoride release (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. 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, all higher than the therapeutic range (1.2-2.8 µg F^-/ring/day) but much lower than the toxic level (51 µg F^-/ring/day). At the 7th 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 with EDX analysis showed successful integration of calcium fluoride on the surface of the O-ring. Instron test revealed no significant change in elastic properties of the O-rings after coating. In summary, 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. This study is promising for the prevention of white spot lesions in orthodontic patients.

Detailed results and inferences:

1. Development of a novel post-processing strategy to load calcium fluoride onto the ordinary O-rings

Briefly, the calcium fluoride powder was dissolved in acetone (400 mg/ml) as Solution A; the Polycaprolactone (PCL) powder was dissolved in acetone to the concentration of 5%, 10% or 20% as Solution B. 0.5 ml of Solution A and B were then mixed to obtain three working solutions: calcium fluoride (400 mg/ml) in 2.5%, 5% or 10% PCL (Fig.1a). The ordinary O-rings (American Orthodontics, Wisconsin) were modified into Ca-F O-rings by dipping the O-rings into one of the working solution for one second. A total of four groups of O-rings were set: 1) ordinary O-rings (controls); 2) Ca-F O-rings coated with 2.5% PCL; 3) Ca-F O-rings coated with 5% PCL; 4) Ca-F O-rings coated with 10% PCL. After the coating, the Ca-F O-rings were air-dried for 24 hours. SEM (n=8), Instron (n=8) and release profiles (n=16) were used to analyze the amount of coated calcium fluoride, the elastic properties, and fluoride release amounts between groups. Pilot studies have been previously completed to determine the basis of the dip concentrations and the necessary effect size. With the proposed sample size (n=8), 80% power can be attained with an effect size of 2.5.

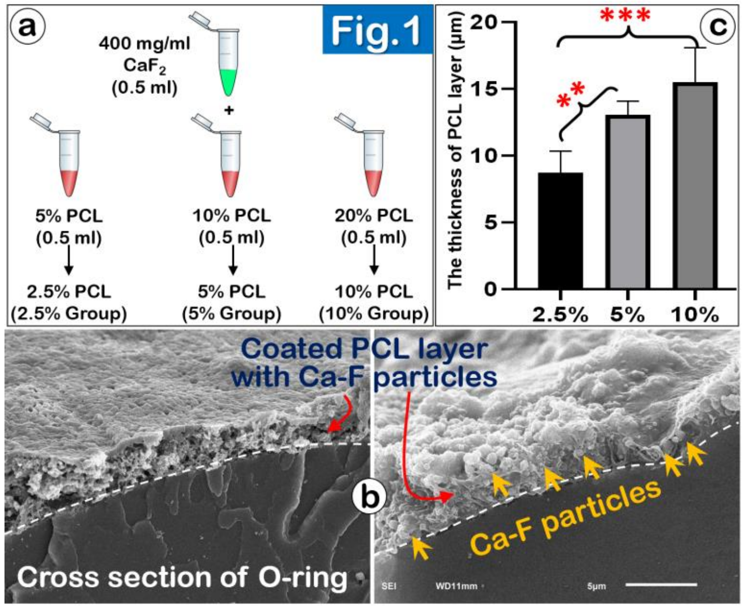
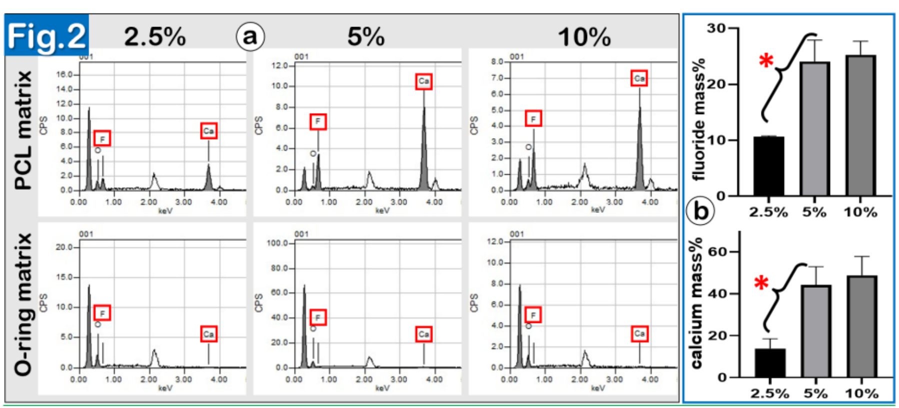


*Fig.1 a) Coating material preparation; b) SEM images for coated PCL layer on O-ring; c) thickness of PCL layers in different Ca-F O-rings.(**P<0.01; ***P<0.001)*

2. Demonstration of the successful coating of calcium fluoride onto the O-rings

We first used a scanning electron microscope (SEM) to characterize the morphology of the O-ring on cross-section. The lower magnification image showed the coated PCL layer on the top of the O-ring matrix (Fig.1b, left); the higher magnification image further displayed the calcium fluoride particles in white color embedded into the PCL layer (Fig.1b, right). We then measured the thickness of coated PCL layers in three groups of Ca-F O-rings on SEM level. The quantitation results showed a gradual increase of PCL thickness from 2.5% to 10%. The 2.5% group was significant thinner that the other two (Fig.1c), while there was no statistical difference between 5% and 10% groups. This data indicated that likely more calcium fluoride was incorporated in the 5% and 10% groups.

To verify this assumption, the amount of calcium fluoride in the PCL layers were tested by energy-dispersive X-ray spectroscopy (EDX) associated with SEM (EDX/SEM) mapping[35]. The images of EDX clearly showed two absorbance peaks for calcium and fluoride ions in the PCL matrix for the three Ca-F O-ring groups, whereas no calcium or fluoride ions were detected in the O-ring matrix (Fig.2a). The



quantitation results further revealed that the percentage of fluoride mass in the 2.5% group was less than half that of the 5% and 10% groups, and there were no significant difference between the 5% and 10% group (Fig.2b, upper). The percentage of calcium mass followed a similar trend (Fig.2b, lower). This data demonstrates that calcium and fluoride ions were successfully incorporated into the PCL layer on the O-ring surface.

3. Ca-F O-rings maintain the features of elastic ligatures

To demonstrate if the modification affects the elastic property of the Ca-F O-ring, we used an Instron to test its elasticity according to ISO 21606:2007[36] and compared it with that of the ordinary O-ring. Briefly, an O-ring was stretched by Instron at a rate of 100 mm/min to the length of 4 folds of the outer diameter (OD) for 5s. Subsequently, the ring was returned to 3 folds of OD at the same rate and the tensile force was recorded after 30secs holding. Tensile failure, which happens when the O-ring cannot be stretched to the length of 4 folds of OD, did not occur in any of the groups of Ca-F O-rings. After the modification (T1), the tensile force in the 2.5% group was slightly decreased, but moderately increased in the 5% and 10% groups with significant difference between controls and 10% group (Fig.3a). After 7-weeks soaking (T2), the tensile force in all four groups decreased compared to the initial stage, and the three groups of Ca-F O-rings were all lower than the controls with significant differences found in the 2.5% and 10% group (Fig.3b). The percentage of decrease from the control to 10% groups are 4%, 12.4%, 11.9% and 26.6%, respectively. This data indicates that the 5% group has the least amount of change on the elastic property after modification and soaking compared to controls.

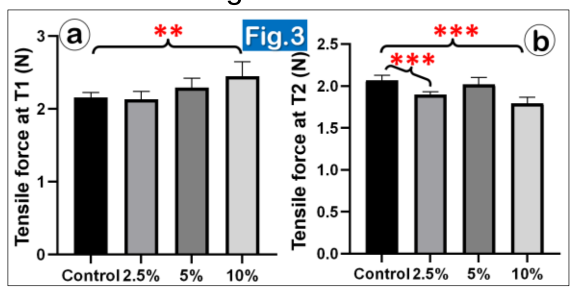


*Fig.3 The tensile forces of O-rings before (a) and after (b) 7-week soaking in distill water. (**P<0.01; ***P<0.001)*

4. Evaluation of the releasing profile for Ca-F O-rings

Next, we evaluated the fluoride-releasing pattern of the Ca-F O-rings. It has been reported that stretching increases the amount of fluoride released from fluoridated elastomeric ligatures[37]. Thus, all O-rings were placed on brackets for the maxillary lateral incisor (American Orthodontics, WI) before measurement to mimic clinical application. Every fourth O-ring was immersed into 10 ml of distilled water in a 50 ml centrifuge tube. Each group had 4 tubes with a total 16 O-rings per group. All tubes were incubated at 37 °C. To assess the release profile of fluoride, measurements were taken at 10 time points (Fig.4a) by Thermo Scientific Orion Dual Star Ion Selective Electrode (ISE) Meter. Calibration was conducted before each measurement. At each time point, the O-rings were removed from the tube, dried, and transferred to a new tube of 10 ml distilled water to incubate at 37 °C.

The cumulative releasing curves showed sustained release in the 5% and 10% groups for 7 weeks, while the rate of the 2.5% group significantly decreased after 3 weeks (Fig.4a). 14%, 8.7% and 7% of total fluoride was released in the first day for the 2.5%, 5% and 10% groups, respectively, which is much slower than Fluor-I-Tie® (35% in first day[15]). The average releasing rate within the first week for the 2.5%, 5% and 10% groups were 21.8, 27.5 and

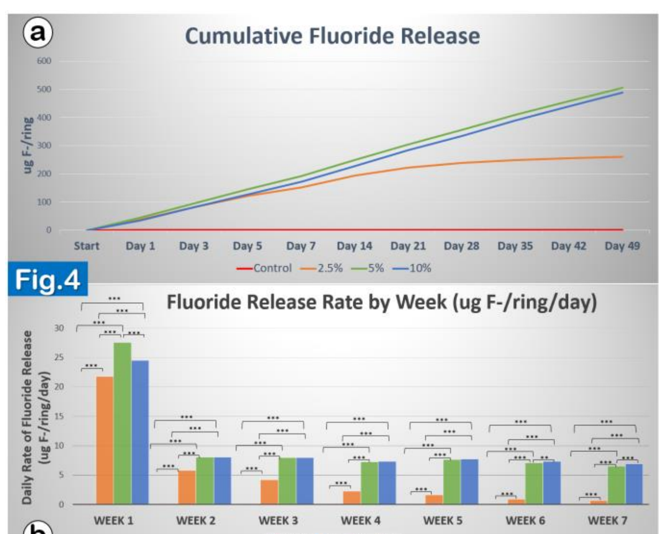


*Fig.4 The cumulative (a) and average by week fluoride release (b) of four groups of O-rings.(***P<0.001)*

24.5 μg F^-/day/ring, respectively (Fig.4b), which is much lower than *toxic level (51 μg F^-/day/ring[18,38])*. The average releasing rate in 7 weeks for the 2.5%, 5% and 10% groups were 5.32, 10.0 and 9.99 μg F^-/day/ring, respectively. These rates are all higher than the *therapeutic range (1.2-2.8 μg F^-/day/ring[18,38])*. The fluoride-releasing rates in the 7th week for the 2.5%, 5% and 10% groups were 0.69, 6.54, and 6.97 μg F^-/day/ring, respectively, indicating a continuous effective fluoride release in the 5% and 10% groups. Reliability was established for fluoride release 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, which demonstrates a high consistency of the coating procedure.

Taken together, our pilot data reveals that 1) the post-processing strategy can effectively and reliably coat PCL incorporated with calcium fluoride particles onto the surface of O-rings; 2) the Ca-F O-rings exhibit long-term sustained and effective fluoride release; 3) the Ca-F O-rings maintain the features of elastic ligatures after modification. This data indicates a potential for Ca-F O-rings to be a promising product for the prevention of WSLs during orthodontic treatment.

Response to the following questions:

1. Were the original, specific aims of the proposal realized?

Yes.

2. Were the results published?

No, but we plan to publish it.

3. Have the results of this proposal been presented?

Yes, and AAOF support was acknowledged

Title: The Development of Sustained Fluoride-Releasing O-Rings for the Prevention of White Spot Lesions: *An In Vitro Study*

Authors: Kativa Strickland, Chi Ma, Peter Buschang, Yan Jing

Year: 2022

Location: 2022 AAO annual meeting at Miami, E-poster

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

We have used the data obtained in this study to apply for a Research Aid Award for one of our residents in Class 2024. This application has been approved to be funded (07/2022-06/2023).

Accounting for Project; (i.e.), any leftover funds, etc.

We may have around $100 leftover when the project ends.