Effects of Cleaning Agents on the Properties of Two Different Thermoplastic Retainer Materials

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EFFECTS OF CLEANING AGENTS ON THE PROPERTIES OF TWO DIFFERENT THERMOPLASTIC RETAINER MATERIALS

by

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ABSTRACT

EFFECTS OF CLEANING AGENTS ON THE PROPERTIES OF TWO DIFFERENT THERMOPLASTIC RETAINER MATERIALS

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Objective:

There are several different thermoplastic retainer materials and cleaning methods available to fabricate and clean clear retainers. Some studies have investigated the changes in properties of clear retainers after being exposed to different staining agents, varying levels of mechanical strain, and different cleaning agents. However, there are few studies that compare different thermoplastic materials to each other when being subjected to these different types of stresses. The aim of this study is to evaluate the changes in properties of two commonly used thermoplastic retainer materials, polyethylene terephthalate (PETG) and ethylene/propylene (EP), after exposure to different retainer cleaning agents over 28 cleaning cycles.

Methods:

Samples were prepared by thermoforming sheets of Clear Splint Biocryl (PETG) and Invisacryl C (EP) with a Biostar, then cut into flat rectangular pieces. These samples were tested for surface roughness, color change, and flexural modulus after they were cleaned with four different types of retainer cleaning materials. Measurements were taken at three different time points for surface roughness and color change, while the flexural modulus was measured at the end of the experiment. Statistical analysis was completed with analysis of variation (ANOVA), t-tests, and post-hoc analysis. P values less than 0.05 was considered statistically significant.

Results:

The color change of both materials increased over time. Most of the color change for Invisacryl occurred between baseline and cleaning cycle 16. Listerine affected the color change of Biocryl the most, whereas there was no difference in cleaning method for the Invisacryl group. There was no significant difference in surface roughness for either material when cleaned with any of the cleaning methods at any time point. However, Invisacryl had a significantly higher surface roughness than Biocryl. Finally, there was no difference in elastic modulus between any of the cleaning methods for each material. There was a significant difference between the two materials, with Biocryl being the stiffer material than Invisacryl.

Conclusion:

Any of the cleaning methods evaluated in this study are recommended for use on PETG and EP materials, except for Listerine due to its ability to significantly impact a

color change on Biocryl. When comparing the two retainer materials, Invisacryl C retainers are not recommended for patients with poor hygiene due to their increased surface roughness. On the other hand, Biocryl is recommended to patients with severe pre-treatment rotations, spaces, and intruded teeth due to its increased elastic modulus.

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CHAPTER 1
INTRODUCTION

Retention is the last phase in orthodontic treatment and can arguably be the most difficult for several reasons. This phase relies on the stability of the occlusion at the end of treatment, growth, biology, and patient compliance. In fact, retention is still considered part of the active phase of treatment to some orthodontists and must be integrated into the original treatment plan (Graber et al, 2017). When fixed appliances are removed, teeth will want to return to their original position (Proffit et al 2019, Graber et al 2017, Johnston et al, 2015). For example, a study found that in four weeks after fixed appliance removal, while marginal ridges and buccal-lingual inclination improved, there was significant relapse in alignment, interproximal contacts, overjet, and intercanine width (Lyotard et al, 2010). While some relapse is inevitable, it is the clinician's job to create a treatment plan that will end in a stable result accompanied by an appropriate and individualized retention protocol that can maintain the teeth in their post treatment position (Johnston et al, 2015).

Historically, there are four main schools of thought that attempt to explain relapse. Kingsley proposed that if the teeth were in proper occlusion, they were protected from relapse (Graber et al, 2017). Alternatively, in the 1922, Rogers argued that soft tissue, or muscle imbalance was the main reason for relapse (Lyotard et al, 2010). Shortly after, Lundstrom suggested that the apical base was the critical factor in retention (Graber et al, 2017). Finally, Tweed maintained that the mandibular incisors must be kept upright in order to prevent relapse (Lyotard et al, 2010). While none of these schools of thought are incorrect, it is now accepted that relapse is a multifactorial problem and does not rely on a single factor alone.

After appliances are removed, it takes 3-4 months for the PDL to reorganize to their new position and this reorganization will not occur until the teeth are no longer splinted together and are freely standing (Proffit et al, 2019). The supracrestal elastic fibers take even longer to settle into their final position and can displace teeth for up to a year after appliance removal if not stabilized with retention (Proffit et al, 2019). Consequently, retention is necessary after orthodontic treatment and the clinician must know what retention devices are available and the correct protocols to follow (Johnston et al, 2015). Broadly, there are two types of retainers – fixed and removable. While many clinicians have their own reasons for choosing one over the other, there is no evidence in favoring one method over the other when considering their effect on periodontal health, cost-effectiveness, survival rates, and patient preference, so the ultimate selection of retainer remains to be due to preference of the clinician (Al-Moghrabi et al, 2018).

There are many designs and wires used to used fixed retention. Fixed retainers are traditionally made of a wire bent to fit the lingual contour of a section of teeth so that it can be passively bonded and will not introduce any unwanted force to teeth. These retainers are typically made for the mandibular canine-to-canine segment but can also be made to keep spaces closed like diastemas. One of the major benefits to fixed retention is the reduced need of patient compliance (Johnston et al, 2015). In addition, fixed retention has been shown to be more effective at maintaining the alignment of the mandibular anterior segment when compared to clear removable retainers (Al Moghrabi et al, 2018). However, fixed retainers are not infallible. They require good hygiene to maintain a healthy periodontium as they can accumulate higher plaque (Al Moghrabi et al, 2018). It is also important for the clinician to be able to successfully repair a fixed retainer without adding any unwanted force to any of the teeth involved (Johnston et al, 2015).

The Hawley and Begg retainers are two types of removable retainers that are made with a hard acrylic palatal or lingual portion and a labial bow. Benefits to these retainers include the ability of the clinician to adjust the labial bow to activate tooth movement if necessary and to improve the intercuspation of posterior teeth by settling into a natural position since there is no occlusal coverage (Johnston et al, 2015). In addition, these retainers can be made with an anterior bite plate that can be beneficial to patients who begin treatment with increased overbite to prevent supraeruption of the anterior teeth (Sauget et al, 1997).

Another type of removable retainer is the vacuum-formed, or clear retainer. These retainers have gained popularity with not only patients, but also orthodontists, as a quick and reliable way to maintain good occlusion and stability post orthodontic treatment. Several studies have evaluated their effectiveness over the traditional Hawley retainer giving varied results. Some studies have found that thermoplastic retainers were better at maintaining arch length and alignment of maxillary teeth compared to the Hawley (Ramazanzadeh et al, 2018, Rowland et al, 2007). On the other hand, a systematic review conducted in 2014 concluded that there is insufficient evidence to claim the clear retainer or the Hawley as the superior appliance for retention (Mai et al, 2014).

Some reasons for the popularity of clear retainers include their superior esthetics, ease of fabrication, the ability for the patient to maintain good hygiene, large tooth contact area to maintain rotations, and ability to act as a mouthguard for patients with mild to moderate bruxism (Graber et al, 2017). These clear retainers are typically made from a sheet of a polymer material that is thermoformed over a stone or printed model of a patient’s dental arch. Similar to the labial bow of the Hawley retainer, small adjustments can be made to produce minor tooth movements if needed (Johnston et al, 2015). Patients are advised not to eat or drink anything other than water when wearing the retainers since distortion due to repeated loading, staining agents, and cleaning agents become apparent overtime (Johnston et al, 2015).

While it is clinically important to evaluate the property changes in thermoplastics after being exposed to both staining and cleaning agents, it is also important to know the changes to these materials that come from cleaning products alone. Currently, there are several options of cleaning solutions available to patients and clinicians, ranging from DIY at home solutions like diluted vinegar and hydrogen peroxide, to tablets and foam. Considering the amount of cleaning products available without a standardized cleaning protocol, it is important for a clinician to be aware of the effects of different cleaning solutions on a variety of thermoplastic materials.

CHAPTER 2
REVIEW OF LITERATURE

Physical Properties of Thermoplastic Retainer Materials

Several different thermoplastic materials are available to use for clear retainers including polyethylene terephthalate glycol (PETG), polyethylene/propylene, polypropylene, copolyesters, and polyurethane. In general, thermoplastics are composed of amorphous or partially crystalline polymers (Ryu et al, 2018). Their use in dentistry have been accepted due to their wide range of applications including good biocompatibility, low cost, chemical stability, and superior esthetics (Porojan et al, 2020). Despite their ease of use, these materials are also susceptible to changes in the thermoforming process and from the oral environment. (Porojan et al, 2020).

Many studies have examined the changes that happen within plastic during the thermoforming stage. One study evaluated the changes in thermoforming in four different plastics from a variety of manufacturers and evaluated the changes in flexural modulus, water absorption, color change, and surface hardness (Ryu et al, 2018). They found after deformation due to thermoforming, the transparency decreased, and water absorption and solubility increased in all materials. The hardness of the copolyesters (Essix A+ and Essix ACE) increased after thermoforming, while the hardness stayed the same in PETG (Duran and eClinger) (Ryu et al, 2018).

Traditionally, orthodontic tooth movement is delivered due to the stress deformation of different wires when tied to orthodontic brackets (Fang et al, 2013). When using thermoplastics for tooth movement, it is important to note the stress relaxation that happens when the material is deformed (Fang et al, 2013). Several studies have shown that one of the reasons that stress relaxation occurs in these materials is due to the plastic's characteristic ability to absorb water. (Fang et al, 2013).

To further evaluate the consequences of water absorption of thermoplastics, one study sought to determine a physical property of different thermoplastics that can be used to demonstrate the ability of exerting an orthodontic force (Inoue et al, 2020). They evaluated four different thermoplastic materials including Essix A+ (polyester), Duran (PETG), Erkodur (PETG), and Essix C+ (polypropylene). In this study, orthodontic force was calculated based on the density, elastic modulus, and Poisson ratio of each material after being exposed to water absorption tests. They found that the elastic modulus of the materials was the best property to evaluate a material for orthodontic force. Furthermore, Essix C+ demonstrated no change in elastic modulus after being exposed to water and is the most advantageous out of the four materials studied to give the most constant orthodontic force (Inoue et al, 2020).

In this study, the thermoplastic materials used were polyethylene terephthalate glycol (PETG) (Biocryl, Great Lakes Dental Technologies) and ethylene-propylene polymer (EP) (Invisacryl C, Great Lakes Dental Technologies). PETG is one of the most common thermoplastics used in orthodontic applications. It is a non-crystallized, amorphous copolyester fabricated from polyglycolic acid (Wang et al, 2021). Some of the reasons for its popularity include high ductility, chemical resistance, processability, and recyclability (Wang et al, 2021). The second material used in this study, EP, is considered as one of the lightest thermoplastics used and have the least resistance to wear due to their lack of material hardness (Gardner et al, 2003). One study compared the wear between two polypropylene based materials to one PETG based material and found that the PETG material was significantly harder than both polypropylene materials (Bernard et al, 2003)

Effect of material aging and staining on transparency

It is common for an orthodontist to recommend full time wear of retainers after fixed appliances are removed for a matter of several days, this includes all day and night except for eating, brushing teeth, or drinking anything other than water. However, much like active orthodontic treatment, compliance is also a problem in retention and patients might consume foods or liquids while wearing the retainers that can stain the materials (Bernard et al, 2020). To evaluate the staining potential of different thermoplastic materials, one study used five different, common staining agents, and exposed them to three different thermoplastics over a matter of seven days. After being stained, the researchers evaluated the cleaning potential of three cleaning solutions on the stained materials. Out of the staining agents used, black tea was the most potent to all of the thermoplastics. In addition, the Invisalign retainers (polyurethane) were the most prone to staining from coffee and red wine, when compared to retainers made by Clear Correct (polyurethane) or Minor Tooth Movement (PETG). Finally, they showed that both cleaning methods, Invisalign Cleaning Crystals alone and Retainer Brite tablets in a sonic cleaner, were equally as effective at removing stains from the black tea (Bernard et al, 2020).

Another study evaluated the *in vivo* color change of two different thermoplastics (Zafeiriadis et al, 2018). They gave patients Essix C+ (polypropylene) or Vivera (polyurethane) retainers after removal of fixed appliances and instructed them to wear 24 hours per day, except for eating, drinking, or brushing teeth. In addition, they instructed the patients to clean the retainers at night with a toothbrush and running water. After a three-month study period, they found that both Essix C+ and Vivera retainers demonstrated similar color stability (Zafeiridais et al, 2018).

A series of three studies were conducted to evaluate the effects of cleaning agents alone on transparency, surface roughness, and flexural modulus of different thermoplastics including polyurethane (Vivera), copolyester (Essix ACE), and polyethylene/propylene (Essix C+) (Agarwal et al, 2018, Wible et al, 2018, 2019). The cleaning products used include Invisalign Cleaning Crystals, Polident Denture Cleaner, Retainer Brite Cleaning Tablets, Listerine Cool Mint, 2.5% vinegar, 0.5% sodium hypochlorite, 3% hydrogen peroxide, and toothbrushing with distilled water. In each study, the samples were cleaned twice a week for 6 months and after the cleaning period, the surface roughness, transparency, and flexural modulus were tested. In respect to the transparency, they found that all cleaning materials caused a similar and consistent decrease in transparency of Vivera, Essix ACE, and Essix C+ (Agarwal et al, 2018, Wible et al, 2019). However, for the Essix ACE material, they found and even greater decrease in transparency when cleaned with Listerine (Wible et al, 2018). This suggests that the clinician must be aware of what thermoplastic they use and how they instruct their patients to care for their clear retainers for greater long-term success and survival.

Effect of material aging and staining on surface roughness

Another study evaluated the changes in color and surface roughness of three different brands of PETG (Duran by Sheu-Dental GmbH, Biolon by Dreve Dentamid

GmbH, and Crystal by Bio Art Dental Equipment) after exposure to different staining and cleaning agents (Porojan et al, 2020). Sheets of PETG were thermoformed over a stone model of a shape that mimicked a central incisor. After thermoforming, the samples were divided into groups and immersed in different staining agents including instant coffee, black tea, Coca-Cola, and distilled water. These samples were further divided into different groups subjected to different cleaning solutions including Cetron Cleaning Power, Corega Cleanser Tablets, and brushing. After soaking in the cleaning solutions for 24 hours, the samples were cleaned to their respective cleaning group. They found that there is a slight color change after 24 hours, but an even larger color change after 48 hours regardless of the material, staining agent, or cleaning method. In addition, they found that all samples demonstrated a clinically acceptable limit of microroughness in all the samples at every time point. Finally, while the color changes were insignificant, there was a difference in nanoroughness between the materials after cleaning with a toothbrush (Porojan et al, 2020).

The second factor of the Agarwal and Wible study series was the effect of cleaning agents on the surface roughness of different thermoplastics. They found no significant difference between the different cleaning agents on the surface roughness of Essix ACE (Wible et al, 2018). There was, however, a significant difference found when cleaning Vivera with 0.6% sodium hypochlorite between baseline and after 6 months. Yet, after further SEM imaging, they found that though it was statistically significant, the results still may night be clinically significant (Agarwal et al, 2108). Similarly, there was a statistically significant difference in surface roughness found when cleaning Essix C+

with Retainer Brite, but after further examination with SEM, the results were not clinically significant (Wible et al, 2019).

Material aging and its effect on flexural modulus

A study by Pascual et al evaluated the essential work fracture of thermoplastics after being exposed to different cleaning products. The products used include distilled water, Listerine, Crest ProHealth, 3% hydrogen peroxide, and Polident, while the thermoplastics tested were PETG (Tru-Tain Splint) and polypropylene/ethylene-propylene rubber blend (Essix C+). They found that there was no difference between the different cleaning products in the energy needed to initiate fracture with either material, except water. They also found that out of all the cleaning products, Crest ProHealth and hydrogen peroxide showed increased resistance to fracture initiation and hydrogen peroxide showed decreased resistance to plastic fracture growth in Essix C+ (Pascual et al, 2010).

The third and final factor of the Agarwal and Wible study series was the effect of cleaning agents on the flexural modulus of different thermoplastics. For Essix ACE, the greatest increase in flexural modulus was found when cleaning with 3% hydrogen peroxide over the 6-month period, but there were no differences found when cleaning with Invisalign Cleaning Crystals and Retainer Brite (Wible et al, 2018). An increase in flexural modulus was found when cleaning Vivera with 2.5% vinegar solution and toothbrushing, but no difference between the other cleaning agents (Agarwal et al, 2018). Finally, an increase in flexural modulus was found in Essix C+ after being cleaned with

3% hydrogen peroxide, but there was no difference when cleaned with Listerine (Wible et al, 2019).

Overall, this series of studies found that to best maintain the transparency and structural integrity of the retainer, Polident, Listerine, and Invisalign cleaning crystals were recommended to clean Vivera retainers twice a week, while toothbrushing and diluted vinegar were not (Agarwal et al 2018). Invisalign cleaning crystals and Retainer Brite tablets were recommended to clean Essix ACE retainers twice a week, while toothbrushing and Listerine were not (Wible et al, 2018). Finally, no particular cleaning solution was superior to clean Essix C+ retainers, however diluted hydrogen peroxide was not recommended due to its oxidizing characteristics (Wible et al, 2019).

Cleaning products

There are a variety of cleaning products available on the market today. This study evaluates the effects of Retainer Brite Cleaning Tablets, Fresh Guard Soak, Listerine Cool Mint Mouthwash, and EverSmile WhiteFoam, compared to an artificial saliva control. These four specific cleaning products were chosen because they are examples of four different forms of cleaning methods – tablet, powder, liquid, and foam. Other tested cleaning methods include Invisalign Cleaning Crystals, 3% hydrogen peroxide, 2.5% vinegar, 0.6% sodium hypochlorite, Polident Denture Cleaner, Crest ProHealth and toothbrushing (Pascual et al, 2010; Chang et al, 2014; Agarwal et al, 2018; Wible et al, 2018 and 2019; Porojan et al, 2020).

Retainer Brite is packaged in tablets. The major active ingredients are two oxidizing and corrosive agents – potassium monopersulfate (37%) and sodium perborate monohydrate (27%) (*Retainer Brite* MSDS No. RB-XXX). This is a popular cleaning method on the market and has been highly studied. One study found that Retainer Brite tablets were just as effective as Invisalign Cleaning Crystals when removing black tea stains off of polyurethane and PETG retainers, but less effective when removing red wine and coffee stains (Bernard et al, 2020). Another study evaluated the changes in copolyester retainers with a variety of cleaning products and found that Retainer Brite and Invisalign Cleaning Crystals did not affect the flexural modulus after a 6-mo period (Wible et al, 2019).

Fresh Guard Soak is packaged in a powder, or crystal form. The company lists their ingredients as a proprietary formula and has not released the major ingredients (*Fresh Guard Soak 24 ct by Efferdent* MSDS No (unknown)). Few studies have evaluated effects of this cleaning method.

Listerine Cool Mint Mouthwash is packaged as a liquid. The major active ingredients include 10-30% ethanol (*Listerine Cool Mint Mouthwash* MSDS No NA-19292-116-A). This is a highly studied cleaning method, as it is a common household item that patients likely have at home. Previous studies have concluded that Listerine significantly decreases light transmittance on copolyester materials when compared to a variety of other cleaning products (Wible et al, 2019). Alternatively, another study showed that polyurethane retainers showed the least amount of change in light transmittance, along with Invisalign Cleaning Crystals and Polident, when cleaning for a 6-month period (Agarwal et al, 2018).

EverSmile White Foam is packed in a bottle as a foam. The major active ingredients include 3.8% hydrogen peroxide and 2% glycerin (*EverSmile WhiteFoam*

MSDS No J03551). There is no known published paper that evaluates the effect of this product on retainer materials. However, hydrogen peroxide in liquid form has been studied and has been found to be destructive to some materials, like copolyesters and polypropylene/ethylene, when soaking for 15 minutes in a 3% solution due to its oxidizing characteristics (Wible et al, 2018 and 2019).

Considering the varied results among different cleaning products and thermoplastics, it is important to continue this research and to examine a variety of materials, considering the type of polymer and the manufacturer. The objective of this study is to evaluate the color change, surface roughness, and flexural modulus of Biocryl (PETG) and Invisacryl C (EP) over 28 cleaning cycles.

CHAPTER 3
MATERIALS AND METHODS

Study Design

All procedures and testing were completed at the Dental Lab at the Marquette University School of Dentistry (MUSoD) and the Biomaterials Lab at Wehr Physics Building at Marquette University. For the purpose of this study, two thermoplastic retainer materials were tested: Clear Splint Biocryl (Great Lakes Dental Technologies; Tonawanda, NY) and Invisacryl C (Great Lakes Dental Technologies; Tonawanda, NY). In addition to the two retainer materials, five different cleaning solutions were also tested: Listerine Cool Mint Mouthwash, Retainer Brite Cleaning Tablets, EverSmile WhiteFoam, Fresh Guard Soak, and artificial saliva. The samples were tested for color change and surface roughness at baseline, cleaning cycle 16, and cleaning cycle 28 and three-point bending test was completed at the end of the 28 cleaning cycles.

Specimen Preparation

The thermoplastic retainer materials used in this study were Clear Splint Biocryl (Great Lakes Dental Technologies; Tonawanda, NY) in 1mm/125mm round sheets, and Invisacryl C (Great Lakes Dental Technologies; Tonawanda, NY) in 1mm/125mm round sheets. Two samples at a time were made by thermoforming individual sheets over two rectangular stainless-steel blocks (1in x 2.5in x 0.25in) spaced apart using a Biostar (SHEU Dental Technologies; Iserlohn, Germany) using the manufacturer recommended heating times (Figures 1 and 2). Rectangular samples were then cut out of the thermoformed sheets using a heated 15 blade scalpel. Samples were labeled using a dental handpiece and bur to indicate the cleaning group where the sample belonged (Figure 3).

Figure 1. Biostar pressure molding machine

Figure 2. Rectangular stainless-steel blocks (1in x 2.5in x 0.25in) spaced apart on Biostar platform

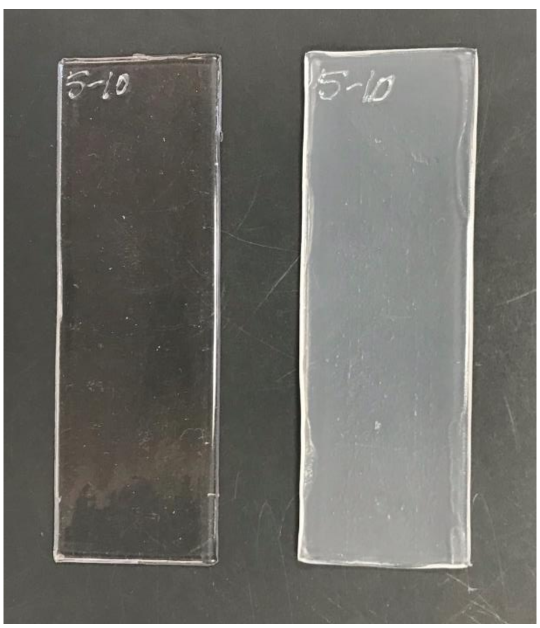

Figure 3. Examples of final sample preparation of Clear Splint Biocryl (left) and Invisacryl C (right)

Experimental Procedure

The samples were stored in an artificial saliva solution in a 37°C incubator between cleanings (Figure 4). The Fusayama-Meyer artificial saliva solution was prepared with 0.4 g/L KCl, 0.4 g/L NaCl, 0.6 g/L CaCl2, 0.690 g/L NaH2PO4, and 1 g/L Urea in a 20 L container with deionized water. The solution was refreshed after each cleaning and was prepared several times as need throughout the experiment. Each sample group was placed in a plastic slide box with the top removed, inside of a Tupperware to contain the artificial saliva.

Figure 4. 37°C incubator (Fischer Scientific)

The samples were cleaned 4 times a week for a total of 7 weeks and 28 total cleaning cycles. The current recommendation to patients is to use cleaning products such as the ones tested as an adjunct to rinsing or brushing daily with water and to use the cleaning solution twice a week. The experimental period was planned to mimic the number of cleanings in a 90-day period when a patient is cleaning twice a week. At each cleaning, the artificial saliva that each sample group was stored in was disposed of and the cleaning solution was prepared. The control group was refreshed with new 600mL artificial saliva for each cleaning cycle. Retainer Brite Cleaning Tablets and Fresh Guard Soak were dissolved in 600 mL water, then placed in the sample group containers (Figures 4 and 5).

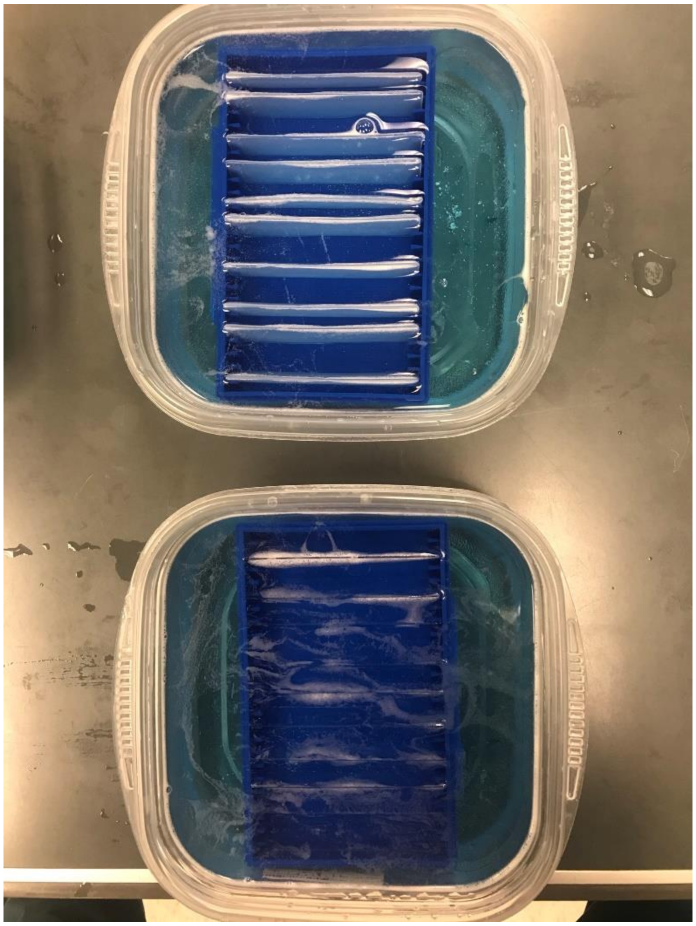

Figure 5. Retainer Brite samples during a single cleaning cycle

Figure 6. Fresh Guard samples during a single cleaning cycle

The Retainer Brite sample groups were cleaned for 15 minutes, while the Fresh Guard sample groups were cleaned for 5 minutes, per each manufacturer's instruction. 600 mL of Listerine was placed into the respective cleaning containers and cleaned for 15 minutes (Figure 6).

Figure 7. Listerine samples during a single cleaning cycle

A single pump of EverSmile WhiteFoam was placed onto the numbered surface of each sample, then spread over the entire sample (Figure 7). The EverSmile WhiteFoam sample groups were cleaned for 5 minutes. After the specified cleaning time of each sample group, the samples were rinsed with water, and the containers were refreshed with new 600 mL of artificial saliva.

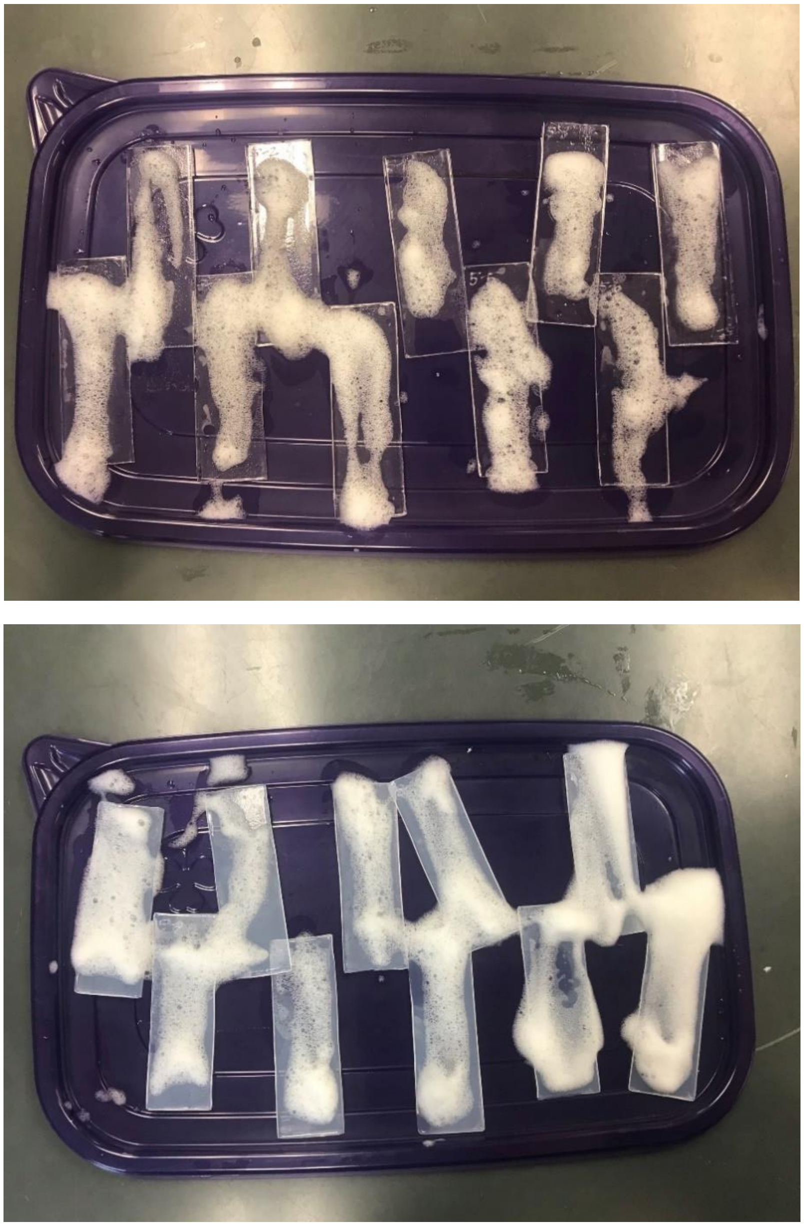

Figure 8. EverSmile WhiteFoam groups during a single cleaning cycle (Biocryl top, Invisacryl bottom)

After 4 weeks of cleaning and a total of 16 cleaning cycles, surface roughness measurements and transparency measurements were taken. Before each measurement was taken, the samples were wiped dry with a thin paper towel to ensure no liquid from the artificial saliva or saliva precipitate skewed measurement numbers. After the measurements were taken, the samples were placed in a new 600 mL solution of artificial saliva, placed back in the incubator, and the experiment resumed. At the end of 7 weeks and a total of 28 cleaning cycles, the samples were dried with a thin paper towel, then surface roughness, transparency, and flexural modulus of the samples were taken.

Morphology and surface roughness the samples were evaluated using a 3D Laser Measuring Microscope (LEXT OLS4000, Olympus, Japan). Samples were imaged at 20X magnification in both laser and color mode. The arithmetic average deviation was used to calculate surface (Sa) roughness. The calculations were performed with no cutoff applied. To measure surface roughness, the sample was loaded in the microscope with the labeled side up. A piece of paper (1in x 2.5in) with a small mark located in the center was placed under each sample in the same orientation to ensure each measurement was taken in the same spot (Figure 8). One measurement was taken per sample. The following data were taken per sample: Sa, Sq, Ssk, Sp, Sku, Sv, and Sz. For the purpose of this study, only Sa values were used. Average Sa values were determined per sample group and per time point. All measurements were made by the same examiner for all three time points.

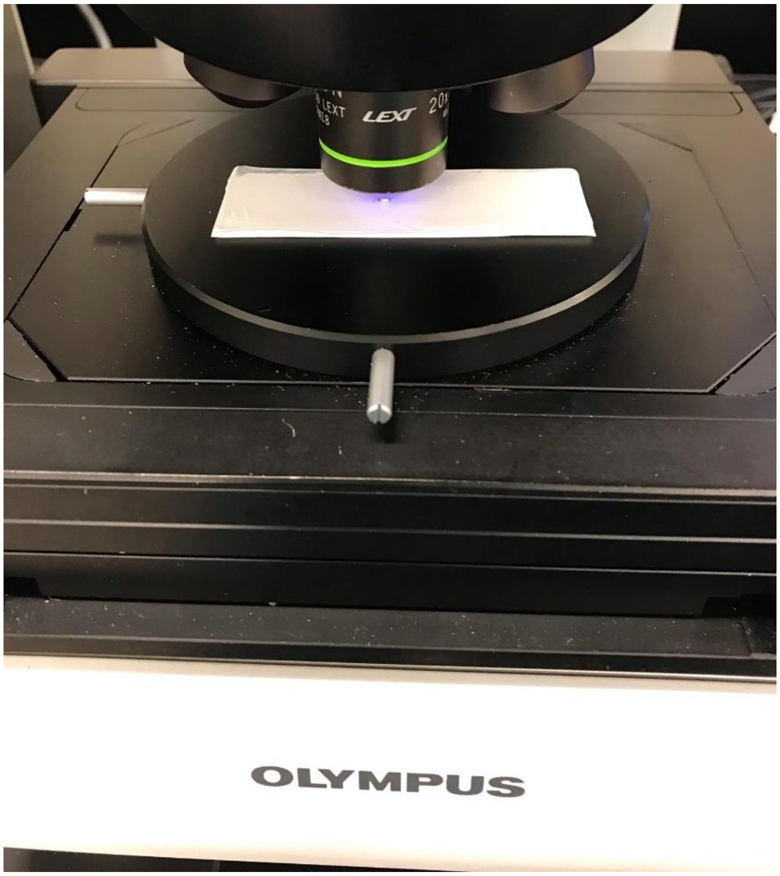

Figure 9. Sample loaded in laser microscope (LEXT OLS4000) during measurement

To measure transparency, the sample was loaded on a Cm-700d spectrophotometer (Konica Minolta, Inc, Ramsey NJ) with the labeled side up. The sample rectangle piece of paper (1in x 2.5in) was placed on the sample to ensure the measurement was taken at the same spot for each time point. The piece of paper was removed before measurements were taken. L*, a*, b* values were recorded for each sample and each time point. The change in transparency was determined with the same protocol as a previously published article: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Porojan et al, 2020).

Elastic modulus was measured with 3-point bending test on a universal testing machine (Model 5500R; Instron Crop., Norwood MA) with the following parameters: beam distance set at 16 mm and 1 mm/min speed according to ATSM standards (Figure 9). Before deforming the samples, the thickness and width of each sample was taken in order to properly evaluate the elastic modulus. The displacement (mm) and Force (kN) of each sample were used to determine the flexural modulus (MPa) using the following equation published in a previous article: $E = [(F_2\text{-}F_1)l^3]/[4bh^3(d_2\text{-}d_1)]$ (Ryu et al, 2018).

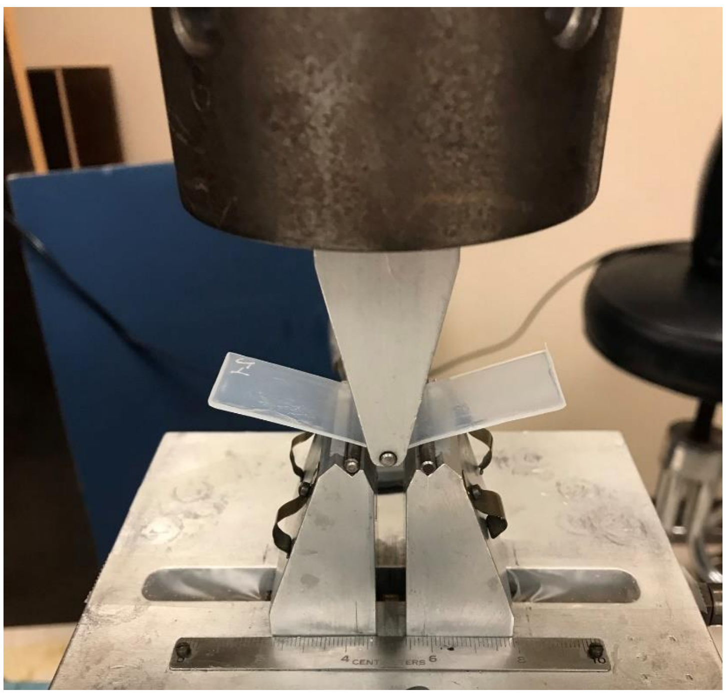

Figure 10. Invisacryl sample loaded in universal testing machine during testing

Statistical Analysis

After the data were collected for each experiment with their respective machines, the raw data were collected in an excel spreadsheet and the proper variables were calculated. These data were used to calculate the mean differences between the samples.

One-way ANOVA and student’s T-test were used to evaluate the differences between the means of the effect of cleaning methods at each time point for each material. Post hoc analysis was used to calculate difference between each experimental method. The significance was set to $p < 0.05$. The calculations and analyses were performed with IBM SPSS Statistics for Windows version 27.

CHAPTER 4
RESULTS

Color Change

Final color change (total ΔE) was analyzed via two-way ANOVA with material and cleaning method as factors. There were significant differences ($p < 0.05$) with respect to material and cleaning method, but also a significant interaction ($p < 0.05$) between the two factors. Due to errors in the measurement of the Control and Fresh Guard groups in the Biocryl material group, these data were excluded from further analysis and one-way ANOVA was used to examine the effect of cleaning method for each material due to the interaction. For Biocryl, Listerine caused a significantly ($p < 0.05$) greater color change than either Retainer Brite or EverSmile WhiteFoam (Figure 10). For Invisacryl, post-hoc analysis via Scheffe found no significant ($p < 0.05$) difference between cleaning groups (Table 1).

Table 1. Color change means (ΔE) at all time points

	Biocryl			Invisacryl		
Cleaning Method	ΔE1	ΔE2	ΔE total	ΔE1	ΔE2	ΔE total
Control	7.172±1.7	0.186±0.1	7.153±1.8	2.088±0.3	0.391±0.1	2.406±0.3
Fresh Guard	6.429±0.2	0.157±0.1	6.373±0.1	1.913±0.3	0.388±0.2	2.243±0.3
Retainer Brite	0.198±0.1	0.140±0.1	0.275±0.1	1.863±0.2	0.32±0.2	2.119±0.3
Listerine	2.444±0.5	1.052±0.4	3.442±0.5	1.772±0.1	0.335±0.2	2.037±0.3
EverSmile WhiteFoam	0.204±0.1	0.142±0.1	0.273±0.1	1.891±0.2	0.261±0.1	2.066±0.2

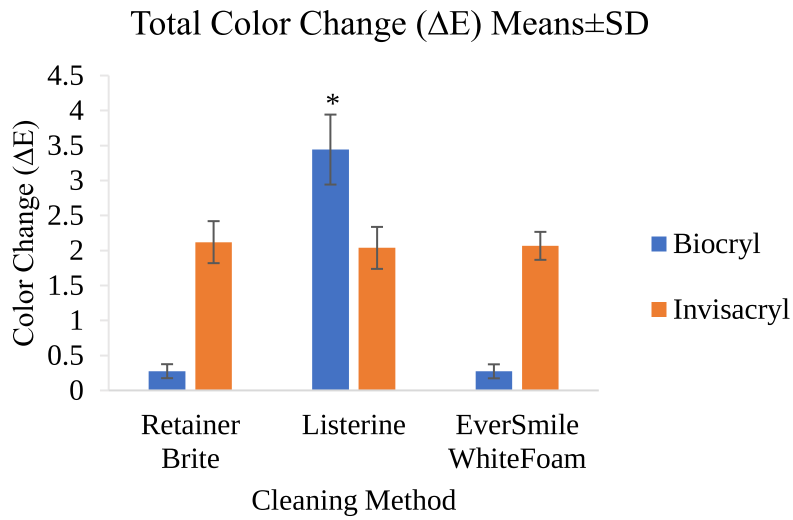

Figure 11. Total color change (ΔE, means±SD) of Biocryl and Invisacryl compared to different cleaning methods. Control and Fresh Guard groups within the Biocryl group were omitted from the study due to measurement errors. ($p < 0.05$)

While there was no significant difference between cleaning methods, one-way ANOVA shows a significant difference ($p < 0.05$) between time points, with the most color change happening between baseline and cycle 16 (Figure 11).

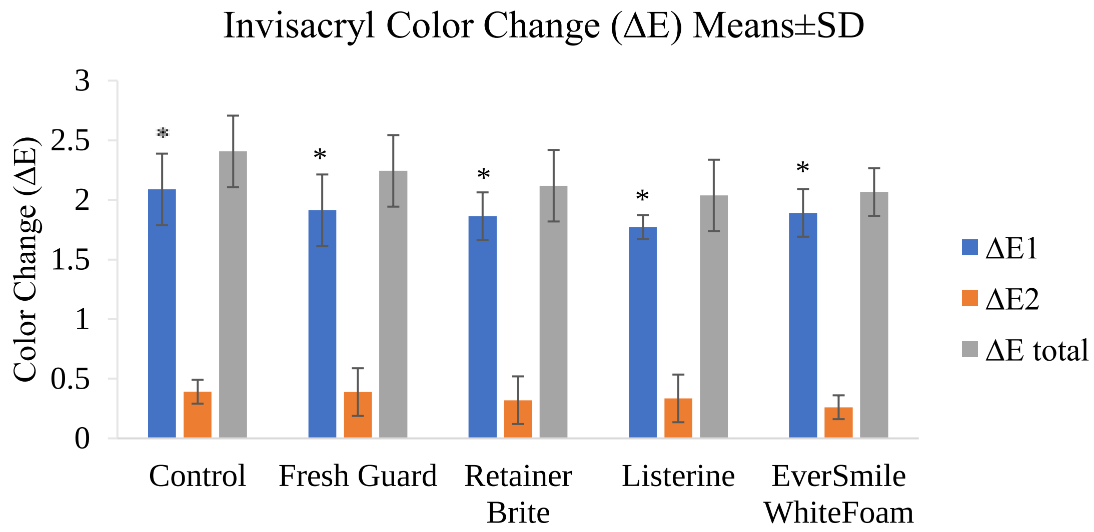

Figure 12. Color change means±SD for Invisacryl with different cleaning methods at all three time points (* $p < 0.05$)

Surface Roughness

Multivariate three-way ANOVA with factors of time, material, and cleaning. No significant difference ($p > 0.05$) in roughness over time and no significant difference ($p > 0.05$) between groups with respect to cleaning method (Figures 11 and 12).

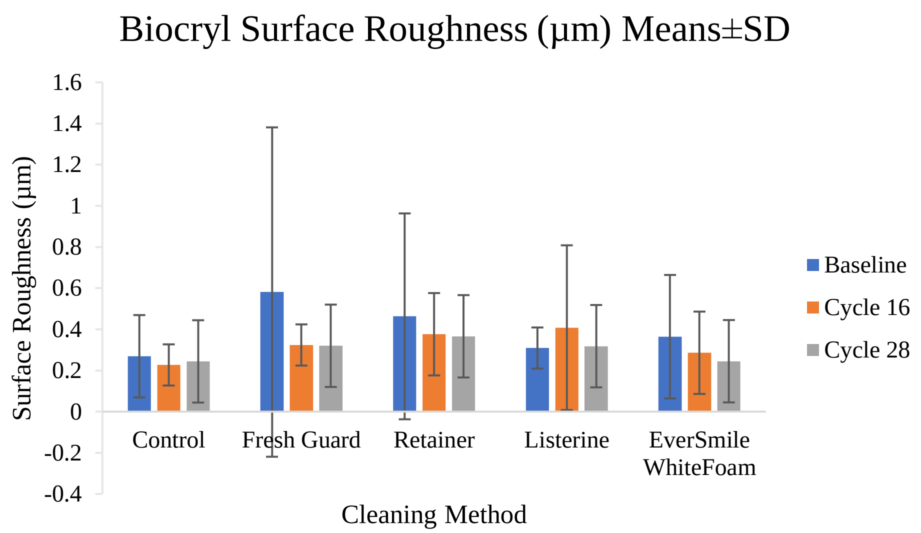

Figure 13. Biocryl surface roughness (MPa) means±SD compared to different cleaning methods at baseline, cycle 16 and cycle 28.

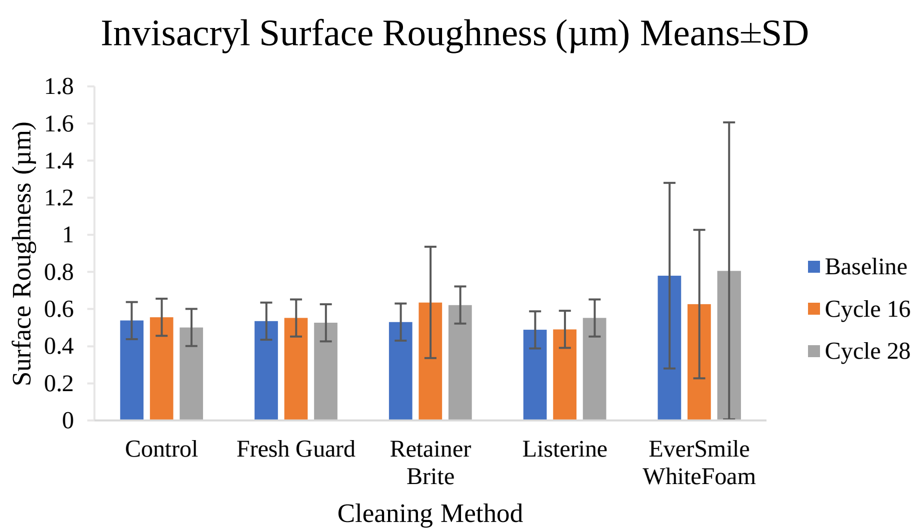

Figure 14. Invisacryl surface roughness (MPa) means±SD compared to different cleaning methods at baseline, cycle 16, and cycle 28.

Overall, Invisacryl was significantly ($p < 0.05$) rougher than Biocryl (Table 2). Laser microscope imaging shows a visibly rougher surface of Invisacryl compared to Biocryl at 20X magnification (Figure 13).

Table 2. Surface Roughness means±SD (µm) at all time points

	Biocryl			Invisacryl		
Cleaning Method	Baseline	Cycle 16	Cycle 28	Baseline	Cycle 16	Cycle 28
Control	0.269±0.2	0.227±0.1	0.244±0.1	0.538±0.1	0.556±0.1	0.501±0.1
Fresh Guard	0.581±0.8	0.324±0.1	0.320±0.2	0.535±0.1	0.552±0.1	0.526±0.1
Retainer Brite	0.463±0.5	0.376±0.2	0.366±0.2	0.53±0.1	0.636±0.3	0.622±0.1
Listerine	0.309±0.1	0.408±0.4	0.318±0.2	0.488±0.1	0.491±0.1	0.552±0.1
EverSmile White Foam	0.364±0.3	0.286±0.2	0.245±0.1	0.780±0.5	0.627±0.4	0.806±0.8

Table 3. Independent T-test of surface roughness means±SD (µm) after cycle 28

Cleaning Method	Biocryl	Invisacryl	*p* value
Control	0.24±0.1	0.5±0.1	<0.001
Fresh Gurad	0.32±0.1	0.53±0.1	0.003
Retainer Brite	0.37±0.2	0.62±0.1	0.002
Listerine	0.32±0.2	0.55±0.1	0.002
EverSmile WhiteFoam	0.25±0.1	0.81±0.7	0.024

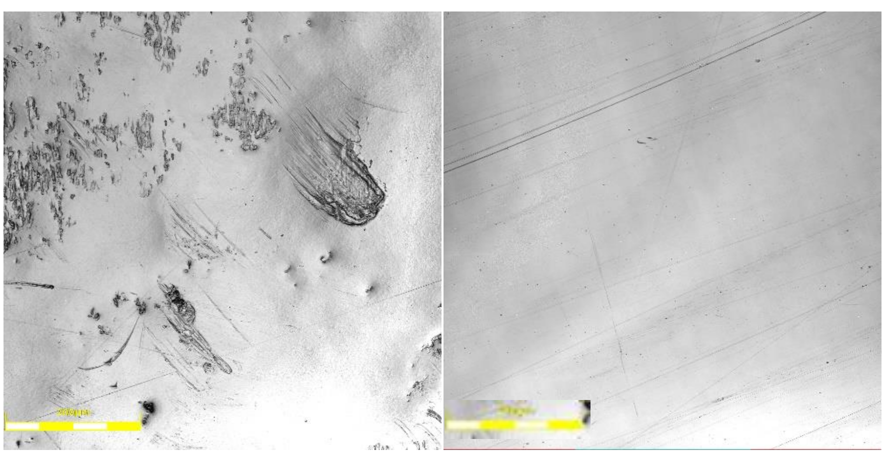

Figure 15. Laser microscope images of Invisacryl (left) and Biocryl (right) at 20X magnification. Bar = 200 µm.

Flexural Modulus

Flexural modulus was analyzed via two-way ANOVA with material and cleaning method as factors. A significant ($p < 0.05$) difference was found with respect to material, with Biocryl being the stiffer material (Table 4, Figure 5). No significant ($p > 0.05$) difference was found between groups with respect to cleaning method. Also, no significant interaction was found ($p > 0.05$) between material and cleaning method (Table 4).

Table 4. Independent T-test of elastic modulus means±SD (MPa) after cycle 28

Cleaning Method	Biocryl	Invisacryl	*p* value
Control	1676.9±409	419±103	<0.001
Fresh Guard	1745.9±244	468.5±88	<0.001
Retainer Brite	1825.8±224	438.7±61	<0.001
Listerine	1935.5±156	437.4±95	<0.001
EverSmile WhiteFoam	1936.9±304	425.5±108	<0.001

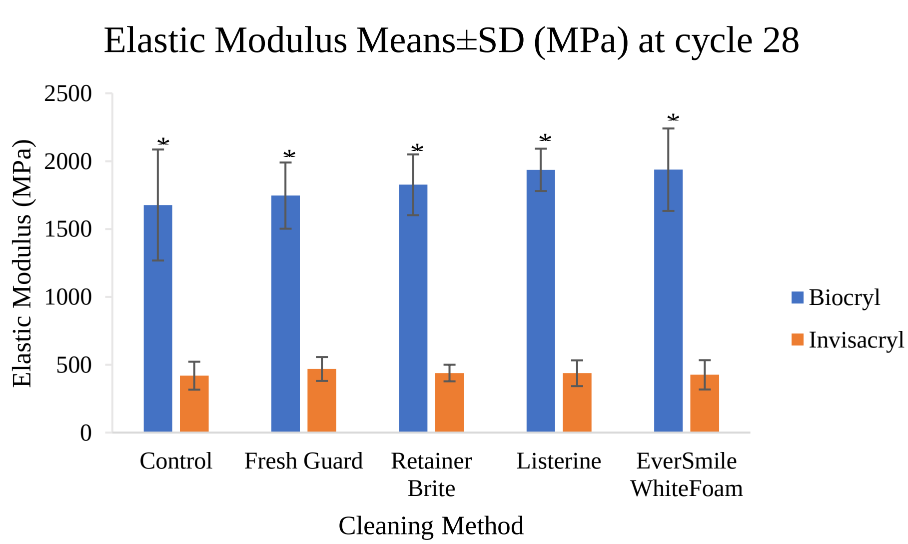

Figure 16. Means±SD of elastic modulus (MPa) at cycle 28. No significant differences were found among the cleaning methods, however significant difference exist between the two materials ($p < 0.05$).

CHAPTER 5
DISCUSSION

Several different thermoplastics are currently available to orthodontists to use as appliances for retention. There is also a variety of products available to patients to use to clean their retainers. This study focuses on the effect of several different cleaning methods on two popular thermoplastic materials – polyethylene terephthalate glycol (PETG) and ethylene propylene (EP). In this study we evaluated the changes in surface roughness, color change, and flexural modulus when Clear Splint Biocryl (PETG) and Invisacryl C (EP) (Great Lakes Dental Technologies) were cleaned with Fresh Guard Soak, Listerine Cool Mint Mouthwash, Retainer Brite Cleaning Tablets, and EverSmile WhiteFoam. Data were obtained to evaluate differences between the effects of the cleaning product on each material, and between each material when cleaned with the same cleaning product.

To patients, the most desirable characteristic of clear retainers is their superior esthetics. If kept clean, these retainers remain relatively unseen, and the patient will feel more comfortable wearing them for longer hours of the day and in social settings. The color and translucency can change due to plaque and tartar buildup, staining agents, or cleaning materials. In this study, color change was tested using a spectrophotometer at baseline, cycle 16, and cycle 28. Due to a measurement error between researchers, the control and Fresh Guard cleaning groups were omitted from the Biocryl group when evaluating color change. Despite the measurement error, a significant difference was found when cleaning Biocryl with Listerine, compared to with Retainer Brite or EverSmile WhiteFoam. The Listerine group showed an average total color difference (ΔE

total) of 3.442, while the other groups showed an average total color difference of less than 0.3. This is a noticeable and clinically significant color change. This finding also agrees with a previous study that found most color change occurred when cleaning a copolyester material with Listerine (Wible et al, 2019). Within the Invisacryl group, no significant difference was found between cleaning methods. Interestingly, most of the color change was seen within the first 16 cleaning cycles. This suggests color changes due to any cleaning agent on Invisacryl retainers can occur within the first 2 weeks of use if cleaning with any of the tested method four days a week.

A previous study found that ethanol alone in mouthwash can cause yellowish discolorations (Zafeiriadis et al, 2018). If it is indeed the ethanol base in the mouthwash that is causing the color change, one can suggest that an alcohol-free mouthwash might not cause a significant change in color. Considering the differences between the EP and PETG materials evaluated in this study and the copolyester and other materials evaluated in previous studies, it is important for clinicians to be aware of what materials they choose for clear retainers and how to advise their patients to keep them clean.

Surface characteristics of appliances in the oral environment are important to evaluate because they can contribute to harboring excess microorganisms and can lead to enamel demineralization and discomfort if the material is excessively rough (Porojan et al, 2020). This study found no difference on the effect of different cleaning methods on both Biocryl and Invisacryl groups. However, there was a difference between the two groups, with Invisacryl being the rougher material. Studies have reported that the tongue can detect differences in surface roughness at 0.5 µm (Porojan et al, 2020). The average roughness of Invisacryl throughout all time points ranged around 0.5-0.7 µm. This is clinically significant since a patient should clinically be able to notice a difference in roughness. Measurements were taken with a laser microscope in this study, whereas other studies used profilometry (Wible et al, 2018 and 2019, Agarwal et al, 2018), SEM, and atomic force microscopy (Ahn, Hyo-Won et al, 2015, Porojan et al 2018). Potential errors in measurement may have occurred when attempting to consistently measure the same surface over each time period.

The final characteristic evaluated in this study was flexural modulus. Unlike color change and surface roughness, testing the flexural modulus destroyed all the samples; so, in this study only the flexural modulus at cycle 28 was tested. No significant difference was found between cleaning methods for Biocryl or Invisacryl, however there was a significant difference between Invisacryl and Biocryl groups as a whole, with Biocryl being the stiffer material. A previous study found that the flexural modulus increased when using cleaning agents like 2.5% vinegar and toothbrushing on polyurethane, and 3% hydrogen peroxide on copolyester and polyethylene/propylene retainers (Agarwal et al, 2018, Wible et al, 2018, 2019). These specific cleaning agents were not involved in this study, however the active ingredient of EverSmile WhiteFoam is 3.8% hydrogen peroxide. Differences in the previously mentioned studies include different thermoplastic compositions and cleaning protocol. In addition, these studies submerged the samples in a liquid solution of 3% hydrogen peroxide for 15 minutes, whereas the current study used a stronger solution in a foam form, only applied to one side of the samples for 5 minutes, then rinsed with water. Oxidation due to hydrogen peroxide exposure has been known to increase the stiffness of polymers as a result of surface porosity changes in polyester

(Caudill et al, 1992). It is possible that if the materials used in this study were submerged in a liquid solution like the previous one, flexural modulus may have increased.

There are many brands and compositions of thermoplastics available to clinicians to use to fabricate thermoplastic retainer materials. This study evaluated the effects of a novel cleaning method, Ever White Smile Foam, against more commonly known cleaning methods. Compared to the other cleaning methods, this novel product was not more detrimental to either thermoplastic material than any other cleaning method. No cleaning method was significantly more detrimental to either material, except for Listerine which was the cleaning method with the greatest color change potential for the Biocryl group.

Limitations to the Study

While laboratory simulated intraoral conditions were attempted to be kept constant, *in vitro* studies are clearly different than what happens in a clinical setting. Limitations to this study include the absence of these clinical scenarios like oral function, natural saliva, microorganisms, and the physiologic variations that exist between patients. This study used samples thermoformed over a rectangular block and cut in a flat, rectangular shape. Realistically, these thermoplastic materials are used for a retainer that is thermoformed over a complex dental arch, with many areas of differing material thickness, cervices, and load bearing areas. In addition, when patients remove their retainers, they likely keep it in a dry case, yet in this study, samples were generally submerged in a solution at all time points. Thermoplastics are subject to deformation due to temperature, humidity, manufacturing, and time after elastic deformation (Ahn, Hyo-Won et al, 2015). Differences in flexural modulus, transparency, and surface roughness may be more abundant in retainers used daily than in the flat, uniform, rectangular samples used in this study.

CHAPTER 6
CONCLUSION

The color change of Biocryl and Invisacryl increase over time, but at different rates. The color change of Biocryl with Retainer Brite and Ever White Smile Foam was minimal and similar over the three time points, however most of the color change for Invisacryl occurred between baseline and day 16. Listerine affected the color change of Biocryl the most, whereas there was no significant difference in cleaning method for the Invisacryl group. There was no significant difference in surface roughness for Biocryl and Invisacryl when cleaned with any of the cleaning methods at any time point. However, Invisacryl seemed to have a significantly higher surface roughness than Biocryl. Finally, there was no significant difference in elastic modulus between any of the cleaning methods for each material. There was, however, a significant difference between the two materials, with Biocryl being the stiffer material than Invisacryl.

Any of the cleaning methods evaluated in this study are recommended for use, except for Listerine. Care must be taken when using Listerine to avoid significantly changing the color of the retainer after repeated cleaning cycles for PETG based retainers. When comparing the two retainer materials, Invisacryl C retainers are not recommended for patients with poor hygiene due to their increased surface roughness. Biocryl, due to its stiffness, is recommended to patients with severe pre-treatment rotations, spaces, and intruded teeth. The stiffness of the material can offer a stronger hold on the post-treatment positions of teeth. This material can also be considered as a good material to use for night guards since it is stiff and seems to be able to significantly resist the wear that can happen due to parafunctional activity.

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