An Experimental Study on Improving Stain Resistance, Bacteria Resistance and Wearing Comfort of a Hawley Retainer Through a Thin Layer of Silicone Surface Coating

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Abstract

Dental retainers have been used to maintain the position of the teeth as aligned after braces. Retainers were meant not only for the purpose of aesthetics but also for medical conditions that require the realigning of the teeth. Although retainers were generally meant for the benefit of braces users, retainers could result in negative consequences if not taken care of, as there could be serious infections related to this. A Hawley retainer is made of an acrylic base and stainless steel wires. Silicone had not been widely tested with dental retainers, even though it had been used in many medical cases. This experiment assessed the differences between the silicone-coated acrylic square/strip samples and the acrylic-only square/strip samples in terms of stain resistance when exposed to coffee, bacterial resistance with *Streptococcus salivarius*, and enhanced wearing comfort. Wearing comfort was determined by the amount of friction that was perceived. Wearing comfort encompasses how comfortable the material will be if scraped against a surface. The less friction involved, the smoother the surface just like how ice has less friction and is smoother than a wool rug. It was important to note that the silicone used in this research was from a mold kit and not medical grade, so the impurities could result in results that may differ from medical grade silicone. We hypothesized that there would be less stain, less bacteria and more smoothness on the surface of a silicone coated acrylic sample than an acrylic only sample. The experiments had differing results based on the particular design criteria. The silicone sample showed less visible stain, indicating a stronger ability to resist stains. It also had similar bacteria resistance as the acrylic only sample. Finally, there was not enough evidence to support silicone coated acrylic samples performing better in the smoothness test.

Introduction

Hawley retainers (Figure 1) were introduced in 1919 for the purpose of preventing relapse from the orthodontic treatment (Ramazanzadeh et al.). Since then, there have been many advancements to retainers, including the development of permanent and clear retainers like Invisalign. They were used to maintain the positions of the teeth after braces. Hawley retainers could be difficult to maintain and keep clean, leading to stains and bacterial growth. This makes oral hygiene a major concern. Stains combining with bacteria on retainers could likely lead to infections, as they create a layer of biofilm on the retainer (Kasibut et al.).

Figure 1: Hawley retainer fitted onto a teeth model.

The number of people wearing braces had been constantly increasing; from 2016 to 2018, 60,000 US adults started wearing braces (Hung et al.), and consequently, more individuals were now using retainers. Retainers are typically worn for a lifetime to ensure that teeth remain in their properly aligned positions after removal of braces. Although mostly worn at night, stain and bacteria from the teeth could still result in the buildup of plaque or biofilm on the retainer. A previous study conducted tried to collect the saliva and plaque samples off of the clear retainer surface to study the bacteria microbiome (Velliyagounder et al.). This shows that bacteria and stain could attach to a retainer when it was put into the mouth at night, highlighting the importance of researching and developing retainers that are resistant to staining and bacterial growth, while also maximizing wearing comfort.

Silicone was chosen to be experimented in this research because it was widely used in medical products, including prosthetics and implants, due to its biocompatibility and safety for human use (Chruściel). It is also water-repellent, a property that was central to this study (Rebane et al.). Dental acrylic is considered hydrophobic (Gad et al.). In addition, acrylic is known to be very structured and difficult to break, making it a very sturdy material; so, including additional wearing comfort to the retainer may also be beneficial to wearers. There will be three design criteria for this experiment study, which are stain resistance, bacteria resistance, and wearing comfort.

Stain Resistance

In terms of stain resistance, coffee and tea are two of the most popular beverages (Mititelu et al.) and are known to stain teeth, making these two drinks crucial for experimentation with retainers.

A study that was conducted more than 10 years ago to identify which material, silicone, copolyamide, or heat-polymerized resins, had the highest discoloration effect in coffee and tea (Lai et al.). Results show that for coffee, silicone had the highest discoloration, followed by copolyamide and acrylic resins. For tea, copolyamide had the highest discoloration while silicone and the acrylic resins had minimal color changes (Lai et al.). In the experiment, we want to find out whether the specific silicone material we used had a different result with regard to discoloration.

Bacterial Resistance

Research had been focused on incorporating different additives to elastomeric silicone to see the effect of the growth and adhesion of *Escherichia coli*, a gram-negative bacteria, onto the surface of the additives (Rebane et al.). It was shown that the silicone foam sample with active carbon additives had the highest growth of *E. coli* while the silicone foam sample with tannic acid had the lowest growth. This research shows that different additives could change how well *E. coli* could stick to the surfaces of silicone foams (Rebane et al.).

Streptococcus salivarius was chosen for this study because of its importance in the oral microbiome, making it a suitable candidate for this research (Kaci et al.). They had inflammatory preventative properties both *in vitro* and *in vivo*. They inhibit activation of NF-κB pathway in human intestinal epithelial cells and inflammation in severe colitis mice (Kaci et al.).

Although there had been research done on bacteria like *Escherichia coli* on silicone materials, there had not been many experiments related to *Streptococcus salivarius*, a very crucial bacteria in the oral microbiome. With this experiment, it could be determined whether the addition of this material will result in more or less bacteria accumulation.

Wearing Comfort

A study focused on identifying whether there would be differences in surface roughness between a silicone-based material (Mucopren Soft) and acrylic resin-based material (Trusoft) (Araújo et al.). Through the Tukey's test, a statistical test to determine which group's mean different from the rest, they found that surface roughness was lower for the silicone-based material (Araújo et al.).

Currently, there were no experiments that found a material that could help with all three of these properties, being stain resistant, bacteria resistant, and comfortable. So in this experiment, silicone was used to test for these properties.

We hypothesized that silicone-coated acrylic samples would (a) exhibit less staining, (b) support fewer bacterial colonies, and (c) had a smoother surface.

Methods

There were three design criteria that this research tests, which were stain resistance, bacteria resistance, and smoothness. There was also an attempt to use Earl Grey Tea to test the stain resistance, but the results were not as obvious as coffee, so the experiment was stopped after the first trial. The independent variable was the number of days tested for the stain and bacteria test and the length pulled for the friction test. The silicone was layered by dipping the samples into the 1:1 ratio mixture and leaving it dry on a clean surface. 2 days later, it was removed from the surface for experiments.

Stain Resistance

The first design criteria was testing the staining resistance of acrylic versus silicone layered acrylic. The coffee (Starbucks Nitro Cold Brew) was poured into a Petri dish and filled to the top. One silicone layered sample and one non-silicone layered sample were put into the coffee. The samples had to be completely submerged into the coffee. The control in this design criteria was distilled water, so one of each sample was placed into the water. After four days, the samples were taken out of the liquid and changes were observed by taking a photo. They were checked again at seven and eleven days. They were then placed back into the Petri dishes. This process was repeated for ten more days. Then, with the red food coloring dye from Market Pantry, the spectrophotometer was calibrated by using 0%, 25%, 50%, 75%, and 100% of the dye. The stock solution was created from 3 drops of the food coloring dye in 100 mL of water. 5 mL taken out would be the 100% the solution, or the stock solution. 75% would be made from 3.75 mL of the stock solution and 1.25 mL of water and so on, as seen in Figure 2.3. Then using the calibration, the concentration of the color of the samples could be found from the pictures and the absorbance could be determined. The concentration of the colors were found by averaging three observers' responses. Understanding that this method was not the most accurate, finding a way to accurately measure the percentage of the stock solution would lead to a more accurate calculation of the absorbances. By using the colors of the different percentages of the stock solution, an estimate for the percentage of stock solution each acrylic sample was recorded. The type of graph collected was the absorbance vs concentration, where concentration was on the x-axis and absorbance was on the y-axis. By using the chart on Google Sheets, a parabola of best fit was created instead of a line of best fit from Figure 3.1 because the concentration used was slightly too high for the spectrophotometer, so the plot was not linear as seen with Figure 3.2.

Test 1 lasted 11 days, where the acrylic only sample was at 33% of the stock solution, or $1.99*10^{-5}$ M. The silicone-coated acrylic samples was at 25% of the stock solution, or $1.51*10^{-5}$ M. Test 2 lasted 7 days. The acrylic sample was at 47% of the stock solution, or $2.83*10^{-5}$ M. The silicone-coated acrylic samples was 28% of the stock solution $1.69*10^{-5}$ M. Test 3 lasted 4 days. The acrylic sample was 40% of the stock solution, or $2.41*10^{-5}$ M. The silicone-coated acrylic samples were 25% of the stock solution, or $1.51*10^{-5}$ M. Higher molar concentrations corresponded to higher absorbance values. The increase in absorbance was greater in the acrylic-only samples than in the silicone-coated acrylic samples (Figure 2.1 and 2.2).

There was both a polynomial of best fit and a line of best fit because the stock solution of 3 drops of red 40 dye in the 100 mL was too concentrated. This resulted in the data points leaning towards a curve rather than a straight line. The absorbance and molar concentrations were calculated with the polynomial of best fit.

Bacteria Resistance

The second test was bacteria resistance. The bacteria used in this research was *Streptococcus salivarius*. To reactivate the bacteria, 1 mL of the solution from the slant, which was a test tube filled with nutrient agar that allows the bacteria to grow, was placed into the container where the bacteria was placed. After mixing, the solution was transferred back into the slant. Then, it was placed in the incubator at 37˚C for 48 to 72 hours. When the bacteria were reactivated, the Tryptic Soy Agar Petri dish was streaked four times using the inoculating tube, each time turning the Petri dish 90˚, ensuring that approximately the same amount of bacteria was added to each sample. The samples, silicone-layered and non-silicone layered acrylic, were placed facing the nutrient agar onto the Petri dish. To make sure that no moisture contacts the nutrient agar, the Petri dish was placed into the incubator upside down. The Petri dish was incubated at 37˚C for 36 to 48 hours. The Petri dishes were then taken out and the number of colonies present were counted.

Wearing Comfort

The third test was wearing comfort, measured by smoothness. A longer strip sample of the acrylic was used, and it was placed on a flat consistent table. Using masking tape, tape was put on one end of the unpolished surface of the non-silicone sample. Then another piece of masking tape was placed on the other end of the force sensor hook. Turning the force sensor on, a 500 g weight was placed on top of the sample. This was to ensure that the force sensor was able to detect the force of the friction when the sample was being dragged as the sample was too light. The force sensor was dragged across the table for a distance of 20 cm. The weight was recorded as what was indicated on the sensor. This process was repeated for the sample coated with silicone.

Each of these tests were repeated three times for the purpose of reducing any possible unknown variables that could had caused a variation in one of the trials. The averages of the stain resistance and smoothness tests were taken.

Results

Stain resistance

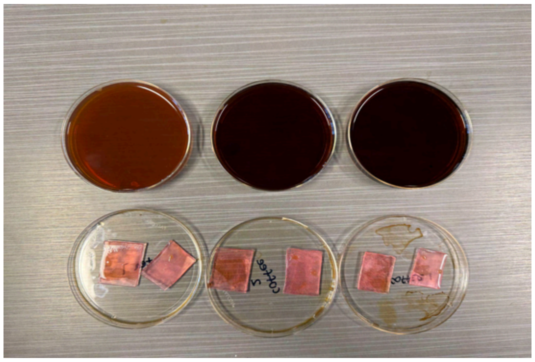

Figure 2.1: Tea after 3 days, Coffee1.1 after 5 days, Coffee2.1 after 3 days.

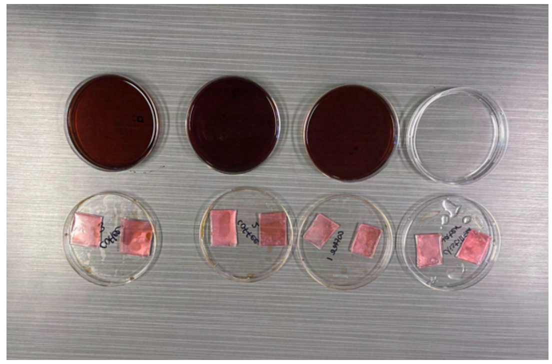

Figure 2.2: Coffee 1.2 after 11 days, coffee 2.2 after 7 days, and coffee 3 after 4 days.

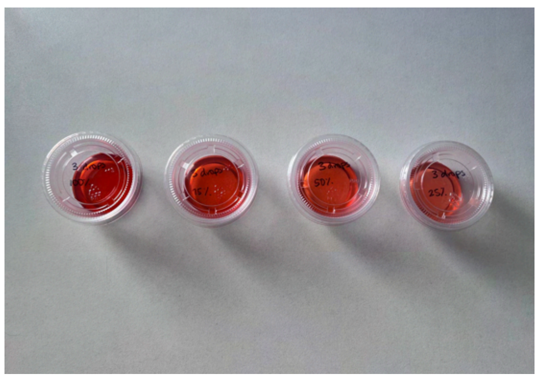

Figure 2.3: Concentration gradient for 3 drops of Red 40.

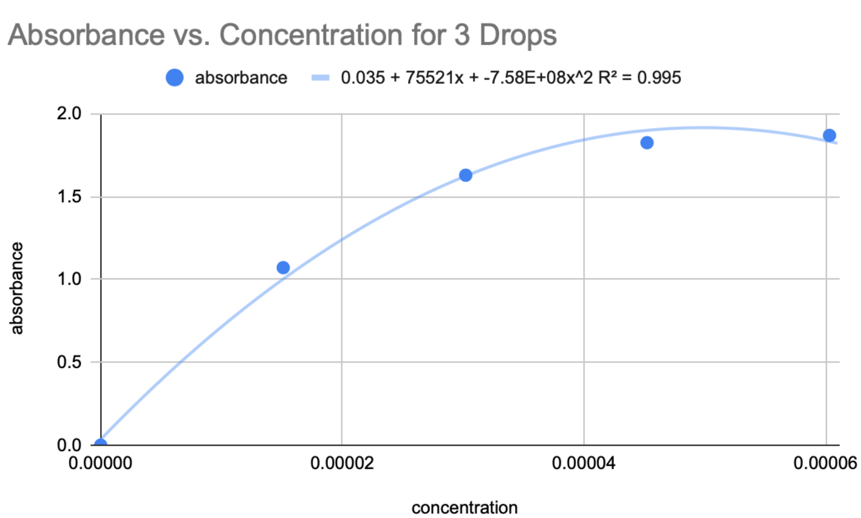

Figure 3.1: Polynomial trendline for the concentration-absorbance graph from the stain resistance experiment.

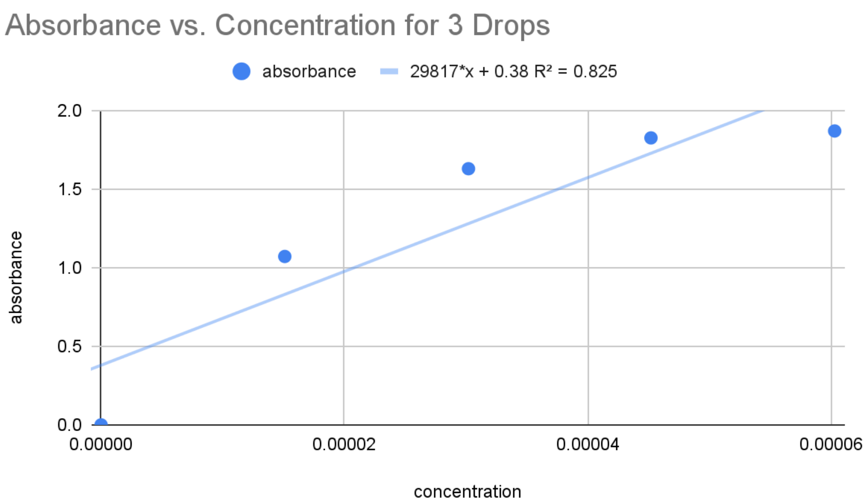

Figure 3.2: Linear trendline for the concentration-absorbance graph from the stain resistance experiment.

	Coffee1.1		Coffee2.1		Water		Coffee1.2		Coffee2.2		Coffee3	
	No silicone	Silicone	No silicone	Silicone	No silicone	Silicone	No silicone	Silicone	No silicone	Silicone	No silicone	Silicone
Observer #1	30%	20%	40%	30%	20%	20%	35%	25%	45%	30%	35%	25%
Observer #2	30%	25%	50%	30%	25%	25%	30%	25%	50%	30%	45%	25%

Observer #3	35%	25%	35%	25%	25%	25%	35%	25%	45%	25%	40%	25%
Avg	32%	23%	42%	28%	23%	23%	33%	25%	47%	28%	40%	25%
Molar Concentrations (M)	1.93×10^{-5}	1.39×10^{-5}	2.53×10^{-5}	1.69×10^{-5}	1.39×10^{-5}	1.39×10^{-5}	1.99×10^{-5}	1.51×10^{-5}	2.83×10^{-5}	1.69×10^{-5}	2.41×10^{-5}	1.51×10^{-5}
Absorbance	1.210	0.938	1.460	1.095	0.938	0.938	1.238	1.003	1.565	1.095	1.415	1.003

Table 1: Percentage of stock solution, molar concentration, and absorbance (λ=504 nm)

Results showed that the acrylic only samples had a higher absorption value at a wavelength of 504 nm (Table 1). Therefore, the silicone-coated acrylic samples worked better in the stain resistance test than the acrylic only samples, and this was consistent with the hypothesis. Higher concentrations corresponded to higher absorbance values. Increased absorbance indicates greater staining by coffee, resulting in a darker surface color present on the acrylic only samples. In Figure 2.1, it could be seen that the samples on the left representing the acrylic only squares were darker in color than the samples on the right representing the silicone-acrylic squares. In Figure 2.2, other than the samples in water since it was the control, the samples on the right representing acrylic only squares had a darker color than samples on the right that are silicone-acrylic squares. These results could be seen in the table, where the molar concentrations were all higher for the silicone-only square samples.

The null hypothesis was that the difference in mean absorption value for non silicone samples was the same as the value for silicone samples. The alternative hypothesis was that the difference in mean values for absorption in silicone samples was less than the value for non-silicone samples. The test done was a 2-sample t-test.

For Independence Groups Assumption, silicone-coated acrylic samples and acrylic-only samples were independent of each other. In Independence Assumption, each trial within the groups were independent of each other. The samples were randomly placed into the Petri dishes. Both models for the two groups were assumed to be Normal although sample size was small, and the alpha level was 0.05. Since the p-value was 0.001948, it was much lower than the significance level of 0.05. Therefore, the null hypothesis was rejected and there was evidence that samples that had silicone resisted stain more than samples that had acrylic only.

Bacterial resistance

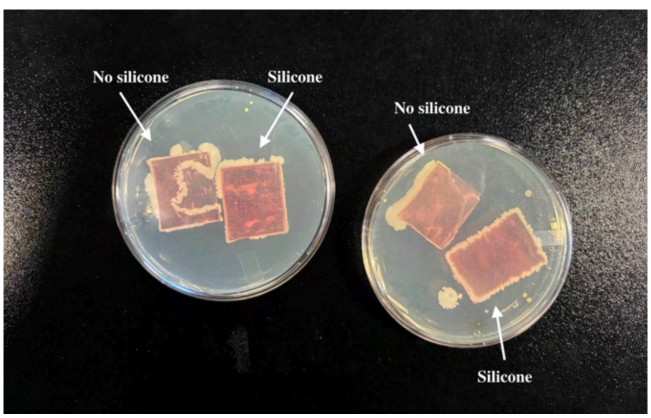

Figure 4.2: Results of bacteria growth after 48 hours.

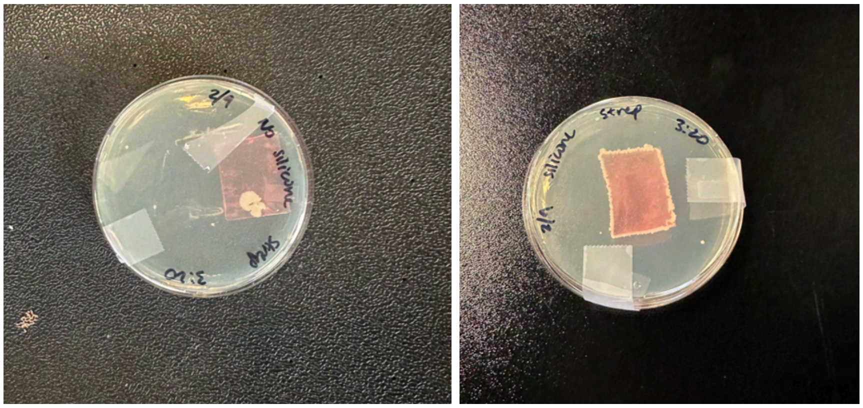

Figure 4.3: Results of the bacteria growth on acrylic only sample after 48 hours.

The number of bacterial colonies could not be counted due to limited equipment, making assumptions and conclusions about the difference in bacterial growth difficult. There was one colony surrounding both types of square samples, silicone-acrylic and acrylic-only, with occasional growth of bacteria on the surface facing the agar for samples without silicone. In Figure 4.2, it could be seen that for both Petri dishes, there was a series of bacteria surrounding the samples. For the Petri dish on the left specifically, there was a ring of bacteria on the surface of the sample without silicone. For Figure 4.3, the photo on the left contained a ring of bacteria on the surface as well for the sample without silicone. The photo on the right had bacteria growing around the sample with silicone.

Wearing Comfort

Figure 5.1: Prototype for acrylic-only sample.

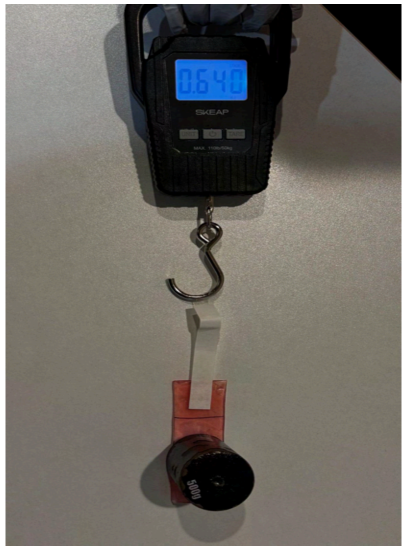

Figure 5.2: Prototype for silicone-coated acrylic samples.

	Without silicone (kg)	With silicone (kg)
Trial 1	0.06	0.535
Trial 2	0.065	0.640
Trial 3	0.07	0.710
Average	0.065	0.628
Standard deviation	0.005	0.0881

Table 2: Force of friction recorded for samples with and without silicone

From the friction test, the average from the force sensor without silicone was 0.065 kg, with the three trials being 0.07 kg, 0.065 kg, and 0.06 kg (Table 2). The average with silicone was 0.628 kg, with the three trials being 0.535 kg, 0.640 kg, and 0.710 kg (Table 2).

The null hypothesis was that the difference in mean friction value for non silicone samples was the same as the value for silicone samples. The alternative hypothesis stated that there was a difference in mean values for friction in silicone samples that was greater than the values for friction non-silicone samples. Independence Groups Assumption was satisfied as silicone-coated acrylic samples and acrylic-only samples were independent from each other. Independence Assumption was also satisfied as each trial within the group was independent from each other. The experiment was not random, but that was one of the purposes of the experiment, so the test would proceed. The Nearly Normal Condition was assumed to be met. The Significance level was 5%. The p-value was 0.996046, which was much greater than the alpha level of 5%, meaning that we failed to reject the null hypothesis and that there was no evidence to prove the friction for acrylic-only samples was higher than silicone-coated acrylic samples.

Friction testing showed that the average friction value for the acrylic-only samples was significantly lower than that of the silicone-coated acrylic samples. In terms of standard deviation, the trials with silicone samples had a much higher value, 0.0881 kg, than without silicone, 0.005 kg (Table 2). This means that there was more variation between the friction force values recorded for samples with silicone than without silicone.

Supplementary Materials

Stain Resistance

The volume of a drop of Red 40 from Market Pantry was approximately 0.05 mL. This meant that for the stock solution of 3 drops, the total volume was 0.15 mL. The concentration of the bottle was about 20 mg/mL. The mass of the 3 drops could be calculated as 0.15 mL x 20 mg/mL, or 3.0 mg. The molar mass of Red 40, C18H14N2Na2O8S2, was 496.42 g/mol. The number of moles was (0.0030 g)/(496.42 g/mol), or 6.043x10-6 mols.

The total volume was 100 mL of water and 0.15 mL of food coloring dye, making it 100.15 mL or 0.10015 L. The molarity was calculated by (6.043x10-6 mols)/(0.10015 L) which was 6.03x10-5.

The rest of the solutions made from the stock solution had a molar concentration of x% multiplied by 6.03x10-5.

Discussion

Stain Resistance

The results from the experiment contradicted the findings reported in the literature review that determined whether silicone, copolyamide, or heat-polymerized resins had the highest discoloration (Lai et al.). However, it was important to note that the results from this test are subjective to a degree; so, further research and experiments were necessary to assess the absolute validity of the statement. This could be due to the fact that the amount of time the results were observed were different. The maximum time observed in this research was 11 days, which was shorter than in the one in the literature review (Lai et al.). This research could have gone for a longer period if not for time restraints, which might be the reason why acrylic ends up having less staining compared with the silicone that they tested with. Also, the type of silicone was different, used was silicone (Gingivamoll) (Lai et al.), and the silicone used here was from a silicone mold kit. The uncertainty introduced by this method could actually result in absorbance differences between silicone-coated acrylic and acrylic-only samples to not be truly meaningful. If more advanced instruments and time was allotted, a way to identify the exact shade of red could be determined; this could lead to a much more accurate absorbance for the samples. In the staining test, sample colors were estimated visually, introducing uncertainty and limiting the accuracy of the calculated concentration values.

Bacterial Resistance

The new retainer design with silicone layer does not impact bacterial accumulation. From the results, it could be seen that we failed to reject the null hypothesis, which stated that the amount of bacteria on silicone-coated acrylic samples and acrylic-only samples are the same. More research was needed to further confirm the results of this experiment as there was not a quantifiable way to count bacteria. Initially, a count of bacterial colonies was intended to happen, but the bacteria ended up growing in one large colony, so only a qualitative estimate could be made. A better way to quantify the number of bacteria on the surface of the sample was to count the individual bacteria, but there were not any instruments that could be used at the time to perform this. Another way to quantify the amount of bacteria would be to measure the surface area, which could be tested in future research. A surprising finding was that of all the samples where the bacteria started growing between the sample and the agar, none of them had a layer of silicone on it. There could be further research done for this phenomenon. This phenomenon was present throughout all of my three trials, meaning that there could be a variable that had not been discovered in this research as to why this occurs; one possibility could be that the acrylic only samples were not as sticky as the silicone-coated acrylic samples, meaning that they were less likely to stay on the Petri dish agar when it was flipped upside down as some gaps were not fully sealed. Better designed and conducted experiments need to be done in order to determine which sample was better for fighting bacteria.

Wearing Comfort

The silicone-coated acrylic samples exhibited higher friction values than the acrylic-only samples. A higher friction value suggested that the retainer may be more resistant to displacement, making it less likely to shift unintentionally. However, there was an initial consideration that the more friction, the rougher the surface; therefore, the values found through this experiment could only show how the null hypothesis could not be rejected. This could be due to the fact that the friction test does not account for the compressibility of the silicone and the stickiness of the surface of the silicone mold kit.

Through these three design criterias, it could be seen that only the stain resistance test supports the hypothesis. The bacteria and smoothness test both reject what the hypothesis states. However, further experiments with different methods and data may bring about a different conclusion than the one mentioned in this research.

With these test results, it could be seen that silicone could act as a possible future add-on to dental Hawley retainers as this and future research could lead to further evidence. As with this experiment, it could already be seen that stain could be greatly reduced with the addition of silicone.

A regular silicone mold kit was used in this study instead of medical-grade silicone because the latter was not accessible due to its high cost and limited availability. A medical grade silicone was more pure, which could affect the amount of bacteria growing on the surface or the ability for this material to resist stain.

Future Research

Future research could involve using medical-grade silicone and evaluating the effects of incorporating *Streptococcus mutans* into the bacterial resistance tests (Kim et al.). Another potential direction would be testing this prototype with human participants.

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