COMIRB Protocol

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COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
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Project Title: Prevention of enamel demineralization in fixed appliance orthodontic patients using UDMA-K18 sealant to prevent microbial attachment. A randomized split mouth clinical trial.

Principal Investigator: Dr. Clifton Carey, Professor and Director of Translational Research
Orthodontics Residents: Dr. Shivani Patel and Dr. Megan Fellows

Dr. Carey is the research mentor for University of Colorado, School of Dental Medicine, Department of Orthodontics residents Drs. Patel and Fellows. Drs. Patel and Fellows will be performing all the sealant and bracket placements. Dr. Carey will assist in the histology and evaluation of the extracted teeth.

I. **Hypotheses and Specific Aims**: The hypothesis is that UDMA-K18 containing smooth surface sealant will be more effective at reducing enamel demineralization than the UDMA control or no treatment. This study will assess the effect of a quaternary ammonium methacrylate (QAM) called K18 antimicrobial compound tethered to urethane dimethacrylate resin (UDMA-K18). This resin will be tested against UDMA sealant and no sealant. This study will evaluate effectiveness of a UDMA-K18 sealant to prevent biofilm attachment to tooth surfaces thereby eliminating the possibility for the tooth to be demineralized.

II. **Background and Significance:** Far too frequently orthodontic patients are unable to perform sufficient oral hygiene to remove all of the bacterial biofilm surrounding orthodontic brackets. When the bacterial biofilm is not removed, tooth demineralization is possible. The incidence of new tooth demineralization, often called white spot lesions (WSL), has been studied at the University of Colorado Department of Orthodontics. Forty-four orthodontic patients ages between 11 and 21 years of age were assessed over the course of their therapy for the development of new WSL. This study reported that 84.6 % of the patients developed new WSL, and the total area of WSLs also increased from 16.6 ± 23.6 mm^2 per patient to 42.6 ± 50.7 mm^2 per patient. The period of therapy where the greatest increase in WSL occurred was between initial banding and 1 year of therapy. The area most susceptible to WSLs during treatment is the cervical third, and both anterior and posterior teeth are equally susceptible to the development of new lesions. (Faranesh et al., 2017). Given that the bacterial biofilm is

hard to remove due to compromised access to all of the tooth surfaces by orthodontic appliances (brackets and wires) a new strategy that limits the ability of bacterial to attach to tooth surfaces may have a protective capacity preventing WSL. The UDMA-K18 dental resin is designed to protect enamel from bacterial attachment and biofilm associated acid demineralization which can lead to the formation of white spot lesions (WSL) on the surface on the tooth. The composition of the experimental sealant is K18, a quaternary ammonium methacrylate linked to a siloxane backbone covalently bonded to branched organic urethane dimethacrylate (UDMA) monomers. See Figure 1 for the chemical structure. These UDMA or UDMA-K18 monomers are mixed with camphorquinone photoinitiators, which when placed on a tooth surface can be polymerized by exposure to a dental blue light.

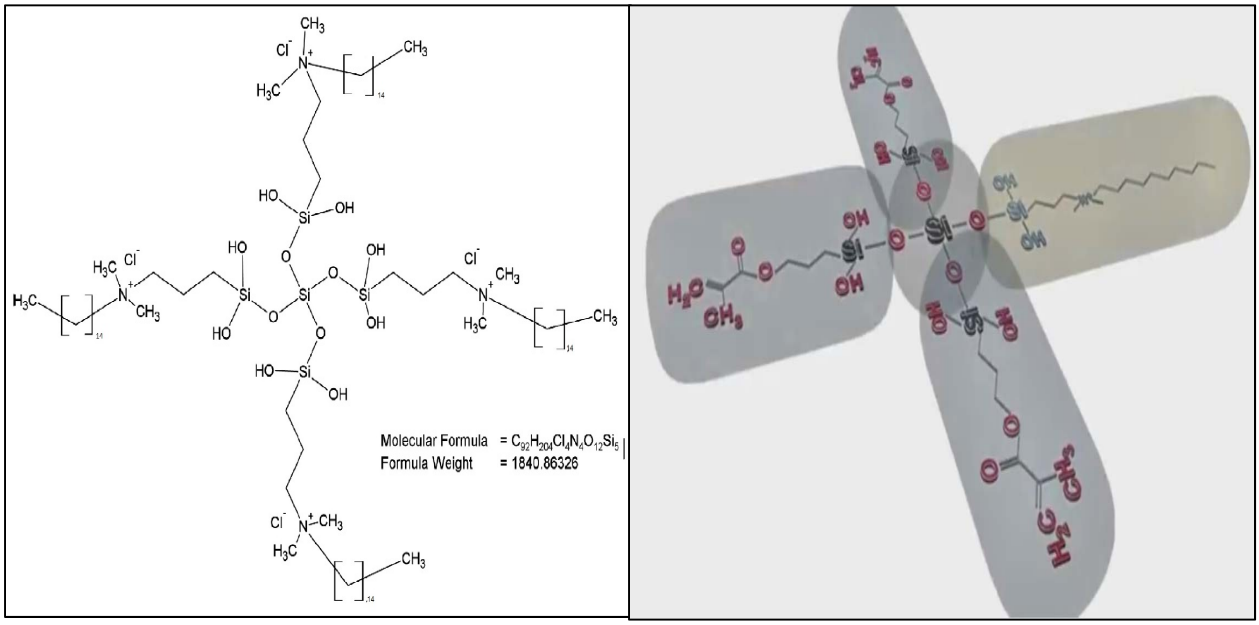

Figure 1. Structure of UDMA-K18.

III. Preliminary Studies/Progress Report: This tethered K18 anti-attachment compound has been tested in clinical studies for antimicrobial accumulation on orthodontic retainers (as well as antimicrobial and cytotoxic activity in clinical split mouth study (Liu *et al.*, 2016) and for cytotoxic and carcinogenic activity (Gong et al, 2012).

a. Liu et al., 2016 studied the antimicrobial activity of a quaternary ammonium methacryloxy silicate-containing acrylic resin in a double blind randomized clinical trial. The quaternary amine (QAM) was synthesized with a sol-gel reaction between a tetraalkoxysilane and

two trialkoxysilanes, creating a methacryloxy functional group and long C-18 carbon chain. Liu et al, mentioned that in previous studies QAMs at a concentration of 4 % to 6 % have been shown to have immediate contact killing antimicrobial properties. Cell viability assays have also been previously completed and the acrylic resin has been deemed noncytotoxic and received 510(K) clearance from the US Food and Drug Administration. This in-vivo experiment of 32 patients reviewed the antimicrobial efficacy of quaternary ammonium methacrylate (QAMs) containing orthodontic acrylic added to orthodontic retainers. There was a statistically significant difference between the QAMs containing side and the control side in the percentage kill in the biovolume. The study found that the QAMs-containing acrylic demonstrates favorable antimicrobial activity against biofilms in vivo experiments.

b. Gong et al., (2012) studied the possible cytotoxicity by use of the MTT assay on a mouse fibroblast cell line (L929). They determined the effects of increasing molar feed ratios of 3-(Trimethoxysilyl) propyldimethyloctadecyl ammonium chloride (SiQAC, a starting material) on the cytotoxicity of a series of silsesquioxane-silica hybrids (SqSHs, which include K18). The results of the cell viability assays indicated that the QAMS-containing orthodontic acrylic resin is relatively non-cytotoxic Therefore, the QAMS-containing orthodontic acrylic has received 510(K) clearance for marketing by the U.S. Food and Drug Administration (FDA) .

c. K18-UDMA has been tested in the Principal Investigator's laboratory for anti-attachment of biofilm (antimicrobial activity) (Stricker *et al.*, 2016). 2.6×10^{-6} All of these studies show that tethered K18 to UDMA or acrylates prevents attachment of microbial biofilms to surfaces.

d. The methods for determination of lesion depth and mineral density have been tested in the Principal Investigator's laboratory (Lee *et al.*, 2015; and Schmuck & Carey, 2010). In the Lee study a total of 64 extracted teeth were cleaned and prepared with an orthodontic bracket. The teeth were randomly divided into 8 experimental groups of 8 samples to evaluate the protection from erosion efficacy of commercial and experimental smooth

surface sealants. The outcome was the amount of erosion that the teeth experienced, which was measured by determining the lesion (tooth loss) depth as seen in the cross-section through the orthodontic bracket. The results of the demineralization were between 44.7 ± 21.9 μm for the negative control to 1.1 ± 4.4 μm lost for one of the fluoride containing smooth surface sealants. The erosive loss for the Transbond XT smooth surface sealant that this study proposes to use as a bonding agent was 29.3 ± 9.6 μm which is significantly less erosion than the control and significantly more erosion than the commercially available fluoride containing smooth surface sealant ($p < 0.05$).

e. The methods described in the paper by Schmuck and Carey (2010) will be used for the transverse microradiography (TMR) of the samples in this study. The extracted teeth will be sectioned and digital images will be taken with incident and transmitted polarized light to visualize any lesion in the sample. TMR will be used to determine surface lost. TMR determination of lesion depth will be used to compare the measurement via polarized light microscopy.

IV. Research Methods:

a. Outcome Measures: mineral density profile and lesion depth of extracted teeth in these experiments. Mineral density will be measured by transverse x-ray microradiography (Schmuck and Carey, 2010), lesion depth will be measured by polarized light microscopy (Wefel and Harless, 1984; Darvishiabyaneh and Carey 2016; and for orthodontic evaluations Lee *et al.*, 2015).

b. Description of Population to be Enrolled:

Research participants will be between 12 and 89 years of age who are subjects undergoing orthodontic treatment at the Department of Orthodontics Clinic, School of Dental Medicine, University of Colorado. The following inclusion criteria will be applied: comprehensive fixed orthodontic treatment with brackets from at least second premolar to second premolar, treatment planned already for the extraction of at least 3 bicuspids (any combination), patient at the University of Colorado School of Dental Medicine Orthodontic Clinic, can place bracket base on teeth to be extracted for at least 1 month

prior to extractions, no previous white spot lesions, caries or restorations present on the facial surface of the teeth being studied, adequate oral hygiene.

In the recruitment process the attending resident will provide information and answer any questions about the study to the potential study participant. If there is interest on behalf of the patient then the consent form will be reviewed and after presentation the form will be signed by all who wish to join the study. The consent form is attached as a separate document. There is no benefit to the patient to participate other than participating in a study that may find a beneficial treatment for others in the future. There will be no effect on the patients treatment or experience should they decide not to participate or to participate.

The requirement of at least 3 biscupids to be extracted is to meet the requirements of the matched pair experimental design. Because each patient is different and their oral biology is different treating and analyzing teeth that do not have matched pairs in the same patient are not useful for this study therefore the inclusion criteria cannot include patients with less than 3 planned extractions.

The inclusion of minors aged 12 and older is because minors are more likely to develop white spot lesions and should this sealant provide protection then the whole orthodontic population will benefit. Also there is no risk associated with the procedures outlined in this trial so there is no reason to exclude minors from this study.

Currently the Department of Orthodontics enrolls 50 to 75 patients per month. The number of patients that meet the above inclusion criteria will be about 30 % of those enrolled. It is anticipated that a minimum of 1 patients per month can reasonably be enrolled into the study. Thus, the study should receive full enrollment in 4 months.

c. Study Design and Research Methods (see CONSORT diagram on last page)

i. The study has a split mouth clinical design where each of two controls and one experimental sealant will be in each person.

1. Each patient will have all three experimental sealant systems including: no sealant (negative control), UDMA sealant (control) and K18-UDMA sealant

(experimental group). The number of patients required for this study was calculated from studies that had a similar design (de Moura *et al.*, 2006; Uysal *et al.*, 2010). De Moura et al. studied the effect of a fluoridated antiplaque dentifrice on the development of caries lesions adjacent to orthodontic brackets. Fourteen orthodontic patients with planned extractions were divided into two groups of 7. The patients used experimental or control toothpastes 3 times per day for 28 days. The teeth were extracted and the mineral loss was determined in the cross-sections by use of microhardness. The results showed that there was a statistically significant ($p < 0.05$) improvement with the experimental antiplaque toothpaste for the first 10 μm of depth. There was no significant difference at deeper distances from the surface. A calculation of their experimental power shows that an n of 7 per group is insufficient ot achieve 80 % power at an α of 0.05. Additionally, the microhardness method is less sensitive to mineral density loss than the TMR methods to be used in this study. Balancing the increased sensitivity via TMR with the insufficient power in the de Moura study to make conclusions about the efficacy of the antiplaque toothpaste to prevent tooth loss; we opt to be more conservative and use a larger n per group. Based on the previously observed variations in mineral density and clinically relevant minimum difference of 10 % mineral density in the first 50 microns of tooth surface between the negative control and the experimental K18-UDMA sealant, the number of subjects to achieve 80 % power at an alpha of 0.05 is 20 subjects. It is anticipated that up to 30 patients may be recruited to this study, with 20 needed for statistical power to answer our research question.

2. If the patient has a fourth bicuspid is planned for extraction, a random treatment will administered to this last tooth. This will provide additional information on the effect of location (upper or lower jaw) on the protective ability of the experimental sealant.

ii. Teeth planned for extraction will be bonded with a bracket pad using one of three systems: no treatment (negative control), UDMA sealant (control) and K18-UDMA sealant (experimental group). The bracket pad does not have the bracket protruding from the base.

1. Blinding:
    a. The patients will be blinded as to which teeth receive the various treatments. All the teeth to be extracted will receive a bracket pad and which will serve to blind the patient.
    b. The dentists will be partially blinded as they will not be able to identify the UDMA from the UDMA-K18 sealants. These are provided to the dentists as Sealant A or Sealant B. The identity of Sealant A and Sealant B will be in a sealed envelope stored in the desk of the Chair of the department of Orthodontics. This drawer is kept locked.
    c. The tooth extraction, preparation of the extracted teeth for analysis and the statistical evaluation will be blinded to the identity of the sealants until the evaluations are completed.
    d. When the patient is to receive their orthodontic bands, the attending dentist (either Dr. Patel or Fellows) will randomly draw an envelope from a container of envelopes that contains a 3 x 5 card which indicates which teeth will receive Sealant A, Sealant B or no treatment. The attending dentist will write the patient number and date of sealant placement on the card and place that card into a study file for this information. The placement allocation indicated on the cards will be determined via computer randomization.
2. Bonding may occur then there is referral for extractions. The bonding protocol will be the standard protocol used in the orthodontic clinic for bonding orthodontic brackets to teeth.
3. The differences will be that instead of bonding a full orthodontic bracket to the bicuspids planned for extraction, a bracket pad will be bonded. The use of a

bracket pad in place of a full bracket is to reduce the possibility of the bracket from interfering with the orthodontic archwires attached to the other teeth as part of the standard orthodontic therapy.

4. The treatment to be used on each of the planned to be extracted bicuspids will be determined by selecting an envelope from a container that has within it a card indicating which teeth are to receive which treatments. The patient ID number will be recorded on the card which will be kept by the study coordinator in a secure location.

iii. All patients will be given standard hygiene instructions.

iv. When the patient reports to the oral surgeon for extractions, the extracted teeth will be placed in Tooth Storage Solution which is antimicrobial and does not alter the surfaces or composition of the teeth. The container will have the patient number and date of extraction written on the label.

v. There is no difference related to the procedures used to extract the teeth that are to be extracted for orthodontic therapy. The standard of care is the same for all patients whether there is a sealant or not on the teeth to be extracted.

vi. These teeth will be returned to the laboratory for histological evaluation of mineral density and lesion depth.

vii. The original location of the extracted teeth can be easily made from the tooth morphology. The source of the extracted tooth (original location and patient number) will be recorded in the laboratory notebook, and when data evaluation is conducted will be used to identify the experimental group for that the tooth.

d. **Description, Risks and Justification of Procedures and Data Collection Tools:**

i. Description: These experiments utilize bicuspids that are treatment planned for extraction to allow for an approximant 30-day period where the experimental treatment is exposed to the environment of the mouth in exactly the same manner as all other teeth in the patients mouth.

1. About 30 % of the patients who receive treatment planning for orthodontic therapy will require extraction of bicuspids.
2. The extractions are usually scheduled to occur at about 30 days after bracket bonding to the other teeth.
3. The experimental design requires that each patient will have all experimental and control conditions (on separate teeth). This split mouth paired samples design increases the power of the experiment and allows for fewer patients to be required for sufficient statistical power. Paired analyses within an individual renders greater sensitivity to experimental effects than population based analyses.
4. Bonding bracket pads instead of full brackets removes the protruding portion of the bracket so that it does not interfere with the orthodontic wires that are placed for the therapy.
5. The use of smooth sealants on the teeth to be extracted is not unique as many patients have a smooth surface sealant placed after the orthodontic brackets are placed. There are several commercial products that release fluoride specifically for the purpose of prevention of WSL. It is unfortunate that there are few well designed studies about the use of these sealants.
6. Should the patient decide to withdraw from planned orthodontic therapy prior to extractions, the bracket pads can be removed following the standard procedures for removing brackets at the same time as the brackets are removed. The sealants will come off at the same time because the standard procedure includes polishing the tooth surfaces when brackets are removed.
7. Patient identification is only to identify which tooth received the specific treatments so that when the teeth are extracted by the oral surgeon the groups can be identified. No patient information is associated with the outcome measures.

ii. Justification of Procedures: There are no procedures delivered to the patient that are not within the scope of the standard procedures for orthodontic patients. All data is gathered from the extracted teeth in the laboratory.

iii. Data Collection Tools: Mineral density and lesion depth will be determined in the laboratory through histological examination of the teeth. Briefly, the teeth are cross-sectioned through the bracket pad to generate a 1 mm thick slab from the middle of the tooth. This slab is observed under polarized light microscopy to measure the lesion depth (if any) from the surface, and subjected to x-ray to determine the mineral density across the lesion.

e. **Potential Scientific Problems:** None are anticipated.

f. **Data Analysis Plan**

i. Statistical analysis will include graphical evaluations, along with descriptive statistics (mean, standard deviation), and one-way ANOVA for the three groups for both mineral density and lesion depth outcomes. If appropriate, there will be follow-up ranked comparison testing at a significance of $P \leq 0.05$. There will be paired-t tests between UDMA and negative controls, UDMA-K18 and negative controls, and paired-t tests between UDMA and UDMA-K18 sealants on mineral density and lesion depth outcomes. Should there be data from a 4^{th} tooth (2 teeth will receive the same treatment) the data from the 2 teeth with the same treatment will be averaged prior to comparison to the controls.

ii. The possibility of regional variations (left vs. right, upper vs. lower) has been taken into account in the calculation of the size of the patient cohort. The sample number calculation was based on differences and variations that are relatively large, and an additional 10 (50 % number) patients were added to the cohort to give the strongest probability of being able to make statistically based conclusions.

iii. Secondary analyses may be possible to assess the ability of microbial biofilm to cause demineralization differences between upper and lower arches. If a patient has a fourth bicuspid that is to be extracted (which is usually the case) the assignment of one of the

three treatments will be to generate a pair (left vs. right or upper vs. lower) for comparison. The statistical analyses will include paired-t tests for the differences that may be observed within each treatment group.

g. **Summarize Knowledge to be Gained:** Whether the use of UDMA-K18 sealant prevents tooth demineralization during orthodontic therapy.

   i. This is not a pilot study, and is intended to provide the clinical efficacy of the UDMA-K18 sealant to prevent white spot lesions around orthodontic brackets. Unless the variation is much larger than anticipated there will be sufficient power in the experimental design to make statistically based conclusions about the sealant efficacy. One of the experimental design features is that each patient will have all the controls and the experimental sealant so paired differences within each patient will be determined. This sort of paired difference experimental design is one of the most sensitive

   ii. The data generated and the conclusions achieved will be useful for the dental industry to decide whether to commercialize the sealant.

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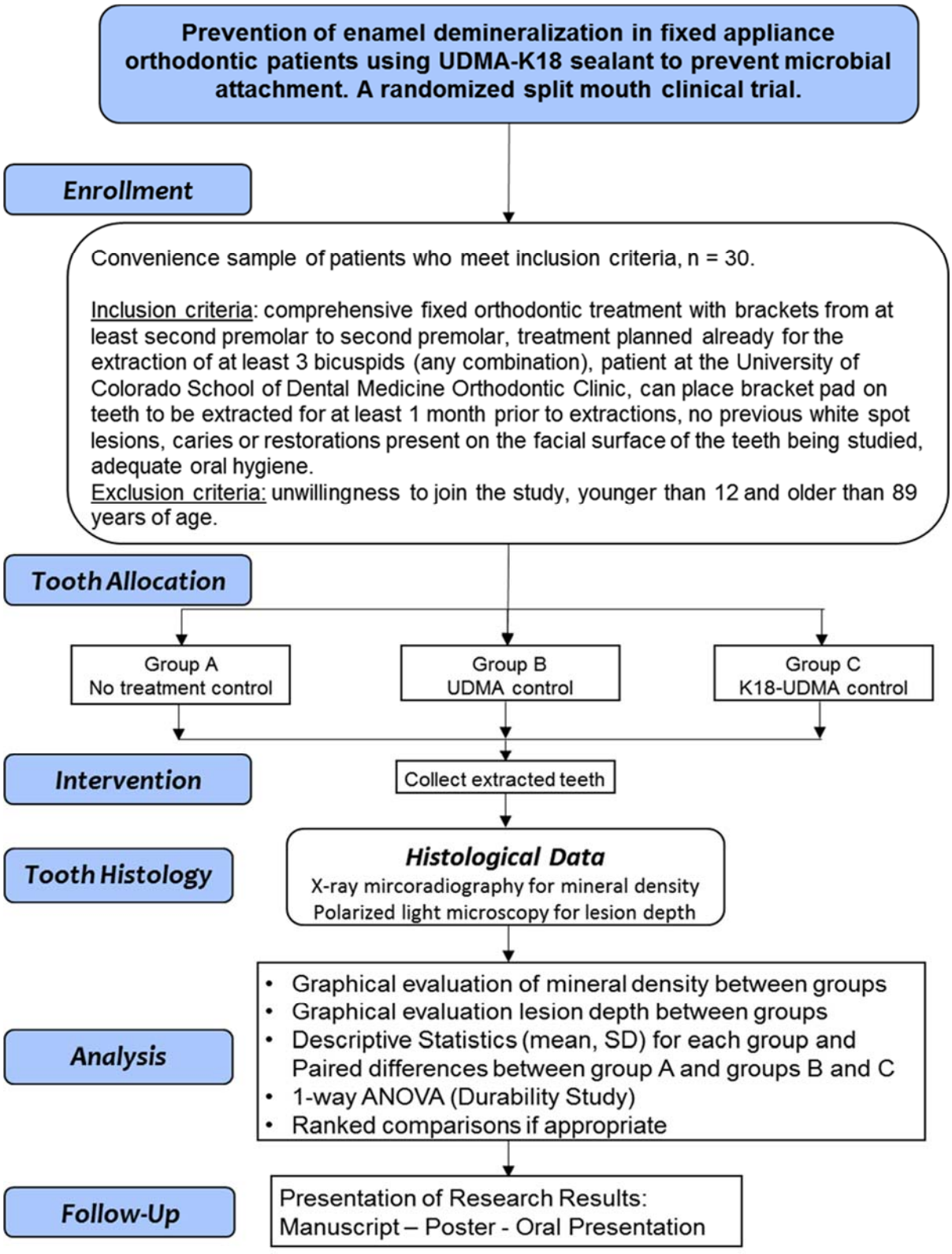
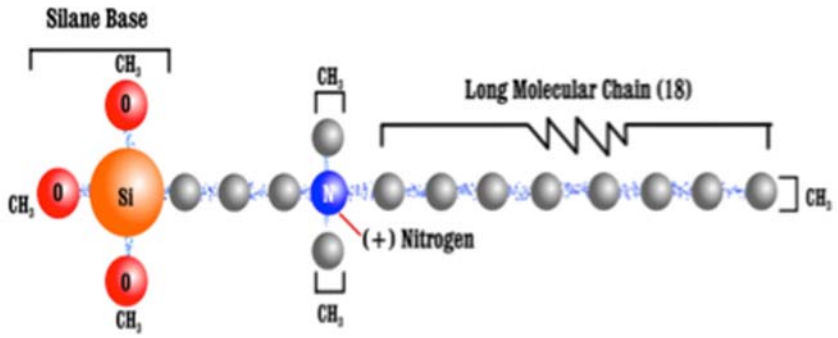
Experimental Diagram.





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