Changes in Esthetic Characteristics of Vacuum-formed Retainers after Staining and Cleaning

Matthew Tsai, D.M.D.

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Orthodontics

Oregon Health & Science University
Portland, Oregon

December 16, 2022

Acknowledgements

Thank you to my research mentor Dr. Jack Ferracane for his advice, feedback, and genius through all stages of this project.

Thank you to my thesis committee members: Dr. Jeff Nickel and Dr. Jonathan Yih for their feedback and support of the project.

Thanks to everyone in the Ferracane, Pfeifer, and Bertassoni lab space for adopting me during my data collection process.

Much gratitude to all orthodontic residents who aided me with fabricating specimens i.e., the dreaded "cutting squares".

Thank you to Yi-Hsien Chang for your help with data collection during many late nights - all the best with your pursuit in dentistry.

Thank you to Chieh-Yun Lo, Matt Logan, and Sarah Patty for their expertise on the potential chemistry behind our findings.

Changes in Esthetic Characteristics of Vacuum-formed Retainers after Staining and Cleaning

Matthew Tsai, D.M.D.[a]
Jeff Nickel, D.M.D., M.Sc., Ph.D.[a]
Jonathan Yih, D.M.D., M.S. [a]
Jack L. Ferracane, Ph.D.[b]

[a] Department of Orthodontics, Oregon Health & Science University, Portland, OR
[b] Department of Restorative Dentistry, Oregon Health & Science University, Portland OR

Corresponding Author:
Matthew Tsai, D.M.D.
E-mail: thew8888@gmail.com
Phone: 909-569-8831

DECLARATION OF INTEREST

None of the authors have any interest, financial or otherwise, in any of the thermoplastic materials and cleaning agents involved in this study.

Table of Contents

Abstract

Objective: To measure and compare initial, post-staining, and post-cleaning color, gloss and surface roughness of five different orthodontic thermoplastic retainer materials.

Materials and Methods: Five different materials were studied; two polyethylene (PE) copolymers: Essix Plus, Invisacryl Ultra; two polypropylene (PP) copolymers: Essix C^+ and Invisacryl C, and one polyurethane (PU) polymer, Zendura. Square specimens were cut from thermoformed discs of the material. Color of the materials was measured with a spectrophotometer against both a white and black tile using the CIELAB color space; ΔE^*_{00} values and contrast ratio were calculated from these measurements. Gloss was measured with a glossmeter. Surface roughness was measured with surface roughness tester. Measurements were taken before and after immersion in either water, coffee, red wine, or turmeric for 96 hours. The same measurements were repeated after cleaning each specimen with either Retainer Brite, Polident, or Invisalign Cleaning Crystals.

Results: In general, materials differed significantly in initial esthetic characteristics except for contrast ratio and gloss for EP and IU; staining and cleaning do not significantly affect these characteristics. Turmeric stains dramatically more than any other staining agent. Coffee stains more than red wine. Polyethylene-based VFR stains the most with turmeric, followed by polyurethane-based materials, then polypropylene-based materials. Essix C^+ stains the most with coffee but least with turmeric.

Conclusion: For all VFR materials, staining and cleaning does not change contrast ratio, gloss, and surface roughness in a clinically significant manner. Turmeric is a powerful staining agent, and ΔE^*_{00} for PE > PP > PU when stained. Invisalign Cleaning Crystals, Polident, and Retainer Brite do not differ significantly in effectiveness of stain-removal except for turmeric in certain cases, where Invisalign Cleaning Crystals were most effective.

Introduction

After orthodontic treatment, full-time retention is necessary for at least 3-4 months before transitioning to part-time retention because teeth will initially be unstable in the face of occlusal and soft tissue pressures that later can be resisted.[1] Under such pressures and without retention, there is a tendency for teeth to return to their initial positions, and any change in tooth position or arch relationship subsequent to the completion of orthodontic treatment constitutes relapse.[2] It was reported that approximately 70% of orthodontic cases result in some degree of relapse, and relapse in the form of crowding has been shown to occur even up to 20 years post-retention.[3,4] The causes of relapse are due to a number of different factors, including the periodontium, occlusion, soft tissue, and growth.[5,6] The reason behind full-time retention is 3-fold: 1) it allows for gingival and periodontal tissue to reorganize as it usually takes 3-4 months before slight mobility of teeth disappears, 2) since teeth may be in an inherently unstable position immediately after treatment, soft tissue pressures constantly produce a relapse tendency if there is no retention, and 3) changes produced by growth may alter orthodontic treatment result.[1]

There is no debate on whether retention is needed after orthodontic treatment. There is, however, no consensus among orthodontists on retainer choice/combination and what regimen for patients to follow during full-time and part-time wear. According to a study in 2008, most orthodontists in the US prescribe less than 9 months of full time wear of removable retainers and thereafter advise part-time, lifelong wear.[7] The same authors also found that maxillary Hawley and Essix retainers and mandibular fixed lingual retainers and Hawley retainers are most commonly used by U.S. orthodontists.[7] A study in 2004 found that the most commonly used retainers among Australian orthodontists were maxillary Essix and mandibular canine to canine bonded retainers.[8] A survey study in Ireland in 2013 found that vacuum-formed retainers (VFRs) were the most common retainer choice for full time wear in the maxillary and mandibular arch, and full occlusal coverage with a thickness of 1.0 mm prescribed by the majority of practioners.[9] Although a more recent survey study in 2018 suggests that the type of retainer that an orthodontist chooses to prescribe to patients varies depending on the country and region within the country he/she practices, it is safe to say that VFR use increased even more over the last decade relative to previous years[10,11] Indeed, VFRs have gained much popularity since their invention due to their desired esthetics and ease of fabrication. [25]

Two most common thermoplastics used for fabricating VFRs are polyethylene (PE) copolymers and polypropylene (PP) copolymers.[12] Polyethylene copolymers are considered more esthetic because the material is virtually transparent. Polypropylene copolymers are considered more durable (wear-resistant) and flexible, but esthetically they are inferior to polyethylene because the polyethylene material is translucent. Other common polymers used to fabricate thermoplastic appliances include polyester and polyurethane (PU).[13] Thermoplastic polyurethane is one of the most versatile engineering thermoplastics with wide applications in various industries.[14] Of polyurethane-based VFR materials, Zendura seems to have gained much popularity in the past few years for its supposed durability.

Because retainers are essential in preventing orthodontic relapse, it is crucial to have an effective cleaning technique to facilitate long-term use of the retainers. Stained retainers appear unesthetic and unsanitary. It is desirable that VFRs remain clear, especially when full-time wear and social interactions overlap. Retainer Brite, Polident, and Invisalign Cleaning Crystals are popular cleaning agents for VFRs on the U.S. market. Currently, there are few studies on staining and cleaning of clear thermoplastic appliances, and even fewer studies that examine pre-staining, post-staining, and post-cleaning esthetic characteristics of VFR materials.

The purpose of this study was to explore the following questions.

1. Do the VFR materials differ in initial esthetic and surface characteristics?
2. Do the materials respond differently when immersed in different staining agents? Further, do changes in esthetic characteristics, if any, depend on material or stain?
3. If materials do stain, what is the best cleaning agent to remove said stain?

Based on the above questions, the following working hypotheses were formed based on consideration of previous literature and anecdotal evidence:

1. Compared to polyurethane and polypropylene-based materials, polyethylene-based materials will have lower initial contrast ratio, higher initial gloss, and lower initial surface roughness values.
2. Aside from turmeric, coffee will produce the most prominent color change. Further, among materials stained by coffee, Essix C^+ (polypropylene copolymer) will exhibit the greatest change in color.
3. The magnitude of color change is not associated with a change in other esthetic characteristics.
4. Different cleaning agents will have similar effectiveness in removing stain from VFR materials.

Materials and Methods

Five thermoplastic materials were chosen for inclusion in the study based on chemical composition: Essix C$^+$ (EC), Essix Plus (EP), Invisacryl Ultra (IU), Invisacryl C (IC), and Zendura (Z) [Table 1]. In terms of general composition, Essix C$^+$ parallels Invisacryl C being polypropylene-based, while Essix Plus parallels Invisacryl Ultra being polyethylene-based. Zendura is made from a polyurethane. Each material was packaged as a circular disc of 125mm diameter x 1mm thickness and were first thermoformed onto resin blocks that were 12mm x 12mm x 5mm using a positive pressure machine (Biostar VI; Scheu-Dental, Iserlohn, Germany) [Figure 1a-1c]. The resin blocks were printed from an iPrint 3D printer (iPrint 3D, LLC, Edmonton, Canada). Thermoforming the material first for this study eliminated confounding factors related to any changes in properties that may be introduced by this step. Each material was thermoformed using the respective manufacturers' instructions (Table 1). The thermoformed pieces were then cut with scissors into pieces of 12x12x1 mm (hereafter referred to as 'specimen') so that 120 specimens per VFR material were prepared (Figure 2a-2b). To orient each specimen during measurements, a corner of each specimen was modified by cutting off the corner which then was identified as the upper right corner (Figure 3).

The experimental design is outlined in Figure 4. For each VFR material, the prepared 120 experimental specimens were divided into 4 staining groups of 30, each placed in a tackle box (Plano Molding Co, Plano, IL) for organization (Figure 5a-5b). Each staining group was then immersed in one of the following under 37°C for 96 hours: deionized water (control), red wine, coffee, and turmeric (Table 2). Previous studies have demonstrated significant staining of VFR materials at the 48 to 72-hour time period.[15,16] For this study, the duration of immersion was decided to be 96 hours so that the effects of staining could be more visible. For each staining group of 30 specimens, each was placed in a test tube (Globe Scientific Inc., Mahwah, NJ), covered with 5ml of staining agent, then put in an incubator (Shel Lab CO_2 Series; Sheldon Mfg. Inc., Cornelius, OR) [Figure 6a-6c]. Four days (96 hours) of immersion time approximates 64 minutes of exposure a day for 3 months or 48 minutes a day for 4 months. This simulates the behavior of a non-compliant patient who consumes beverages with retainers in place.

After immersion in their designated staining agents, each staining group of 30 specimens was then further divided into 3 cleaning groups of 10, each cleaned by either Polident, Retainer Brite, or Invisalign Cleaning crystals according to manufacturer's instructions (modified as needed for standardization of procedures) [Table 3]. Each specimen was cleaned in the same test tube in which they were stained (Figure 7a-7b). Measurements were taken at three time points: T_1 (initial), T_2 (after staining), and T_3 (after cleaning). At T_1, specimens were measured as is. At T_2 and T_3, stained and cleaned specimens were measured for color, gloss, and surface roughness after being rinsed with deionized water and dried with gauze.

To measure color, all specimens were placed on the target mask (3mm diameter) of a spectrophotometer (CM-700d; Konica Minolta, Osaka, Japan) via a guide fabricated from Memosil® 2 (Kulzer GmbH, Hanau, Germany) that allowed for consistency in positioning when taking measurements. Each specimen was placed such that the glossy side (the side that did not come in contact with the calibration block during the thermoforming process) was facing the sensor with the modified corner towards a red mark placed with a sharpie pen (Figure 8a). Initial color readings were made against a white tile with the spectrophotometer according to the Commission Internationale de l'Eclairage (CIE) L*, a*, b* (LAB) color scale; the same measurements were then taken with the same specimen against a black tile. The spectrophotometer was placed on its base, with the specimen over the sensor and the tile balanced atop the specimen (Figure 8b). The spectrophotometer was calibrated using a known standard (a white surface located on the inside of the cap), supplied by the manufacturer, prior to taking readings. ΔE^*_{00} was calculated between each timepoint using the formula in Figure 9. All ΔE_{00} values reported are based on L*a*b* measurements taken against the white tile as this better simulates the intraoral environment with retainer material against labial surface of teeth. Contrast ratio within each timepoint was calculated by dividing the L* value of color measurements against the black tile by the L* value of color measurements against the white tile.

The gloss of each specimen was measured with a glossmeter (NOVO-CURVE; Rhopoint Americas Inc., Troy, MI). Three gloss measurements were taken for each specimen at a 60° angle. For the first measurement, each specimen was centered at the measuring window of the glossmeter, glossy side down, aligned such that the modified corner was on the upper right (Figure 10). After the first measurement was recorded, the specimen was then rotated 90° clockwise for the second measurement to be taken. Lastly, the specimen was once again rotated 90° clockwise for the third gloss measurement to be taken. All measurements were performed while each specimen was fully covered by a paper lid wrapped in aluminum foil in order to ensure that ambient light did not interfere.

Surface roughness (Ra) was measured with a handheld surface roughness tester (TR-200; INNOVATEST, Maastricht, Netherlands). The settings were as follows: 0.8 mm sampling length, 2mm evaluation length, and range of +/- 40 μm. Surface roughness values were measured at three locations across the center of each specimen (Figure 11). All measurements were taken with the glossy side of each specimen facing up and the modified corner on the upper right. For the first measurement, the stylus of the TR-200 was positioned directly on the left edge of the specimen and at the mid-point between the top and bottom edge. After the first measurement was recorded, the specimen was then rotated 90° clockwise for the second measurement to be taken. Lastly, the specimen was once again rotated 90° clockwise for the third gloss measurement to be taken. For both the gloss and surface roughness, the multiple readings ensured that variation due to the orientation of the specimen, which can significantly affect the measured value, was taken into account.

Data Analysis

The SigmaPlot (SPSS Inc., Chicago, IL) statistical software was utilized for all data analysis performed in this study. One-way ANOVA tests were performed across all materials to compare initial contrast ratio, gloss, and surface roughness. Two-way ANOVA tests were performed on contrast ratio, gloss, surface roughness, and ΔE^*_{00} (T_2-T_1) values for material and stain, using the T_2 measurements. Three-way ANOVA tests were performed on contrast ratio, gloss, surface roughness, and ΔE^*_{00} (T_3-T_1) for material, stain, and cleaner after the T_3 timepoint. All of the tests were conducted at $\alpha = 0.05$. Where significance was found, further comparisons between materials were conducted using Tukey's post-hoc test with $\alpha = 0.05$. Whenever the conditions of equal variance and normality were violated by the data, which was true in most cases, the software performed non-parametric statistics, as noted in the Results section.

Results

The results section is organized as follows. First, observations from a visual inspection of specimens will be described. Then, findings related to esthetic characteristics will be discussed in the order of contrast ratio, gloss, surface roughness, and color. Where statistically significant differences were found, the p-values were generally less than 0.001; for a more succinct presentation of the data, all p-values have been omitted from the following optimized results section. The full version of the results section containing all p-values is presented in Appendix A with supplementing tables in Appendices B-E. Color is divided into two sections: ΔE^*_{00} (T_2-T_1), which represents the magnitude of color change from the staining agents, and ΔE^*_{00} (T_3-T_1), which represents how much a specimen has changed in color from its original color after the staining and cleaning procedures.

VISUAL INSPECTION OF SPECIMENS

Photographs showing the color of specimens after immersion in various staining and cleaning solutions are provided in Figures 12-13. Visual inspection indicated that turmeric produced the most prominent change in color across all materials, followed by coffee. Red wine and water produced less noticeable color changes, if any. For all samples stained by turmeric, it was evident that EP and IU stained to a similar extent, followed by Z, IC, and EC. In general EP, IU, and Z stained significantly more than EC and IC.

When all specimens were compared side by side (Figure 12), it was difficult to discern that EP, IU, and Z were stained by anything except for turmeric. For EC and IC, it was difficult to visually distinguish between specimens stained by red wine and water; for coffee, a faint stain could be observed, with a hint that EC stained more than IC. For polypropylene-based materials, it was evident that EC stained less than IC with turmeric.

CONTRAST RATIO

Among the different VFR materials, IC had the highest contrast ratio while EP had the lowest. In general, PP-based materials have higher contrast ratios than EP-based materials. To determine whether initial contrast ratio differed significantly among materials, a Kruskal-Wallis one way ANOVA on ranks was performed. Results showed a significant variation in medians among materials. Further, a Tukey Test indicated that all materials differed significantly from each other in terms of contrast ratio, except for IU and EP. The full data set is described in Table 4 and Figure 14. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

IC (0.41) > EC (0.39) > Z (0.35) > IU (0.24)[a] > EP (0.23)[a]

To determine whether post-staining contrast ratio differed significantly between materials and whether this depended on staining agent, a 2-way ANOVA between material and stain was performed. Results indicated significant main effects of material and stain. No significant interaction was found between materials and stain. Results indicated significant main effects of material and stain, i.e. there was a difference between the materials and a difference between the staining agents. However, there was no significant interaction between the main variables. The full data set is described in Table 5 and Figure 17. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

Comparison of Staining Agents for Different Materials

EC x Staining Agents - T (0.42) > W (0.40)[a] > RW (0.40)[a] > C (0.40)[a]

EP x Staining Agents - T $(0.25)^a$ > C $(0.24)^a$ > W $(0.23)^b$ > RW $(0.23)^b$

IC, IU, Z x Staining Agents - No significant differences were found within IC, IU, and Z specimens immersed in various staining agents.

Comparison of Materials for Different Staining Agents

Coffee x Materials - IC (0.42) > EC (0.40) > Z (0.34) > IU $(0.25)^a$ > EP $(0.24)^a$

Red Wine x Materials - IC (0.43) > EC (0.40) > Z (0.34) > IU $(0.24)^a$ > EP $(0.23)^a$

Turmeric x Materials - IC $(0.43)^a$ > EC $(0.42)^a$ > Z (0.35) > IU $(0.25)^b$> EP $(0.25)^b$

Water x Materials - IC (0.43) > EC (0.40) > Z (0.35) > IU $(0.25)^a$ > EP $(0.23)^a$

Results of a 3-way ANOVA between material, staining agent, and cleaning agent relative to contrast ratio is available in the appendix only, as it did not provide any significant additional information (no main effect of cleaning agent was found) [Table 9].

GLOSS

Among all VFR materials, IU had the highest gloss while EC had the lowest. PE-based materials had higher gloss than PP-based materials in general. To determine whether initial gloss differed significantly between materials, a Kruskal-Wallis one way ANOVA on ranks was performed. Results showed a significant median variation among materials. Further, a Tukey Test indicated that all materials differ significantly from each other in terms of contrast ratio except for IU and EP. The full data set is described in Table 4 and Figure 15. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

IU $(84.9)^a$ > EP $(83.0)^a$ > Z (75.2) > IC (59.4) > EC (54.3)

To determine whether post-staining gloss differed significantly between materials and whether this depended on staining agent, a 2-way ANOVA was performed. Results indicated significant main effects of material and stain, i.e. there was a difference between the materials and a difference between the staining agents. In addition, there was a significant interaction between the main variables. The full data set is described in Table 5 and Figure 18. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

Comparison of Staining Agents for Different Materials

EC x Staining Agents – C (54.1) > RW (53.1)^{a} > W (51.9)^{a} > T (45.9)^{a}

EP x Staining Agents – No significant differences were found between staining agents.

IC x Staining Agents – RW (62.0)^{a} > W (59.6)^{a,b} > T (58.2)^{a,b} > C (55.6)^{b}

IU x Staining Agents – T (87.1)^{a} > RW (84.1)^{a} > C (80.9) > W (80.5)

Z x Staining Agents – T (88.4) > RW (73.5)^{a} > W (72.0)^{a} > C (70.6)^{a}

Comparison of Materials for Different Staining Agents

Coffee x Materials – EP (83.9)^{a} > IU (80.9)^{a} > Z (70.6) > IC (55.6)^{b} > EC (54.1)^{b}

Red Wine x Materials – IU (84.7)^{a} > EP (79.4)^{a,b} > Z (73.5)^{b} > IC (62.0) > EC (53.1)

Turmeric x Materials – Z (88.4)^{a} > IU (87.1)^{a} > EP (81.7) > IC (58.2) > EC (45.9)

Water x Materials – EP (82.2)^{a} > IU (80.5)^{a} > Z (72.0) > IC (59.6) > EC (51.9)

Results of a 3-way ANOVA between material, staining agent, and cleaning agent relative to gloss is available in the appendix only, as it did not provide any significant additional information (no main effect of cleaning agent was found) [Table 10].

SURFACE ROUGHNESS

Among all VFR materials tested, IC had the highest surface roughness and EP the lowest. To determine whether initial surface roughness differed significantly between materials, a Kruskal-Wallis one-way ANOVA on ranks was performed. Results showed a significant variation in medians among materials. Further, a Tukey Test indicated that all materials differed significantly from each other in terms of surface roughness, except for IC and EC. The full data set is described in Table 4 and Figure 16. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

IC (0.438)[a] > EC (0.409)[a] > Z (0.335) > IU (0.222) > EP (0.141)

To determine whether post-staining surface roughness differed significantly between materials and whether this depended on staining agent, a 2-way ANOVA between material and staining agent was performed. Results indicated significant main effects of material and stain, i.e. there was a difference between the materials and a difference between the staining agents. However, there was no significant interaction between the main variables. The full data set is described in Table 5 and Figure 19. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

Comparison of Staining Agents for Different Materials

EC x Staining Agents – T (0.465)[a] > C (0.441)[a,b] > RW (0.386)[b,c] > W (0.349)[c]

EP, IC, IU x Staining Agents – No significant differences between staining agents were found within EP, IC, and IU.

Z x Staining Agents – W (0.383)[a] > RW (0.344)[a,b] > C (0.311)[b,c] > T[c] (0.250)

Comparison of Materials for Different Staining Agents

Coffee x Materials – EC (0.441)^{a} > IC (0.431)^{a} > Z (0.311) > IU (0.231)^{b} > EP (0.192)^{b}

Red wine x Materials – IC (0.483) > EC (0.386)^{a} > Z (0.344)^{a} > IU (0.199)^{b} > EP (0.169)^{b}

Turmeric x Materials – EC (0.465)^{a} > IC (0.457)^{a} > Z (0.250) > IU (0.241) > EP (0.159)

Water x Materials – IC (0.442)^{a} > Z (0.383)^{a,b} > EC (0.349)^{b} > IU (0.217)^{c} > EP (0.170)^{c}

Results of a 3-way ANOVA between material, staining agent, and cleaning agent relative to surface roughness is available in the appendix only, as it did not provide any significant additional information (no main effect of cleaning agent was found) [Table 11].

COLOR - ΔE^*_{00} (T_2-T_1)

Across all materials, turmeric resulted in the largest average change in color in terms of ΔE_{00} (19.58), followed by coffee (1.61), red wine (0.56), and water (0.22). To determine whether post-staining color differed significantly between materials and whether this depended on staining agent, a 2-way ANOVA between material and staining agent was performed. Results indicated significant main effects of material and stain, i.e. there was a difference between the materials and a difference between the staining agents. In addition, a significant interaction between material and stain as found. The full data set is described in Table 6 and Figure 20-21. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

Comparison of Staining Agents for Different Materials

EC x Staining Agents – T (8.25) > C (2.25) > RW (0.62) > W (0.25)

EP x Staining Agents – T (27.63) > C (1.59) > RW (0.62) > W (0.18)

IC x Staining Agents – T (9.39) > C (1.19) > RW (0.73) > W (0.29)

IU x Staining Agents – T (27.68) > C (1.23) > RW (0.39)[a] > W (0.20)[a]

Z x Staining Agents – T (24.95) > C (1.77) > RW (0.44)[a] > W (0.19)[a]

Comparison of Materials for Different Staining Agents

Coffee x Materials – EC (2.25) > Z (1.77)[a] > EP (1.59)[a,b] > IU (1.23)[b] > IC (1.19)[b]

Turmeric x Materials – IU (27.68)[a] > EP (27.63)[a] > Z (24.95) > IC (9.39) > EC (8.25)

Red Wine and Water x Materials – Within specimens immersed in red wine and water, no significant differences between materials were found.

COLOR - ΔE^*_{00} (T_3-T_1)

To determine whether post-cleaning surface color differed significantly between materials and whether this depended on cleaning agent, 2-way ANOVA tests between staining agent and cleaning agent were performed for each material. The full data set is described in Table 7 and Figure 22-26. Findings are summarized in the relationship below (materials ranked in order); values with the same superscript were not significantly different.

Comparison of Cleaning Agents for Different Staining Agents

Essix C+ (EC) - Turmeric x Cleaning Agents

R (5.94)[a] > P (5.58)[a] > I (4.09)

Essix Plus (EP) - Red Wine x Cleaning Agents

R (0.84)[a] > P (0.66)[a,b] > I (0.37)[b]

Invisacryl C (IC) - <u>Turmeric x Cleaning Agents</u>

P (6.98) > R (6.52) > I (6.02)

Invisacryl Ultra (IU) - No significant differences between cleaning agents were found.

Zendura (Z) - <u>Turmeric x Cleaning Agents</u>

P (24.96)[a] > R (24.90)[a] > I (23.38)

To determine whether color change differed among materials, and whether this change depended on the staining and cleaning agents, a 3-way ANOVA was performed between materials, cleaning agent, and stain. Results indicated main effects of material, staining agent, and cleaning agent i.e. there was a difference between the materials, a difference between the staining agents, and a difference between the staining agents. In addition, interactions between all 3 variables were found. The full data set is described in Table 7. Findings are summarized in the relationships below (materials ranked in order); values with the same superscript were not significantly different.

The 3-way ANOVA between material, staining agent, and cleaning agent indicated that on average, Invisalign Cleaning Crystals lead to statistically lower ΔE^*_{00} (T_3-T_1) values, as shown in the relationship below. Values with the same superscript were not significantly different.

R (4.96)[a] > P (4.95)[a] > I (4.69)

<u>Invisalign Cleaning Crystals</u>

<u>Comparison of Materials for Different Staining Agents</u>

Coffee x Materials - EC (1.00)^{a} > IC (0.93)^{a} > EP (0.36)^{b} > IU (0.30)^{b} > Z (0.29)^{b}

Turmeric x Materials - IU (27.81)^{a} > EP (27.47)^{a} > Z (23.38) > IC (6.02) > EC (4.09)

Red wine and Water x Materials - For all specimen immersed in red wine and water, then cleaned by Invisalign Cleaning Crystals, no significant difference between materials was found.

Comparison of Staining Agents for Different Materials

EC x Staining Agents - T (4.09) > C (1.00) > RW (0.28)^{a} > W (0.21)^{a}

EP x Staining Agents - T (27.36) > RW (0.37)^{a} > C (0.36)^{a} > W (0.14)^{a}

IC x Staining Agents - T (6.02) > C (0.93) > W (0.28)^{a} > RW (0.27)^{a}

IU x Staining Agents - T (27.47) > C (0.30)^{a} > RW (0.29)^{a} > W (0.21)^{a}

Z x Staining Agents - T (23.38) > W (0.33)^{a} > RW (0.33)^{a} > C (0.29)^{a}

Polident (P)

Comparison of Materials for Different Staining Agents

Coffee x Materials - EC (1.11)^{a} > IC (1.02)^{a} > IU (0.38)^{b} > Z (0.37)^{b} > EP (0.36)^{b}

Turmeric x Materials - IU (27.55)^{a} > EP (27.46)^{a} > Z (24.96) > IC (6.98) > EC (5.58)

Red wine and Water x Materials - For all specimen immersed in red wine and water, then cleaned by Polident, no significant difference between materials was found.

Comparison of Staining Agents for Different Materials

EC x Staining Agents - T (5.58) > C (1.11) > RW (0.37)^{a} > W (0.20)^{a}.

EP x Staining Agents - T (27.46) > RW (0.66)^{a} > C (0.36)^{a} > W (0.15)^{a}

IC x Staining Agents - T (6.98) > C (1.02)^{a} > RW (0.58)^{a,b} > W (0.15)^{b}

IU x Staining Agents - T (27.55) > C (0.38)[a] > RW (0.31)[a] > W (0.26)[a]

Z x Staining Agents - T (24.96) > C (0.37)[a] > W (0.36)[a] > RW (0.23)[a]

Retainer Brite (R)

Comparison of Materials for Different Staining Agents

Coffee x Materials - EC (1.11)[a] > IC (0.94)[a,b] > Z (0.74)[a,b] > EP (0.47)[b] > IU (0.33)[b]

Turmeric x Materials - IU (27.81)[a] > EP (27.19)[a] > Z (24.90) > IC (6.52) > EC (5.94)

Red wine and Water x Materials - For all specimen immersed in red wine and water, then cleaned by Invisalign Cleaning Crystals, no significant difference between materials was found.

Comparison of Staining Agents for Different Materials

EC x Staining Agents - T (5.94) > C (1.11) > RW (0.35)[a] > W (0.23)[a]

EP x Staining Agents - T (27.19) > RW (0.84)[a] > C (0.47)[a,b] > W[b] (0.14)

IC x Staining Agents - T (6.52) > C (0.94) > RW (0.40)[a] > W (0.17)[a]

IU x Staining Agents - T (27.81) > RW (0.36)[a] > C (0.33)[a] > W (0.22)[a].

Z x Staining Agents - T (24.90) > C (0.74)[a] > W (0.25)[a] > RW (0.25)[a]

Discussion

From a review of the literature, very few studies have investigated pre-staining, post-staining, and post-cleaning esthetic characteristics of VFR materials. With the increasing prevalence of clear retainer use after orthodontic treatment, proper maintenance and cleaning of the retainers will continue to be critical. Typically, VFRs are commonly provided to patients directly after removal of braces due to ease of fabrication. Following removal of braces, VFRs are then often worn full-time for the 3-4 months thereafter unless the use of a different type of retainer is indicated. During this period of full-time wear, it is important that these retainers remain optically clear and free from staining. To this end, we sought to answer the following questions:

1. Do the VFR materials differ in initial esthetic and surface characteristics?
2. Do the materials behave differently when immersed in different staining agents? Further, do changes in esthetic characteristics, if any, depend on material or stain?
3. If materials do stain, what is the best cleaning agent to remove said stain?

For intrinsic polymers, without additives and impurities, the degree of translucency is influenced primarily by the extent of crystallinity. Some scattering of light occurs at the boundaries between crystalline and amorphous regions as a result of different indices of refraction.[17] For highly crystalline specimens, this degree of scattering is extensive, which leads to translucency, and in some cases even opacity. Highly amorphous polymers are completely transparent.[18] The polypropylene polymers are semicrystalline; therefore, one would expect them to be less transparent than the relatively more amorphous polyurethane and polyethylene copolymers.[19] In our study, the relative lack of transparency is reflected as a higher value for contrast ratio.

CONTRAST RATIO

This study showed that polyethylene copolymers (PE) consistently had lower contrast ratios than polypropylene polymers (PP), consistent with the initial hypothesis (Figure 13). The contrast ratio of the polyurethane (PU)-based VFR, Zendura, was between the PE and PP copolymers, but was closer to that of PP polymers in terms of value. Consistent with these results is the previous study by Knapp et al., who compared the initial clarity of various VFR materials by calculating the color difference between VFR specimens measured against a white photo paper and clear glass microscope slides measured against the same white photo paper; they found that PE copolymers (namely, EP and IU) had significantly better initial clarity than PP polymers (namely, EC and IC).[20]

The one-way ANOVA showed that initial contrast ratios of IU and EP did not differ significantly. The general trend in the two-way ANOVA, similar to that of the one-way ANOVA, indicated that contrast ratio of IU and EP were not significantly different, regardless of what staining agent they were immersed in. This is reasonable since IU and EP should be of similar composition. The same reasoning applies to the findings that IC and EC showed no difference in contrast ratio after immersion in turmeric. It is interesting that although the color of the specimens changed dramatically in turmeric, light penetration through the material and reflecting from the background was not affected significantly. One may have expected greater absorption of certain wavelengths of the light based on the color change, but the effect was similar for the two backgrounds, and ultimately the contrast ratio was the same as in the pre-stained state. In other words, despite a change in color, the clarity of the materials did not change.

For EC specimens immersed in turmeric, a statistically significant increase (0.02) in contrast ratio was found relative to EC specimens immersed in water. While this increase is not easily explained, this subtle difference is deemed unlikely to be clinically significant. Similarly, for EP specimens immersed in turmeric, a similar statistically significant increase (0.02) in contrast ratio was found relative to EP specimens immersed in water; again, this is unlikely to be clinically significant. For IC, IU, and Z, staining agent does not have a statistically significant effect on contrast ratio, suggesting that light transmission through the material was not impeded or affected at all by subtle surface staining.

GLOSS

Data from this study show that in general, polyethylene copolymers had higher gloss than polypropylene polymers (Figure 15), with the polyurethane in between. This is consistent with our working hypothesis.

The one-way ANOVA showed that gloss of IU and EP did not differ significantly, which is reasonable since IU and EP should be of similar composition and have similar surface properties. The general trend in the two-way ANOVA, which is similar to that of the one-way ANOVA, indicated that gloss of IU and EP also were not significantly different after immersion in coffee, red wine, turmeric, or water.

A previous study suggested that gloss values of 40 thorough 50 GU are considered to be clinically acceptable for resin composites.[21] Within EC, specimen stained by turmeric showed a surprising and statistically significant decrease (6.0) in gloss when compared to water; although this could be clinically significant, the resultant average gloss of 45.9 would still be considered to be clinically acceptable. Conversely, within IU and Z, samples stained by turmeric showed a statistically significant increase in gloss (6.6 and 16.4, respectively) when compared to water;

this could be clinically significant depending on whether such an increase in desirable. There is no ready explanation for this large increase in gloss for IU and Z after staining with turmeric. For IC, no staining agent showed a statistically significant difference against water in terms of gloss. Similarly, for EP, staining agent did not seem to affect gloss as no statistically significant difference was found between stains. In general, the gloss of the specimens did not change very substantially, with the exception of the noted cases, which suggests that the staining agents had little effect on the surface structure of the materials, i.e. there was no surface erosion or deposition of staining components. This is logical since these materials tend to be fairly hydrophobic and would not be expected to chemically deteriorate significantly with such a short exposure in relatively benign solutions.

SURFACE ROUGHNESS

In general, polyethylene copolymers had lower surface roughness than polypropylene polymers (Figure 16). This is consistent with our working hypothesis. The surface roughness of PU was between PE and PP. The one-way ANOVA showed that surface roughness of IC and EC did not differ significantly. This similarity in surface roughness is reasonable since IC and EC should be of similar composition, and therefore are expected to have similar surface properties as noted earlier.

A few studies help inform the clinical relevance of the results from the present study. A previous study suggests that patients are able to distinguish between differences in surface roughness values (Ra) of at least 0.5 μm with their tongue.[22] Further, it has been suggested that the threshold surface roughness below which no further reduction for bacteria accumulation can be expected is 0.2 μm, and an increase of surface roughness above an Ra value of 2 μm resulted in a dramatic increase in bacterial colonization.[23] Lastly, when roughened (0.81μm) implant abutments were compared to smooth (0.35μm) ones, it was shown that the rough abutments harbored 20 times more bacteria subgingivally.[23]

Interestingly, EC specimens stained by turmeric showed a statistically significant increase (0.116) in surface roughness when compared to water; this could be clinically significant given that the average initial surface roughness value for EC specimens is 0.409μm, which is greater than 0.2μm; therefore, it is possible that an increase in surface roughness from the 0.2μm threshold may lead to more accumulation of bacteria. However, it should also be considered that VFRs are not located subgingivally, but rather reside over teeth, and the 0.2 μm threshold may not necessarily apply to this case. Conversely, within Z specimens, samples stained by turmeric and coffee show a statistically significant decrease (0.133 and 0.072, respectively) in surface roughness when compared to water; this could be clinically significant considering that a decrease in surface roughness brings the values closer to the 0.2 μm threshold. It Is not clear why the Z material surface roughness was reduced by staining in turmeric, but it could be that the components of the stain deposited onto the surface and "smoothed out" some of the surface variability. These components were adhered well enough to not be removed during the rinsing and drying process prior to being evaluated. In contrast, for EP, IC, and IU, staining agents did not affect surface roughness as no significant difference was found between samples immersed in the various staining agents. To put the above findings further into perspective, as none of the changes in surface roughness discovered in this study exceed the reported tongue-detection threshold of 0.5μm, patients should not be able to feel these changes associated with staining of retainers.

COLOR - ΔE^*_{00} (T_2-T_1)

The proposed clinical perceptible limit for color matching with the human eye is 3.7 ΔE units based on resin composite veneer restorations.[24] However, this is not an "absolute value", with different lighting conditions having an effect on color perception and a light source that approximates standard daylight being ideal for color analysis.[17] For the perceptible limit of reflected color changes in esthetic dentistry, a color difference value of greater than 2 ΔE^*ab units was perceived by all observers, and values between 1 and 2 ΔE^*ab units were perceived frequently.[25] There are no proposed perceptibility or acceptability thresholds for the changes in transmitted color.[26]

The frequently accepted "50:50" clinical perceptibility threshold for color matching in dentistry is 1 ΔE^*_{ab}units.[17] This is interpreted that given a color difference of 1ΔE^*_{ab} units, 50% of observers will notice the color difference, and 50% will see no difference between compared objects. The frequently accepted "50:50" acceptability threshold for color matching in dentistry is approximately 3 ΔE^*_{ab}units.[17] This is interpreted that given a color difference of approximately 3 units, 50% of observers will say the color difference is still acceptable, and 50% would consider it unacceptable. For the purposes of this study, $\Delta E^*_{00} > 3$ was considered clinically significant.

On average and regardless of material, immersion of specimen in turmeric resulted in the largest average change in color in terms of ΔE^*_{00} (19.58), followed by coffee (1.61), red wine (0.56), and water (0.22). Thus, one might say that red wine and water immersion for four days was insufficient to cause a noticeable color change on any of the materials, and coffee produce some change that would be noticeable to only some observers. Turmeric, in contrast, produced drastic observable color changes in all materials.

Within EC specimens, turmeric and coffee resulted in the most change in color (ΔE^*_{00} > 3); specifically, turmeric stained significantly more than coffee. Since specimen immersed in red wine are not significantly different from those immersed in water, the color change is not clinically significant.

Within EP specimens, turmeric stained dramatically more compared to other agents, while coffee did not stain significantly more versus red wine; only turmeric resulted in a ΔE_{00} greater than 3. Within IC, IU, Z and specimens, turmeric stained the most with a ΔE_{00} greater than 3; on the other hand, ΔE^*_{00} of coffee stain for all three materials was less than 3, so these color changes are not deemed to be clinically significant.

When comparing materials, red wine did not produce a statistically significant color change than water for all materials except for EP; in this case of EP, red wine stained more than water statistically; however, this is unlikely clinically significant given the very low ΔE_{00} value. Visually, it is indeed very difficult to discern red wine stains from unstained specimen and specimen stained by water.

Of specimens immersed in coffee, EC stained most significantly ($\Delta E^*_{00} > 3$); EP, IU, and IC seem most resistant to coffee stains given the relatively low ΔE^*_{00} compared to other materials. Our findings are consistent with that of Knapp et al. who reported that after immersion in coffee for 3 weeks, EC produced the largest color change compared to EP, IU, and IC.

For materials stained by turmeric, EC and IC stained significantly less than the rest of the materials (given the relatively low ΔE^*_{00}) while IU and EP stained the most; specifically, EC seemed most resistant to turmeric staining, given that the ΔE_{00} value is statistically lower even compared to that of IC, which should be of similar composition. For materials stained by red wine and water, while statistically significant differences in color change were found, none were clinically significant.

A previous study by Zafeiriadis et al. showed that coffee, tea, and red wine caused visible changes in color of Vivera retainers, which are PU-based.[15] Further, coffee was found to stain more than red wine. More recently, in a study by Liu et al., coffee was identified as the strongest staining agent among common drinking beverages for Invisalign aligners, which are PU-based. Liu et al. postulated that since PU-based polymers contain the ‘NHCOO’ group (carbamate), which is more polar relative to ‘-COO-‘ and ‘C-O-C’ groups in the PC (polycarbonate) and PETG (polyethylene glycol) polymers tested in their study, PU-based polymers are more prone to formation of hydrogen bonds that interact with hydrophilic pigments in solutions; this facilitates more pigment adsorption and leads to more staining. [27] Our findings are in accordance with that of Zafeiriadis et al. and Liu et al in that Zendura, the PU-based material in our study, showed a greater change in color when immersed in coffee compared to red wine. Our results are closer to Zafeiriadis et al’s in terms of raw ΔE values, which is reasonable since Vivera is also a PU-based retainer material. It is interesting that despite having semipolar carbamate groups in its structure, Zendura was significantly stained by turmeric, which is supposedly hydrophobic.

Turmeric’s propensity to stain anything it comes into contact with can be attributed to the high colorant nature of turmeric due to the presence of curcumin. Conjugated diarylheptanoids in curcumin are responsible for the orange color and higher staining ability of turmeric.[28] Coffee also resulted in considerable staining of all specimens. The less polar colorants and water-soluble polyphenols in coffee such as tannin, caffeine, and caffeic acid might have penetrated deeper into the surface of the material, possibly because such colorants are more compatible with polymer matrices and have been shown to cause staining of dental resin composites.[28] In the context of this specific study, the hydrophobicity of the color components of turmeric relative to coffee and wine is likely why it stained VFR materials significantly more. The color components of both wine (anthocyanins) and coffee (melanoidins) are essentially catechols bound to sugars, which makes them more aqueous soluble. As none of the VFR materials have a lot of polar character to them, the minimal staining is logical because the staining components are more likely to remain in the aqueous solution rather than deposit on the VFR materials. If left long enough, they may intercalate deeper into the VFR materials where it might have a better chance of staying and appearing stained. On the other hand, the color component of turmeric (curcumin) is two catechols bound together without a sugar to make it more water soluble. Therefore, turmeric is essentially insoluble at room temperature in deionized water (solubility may increase only slightly in hot or alkaline water). As a result, when the VFRs are exposed to turmeric, the curcumin strongly favors binding to the VFRs through hydrophobic effects compared to dissolving into water.

One question that is difficult to answer is why PE- and PU-based materials stain dramatically more than PP-based materials when exposed to turmeric. One possible explanation is that there is a difference in the manufacture's processing and/or additives added to these proprietary materials that modified their chemical structures. Another possible reason would be that there is a difference in the porosity of the materials that allowed turmeric to penetrate to different degrees. Along the same logic, it is plausible that PE-based materials were stained by turmeric more intrinsically, whereas PP-based materials were stained more extrinsically.

Aside from the above postulations, the fact that PP stained significantly less compared to PE and PU when exposed to turmeric may be attributed to the relative hydrophobicity of the plastic fibers themselves rather than the relative hydrophobicity of the color components of staining agents. Regardless of the relative hydrophobicity of the chromophores in wine and coffee versus turmeric, polypropylene is reportedly such a hydrophobic material that post-modification with any chromophore is nearly impossible, to the point that it requires a special process of entrapping chromophores within the hydrophobic polymer matric during the PP molding process; no such process that the authors are aware of is required for PE and PU. In addition, the tacticity of the base polypropylene in EC and IC may also contribute probability of staining. For instance, a low molecular weight, low melting point atactic PP would likely have more intercalated chromophore molecules than a high molecular weight syndiotactic PP when exposed to the same staining agent. Lastly, the higher free volume in PU and the nature of the PU's hard segment (NC=O-O) to soft segment (polyol) ratio could allow for more intercalation of chromophore molecules over time relative to a syndiotactic PP.

COLOR - ΔE^*_{00} (T_3-T_1)

In general, the lower the ΔE^*_{00} (T_3-T_1) value is for a particular specimen, the closer to the pre-staining color the specimen is after having been cleaned of the stains. Conversely, the higher the ΔE^*_{00} (T_3-T_1) value, the more it has deviated from the original color and maintained the stained color, suggesting that the cleaning agent was less effective. We did not include a comparison of T_3-T_2 because the most important aspect is whether the material retains its original color after staining and cleaning, and not how much a cleaner removes a stain but cannot reproduce the original material.

Comparing Cleaning Agents

The results from five (one for each material) 2-way ANOVAs between staining agent and cleaning agent suggested that for EP and IU, no one cleaning agent was more effective than another. For EC, IC, and Z, Invisalign cleaning crystals (I) resulted in a statistically significant reduction in ΔE^*_{00} (T_3-T_1) when compared to the other cleaning agents. Similarly, a 3-way ANOVA between material, staining agent, and cleaning agent indicated that on average, cleaner I was more effective than P and R; this is consistent with findings from the 2-way ANOVAs discussed above. Although statistically significant findings were noted, the difference in ΔE_{00} did not exceed 1, suggesting that it should not be detectable by the eye.

Why did Invisalign Cleaning Crystals clean turmeric more effectively? Although the exact answer is beyond the scope of this study, at a very basic glance at its active ingredients, sodium tripolyphosphate and sodium dichloroisocyanurate are not present in the other two cleaners (Table 3). In one of the only studies found in the literature that explored the stain removal capability of denture cleansers, results showed that products containing sodium perborate along with trisodium phosphate had a comparatively greater stain removal capability than products containing sodium perborate along with sodium bicarbonate, followed by products containing sodium hypochlorite.[29]

Trisodium phosphate was at one time widely used in formulations for a variety of consumer grade soaps and detergents as its solution is sufficiently alkaline to saponify grease and oils. On the other hand, in addition to moisture preserving for seafood, sodium tripolyphosphate is widely used as a chelating agent to remove mineral cations from water so that other components of a detergent can work properly. The above suggests that these two compounds do not serve the same purpose.

Dichloroisocyanurate is widely used as a cleansing agent and disinfectant for water. Given that deionized water was used in this experiment, chelating agents should not be as relevant. Combining all the above information, we postulate that perhaps the ratio or nature of other active ingredients in Invisalign cleaning crystals relative to the other two cleaners resulted in more effective removal of turmeric staining. However, as the exact ratios of the active ingredients of the cleaners used in this study are unknown, it is not possible to draw any definitive conclusions.

Invisalign Cleaning Crystals (I), Polident (P), and Retainer Brite (R) – Similar trend found for all cleaners

For all materials, turmeric was the only staining agent that resulted in ΔE^*_{00} (T_3-T_1) values greater than 2, regardless of cleaning agent. For specimens stained with turmeric and then cleaned with I, P, or R, IC and EC had ΔE^*_{00} (T_3-T_1) values that were significantly lower (more than 15-20) than those of Z, IU, and EP; however, since the magnitude of ΔE^*_{00} (T_3-T_1) was greater than 2, the color change was still clinically significant.

For specimens stained with coffee and then cleaned with I, P or R, none had a ΔE^*_{00} (T_3-T_1) greater than 2. For specimens stained with RW and then cleaned with I, P, or R, no difference was found compared to water, suggesting that there was no clinically significant color change. Apparently, the cleaning agents tended to be similar in terms of their overall effectiveness, and it is not possible to provide a definitive recommendation to use one over another under normal conditions. It should be noted that the staining with the agents in this study was not suggested to be a normal situation, i.e. soaking in the stain for four solid days, but such a protocol provides an accelerated test to produce at least some color change for the most aggressive agents.

Limitations

The staining protocol used in this study is not fully representative of how orthodontic thermoplastic retainers stain in the clinical environment. In clinical use, the plastics are subjected to two major elements that were not simulate in the current study: oral bacteria and their byproducts, and abrasive forces. Both of these elements likely contribute to the staining seen clinically. The staining protocol used in this study was designed to gauge a material's relative resistance to staining in a consistent manner; as such specimens are flat and do not reflect the actual shape of thermoplastic retainers. For the purpose of this study, flat standard specimens with uniform cross-sectional areas were necessary for measurement of color, gloss, and surface roughness, and they provide standard results that can be used in future studies. Though specimens were flat, they were processed similarly to orthodontic retainers, which ensured that the clinically relevant thermoforming step was not a missing variable in the study. Samples were not exposed to intraoral forces such as chewing, insertion/removal or parafunctions. The use of deionized water in all stages of the study may lead to measurements that are different than if tap water was used; however, this removes confounding factors and allows for more standardized measurements. Lastly, the exact chemistry behind the findings reported is beyond the scope of this study and were not possible due to the proprietary nature of the materials and compounds used.

Conclusions

Within the limitations of this study, the following conclusions can be drawn:

1. PE-based VFRs have the highest contrast ratio, followed by PU, then PP.
2. PE-based VFRs have the greatest gloss, followed by PU, then PP.
3. PE-based VFRs have lowest surface roughness, followed by PU, then PP.
4. For PE-, PU-, and PP-based VFRs, the staining process does not significantly change contrast ratio, gloss, and surface roughness in a clinically significant manner
5. For PE-, PU-, and PP-based VFRs, the cleaning process does not significantly change contrast ratio, gloss, and surface roughness in a statistically and clinically significant manner
6. Turmeric is a very powerful staining agent. PE- and PU-based VFRs stain dramatically more than their PP counterpart when exposed to turmeric. EC stains the least with turmeric.
7. In general, coffee stained more than red wine. Among all VFR materials, EC stains the most with coffee.
8. Statistically, Invisalign cleaning crystals are more effective in removal of only the most aggressive stain, turmeric, from VFRs. However, the difference may not be clinically significant.

Literature Review

Retention

After orthodontic treatment, full-time retention is necessary for at least 3-4 months before transitioning to part-time retention because teeth will initially be unstable in the face of occlusal and soft tissue pressures that later can be resisted.[1] Under such pressures and without retention, there is a tendency for teeth to return to their initial positions, and any change in tooth position or arch relationship subsequent to the completion of orthodontic treatment constitutes relapse.[2] It was reported that approximately 70% of orthodontic cases result in some degree of relapse, and relapse in the form of crowding has been shown to occur even up to 20 years post-retention.[3,4] The causes of relapse are due to a number of different factors, including the periodontium, occlusion, soft tissue, and growth.[5,6] The reason behind full-time retention is 3-fold: 1) it allows for gingival and periodontal tissue to reorganize as it usually takes 3-4 months before slight mobility of teeth disappears, 2) since teeth may be in an inherently unstable position immediately after treatment, soft tissue pressures constantly produce a relapse tendency if there is no retention, and 3) changes produced by growth may alter orthodontic treatment result.[1]

Retainers - Overview

Several retainer designs have evolved over time and can be broadly categorized as either fixed or removable. Fixed retainers lie lingual to anterior teeth and are usually wires bonded to teeth via cement or flowable composite. In theory, these should be the best at preventing return of crowding in the region of interest and maximizing posterior occlusal contact due to their design. Since the wires are physically and constantly in contact with the teeth to be retained, the positions of teeth should not change unless the retainer detaches; as there is no interference between occlusal surfaces of posterior teeth at all, posterior teeth are allowed to "settle", thus maximizing posterior occlusal contact. However, being fixed retainers, they also lead to the most plaque accumulation due to increased difficulty in cleaning around the retainer. Hawley and vacuum formed (VFRs) are the most commonly used retainers in the removable category. Hawley retainers (HRs) are composed of an acrylic plate and steel wire, more specifically involving clasps on molar teeth and a characteristic outer bow with adjustment loops usually spanning from canine to canine. It is suggested that Hawley retainers are better at holding the results of maxillary skeletal expansion and that certain Hawley (e.g. wraparound) designs hold closed extraction spaces better compared to VFRs.[1,30] VFRs are made of thermoplastic material and are thin, transparent, and usually inconspicuous when placed in the mouths of patients, despite the fact that they cover all of the teeth in an arch. Therefore, from an esthetic standpoint, VFRs are preferred by most patients over Hawley retainers that have a visible labial bow that lies right on the labial surfaces of the anterior teeth. It is important to note that a combination of the aforementioned retainers can be used separately for the upper and lower arch depending on the patient's initial clinical presentation and esthetic needs.

Retention Regimen/Choice/Effectiveness

There is no debate on whether retention is needed after orthodontic treatment. There is, however, no consensus among orthodontists on retainer choice/combination and what regimen for patients to follow during full time and part time wear. According to a study in 2008 by Valiathan et al., most orthodontists in the US prescribe less than 9 months of full time wear of removable retainers and thereafter advise part time, life-long wear.[7] The same authors also found that maxillary Hawley and Essix retainers and mandibular fixed lingual retainers and Hawley retainers are most commonly used by U.S. orthodontists.[7] In the UK, it appears that Hawley retainers and VFRs are the 2 most commonly prescribed removable retainers (Dental Practice Board. Annual digest of statistics—orthodontic treatment in England and Wales). A study in 2004 found that the most commonly used retainers among Australian orthodontists were maxillary Essix and mandibular canine to canine bonded retainers.[8] A survey study in Ireland in 2013 found that VFRs were the most common retainer choice for full time wear in the maxillary and mandibular arch, and full occlusal coverage with a thickness of 1.0 mm prescribed by the majority.[9] Although a more recent survey study in 2018 suggests that the type of retainer that an orthodontist chooses to prescribe to patients varies depending on the country and region within the country he/she practices, it is safe to say that VFR use increased even more over the last decade relative to previous years[10,11] Indeed, VFRs have gained much popularity since their invention due to their desired esthetics and ease of fabrication. [25]

Most literature indicates that there are no significant differences in effectiveness in retaining orthodontic treatment results between fixed retainers, HRs, and VFRs. Tylenius et al. in 2010 reported equivalent results after 1 year of retention capacity for fixed, HRs, and VFRs.[31] Similarly, Barlin et al. in 2011 found that the degree of relapse that is likely to occur following a course of fixed appliance therapy is unlikely to be affected by the choice of retainer, vacuum-formed or Hawley.[32] Mai et al. in 2014 found that additional high-quality, randomized, controlled trials concerning these retainers are necessary to determine which retainer (VFR vs Hawley) is better for orthodontic procedures.

For VFRs in particular, Thickett et al. in 2010 found no significant difference in tooth irregularity i.e. degree of relapse between full-time and part-time wear of VFRs.[33] Jaderberg et al. in 2012 suggested that Essix retainer is sufficient for maintaining orthodontic treatment results and that night time wear is adequate.[34] Furthermore, a systematic review by Littlewood et al. in 2016 did not find any evidence that wearing thermoplastic retainers full-time vs. part-time provides greater stability, though this was assessed in only a small number of participants. They concluded that overall, there is insufficient high-quality evidence to make recommendations on retention procedures for stabilizing tooth position after treatment with orthodontic braces, and that further high quality randomized control trials are needed.[35]

It appears that choice of retainer, duration, full time versus nighttime wear, and duration of wear is largely based on clinician preference that is not necessarily supported by robust literature. As such, the most appropriate retention method should be selected on an individual, case to case basis, taking into account such variables as orthodontic diagnosis, the expected level of patient compliance, patient preferences and financial considerations.[31]

Fixed Retainers

A study by Al-Moghrabi et al. in 2018 claims to be the first to suggest that fixed retention offers the potential benefit of improved preservation of alignment of the mandibular labial segment in the long term.[36] Fixed retainers also allow for the greatest increase in posterior tooth contact i.e. "settling" when compared to other forms of retention because its design does not include anything such as acrylic, metal clasps, or in the case of VFRs, the materials themselves, that interferes between the occlusal surfaces of posterior teeth.[37] Disadvantages of fixed retainers are that they are often unintentionally active and that they can debond – both of these unwanted occurrences can lead to changes in alignment of teeth and therefore relapse. [10,38] A study investigating the effectiveness of bonded multistrand lingual retainers showed them to be no more effective in retaining tooth position than a removable Hawley-type retainer.[39] A study in 2016 found that bonded retainers have a better ability to hold the mandibular incisor alignment in the first 6 months after treatment than do vacuum-formed retainers.[40] According to a study in 2017, however, occlusal relapse can be expected after active orthodontic treatment irrespective of long-term use of fixed retainers. Fixed canine-to-canine retainers appear to be effective to maintain mandibular incisor alignment, whereas in the maxilla a fixed retainer may not make any difference in the long term.[41]

VFR History/Background

The earliest accounts of fabrication of appliances with thermoplastic material may be from Nahoum and Ponitz. Nahoum introduced the concept of clear appliances used to move teeth in 1964, whereas Ponitz introduced the idea of clear appliances as retainers in 1971, where he thoroughly described the process of fabrication involving a machine that adapts heat-softened thermoplastic material to a cast in a compartment under vacuum.[42,43] In the same paper, Ponitz also described the concept of an active retainer and attaching denture teeth for edentulous spaces. Two decades later, Sheridan introduced the Essix (DENTSPLY Raintree Essix Glenroe, Sarasota, FL, USA); since then, these polyvnyl siloxane sheets have been so extensively used that the term Essix is still to this day synonymous with VFRs.[44] VFRs can also be used as bleaching trays, though the utilization of bleach may reduce surface hardness and increase roughness of the material, potentially decreasing durability.[45,46] Two most common thermoplastics used for fabricating VFRs are polyethylene copolymers and polypropylene copolymers.[47]

Hawley vs VFRs

At present, VFRs are the most widely used retainers for the maxillary arch, and patients using a clear retainer report greater satisfaction with their treatment than those with other types of retainers.[48] It was shown by Pratt et al. in 2011 that patients delivered with VFRs have greater compliance for the first 2 years after debonding.[49] According to a study by Vagdouti et al in 2019, VFRs are better accepted by adolescents.[50] In general, patients prefer VFRs over Hawley retainers because they are more comfortable.[48,51-53] According to a study by Wan et al. in 2016, this is due to the negative impact of HRs on speech.[54] It was also reported in a recent study comparing wraparound Hawley retainers and VFRs that thermoplastic retainers were more preferable when swallowing liquids than the wraparound Hawley appliance. In addition, it was found that level of overall satisfaction and preference was similar between the two types of retainers.[55]

In terms of effectiveness, Rowland et al. found in 2007 \ that VFRs are more effective than Hawley retainers at holding the correction of the maxillary and mandibular labial segments, though this is likely clinically significant only in the mandibular arch if located to a single tooth replacement.[56] A retrospective, randomized, double-blind comparison study by Barlin et al. in 2011 reported no statistical or clinical significant difference in the effectiveness of Hawley retainers and VFRs in maintaining specific arch-form features after orthodontic treatment.[32] Demir et al. in 2012 found that Essix appliances were more efficient in retaining the anterior teeth in the mandible during a 1-year retention period compared to Hawley retainers.[57] Kaya et al. recently found that the clinical effectiveness of Essix and Hawley retainers was similar during the retention period when assessed by overjet, overbite, maxillary and mandibular intercanine widths, intermolar widths, and arch lengths from lateral cephalometric measurements.[58] In terms of failure rate, one year post-treatment, the failure rate for Hawley and thermoplastic retainers was equal.[59] There seems to be evidence that VFRs are at least equally as effective in preventing relapse compared to Hawley retainers.

The advantages of VFRs over Hawley retainers include esthetic superiority (transparent and thin), ease of fabrication, decreased interference with speech, cost-effectiveness (approx. 1/3 less expensive than Hawley).[33,51,60] Therefore, the potential cost-saving for healthcare and business is significant.

The limitations of VFRs are as follows. The thickness of the tray material itself, albeit thin, makes it so that posterior teeth cannot fully come in contact, and therefore cannot fully "settle" during post-treatment wear, especially when used in both the upper arch and lower arch at the same time.[61] Therefore, Hawley retainers might be the retainer of choice when a lateral open bite is present before debond. A study in 1997 showed that Hawley retainers allow more vertical movement (settling) of the posterior teeth than a VFR.[62] However, a systematic review by Mai et al. in 2014 that compared VFRs and Hawley retainers concluded that posterior occlusion was better at 6 months with a Hawley retainer than a vacuum-formed retainer for the maxillary arch only, but there was no difference at longer-term recall.[63]

In addition, there have been concerns about durability of the material, particularly with regard to occlusal wear of the appliance with cracking in the longer term.[64] VFRs have poor wear resistance along incisal and occlusal surfaces.[65] Two previous in-vitro wear resistance studies using steatite abraders concluded that the wear resistance of polyethylene copolymers is superior to that of polypropylene-based thermoplastics.[47,64]

With regard to periodontal health, a study in 2017 found that use of VFRs resulted in slight periodontal attachment loss that seemed to be clinically insignificant.[66] VFRs may cause greater accumulation of plaque on both teeth and retainers compared to Hawley retainers, presumably because of inhibition of the cleaning effect of saliva caused by VFR or reduced opportunity for good hygiene on and around the retainer.[67] A recent metanalysis suggested that patients using the Hawley retainer had better periodontal health compared with those using VFRs, though more research is still required.[68]

Lastly, clear materials are prone to staining. Since one of the main reasons that patient's prefer VFRs over other forms of retainers is their clarity, color stability and translucency are extremely important properties. If the color of the transparent material is not stable, then it would be hard for it to remain clear over the 3-4 month period full time wear.

VFR Material

Two of the most common thermoplastics used for fabricating VFRs are polyethylene copolymers and polypropylene polymers.[12] Common polymers used to fabricate thermoplastic appliances are polyester, polypropylene, and polyurethane.[13] Polyethylene polymers are considered more esthetic because the material is virtually transparent. Polypropylene/ethylene copolymers are considered more durable (wear-resistant) and flexible, but esthetically they are inferior to polyethylene because the polyethylene material is translucent. Thermoplastic polyurethane is one of the most versatile engineering thermoplastics with wide applications in various industries.[14]

Essix ACE, made from copolyester, is considered esthetically pleasing because the material is virtually transparent while being wear-resistant when exposed to chemicals.

Copolyester is generated by modifications of a polyester, such as polyethylene terephthalate, with isophthalic acid or other diols. Compared to polypropylene polymers, copolyester has been reported to be more transparent and to show less wear.[47,64] Because retainers are essential in preventing orthodontic relapse, it is crucial to have an effective cleaning technique to facilitate long-term use of the retainers. There are, however, some problems associated with long-term use of clear retainers, including loss of translucency and material integrity, discoloration, and plaque and calculus retention.[15,64]

VFR Staining

There is a myriad of different brands and compositions for VFR material. Most companies do not fully disclose the exact composition of their thermoplastic materials as some are patent protected. The only study on staining of thermoplastic retainers by Zafeiriadis et al. in 2014 indicated that coffee, tea, and red wine caused visible changes in color of Vivera (polyurethane) retainers.[15] Overall, the literature is very scarce pertaining to VFR material and staining agents, suggesting that studies of similar materials used for other purposes may be useful in predicting outcomes for retainers.

Clear aligners are also made from thermoplastic materials, similar to VFRs. These materials have a different purpose and are usually replaced every 7-14 days (instead of 3-4 month of full-time wear and then transitioning to nighttime). Perhaps for this reason, a thinner and less durable material is typically used in clear aligner therapy, as compared to the heavier material designed specifically for orthodontic retention used to fabricate retainers; this also explains why using the final aligner in an Invisalign sequence as a retainer is not as effective as other retainer types.[1] For this reason, thermoplastic retainer material and aligner material may not behave exactly the same to physical and chemical stresses. Liu et al. in 2016 found that Invisalign aligners (polyurethane + modification) are more prone to pigmentation than other brands of aligners (PETG) after exposure to wine and coffee[27]. Daniele et al. in 2020 found that polyurethane exhibited the highest mechanical and thermal resistance, possibly due to their high level of crystallinity, whereas PETG presented better transparency and less ability to absorb water[69]. Red wine and coffee produced noticeable color variations after 14 days of immersion, as well as a slight reduction of transparency for both materials. Porojan et al. found a slight change in color for polyethylene terephthalate glycol (PET-G) material at 24 hours exposure to coffee, tea, wine, and distilled water, and a marked color change after 48 hours and 7 days.[16] Lastly, Bernard et al. in 2020 found that Invisalign aligners (multilayer polyurethane + integrated elastomer) are more prone to pigmentation than Clearcorrect (Zendura – polyurethane resin) or Minor Tooth Movement (copolyester + trade secret) after exposure to coffee or red wine, and that black tea caused marked extrinsic stains on the surface of the three tested brands.[70]

In the prosthodontic literature, there have been staining and cleaning experiments on materials such as denture acrylic and restorative resin materials. Staining agents utilized include coffee, tea, wine, coke, grape juice, orange juice, yerba mate, and turmeric (Table 8). Several studies found that coffee caused more staining than tea.[71-76]

It would appear then that despite the limited literature about the color changes or staining of orthodontic retainers, that being made from similar materials and needing to be in place for long periods of time, that esthetic issues are likely and should be more completely investigated.

VFR Cleaning

In general, brushing without toothpaste (to avoid the effects of abrasive particles) and rinsing with water is recommended for cleaning VFRs, though there is no definitive data behind this suggestion. Cleansing agents are also widely used for removing stain and plaque. In general, an effervescent tablet is dissolved in water and retainers are placed in the solution for a short duration according to manufacturer's instructions, similar to the routine cleaning of partial or complete dentures.

Though there has been some published data on clear aligners and cleaning methods, data on clear retainers in this regard remains very scarce. In a study by Agarwal et al in 2018, Vivera retainers (polyurethane) were exposed to seven different cleaning agents, and results suggest that light transmittance was the only tested property that significantly changed from baseline to 6-months. In a similar study by the same group on Essix C+ retainers (polypropylene/ethylene copolymer), Retainer Brite showed the most change in surface roughness and hydrogen peroxide resulted in the greatest effect on flexural modulus, likely due to its strong oxidizing abilities.[77] In another study of similar design on Essix ACE retainers (copolyester), conducted by the same group, results indicated that light transmittance through the specimens decreased significantly from baseline for all cleaning methods at 6 months (one exposure every 2 weeks), and that flexural modulus of the specimens decreased significantly for all cleaning methods except Invisalign crystals and Retainer Brite. In this study, the Listerine group demonstrated the worst light transmittance change while hydrogen peroxide demonstrated the greatest change in flexural modulus of the specimens compared with other cleaning methods. It seems that different thermoplastic materials behave differently once exposed to different cleaning solutions.

Bernard et al in 2020 found that both Invisalign cleaning crystals and Retainer Brite performed similarly in terms of removal of stains from coffee, tea, and red wine.[78] Porojan et al in 2020 reported that when comparing all tested materials (thermoplastic PETG), cleaning methods (powder, tablet, brushing) did not influence the mean roughness value significantly.[16]

Agrawal et al. only exposed retainer materials to cleaning agents, i.e.no staining agents were involved. Porojan et al. and Bernard et al. were the only two groups that conducted studies involving both staining and cleaning thermoplastic material and measuring changes in properties.

CONTRAST RATIO

Contrast ratio is a proxy for translucency. In this study, it is calculated by dividing the L* value measured against a white tile by the L* value measured against a black tile. The higher the contrast ratio, the less translucent a sample is. Conversely, the lower the contrast ratio, the more translucent a sample is, meaning that more light is allowed to pass through. In the context of VFRs, a material with lower contrast ratio should be less noticeable when placed against teeth compared to one with higher contrast ratio.

GLOSS[79]

A gloss meter is an instrument which measures specular reflection of a surface. Gloss is determined by projecting a beam of light at a fixed intensity and angle onto a surface and measuring the amount of reflected light at an equal but opposite angle. The measurement scale, Gloss Units (GU), of a glossmeter is a scaling based on a highly polished reference black glass standard with a defined refractive index having a specular reflectance of 100GU at the specified angle. This standard is used to establish an upper point calibration of 100 with the lower end point established at 0 on a perfectly matt surface. This scaling is suitable for most non-metallic coatings and materials (paints and plastics) as they generally fall within this range. For other materials, highly reflective in appearance (mirrors, plated /raw metal components), higher values can be achieved reaching 2000 Gloss Units. For transparent materials, these values can also be increased due to multiple reflections within the material.

SURFACE ROUGHNESS

Surface roughness (Ra) is typically measured using a surface roughness meter. The device works with an inductive transducer (referred to as "pickup" in the manual) having a diamond tip, similar to the pick-up of a record player. As the diamond tip traverses a target's surface, voltages are generated when the sensor is deflected and are converted into the different roughness parameters by the instrument electronic system as well as being used for profile presentation.[80,81] Clinically, a 0.2 Ra value is said to be the threshold at which no more bacteria will adhere to materials that are placed subgingivally; this threshold may have implications for the accumulation of plaque and other microorganisms.[23,82,83] It is reported that patients are able to distinguish between differences in roughness values of at least 0.5 microns.[22]

COLOR- Quantifying and Interpreting Color Change

The investigation of color changes can involve either one or a combination of three tools: spectrophotometers, colorimeters, and digital photographic analysis.[17] The analysis of color in dentistry is generally defined by the Commision international de l'E'clairage (CIE) L*, a*, b* (LAB) color space. In this three-dimensional color space, with three axes being L*, a*, and b*, the L* refers to the lightness. The L* values ranges from 0 for perfect black to 100 for perfect white. The a* value refers to the chromaticity coordinates in the red-green axis. Positive a* values reflect the red color range and the negative values indicate the green color range. The b* value corresponds to the chromacity coordinate in the yellow-blue axis. Positive b* values indicate a yellow color range while negative values indicate the blue color range. Total color change is measured in terms of ΔE and is calculated by the equation: $\Delta \mathrm{E}_{ab}^{*} = \sqrt{[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})]}$; this equation has evolved significantly since its introduction in 1976 and is now considerably more sophisticated and computationally involved, adjusting for factors such as saturation of a color and perceived lightness for increased accuracy.[84]

The proposed clinical perceptible limit for color matching with the human eye is 3.7 ΔE units based on resin composite veneer restorations.[24] However, this is not an "absolute value", with different lighting conditions having an effect on color perception and a light source that approximates standard daylight being ideal for color analysis.[17] For the perceptible limit of reflected color changes in esthetic dentistry, a color difference value of greater than 2 ΔE*ab units was perceived by all observers, and values between 1 and 2 ΔE*ab units were perceived frequently.[25] There are no proposed perceptibility or acceptability thresholds for the changes in transmitted color.[26]

The frequently accepted "50:50" clinical perceptibility threshold for color matching in dentistry is 1 $\Delta \mathrm{E}_{ab}^{*}$units.[17] This is interpreted that given a color difference of 1$\Delta \mathrm{E}_{ab}^{*}$ units, 50% of observers will notice the color difference, and 50% will see no difference between compared objects. The frequently accepted "50:50" acceptability threshold for color matching in dentistry is approximately 3 $\Delta \mathrm{E}_{ab}^{*}$units.[17] This is interpreted that given a color difference of approximately 3 units, 50% of observers will say the color difference is still acceptable, and 50% would consider it unacceptable.

List of Tables

List of Figures

Table 1: VFR materials chosen for this study

Product Name	Manufacturer	Composition	Biostar Code
Essix C^+ (EC)	Dentsply Raintree Essix, Sarasota, FL	Polypropylene copolymer	163
Essix Plus (EP)	Dentsply Raintree Essix, Sarasota, FL	Polyethylene copolymer	143
Invisacryl C (IC)	Great Lakes Orthodontics, Ltd., Tonawanda, NY	Polypropylene copolymer	202
Invisacryl Ultra (IU)	Great Lakes Orthodontics, Ltd., Tonawanda, NY	Polyethylene copolymer	153
Zendura (Z)	Bay Materials LLC, Fremont, CA	Polyurethane	172

Table 2: Preparation of staining agents

Staining Agent	Product Name	Manufacturer	Preparation
Coffee (C)	Folgers Black Silk	The Golger Coffee Company, Orrville, OH	Brew in a drip coffeemaker (Mr. Coffee TF Series 5-cup Switch Coffeemaker, Jarden Consumer Solutions, Boca Raton, FL) with a ratio of 3 level tablespoons of coffee grounds per 3 cups of water.
Red Wine (RW)	Charles Shaw Shiraz	Bronco Wine Company, Antlanta, GA	Ready-made.
Turmeric (T)	McCormick Gourmet Organic Ground Turmeric	McCormick & Company, Inc., Hunt Valley, MD	1-level teaspoon of turmeric powder into 250ml of boiled water (deionized), mix well with stirring rod.
Water (deionized) (W)	n/a	n/a	From lab faucet

Table 3: Preparation of cleaning agents

Product Name	Manufacturer	Ingredients	Instruction (modified)
Invisalign Cleaning Crystals (I)	Align Technology, San Jose, CA	Sodium sulfate, sodium carbonate, sodium tripolyphosphate, sodium dichloroisocyanurate, sodium lauryl sulfate	1 pack to 100 ml, place appliance, let soak for 15 minutes, rinse with water
Retainer Brite ®	Dentsply Raintree Essix, Sarasota, FL	Sodium Bicarbonate, Citric Acid, Sodium Carbonate, Potassium Persulfate Compound, Corn Syrup Solids, Sodium Percarbonate, Sodium Sulfate (may contain Silica), Sorbitol, Tetraacetylethylenediamine (TAED), PEG-180, Sodium Lauryl Sulfoacetate, Flavor, PEG-8, Magnesium Stearate, FD&C Blue #1 Aluminum Lake (CI 42090), FD&C Blue #2 (CI 73015)	Drop one tablet into a cup of warm water (1 tablet to 100 ml), drop dental appliance into cup, let appliance soak for 15 minutes, rinse appliance thoroughly
Polident (P)	GSK (GlaxoSmithKline), London, UK	Potassium monopersulfate, sodium percarbonate, tetra acetyl ethylene diamine (TAED), Sodium lauryl sulfate	One tablet into cup of warm water (1 tablet to 100 ml), leave for 3 minutes), rinse thoroughly

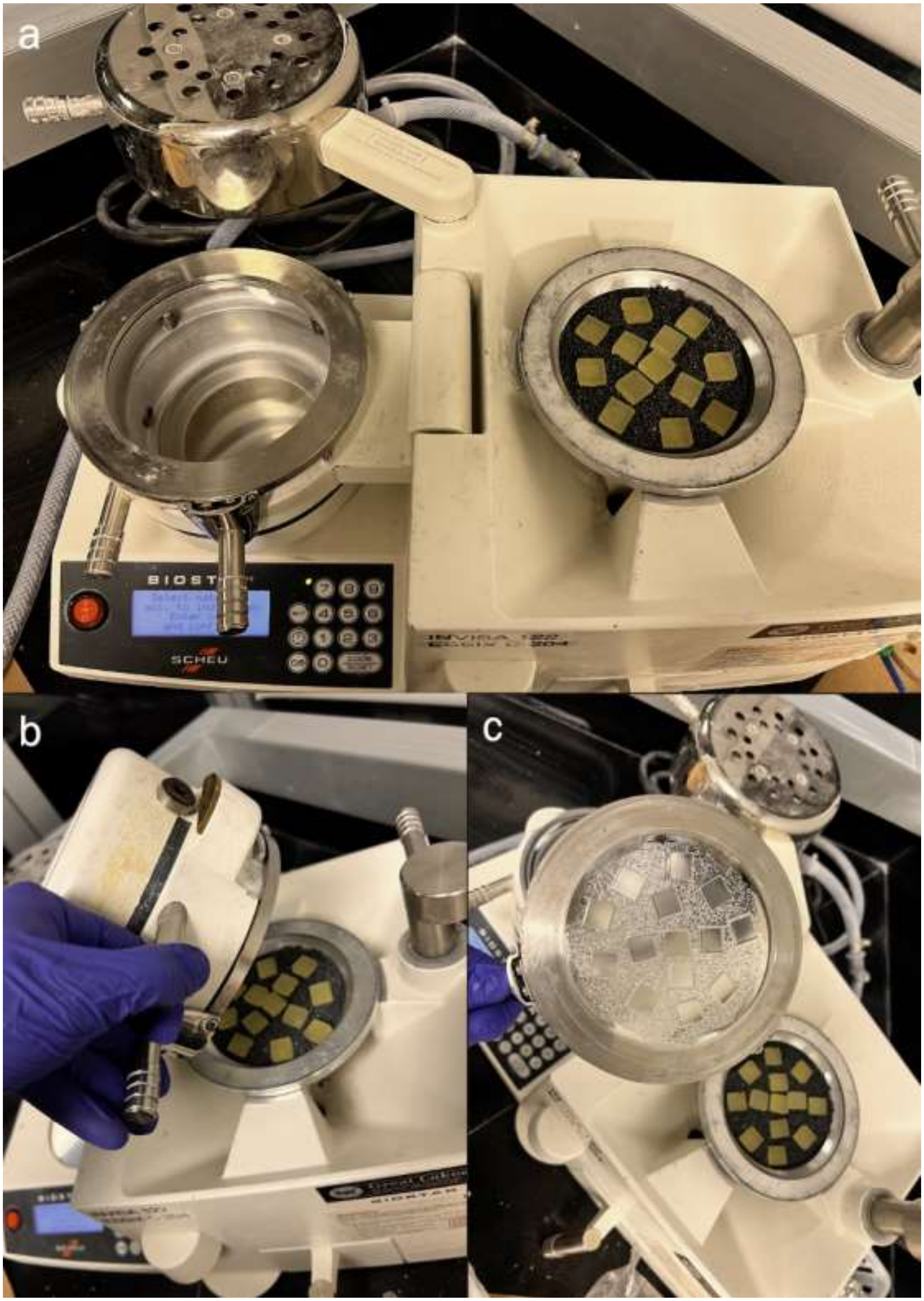

Figure 1: Thermoforming of VFR materials onto resin blocks

(a) Biostar® Positive Pressure Machine, (b) VFR material thermoformed over resin blocks according to manufacturer's instructions, (c) VFR material after thermoforming process.

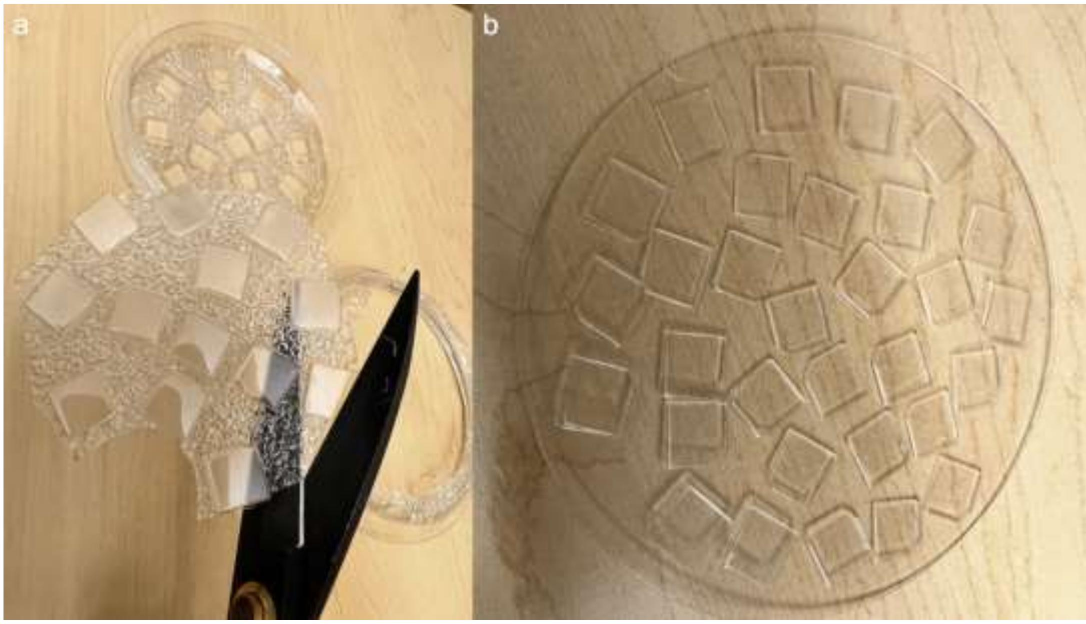

Figure 2: Shaping of VFR specimens

(a) After the thermoforming process, scissors were used to cut out 12mm x 12mm x1mm specimens from VFR material, (b) Specimens placed on pre-thermoformed VFR disc for illustration.

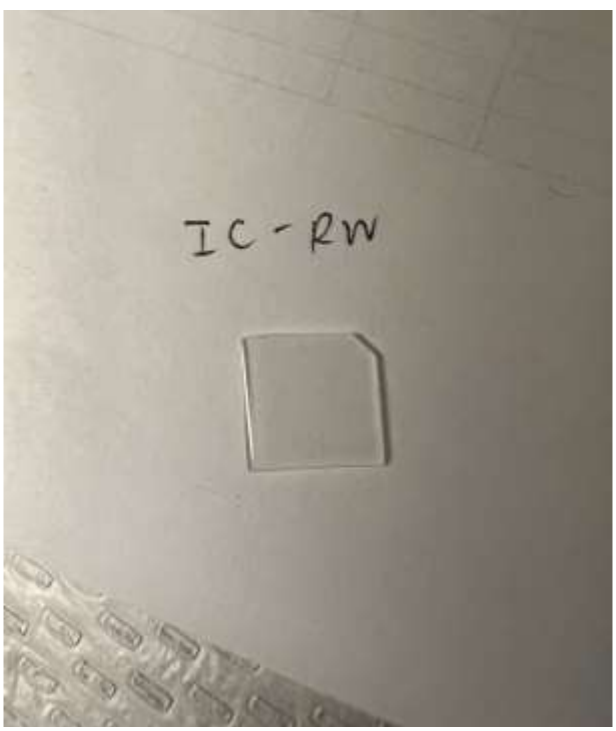

Figure 3: A single specimen ready for initial measurements

A corner is removed from each specimen for consistent orientation.

Figure 4: Experimental design per each of five VFR materials

Experimental design. 120 Specimens per VFR material are exposed to one of four staining agents or control. Stained specimens are then exposed to one of three cleaning agents. Measurements for color, gloss, and surface roughness are collected at three time points: before exposure to staining agents (T1), after exposure to staining agents (T2), and after exposure to cleansing agents (T3).

Figure 5: Tackle box

A tackle box is used to organize specimens so that data can be collected in a systematic fashion.

Figure 6: Staining of specimen

(a) Specimen were placed in individual test tubes and covered with 5ml of staininig agent, (b) Specimens are to be placed in the incubator set at 37 ℃ for 96 hours, (c) Insertion of specimens into the incubator.

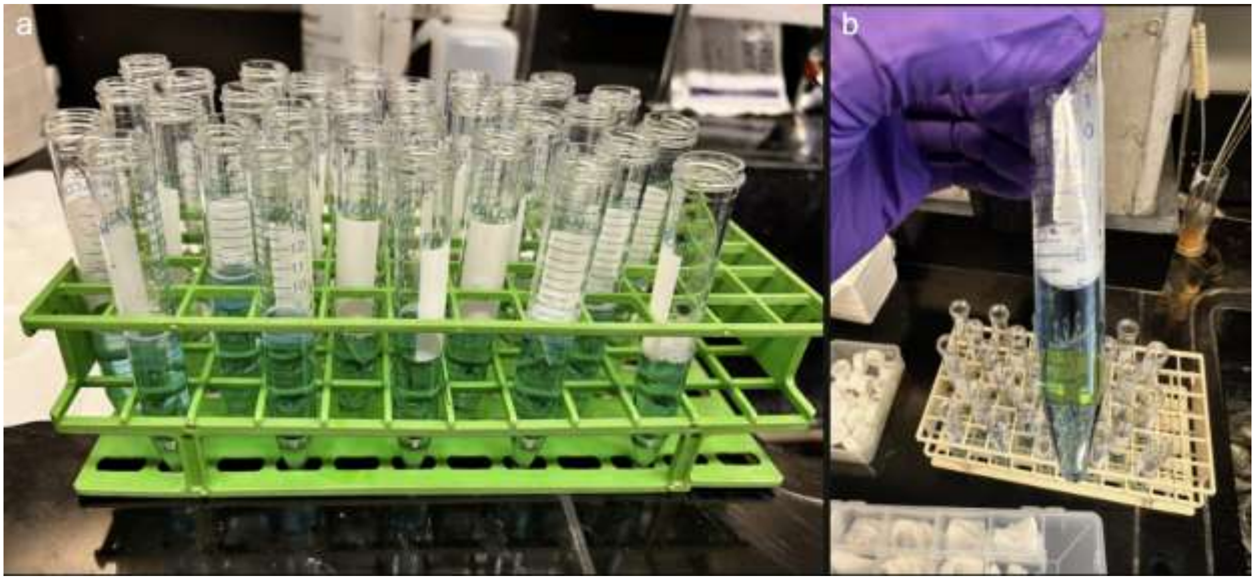

Figure 7: Cleaning of specimen

(a) Cleaning of specimen, (b) A closer look at a specimen stained by turmeric and cleaned by Retainer Brite

Figure 8: Measuring of color

(a) A specimen inserted on the silicon placement guide for consistent measuring, (b) The black tile placed against a specimen to be measured and a white tile in the background.

$$\Delta E^*_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C}\frac{\Delta H'}{k_H S_H}}$$

$$\Delta L' = L^*_2 - L^*_1$$
$$\bar{L} = \frac{L^*_1 + L^*_2}{2} \quad \bar{C} = \frac{C^*_1 + C^*_2}{2}$$
$$a'_1 = a^*_1 + \frac{a^*_1}{2}\left(1 - \sqrt{\frac{\bar{C}^7}{\bar{C}^7 + 25^7}}\right) \quad a'_2 = a^*_2 + \frac{a^*_2}{2}\left(1 - \sqrt{\frac{\bar{C}^7}{\bar{C}^7 + 25^7}}\right)$$
$$\bar{C}' = \frac{C'_1 + C'_2}{2} \text{ and } \Delta C' = C'_2 - C'_1 \quad \text{where } C'_1 = \sqrt{a'^2_1 + b^{*2}_1} \quad C'_2 = \sqrt{a'^2_2 + b^{*2}_2}$$
$$h'_1 = \text{atan2}(b^*_1, a'_1) \mod 360^\circ, \quad h'_2 = \text{atan2}(b^*_2, a'_2) \mod 360^\circ$$

$$\Delta h' = \begin{cases} h'_2 - h'_1 & |h'_1 - h'_2| \le 180^\circ \\ h'_2 - h'_1 + 360^\circ & |h'_1 - h'_2| > 180^\circ, h'_2 \le h'_1 \\ h'_2 - h'_1 - 360^\circ & |h'_1 - h'_2| > 180^\circ, h'_2 > h'_1 \end{cases}$$

$$\Delta H' = 2\sqrt{C'_1 C'_2}\sin(\Delta h'/2), \quad \bar{H}' = \begin{cases} (h'_1 + h'_2 + 360^\circ)/2 & |h'_1 - h'_2| > 180^\circ \\ (h'_1 + h'_2)/2 & |h'_1 - h'_2| \le 180^\circ \end{cases}$$

$$T = 1 - 0.17\cos(\bar{H}' - 30^\circ) + 0.24\cos(2\bar{H}') + 0.32\cos(3\bar{H}' + 6^\circ) - 0.20\cos(4\bar{H}' - 63^\circ)$$
$$S_L = 1 + \frac{0.015\left(\bar{L} - 50\right)^2}{\sqrt{20 + \left(\bar{L} - 50\right)^2}} \quad S_C = 1 + 0.045\bar{C}' \quad S_H = 1 + 0.015\bar{C}'T$$
$$R_T = -2\sqrt{\frac{\bar{C}'^7}{\bar{C}'^7 + 25^7}}\sin\left[60^\circ \cdot \exp\left(-\left[\frac{\bar{H}' - 275^\circ}{25^\circ}\right]^2\right)\right]$$

dE00 Formula

Figure 9: ΔE^*_{00} Formula

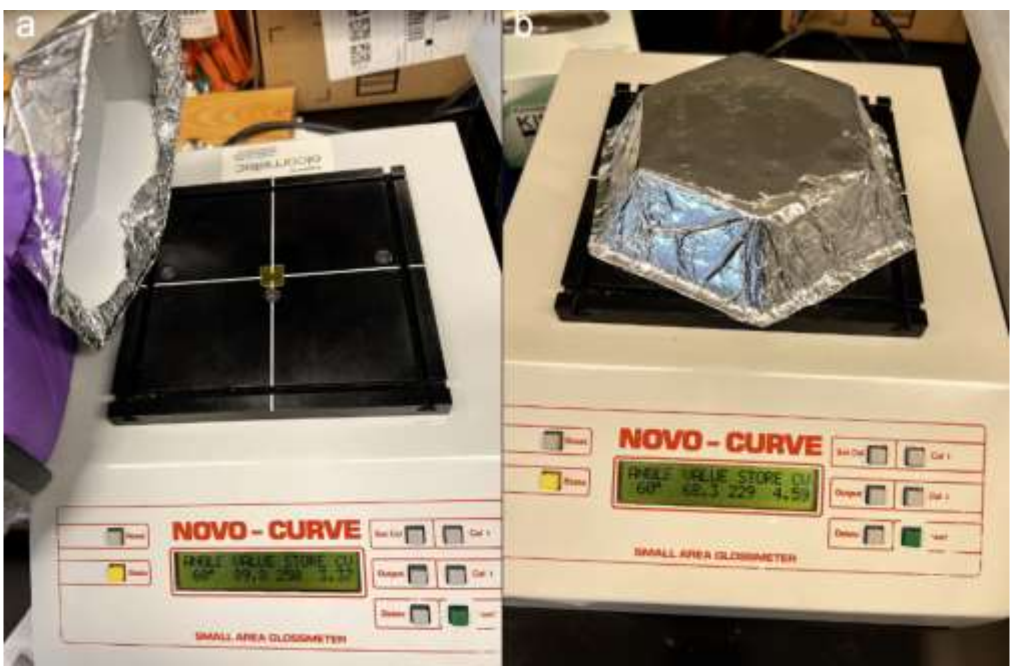

Figure 10: Gloss measurement

(a) A specimen placed on the gloss meter, glossy side facing the aperture,(b) An aluminum-wrapped paper lid placed over specimens to block ambient light during measurements.

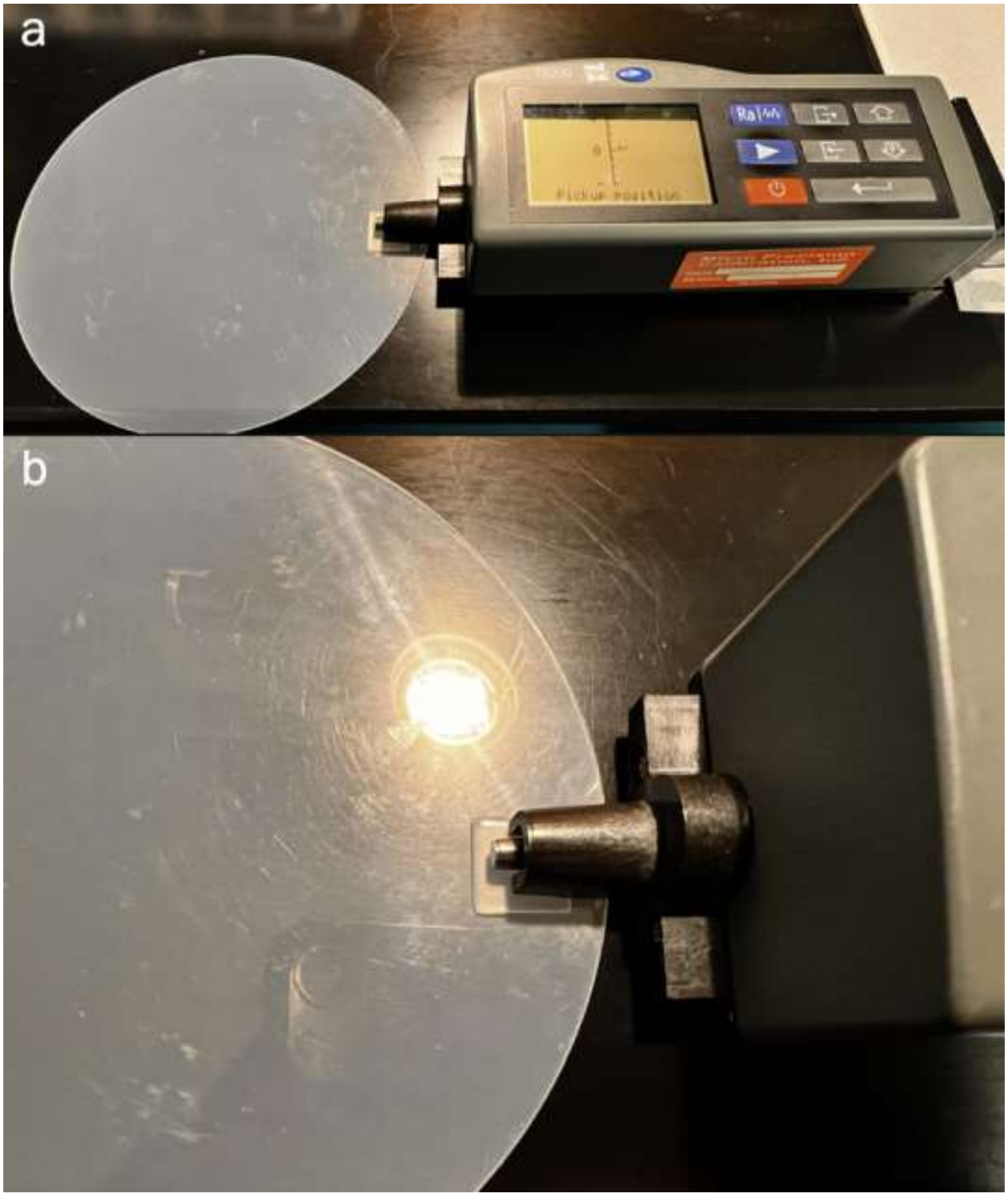

Figure 11: Surface roughness measurement

(a) Surface roughness meter placed on a specimen, (b) A closer look at the stylus of the surface roughness meter placed on a specimen.

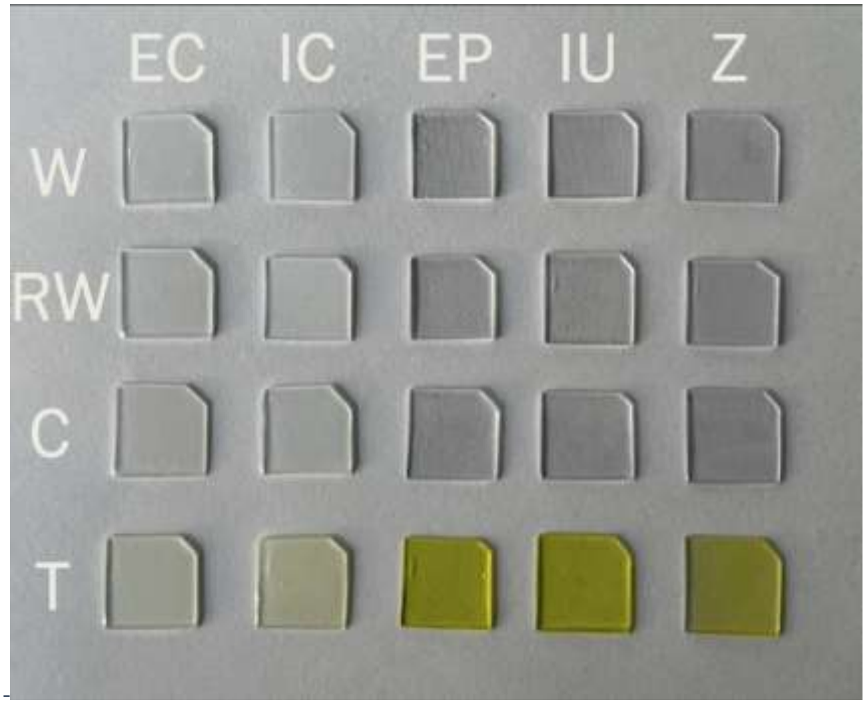

Figure 12: Specimens of 5 materials stained by 4 staining agents and cleaned by Invisalign Cleaning Crystals

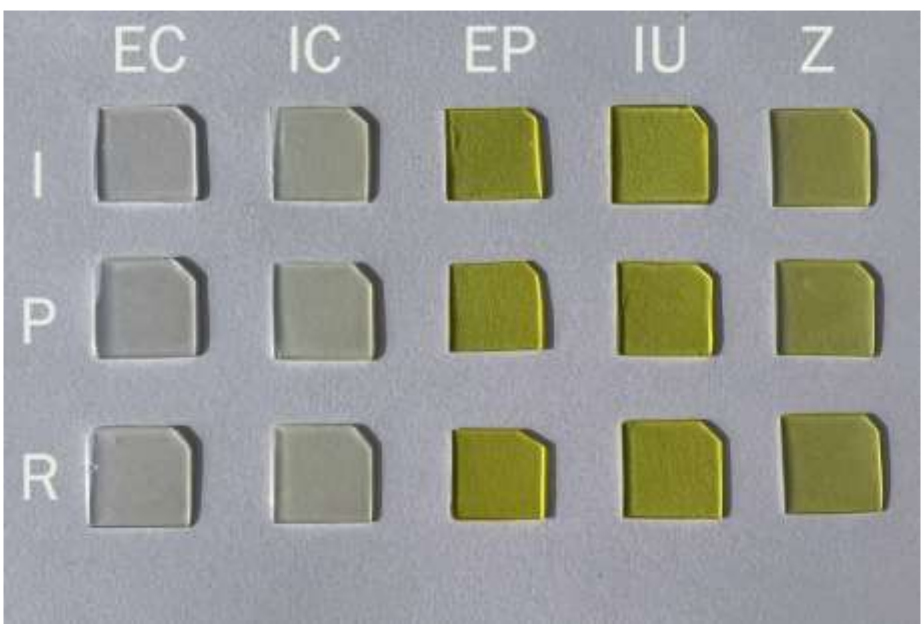

Figure 13: Specimens of 5 materials stained by turmeric then exposed to 3 cleaning agents

Table 4: Descriptive statistics for contrast ratio, gloss, and surface roughness for all materials at T1

Each group contains 120 samples.

T1-Initial	CR AVG	CR SD	Gloss AVG	Gloss SD	SR AVG	SR SD
EC	0.39	0.02	54.3	5.93	0.409	0.08
EP	0.23	0.03	83.0	8.88	0.141	0.06
IC	0.41	0.03	59.4	6.15	0.438	0.08
IU	0.24	0.02	84.9	7.21	0.222	0.07
Z	0.35	0.04	75.2	12.37	0.335	0.18

Table 5: Descriptive statistic for contrast ration, gloss, and surface roughness for all materials at T2

Each group contains 30 samples.

T2-Stained	CR AVG	CR SD	Gloss AVG	Gloss SD	SR AVG	SR SD
EC-W	0.40	0.02	51.9	5.5	0.349	0.038
EC-RW	0.40	0.02	53.1	6.2	0.386	0.076
EC-C	0.40	0.01	54.1	6.0	0.441	0.101
EC-T	0.42	0.02	45.9	3.3	0.465	0.064
EP-W	0.23	0.03	82.2	6.4	0.170	0.053
EP-RW	0.23	0.02	79.4	8.2	0.169	0.055
EP-C	0.24	0.03	83.9	6.8	0.192	0.063
EP-T	0.25	0.03	81.7	9.7	0.159	0.047
IC-W	0.43	0.02	59.6	5.6	0.442	0.085
IC-RW	0.43	0.02	62.0	5.8	0.483	0.082
IC-C	0.42	0.01	55.6	5.5	0.431	0.072
IC-T	0.43	0.04	58.2	6.8	0.457	0.092
IU-W	0.25	0.02	80.5	8.8	0.217	0.088
IU-RW	0.24	0.02	84.7	6.8	0.199	0.058
IU-C	0.25	0.02	80.9	9.3	0.231	0.060
IU-T	0.25	0.02	87.1	11.3	0.241	0.086
Z-W	0.35	0.04	72.0	12.1	0.383	0.194
Z-RW	0.34	0.03	73.5	14.0	0.344	0.227
Z-C	0.34	0.03	70.6	10.6	0.311	0.189
Z-T	0.35	0.03	88.4	12.4	0.250	0.138

Table 6: ΔE^*_{00} T_2-T_1, descriptive statistics grouped by staining agent and material

By Staining Agent		
	AVG	SD
EC-W	0.25	0.08
EP-W	0.18	0.10
IC-W	0.29	0.16
IU-W	0.20	0.16
Z-W	0.19	0.17
EC-RW	0.62	0.17
EP-RW	0.62	0.15
IC-RW	0.73	0.16
IU-RW	0.39	0.18
Z-RW	0.44	0.15
EC-C	2.25	0.40
EP-C	1.59	0.47
IC-C	1.19	0.07
IU-C	1.23	0.27
Z-C	1.77	0.42
EC-T	8.25	1.26
EP-T	27.63	0.48
IC-T	9.39	1.42
IU-T	27.68	0.61
Z-T	24.95	1.41

By Material		
	AVG	SD
EC-W	0.25	0.08
EC-RW	0.62	0.17
EC-C	2.25	0.40
EC-T	8.25	1.26
EP-W	0.18	0.10
EP-RW	0.62	0.15
EP-C	1.59	0.47
EP-T	27.63	0.48
IC-W	0.29	0.16
IC-RW	0.73	0.16
IC-C	1.19	0.07
IC-T	9.39	1.42
IU-W	0.20	0.16
IU-RW	0.39	0.18
IU-C	1.23	0.27
IU-T	27.68	0.61
Z-W	0.19	0.17
Z-RW	0.44	0.15
Z-C	1.77	0.42
Z-T	24.95	1.41

Table 7: ΔE^*_{00} T_3-T_1 descriptive statistics comparing cleaner

	AVG	SD
EC-W-I	0.21	0.08
EC-W-P	0.20	0.07
EC-W-R	0.23	0.08
EC-RW-I	0.28	0.11
EC-RW-P	0.37	0.16
EC-RW-R	0.35	0.10
EC-C-I	1.00	0.06
EC-C-P	1.11	0.12
EC-C-R	1.11	0.09
EC-T-I	4.09	0.33
EC-T-P	5.58	0.67
EC-T-R	5.94	0.96
EP-W-I	0.14	0.04
EP-W-P	0.15	0.06
EP-W-R	0.14	0.06
EP-RW-I	0.37	0.23
EP-RW-P	0.66	0.31
EP-RW-R	0.84	0.61
EP-C-I	0.36	0.12
EP-C-P	0.36	0.09
EP-C-R	0.47	0.30
EP-T-I	27.36	0.48
EP-T-P	27.46	0.49
EP-T-R	27.19	0.69

	AVG	SD
IC-W-I	0.28	0.15
IC-W-P	0.15	0.06
IC-W-R	0.17	0.12
IC-RW-I	0.27	0.10
IC-RW-P	0.58	0.22
IC-RW-R	0.40	0.06
IC-C-I	0.93	0.03
IC-C-P	1.02	0.15
IC-C-R	0.94	0.04
IC-T-I	6.02	0.81
IC-T-P	6.98	0.62
IC-T-R	6.52	0.64
IU-W-I	0.21	0.12
IU-W-P	0.26	0.26
IU-W-R	0.22	0.10
IU-RW-I	0.29	0.20
IU-RW-P	0.31	0.10
IU-RW-R	0.36	0.13
IU-C-I	0.30	0.07
IU-C-P	0.38	0.10
IU-C-R	0.33	0.12
IU-T-I	27.47	0.67
IU-T-P	27.55	0.65
IU-T-R	27.81	0.45

	AVG	SD
Z-W-I	0.33	0.21
Z-W-P	0.36	0.23
Z-W-R	0.25	0.17
Z-RW-I	0.33	0.31
Z-RW-P	0.23	0.16
Z-RW-R	0.25	0.15
Z-C-I	0.29	0.09
Z-C-P	0.37	0.23
Z-C-R	0.74	0.62
Z-T-I	23.38	1.64
Z-T-P	24.96	1.60
Z-T-R	24.90	0.92

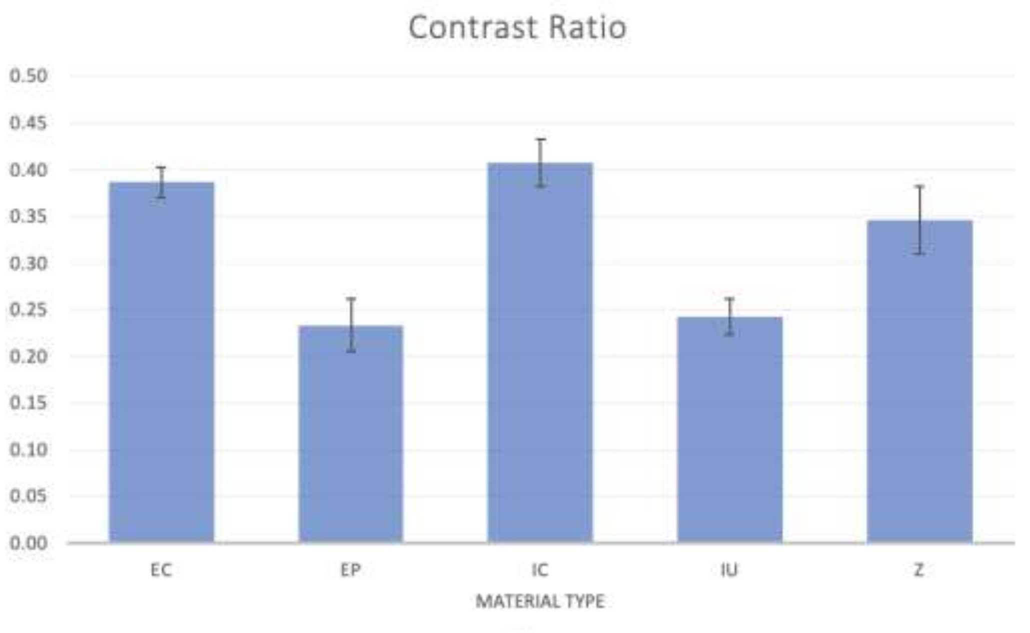

Figure 14: Comparison of average initial contrast ratio values of all materials

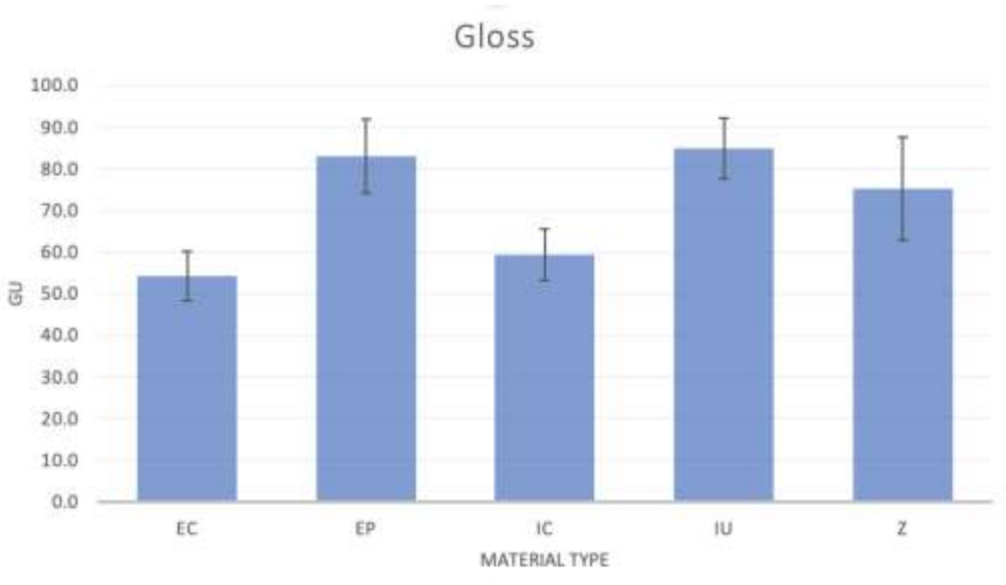

Figure 15: Comparison of average initial gloss values of all materials

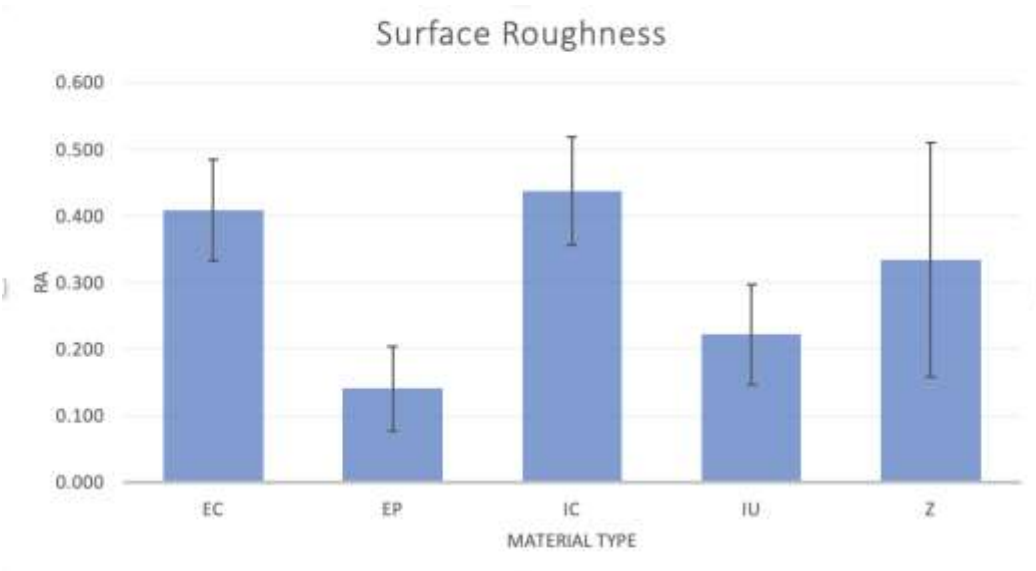

Figure 16: Comparison of average initial surface roughness values of all materials

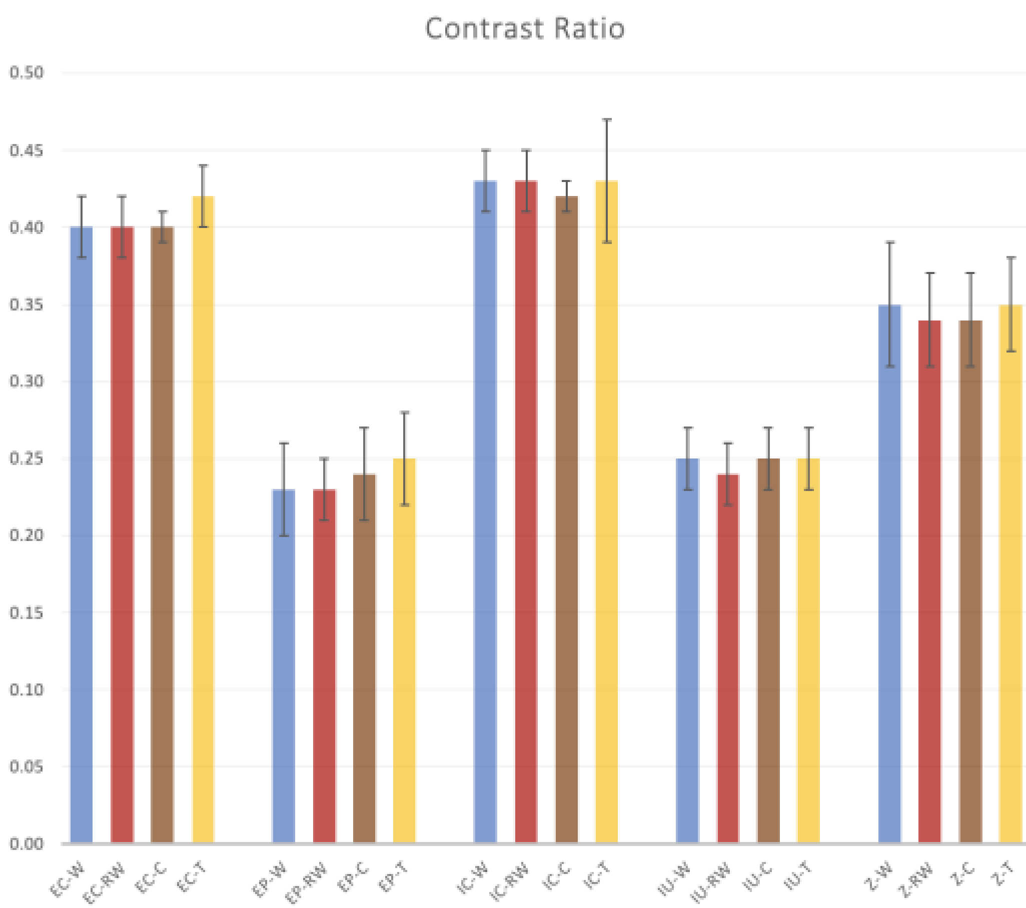

Figure 17: Comparison of average contrast ratio values between staining agents, grouped by material type.

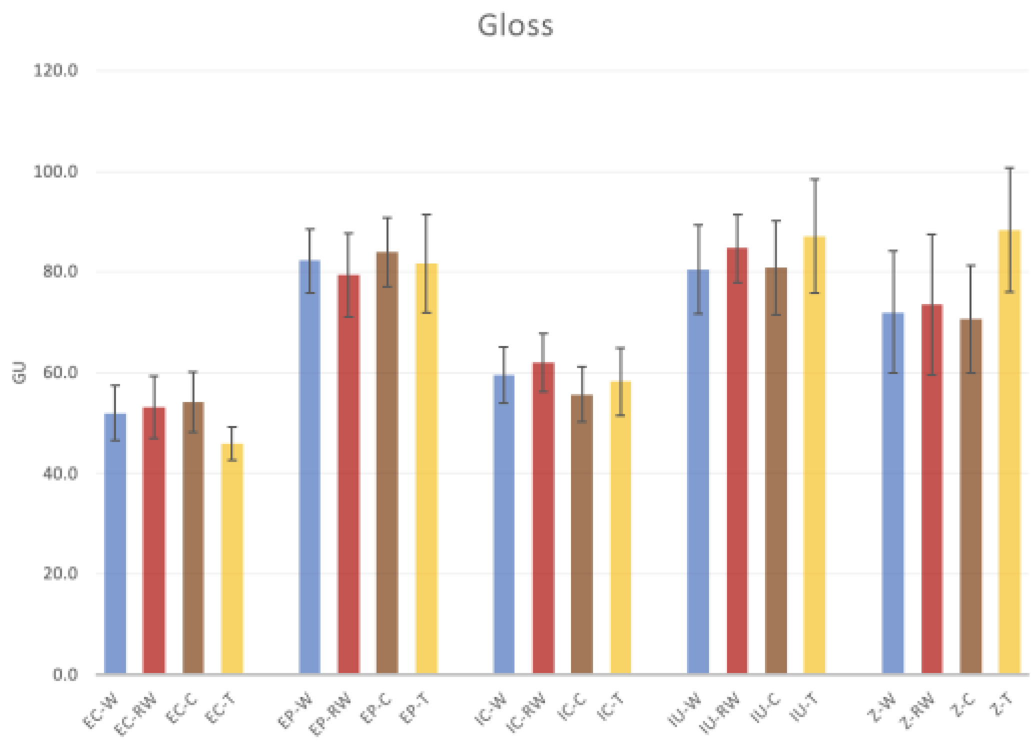

Figure 18: Comparison of average gloss values between staining agents, grouped by material type

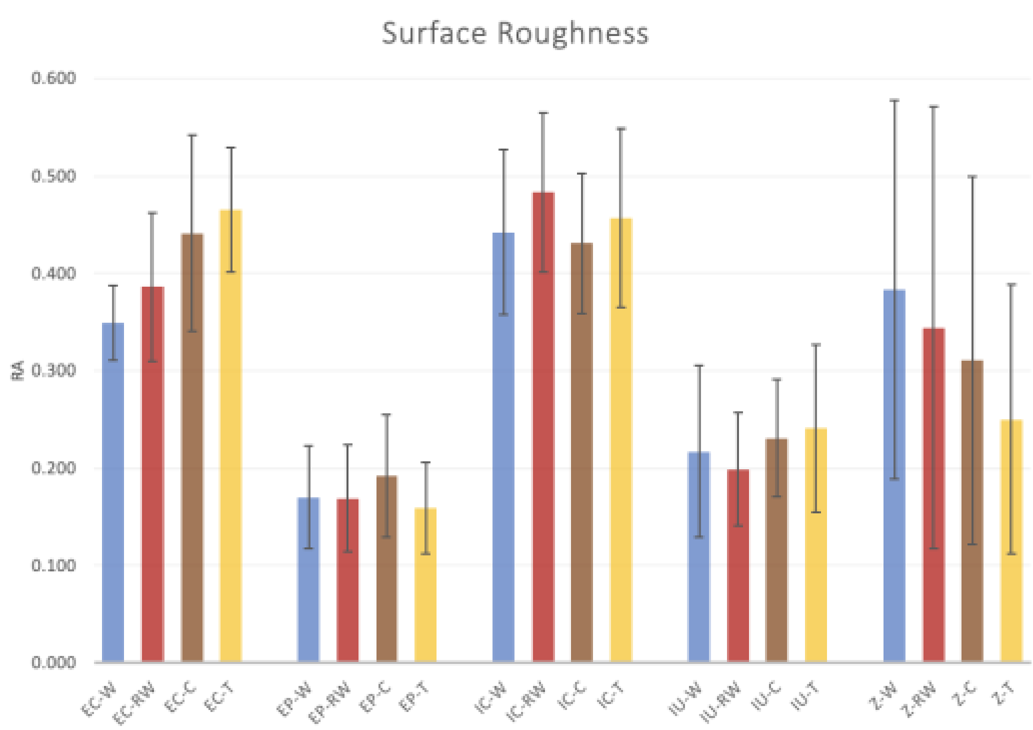

Figure 19: Comparison of average surface roughness values between staining agents, grouped by material type

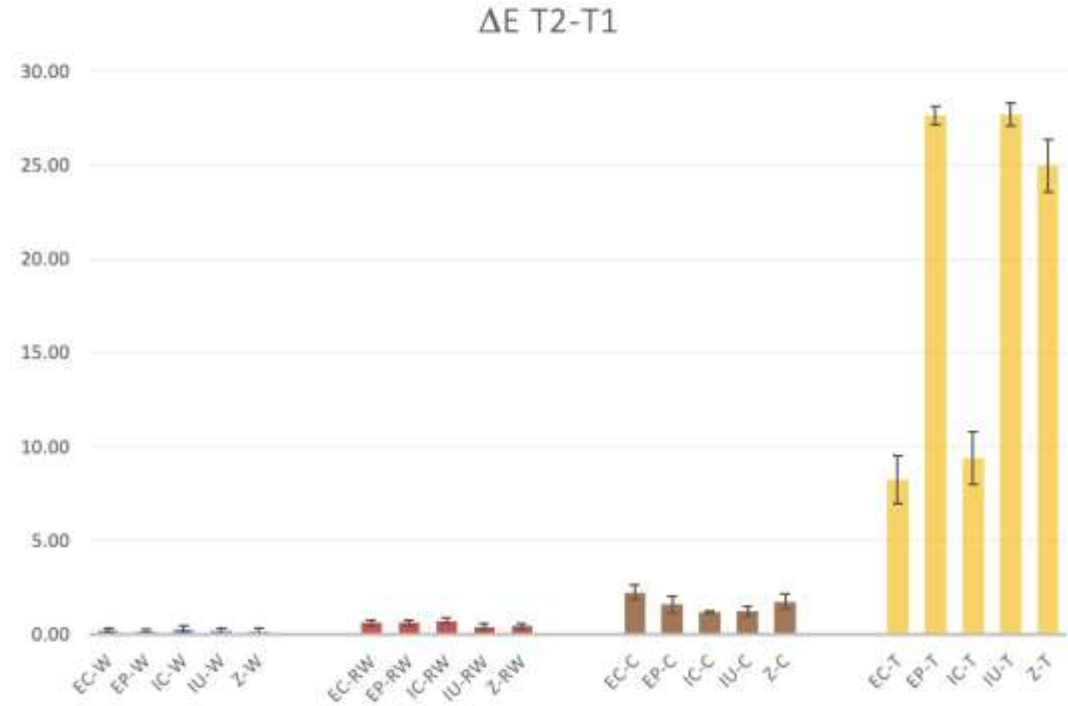

Figure 20: Comparison of average ΔE^*_{00} T_2-T_1 values between different materials, grouped by staining agent

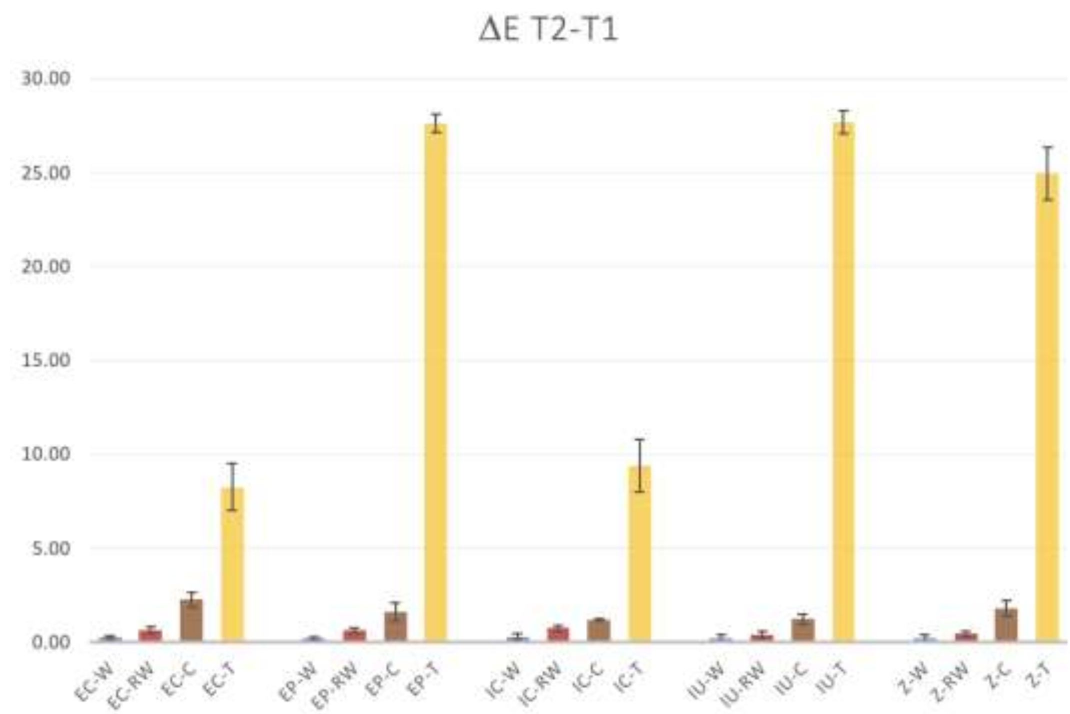

Figure 21: Comparison of average ΔE^*_{00} T_2-T_1 values between different staining agents, grouped by material

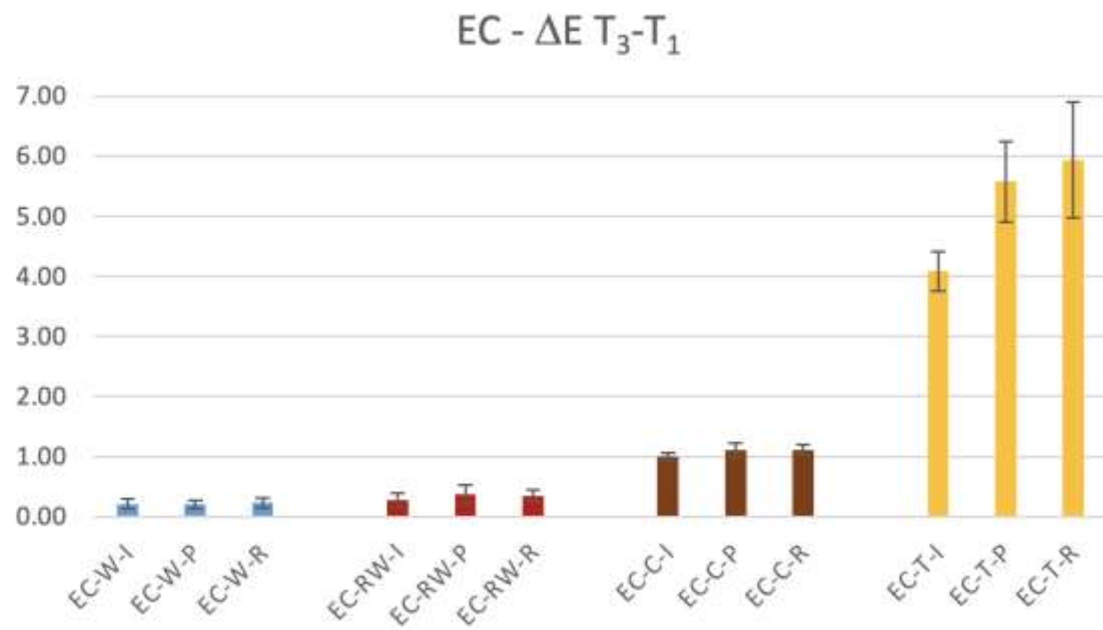

Figure 22: Comparison of average ΔE^*_{00} T_3-T_1 values within the EC material between different cleaning agents, grouped by staining agent

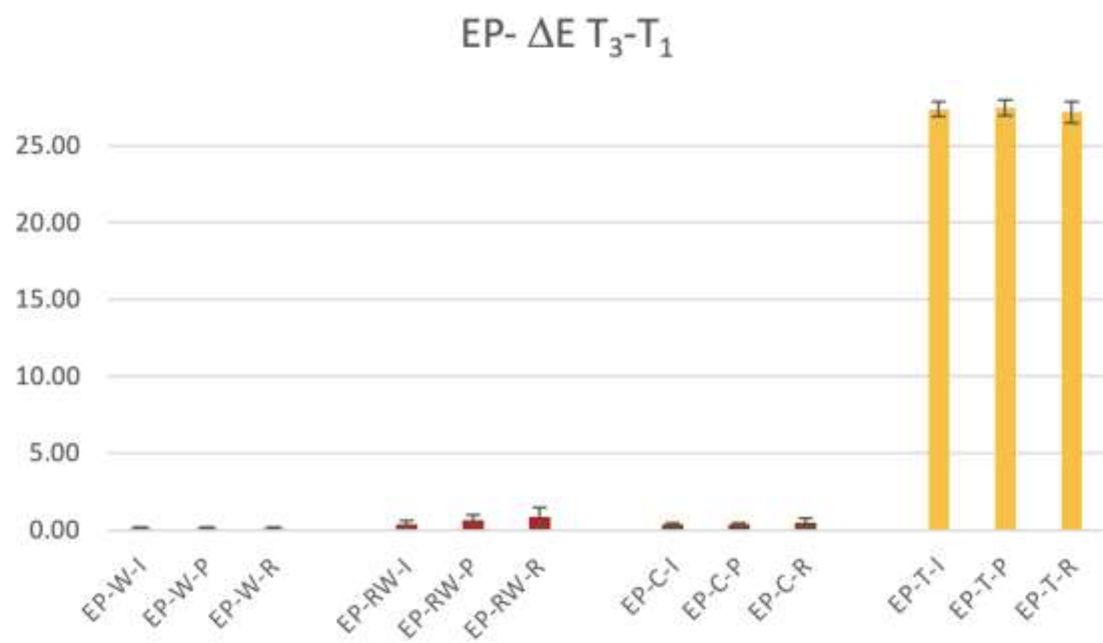

Figure 23: Comparison of average ΔE^*_{00} T_3-T_1 values within the EP material between different cleaning agents, grouped by staining agent

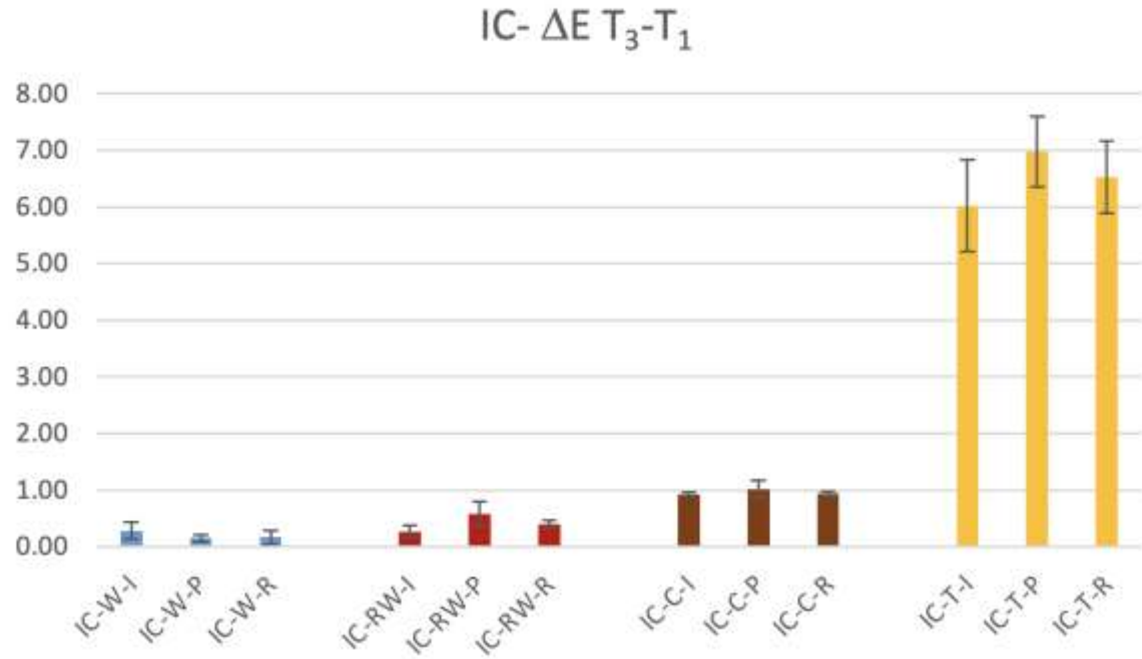

Figure 24: Comparison of average ΔE^*_{00} T_3-T_1 values within the IC material between different cleaning agents, grouped by staining agent

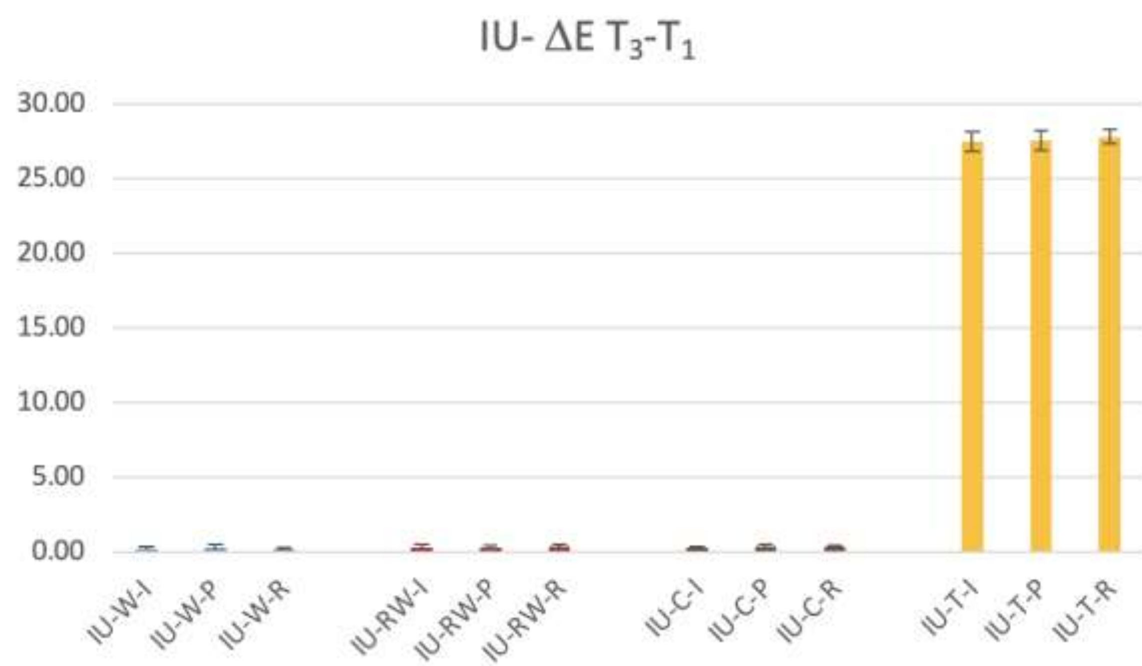

Figure 25: Comparison of average ΔE^*_{00} T_3-T_1 values within the IU material between different cleaning agents, grouped by staining agent

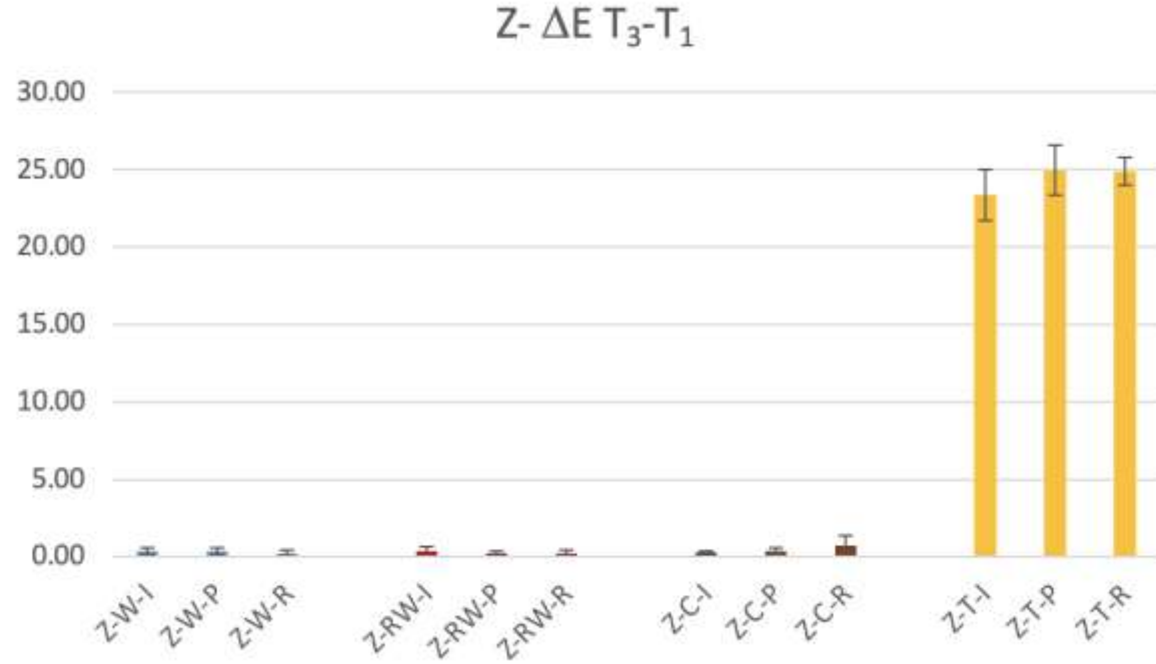

Figure 26: Comparison of average ΔE^*_{00} T_3-T_1 values within the Z material between different cleaning agents, grouped by staining agent

Table 8: Summary of different materials, staining agents, and cleansing agents from literature search

Authors	Year	Title	Material	Staining Agents	Duration of staining/ immersion	Cleaning Agents
Zafeiriadis	2014	In vitro spectrophotometric evaluation of Vivera clear thermoplastic retainer discoloration	Vivera (polyurethane) retainer	Coffee, tea, red wine	12 hr, 3 days, 7 days	N/A
Agarwal	2019	Long-term effects of various cleaning methods on polypropylene/ethylene copolymer retainer material	Polypropylene/ethylene copolymer (EssixC+)	N/A	6 months, cleaned once every 2 weeks	Invisalign Cleaning Crystals, Retainer Brite, Polident, Listerine, vinegar, sodium hypochlorite, hydrogen peroxide, toothbrushing w/ distilled water
Agarwal	2018	Long-term effects of seven cleaning methods on light transmittance, surface roughness, and flexural modulus of polyurethane retainer material	Polyurethane retainer (Vivera)	N/A	6 months, cleaned once every 2 weeks	Invisalign Cleaning Crystals, Polident, Listerine, Retainer Brite, vinegar, sodium hypochlorite, hydrogen peroxide
Agarwal	2019	Long-term effects of different cleaning methods on copolyester retainer properties	Essix Ace (copolyester)	N/A	6 months, cleaned once every 2 weeks	Invisalign Cleaning Crystals, Polident, Listerine, Retainer Brite, vinegar, sodium hypochlorite, hydrogen peroxide
Daniele	2020	Thermoplastic Disks Used for Commercial Orthodontic Aligners: Complete Physicochemical and Mechanical Characterization	PETG and Polyurethane	Red wine, coffee	7 days, 14 days	N/A
Liu	2016	Colour stabilities of three types of orthodontic clear aligners exposed to staining agents	Invisalign (PU+modification), PETG	Coffee, tea, wine	12 hr, 7 days	N/A
Porojan	2020	Surface Quality Evaluation of Removable	PETG	Coffee, cola, tea	24 hr, 48 hr, 7 days	Powder, tablet, brushing

		Thermoplastic Dental Appliances Related to Staining Beverages and Cleaning Agents				
Bernard	2020	Colorimetric and spectrophotometric measurements of orthodontic thermoplastic aligners exposed to various staining sources and cleaning methods	Invisalign aligners (multilayer polyurethane + integrated elastomer), Clearcorrect (Zendura – polyurethane resin), Minor Tooth Movement (copolyester + trade secret)	Coffee, black tea, red wine, cola	12 hr, 7 days	Invisalign cleaning crystals, Retainer Brite + cordless sonic cleaner
Waldemarin	2013	Color change in acrylic resin processed in three ways after immersion in water, cola, coffee, mate and wine	Acrylic resin	Water, cola, coffee, yerba mate and wine	30 days	N/A
Azmy	2021	Influence of Different Beverages on the Color Stability of Nanocomposite Denture Base Materials	Nanocomposite denture base material	Coffee, tea, cola, mineral water	6 days (simulates 6 months consumption)	N/A
Turker	2006	Effect of five staining solutions on the colour stability of two acrylics and three composite resins based provisional restorations	Acrylic and composite resins	Coffee, tea, coca-cola, orange juice, red wine	30 days	N/A
Babanouri	2021	Influence of bleaching agent on surface and mechanical properties of orthodontic thermoplastic retainer materials: An in vitro study	PETG (polyethylene terephthalate glycol)	N/A		Bleach (5h daily for 14 days)
Gregorius[85]	2012	Effects of aging and staining on color of acrylic resin denture teeth	Acrylic resin denture teeth	Distilled water, coffee, red wine	7 days	N/A
Hollis[86]	2015	Color stability of denture resins after staining and exposure to cleansing agents	Denture resins	Coffee, cola, grape juice	8 hours in staining agent followed by 12 hour cleansing, repeated every 24 hours for 2, 4, 6, 8 wks	Polident, Efferdent, GlaxoSmithKline, Prestige Brands Inc
Makhija[87]	2016	Evaluating the efficacy of denture cleansing materials in removal of tea and turmeric stains: An in vitro study	Heat-cured acrylic resins	Turmeric, tea	1 hr tea then 1 hr turmeric	Sodium hypochlorite, Safe plus, clinsodent

Vaddamanu[88]	2021	Effect of Food Colorants on Color of Denture Base Acrylic Resins	Heat cure denture base resins	Tea, coffee, turmeric, betal leaf	30 days	N/A
Oguz	2007	Color Change Evaluation of Denture Soft Lining Materials in Coffee and Tea	Soft lining material	Tea, Coffee	1, 3, 9, 24, 48, 96 hrs	N/A

References

1 Proffit, W. R., Fields, H. W., Larson, B. E. & Sarver, D. M. *Contemporary orthodontics*. (Elsevier, 2019).

2 Littlewood, S. J., Millett, D. T., Doubleday, B., Bearn, D. R. & Worthington, H. V. Retention procedures for stabilising tooth position after treatment with orthodontic braces. *Cochrane Database Syst Rev*, CD002283, doi:10.1002/14651858.CD002283.pub2 (2004).

3 Sadowsky, C. & Sakols, E. I. Long-term assessment of orthodontic relapse. *Am J Orthod* **82**, 456-463, doi:10.1016/0002-9416(82)90312-8 (1982).

4 Little, R. M., Riedel, R. A. & Artun, J. An evaluation of changes in mandibular anterior alignment from 10 to 20 years postretention. *Am J Orthod Dentofacial Orthop* **93**, 423-428, doi:10.1016/0889-5406(88)90102-3 (1988).

5 Littlewood, S. J., Millett, D. T., Doubleday, B., Bearn, D. R. & Worthington, H. V. Orthodontic retention: a systematic review. *J Orthod* **33**, 205-212, doi:10.1179/146531205225021624 (2006).

6 Melrose, C. & Millett, D. T. Toward a perspective on orthodontic retention? *Am J Orthod Dentofacial Orthop* **113**, 507-514, doi:10.1016/s0889-5406(98)70261-6 (1998).

7 Valiathan, M. & Hughes, E. Results of a survey-based study to identify common retention practices in the United States. *Am J Orthod Dentofacial Orthop* **137**, 170-177; discussion 177, doi:10.1016/j.ajodo.2008.03.023 (2010).

8 Wong, P. M. & Freer, T. J. A comprehensive survey of retention procedures in Australia and New Zealand. *Aust Orthod J* **20**, 99-106 (2004).

9 Meade, M. J. & Millett, D. Retention protocols and use of vacuum-formed retainers among specialist orthodontists. *J Orthod* **40**, 318-325, doi:10.1179/1465313313Y.0000000066 (2013).

10 Padmos, J. A. D., Fudalej, P. S. & Renkema, A. M. Epidemiologic study of orthodontic retention procedures. *Am J Orthod Dentofacial Orthop* **153**, 496-504, doi:10.1016/j.ajodo.2017.08.013 (2018).

11 Chang, C. S., Al-Awadi, S., Ready, D. & Noar, J. An assessment of the effectiveness of mechanical and chemical cleaning of Essix orthodontic retainer. *J Orthod* **41**, 110-117, doi:10.1179/1465313313Y.0000000088 (2014).

12 Wible, E. *et al.* Long-term effects of various cleaning methods on polypropylene/ethylene copolymer retainer material. *Angle Orthod* **89**, 432-437, doi:10.2319/060818-429.1 (2019).

13 Zhang, N., Bai, Y., Ding, X. & Zhang, Y. Preparation and characterization of thermoplastic materials for invisible orthodontics. *Dent Mater J* **30**, 954-959, doi:10.4012/dmj.2011-120 (2011).

14 Zhang, N., Fang, D. Y., Bai, Y. X., Ding, X. J. & Zhang, Y. [A comparative study of mechanical properties of commercialized dental thermoplastic materials]. *Zhonghua Kou Qiang Yi Xue Za Zhi* **46**, 551-553, doi:10.3760/cma.j.issn.1002-0098.2011.09.010 (2011).

15 Zafeiriadis, A. A., Karamouzos, A., Athanasiou, A. E., Eliades, T. & Palaghias, G. In vitro spectrophotometric evaluation of Vivera clear thermoplastic retainer discolouration. *Aust Orthod J* **30**, 192-200 (2014).
16 Porojan, L., Vasiliu, R. D., Porojan, S. D. & Birdeanu, M. I. Surface Quality Evaluation of Removable Thermoplastic Dental Appliances Related to Staining Beverages and Cleaning Agents. *Polymers (Basel)* **12**, doi:10.3390/polym12081736 (2020).
17 Chu, S. J., Trushkowsky, R. D. & Paravina, R. D. Dental color matching instruments and systems. Review of clinical and research aspects. *J Dent* **38 Suppl 2**, e2-16, doi:10.1016/j.jdent.2010.07.001 (2010).
18 <1965 Schudy - The Rotation Of The Mandible Resulting From Growth- Its Implications In Orthodontic Treatment.pdf>.
19 Ryokawa, H., Miyazaki, Y., Fujishima, A., Miyazaki, T. & Maki, K. The mechanical properties of dental thermoplastic materials in a simulated intraoral environment. *Orthodontic Waves* **65**, 64-72, doi:10.1016/j.odw.2006.03.003 (2006).
20 Knapp, B. *Fracture toughness, clarity, and staining of thermoplastic orthodontic retainer materials*. (Oregon Health and Science University, 2014).
21 da Costa, J. B., Ferracane, J. L., Amaya-Pajares, S. & Pfefferkorn, F. Visually acceptable gloss threshold for resin composite and polishing systems. *The Journal of the American Dental Association* **152**, 385-392, doi:https://doi.org/10.1016/j.adaj.2020.09.027 (2021).
22 Jones, C. S., Billington, R. W. & Pearson, G. J. The in vivo perception of roughness of restorations. *Br Dent J* **196**, 42-45; discussion 31, doi:10.1038/sj.bdj.4810881 (2004).
23 Bollen, C. M., Lambrechts, P. & Quirynen, M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dent Mater* **13**, 258-269, doi:10.1016/s0109-5641(97)80038-3 (1997).
24 Johnston, W. M. & Kao, E. C. Assessment of appearance match by visual observation and clinical colorimetry. *J Dent Res* **68**, 819-822, doi:10.1177/00220345890680051301 (1989).
25 Seghi, R. R., Hewlett, E. R. & Kim, J. Visual and instrumental colorimetric assessments of small color differences on translucent dental porcelain. *J Dent Res* **68**, 1760-1764, doi:10.1177/00220345890680120801 (1989).
26 Lee, Y. K. Changes in the reflected and transmitted color of esthetic brackets after thermal cycling. *Am J Orthod Dentofacial Orthop* **133**, 641 e641-646, doi:10.1016/j.ajodo.2007.10.035 (2008).
27 Liu, C. L. *et al.* Colour stabilities of three types of orthodontic clear aligners exposed to staining agents. *Int J Oral Sci* **8**, 246-253, doi:10.1038/ijos.2016.25 (2016).
28 Telang, A. *et al.* Effect of Staining and Bleaching on Color stability and Surface Roughness of Three Resin Composites: An in vitro study. *Contemp Clin Dent* **9**, 452-456, doi:10.4103/ccd.ccd_297_18 (2018).
29 Makhija, P., Shigli, d. k. & Awinashe, V. Evaluating the efficacy of denture cleansing materials in removal of tea and turmeric stains: An in vitro study. *Indian Journal of Dental Research* **27**, 528, doi:10.4103/0970-9290.195643 (2016).
30 Blake, M. & Garvey, M. T. Rationale for retention following orthodontic treatment. *J Can Dent Assoc* **64**, 640-643 (1998).

31 Tynelius, G. E. ORTHODONTIC RETENTION. Studies of retention capacity, cost-effectiveness and long-term stability. *Swed Dent J Suppl*, 9-65 (2014).

32 Barlin, S., Smith, R., Reed, R., Sandy, J. & Ireland, A. J. A retrospective randomized double-blind comparison study of the effectiveness of Hawley vs vacuum-formed retainers. *Angle Orthod* **81**, 404-409, doi:10.2319/072610-437.1 (2011).

33 Thickett, E. & Power, S. A randomized clinical trial of thermoplastic retainer wear. *Eur J Orthod* **32**, 1-5, doi:10.1093/ejo/cjp061 (2010).

34 Jaderberg, S., Feldmann, I. & Engstrom, C. Removable thermoplastic appliances as orthodontic retainers--a prospective study of different wear regimens. *Eur J Orthod* **34**, 475-479, doi:10.1093/ejo/cjr040 (2012).

35 Littlewood, S. J., Millett, D. T., Doubleday, B., Bearn, D. R. & Worthington, H. V. Retention procedures for stabilising tooth position after treatment with orthodontic braces. *Cochrane Database Syst Rev*, CD002283, doi:10.1002/14651858.CD002283.pub4 (2016).

36 Al-Moghrabi, D. *et al.* Effects of fixed vs removable orthodontic retainers on stability and periodontal health: 4-year follow-up of a randomized controlled trial. *Am J Orthod Dentofacial Orthop* **154**, 167-174 e161, doi:10.1016/j.ajodo.2018.01.007 (2018).

37 Sari, Z., Uysal, T., Basciftci, F. A. & Inan, O. Occlusal contact changes with removable and bonded retainers in a 1-year retention period. *Angle Orthod* **79**, 867-872, doi:10.2319/101608-536.1 (2009).

38 Schneider, E. & Ruf, S. Upper bonded retainers. *Angle Orthod* **81**, 1050-1056, doi:10.2319/022211-132.1 (2011).

39 Atack, N., Harradine, N., Sandy, J. R. & Ireland, A. J. Which way forward? Fixed or removable lower retainers. *Angle Orthod* **77**, 954-959, doi:10.2319/103106-449.1 (2007).

40 O'Rourke, N., Albeedh, H., Sharma, P. & Johal, A. Effectiveness of bonded and vacuum-formed retainers: A prospective randomized controlled clinical trial. *Am J Orthod Dentofacial Orthop* **150**, 406-415, doi:10.1016/j.ajodo.2016.03.020 (2016).

41 Steinnes, J., Johnsen, G. & Kerosuo, H. Stability of orthodontic treatment outcome in relation to retention status: An 8-year follow-up. *Am J Orthod Dentofacial Orthop* **151**, 1027-1033, doi:10.1016/j.ajodo.2016.10.032 (2017).

42 Ponitz, R. J. Invisible retainers. *Am J Orthod* **59**, 266-272, doi:10.1016/0002-9416(71)90099-6 (1971).

43 Nahoum, H. I. The vacuum formed dental contour appliance. *NY State Dent J* **9**, 385-390 (1964).

44 Sheridan, J. J., LeDoux, W. & McMinn, R. Essix retainers: fabrication and supervision for permanent retention. *J Clin Orthod* **27**, 37-45 (1993).

45 Sheridan, J. J. & Armbruster, P. Bleaching teeth during supervised retention. *J Clin Orthod* **33**, 339-344 (1999).

46 Babanouri, N., Ahmadi, N., Pakshir, H. R., Ajami, S. & Habibagahi, R. Influence of a bleaching agent on surface and mechanical properties of orthodontic thermoplastic retainer materials : An in vitro study. *J Orofac Orthop*, doi:10.1007/s00056-021-00312-3 (2021).

47 Raja, T. A., Littlewood, S. J., Munyombwe, T. & Bubb, N. L. Wear resistance of four types of vacuum-formed retainer materials: a laboratory study. *Angle Orthod* **84**, 656-664, doi:10.2319/061313-448.1 (2014).

48 Mollov, N. D., Lindauer, S. J., Best, A. M., Shroff, B. & Tufekci, E. Patient attitudes toward retention and perceptions of treatment success. *Angle Orthod* **80**, 468-473, doi:10.2319/102109-594.1 (2010).

49 Pratt, M. C., Kluemper, G. T., Hartsfield, J. K., Jr., Fardo, D. & Nash, D. A. Evaluation of retention protocols among members of the American Association of Orthodontists in the United States. *Am J Orthod Dentofacial Orthop* **140**, 520-526, doi:10.1016/j.ajodo.2010.10.023 (2011).

50 Vagdouti, G., Karvouni, E., Bitsanis, E. & Koletsi, D. Objective evaluation of compliance after orthodontic treatment using Hawley or vacuum-formed retainers: A 2-center randomized controlled trial over a 3-month period. *Am J Orthod Dentofacial Orthop* **156**, 717-726 e712, doi:10.1016/j.ajodo.2019.07.008 (2019).

51 Hichens, L. *et al.* Cost-effectiveness and patient satisfaction: Hawley and vacuum-formed retainers. *Eur J Orthod* **29**, 372-378, doi:10.1093/ejo/cjm039 (2007).

52 Saleh, M., Hajeer, M. Y. & Muessig, D. Acceptability comparison between Hawley retainers and vacuum-formed retainers in orthodontic adult patients: a single-centre, randomized controlled trial. *Eur J Orthod* **39**, 453-461, doi:10.1093/ejo/cjx024 (2017).

53 Kumar, A. G. & Bansal, A. Effectiveness and acceptability of Essix and Begg retainers: a prospective study. *Aust Orthod J* **27**, 52-56 (2011).

54 Wan, J. *et al.* Speech effects of Hawley and vacuum-formed retainers by acoustic analysis: A single-center randomized controlled trial. *Angle Orthod* **87**, 286-292, doi:10.2319/012716-76.1 (2017).

55 Chagas, A. S. *et al.* Level of satisfaction in the use of the wraparound Hawley and thermoplastic maxillary retainers. *Angle Orthod* **90**, 63-68, doi:10.2319/031319-197.1 (2020).

56 Rowland, H. *et al.* The effectiveness of Hawley and vacuum-formed retainers: a single-center randomized controlled trial. *Am J Orthod Dentofacial Orthop* **132**, 730-737, doi:10.1016/j.ajodo.2006.06.019 (2007).

57 Demir, A., Babacan, H., Nalcaci, R. & Topcuoglu, T. Comparison of retention characteristics of Essix and Hawley retainers. *Korean J Orthod* **42**, 255-262, doi:10.4041/kjod.2012.42.5.255 (2012).

58 Kaya, Y., Tunca, M. & Keskin, S. Comparison of Two Retention Appliances with Respect to Clinical Effectiveness. *Turk J Orthod* **32**, 72-78, doi:10.5152/TurkJOrthod.2019.18045 (2019).

59 Sun, J. *et al.* Survival time comparison between Hawley and clear overlay retainers: a randomized trial. *J Dent Res* **90**, 1197-1201, doi:10.1177/0022034511415274 (2011).

60 Fudalej, P. S. & Renkema, A. M. A brief history of orthodontic retention. *Br Dent J* **230**, 777-780, doi:10.1038/s41415-021-2955-6 (2021).

61 Dincer, M. & Isik Aslan, B. Effects of thermoplastic retainers on occlusal contacts. *Eur J Orthod* **32**, 6-10, doi:10.1093/ejo/cjp062 (2010).

62 Sauget, E., Covell, D. A., Jr., Boero, R. P. & Lieber, W. S. Comparison of occlusal contacts with use of Hawley and clear overlay retainers. *Angle Orthod* **67**, 223-230, doi:10.1043/0003-3219(1997)067<0223:COOCWU>2.3.CO;2 (1997).

63 Mai, W. *et al.* Comparison of vacuum-formed and Hawley retainers: a systematic review. *Am J Orthod Dentofacial Orthop* **145**, 720-727, doi:10.1016/j.ajodo.2014.01.019 (2014).

64 Gardner, G. D., Dunn, W. J. & Taloumis, L. Wear comparison of thermoplastic materials used for orthodontic retainers. *Am J Orthod Dentofacial Orthop* **124**, 294-297, doi:10.1016/s0889-5406(03)00502-x (2003).

65 Lindauer, S. J. & Shoff, R. C. Comparison of Essix and Hawley retainers. *J Clin Orthod* **32**, 95-97 (1998).

66 Cifter, M., Gumru Celikel, A. D. & Cekici, A. Effects of vacuum-formed retainers on periodontal status and their retention efficiency. *Am J Orthod Dentofacial Orthop* **152**, 830-835, doi:10.1016/j.ajodo.2017.05.029 (2017).

67 Manzon, L., Fratto, G., Rossi, E. & Buccheri, A. Periodontal health and compliance: A comparison between Essix and Hawley retainers. *Am J Orthod Dentofacial Orthop* **153**, 852-860, doi:10.1016/j.ajodo.2017.10.025 (2018).

68 Li, B. *et al.* Assessment of the effect of vacuum-formed retainers and Hawley retainers on periodontal health: A systematic review and meta-analysis. *PLoS One* **16**, e0253968, doi:10.1371/journal.pone.0253968 (2021).

69 Daniele, V. *et al.* Thermoplastic Disks Used for Commercial Orthodontic Aligners: Complete Physicochemical and Mechanical Characterization. *Materials (Basel)* **13**, doi:10.3390/ma13102386 (2020).

70 Sable, D. L. & Woods, M. G. Growth and treatment changes distal to the mandibular first molar: a lateral cephalometric study. *Angle Orthod* **74**, 367-374, doi:10.1043/0003-3219(2004)074<0367:GATCDT>2.0.CO;2 (2004).

71 Chan, K. C., Fuller, J. L. & Hormati, A. A. The ability of foods to stain two composite resins. *J Prosthet Dent* **43**, 542-545, doi:10.1016/0022-3913(80)90328-5 (1980).

72 Ertas, E., Guler, A. U., Yucel, A. C., Koprulu, H. & Guler, E. Color stability of resin composites after immersion in different drinks. *Dent Mater J* **25**, 371-376 (2006).

73 Luce, M. S. & Campbell, C. E. Stain potential of four microfilled composites. *J Prosthet Dent* **60**, 151-154, doi:10.1016/0022-3913(88)90305-8 (1988).

74 Koksal, T. & Dikbas, I. Color stability of different denture teeth materials against various staining agents. *Dent Mater J* **27**, 139-144, doi:10.4012/dmj.27.139 (2008).

75 Chan, K. C., Hormati, A. A. & Kerber, P. E. Staining calcified dental tissues with food. *J Prosthet Dent* **46**, 175-178, doi:10.1016/0022-3913(81)90304-8 (1981).

76 Scotti, R., Mascellani, S. C. & Forniti, F. The in vitro color stability of acrylic resins for provisional restorations. *Int J Prosthodont* **10**, 164-168 (1997).

77 Agrawal, J. M., Agrawal, M. S., Nanjannawar, L. G. & Adaki, R. V. Non-syndromic multiple supernumerary teeth: a rare entity. *BMJ Case Rep* **2013**, doi:10.1136/bcr-2012-007796 (2013).

78 Bernard, G., Rompre, P., Tavares, J. R. & Montpetit, A. Colorimetric and spectrophotometric measurements of orthodontic thermoplastic aligners exposed to various staining sources and cleaning methods. *Head Face Med* **16**, 2, doi:10.1186/s13005-020-00218-2 (2020).

79 Rhopoint Americas. *How is Gloss Measured?*, <https://www.rhopointamericas.com/faqs/how-is-gloss-measured/#:~:text=A%20glossmeter%20(also%20gloss%20meter,an%20equal%20but%20opposite%20angle.> (n.d.).

80 INNOVATEST. MANUAL, TR-200. (n.d.).

81 Checkline Europe. *TR-200 Handheld Surface Roughness Tester With Graphic Display*, <https://www.checkline.eu/prod/surface-roughness-testers/tr-200> (2022).

82 Ahn, H. W., Ha, H. R., Lim, H. N. & Choi, S. Effects of aging procedures on the molecular, biochemical, morphological, and mechanical properties of vacuum-formed retainers. *J Mech Behav Biomed Mater* **51**, 356-366, doi:10.1016/j.jmbbm.2015.07.026 (2015).

83 Kakaboura, A., Fragouli, M., Rahiotis, C. & Silikas, N. Evaluation of surface characteristics of dental composites using profilometry, scanning electron, atomic force microscopy and gloss-meter. *J Mater Sci Mater Med* **18**, 155-163, doi:10.1007/s10856-006-0675-8 (2007).

84 Schuessler, Z. *Delta E 101*, <http://zschuessler.github.io/DeltaE/learn/> (n.d.).

85 Gregorius, W. C. *et al.* Effects of ageing and staining on color of acrylic resin denture teeth. *J Dent* **40 Suppl 2**, e47-54, doi:10.1016/j.jdent.2012.09.009 (2012).

86 Hollis, S., Eisenbeisz, E. & Versluis, A. Color stability of denture resins after staining and exposure to cleansing agents. *J Prosthet Dent* **114**, 709-714, doi:10.1016/j.prosdent.2015.06.001 (2015).

87 Makhija, P. P., Shigli, K. & Awinashe, V. Evaluating the efficacy of denture cleansing materials in removal of tea and turmeric stains: An in vitro study. *Indian J Dent Res* **27**, 528-534, doi:10.4103/0970-9290.195643 (2016).

88 Vaddamanu, S. K. *et al.* Effect of Food Colorants on Color of Denture Base Acrylic Resins. *J Pharm Bioallied Sci* **13**, S664-S666, doi:10.4103/jpbs.JPBS_759_20 (2021).

Appendices

Appendix A

Results

<u>CONTRAST RATIO</u>

Among the different VFR materials tested, IC had the highest contrast ratio while EP had the lowest. In general, PP materials have higher contrast ratios than EP materials.

A Kruskal-Wallis one way ANOVA on ranks [$H(4) = 494.922$ ($p < 0.001$)] showed a statistically significant median variation among materials. Further, a Tukey Test indicated that all materials differ from each other in terms of contrast with one exception: no significant difference was found between IU and EP ($p = 0.631$). The averages compared are as follows: IC (0.41), EC (0.39), Z (0.35) ,IU (0.24), EP (0.23) [Table 4 and Figure 14]. Specifically, results show that IC > EP ($p <0.001$), IU ($p <0.001$), Z ($p <0.001$) and EC ($p = 0.003$). EC > EP ($p <0.001$), IU ($p <0.001$), and Z ($p <0.001$). Z > EP ($p <0.001$) and IU ($p <0.001$). Again, there is no difference between IU and EP ($p = 0.631$).

IC (0.41) > EC (0.39) > Z (0.35) > IU (0.24)[a] > EP (0.23)[a]

A 2-way ANOVA between material and stain found a significant main effect of material ($p < 0.001$) and stain ($p < 0.001$). No significant interaction was found between materials and stain ($P = 0.067$)]

<u>Comparison of Staining Agents for Different Materials</u>

<u>EC x Staining Agents -</u> Within EC specimens immersed in various staining agents, the average contrast ratio compared are as follows: T (0.42), W (0.40), RW (0.40), C (0.40) [Table 5 and

Figure 17]. It was found that T > C ($p < 0.001$), RW ($P = 0.003$) and W ($p = 0.0013$). No other significant differences were found.

T (0.42) > W (0.40)^{a} > RW (0.40)^{a} > C (0.40)^{a}

EP x Staining Agents – Within EP specimens immersed in various staining agents, the average contrast ratio compared are as follows: T (0.25), C (0.24), W (0.23), RW (0.23) [Table 5 and Figure 17]. It was found that T > RW ($p = 0.033$), W ($p = 0.044$) but not C ($p = 0.058$). No other significant differences were found.

T (0.25)^{a} > C (0.24)^{a} > W (0.23)^{b} > RW (0.23)^{b}

IC, IU, Z x Staining Agents - No significant differences of effect of staining agent were found within IC, IU, and Z.

Comparison of Materials for Different Staining Agents

Coffee x Materials - Within specimens immersed in coffee, the average contrast ratio values compared are as follows: IC (0.42), EC (0.40), Z (0.34), IU (0.25), EP (0.24 [Table 5 and Figure 17]. It was found that that IC >EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$), and EC ($p < 0.001$). EC > EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$). Z > EP ($p < 0.001$) and IU ($p < 0.001$). No significant difference between IU and EP was found ($p = 0.266$).

IC (0.42) > EC (0.40) > Z (0.34) > IU (0.25)^{a} > EP (0.24)^{a}

Red Wine x Materials - Within specimens immersed in red wine, the average contrast ratio values compared are as follows: IC (0.43), EC (0.40), Z (0.34), IU (0.24), EP (0.23) [Table 5 and

Figure 17]. It was found that IC >EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$), EC ($p < 0.001$). EC > EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$). Z > EP ($p < 0.001$) and IU ($p < 0.001$). No significant difference between IU and EP was found ($p = 1.000$).

IC (0.43) > EC (0.40) > Z (0.34) > IU $(0.24)^a$ > EP $(0.23)^a$

Turmeric x Materials - Within specimens immersed in turmeric, the average contrast ratio values compared are as follows: IC (0.43), EC (0.42), Z (0.35), IU (0.25), EP (0.25) [Table 5 and Figure 17]. It was found that IC > IU ($p < 0.001$), EP ($p < 0.001$), Z ($p < 0.001$) but not EC ($p = 0.844$). EC > IU ($p < 0.001$), EP ($p < 0.001$), Z ($p < 0.001$). Z > IU ($p < 0.001$) and EP ($p < 0.001$). Lastly, there was no difference between IU and EP ($p = 0.888$). To reiterate, no significant difference between IU and EP nor IC and EC were found.

IC $(0.43)^a$> EC $(0.42)^a$ > Z (0.35) > IU $(0.25)^b$> EP $(0.25)^b$

Water x Materials - Within specimens immersed in water, the average contrast ratio values compared are as follows: IC (0.43), EC (0.40), Z (0.35), IU (0.25), EP (0.23) [Table 5 and Figure 17]. It was found that IC >EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$) and EC ($p < 0.001$). EC > EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$). Z > EP ($p < 0.001$) and IU ($p < 0.001$). No significant difference between IU and EP was found ($p = 0.292$).

IC (0.43) > EC (0.40) > Z (0.35) > IU $(0.25)^a$ > EP $(0.23)^a$

Results of a 3-way ANOVA between material, staining agent, and cleaning agent is available in the appendix as it does not provide us with any significant additional information (no main effect of cleaning agent was found) [Table 9].

GLOSS

Among all VFR materials tested. IU had the highest gloss while EC had the lowest. PE materials have higher gloss than PP materials in general.

Kruskal-Wallis one way ANOVA on ranks showed a statistically significant median variation [H (4) = 393.689, ($p < 0.001$)] among materials. Further, a Tukey Test indicated that all materials differ from each other in terms of gloss with one exception: there is no significant difference between IU and EP ($p = 0.867$). The averages compared are as follows: IU (84.9), EP (0.83.0), Z (75.2), IC (59.4), EC (54.3) [Table 4 and Figure 15]. It was found that IU > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$) but not EP ($p = 0.867$). EP > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$). Z > EC ($p < 0.001$) and IC ($p < 0.001$). IC > EC ($p = 0.029$). To reiterate, IU is no different than EP.

IU (84.9)[a] > EP (83.0)[a] > Z (75.2) > IC (59.4) > EC (54.3)

A 2-way ANOVA found a significant main effect of material ($p < 0.001$) and of stain ($p < 0.004$), as well as a significant interaction found between materials and stain ($p < 0.001$)

Comparison of Staining Agents for Different Materials

EC x Staining Agents- Within EC specimens immersed in various staining agents, the average gloss values compared are as follows: C (54.1), RW (53.1), W (51.9), T (45.9) [Table 5 and Figure 18]. It was found that C > T ($p = 0.001$), RW ($p = 0.006$) and W ($p = 0.032$). No other significant differences found.

C (54.1) > RW (53.1)^{a} > W (51.9)^{a} > T (45.9)^{a}

EP x Staining Agents- Within EP specimens immersed in various staining agents, the average gloss values compared are as follows: C (83.9), W (82.2), RW (79.4), T (81.7) [Table 5 and Figure 18]. No significant differences were found.

IC x Staining Agents - Within IC specimens immersed in various staining agents, the average gloss values compared are as follows: RW (62.0), W (59.6), T (58.2), C (55.6) [Table 5 and Figure 18]. It was found that RW > C ($p = 0.020$) but not T ($p = 0.302$) and W (0.703). No other significant differences were found.

RW (62.0)^{a} > W (59.6)^{a,b} > T (58.2)^{a,b} > C (55.6)^{b}

IU x Staining Agents - Within IU specimens immersed in various staining agents, the average gloss values compared are as follows: T (87.1), RW (84.1), C (80.9), W (80.5) [Table 5 and Figure 18]. It was found that T > W ($p = 0.014$), C ($p = 0.025$) but not RW ($p = 0.714$). No other significant differences were found.

T (87.1)^{a} > RW (84.1)^{a} > C (80.9) > W (80.5)

Z x Staining Agents - Within Z specimens immersed in various staining agents, the average gloss values compared are as follows: T (88.4), RW (73.5), W (72.0), C (70.6) [Table 5 and Figure 18]. It was found that T > C ($p < 0.001$), W ($p < 0.001$) and RW ($p < 0.001$). No other significant differences were found.

T (88.4) > RW (73.5)^{a} > W (72.0)^{a} > C (70.6)^{a}

Comparison of Materials for Different Staining Agents

Coffee x Materials – Within specimens immersed in coffee, the average gloss values compared are as follows: EP (83.9), IU (80.9), Z (70.6), IC (55.6), EC (54.1) [Table 5 and Figure 18]. It was found that EP > EC ($p < 0.001$), IC ($p < 0.001$), Z ($p < 0.001$) but not IU ($p = 0.632$). IU > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$). Z > EC ($p < 0.001$) and IC ($p < 0.001$). No significant difference between IC and EC was found ($p = 0.955$).

EP (83.9)^{a} > IU (80.9)^{a} > Z (70.6) > IC (55.6)^{b} > EC (54.1)^{b}

Red Wine x Materials – Within specimens immersed in red wine, the average gloss values compared are as follows: IU (84.7), EP (79.4), Z (73.5), IC (62.0), EC (53.1) [Table 5 and Figure 18]. It was found that IU > EC ($p < 0.001$), IC ($p < 0.001$), Z ($p < 0.001$) but not EP ($p = 0.111$). In addition, EP > EC ($p < 0.001$), IC ($p < 0.001$) but not Z ($p < 0.054$). Further, Z > EC ($p < 0.001$) and IC ($p < 0.001$). Lastly, IC > EC ($p < 0.001$).

IU (84.7)^{a} > EP (79.4)^{a,b} > Z (73.5)^{b} > IC (62.0) > EC (53.1)

Turmeric x Materials - Within specimens immersed in turmeric, the average gloss values compared are as follows: Z (88.4), IU (87.1), EP (81.7), IC (58.2), EC (45.9) [Table 5 and Figure 18]. It was found that Z > EC ($p < 0.001$), IC ($p < 0.001$), EP ($p = 0.019$), but not IU ($p = 0.976$). In addition, IU > EC ($p < 0.001$), IC ($p < 0.001$) but not EP ($p = 0.098$). Further, EP > IC ($p < 0.001$) and EC ($p < 0.001$). Lastly, IC > EC ($p < 0.001$).

Z (88.4)^{a} > IU (87.1)^{a} > EP (81.7) > IC (58.2) > EC (45.9)

Water x Materials - Within specimens immersed in water. The average gloss values compared are as follows: EP (82.2), IU (80.5), Z (72.0), IC (59.6), EC (51.9) [Table 5 and Figure 18]. It was found that EP > EC ($p < 0.001$), IC ($p < 0.001$), and Z ($p < 0.001$) but not IU ($p = 0.933$). In addition, IU > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$). Further, Z > EC ($p < 0.001$) and IC ($p < 0.001$). Lastly, IC > EC ($p = 0.004$).

EP (82.2)[a] > IU (80.5)[a] > Z (72.0) > IC (59.6) > EC (51.9)

Results of a 3-way ANOVA between material, staining agent, and cleaning agent is available in the appendix as it does not provide us with any significant additional information (no main effect of cleaning agent was found) [Table 10].

SURFACE ROUGHNESS

A Kruskal-Wallis one way ANOVA on ranks showed a statistically significant median variation [$H(4) = 379.133$, ($p < 0.001$)] among materials. Further, a Tukey Test indicated that all materials differ from each other in terms of surface roughness with one exception: no significant difference between IC and EC was found ($p = 0.658$). The averages compared are as follows: IC (0.438), EC (0.409), Z (0.335), IU (0.222), EP (0.141) [Table 4 and Figure 16]. It was found that IC > EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$) but not EC ($p = 0.658$). In addition, EC > EP ($p < 0.001$), IU ($p < 0.001$) and Z ($p < 0.001$). Further, Z > EP ($p < 0.001$) and IU ($p < 0.001$). Lastly, IU > EP ($p < 0.001$).

IC (0.438)[a] > EC (0.409)[a] > Z (0.335) > IU (0.222) > EP (0.141)

A 2-way ANOVA between material and staining agent indicated significant main effects of material ($p < 0.001$) and stain ($p < 0.001$), though no significant interaction was found between material and stain ($p = 0.901$).

Comparison of Staining Agents for Different Materials

EC x Staining Agents - Within EC specimens immersed in various staining agents, the average surface roughness values compared are as follows: T (0.465), C (0.441), RW (0.386), W (0.349) [Table 5 and Figure 19]. It was found that T > W ($p = 0.001$), RW ($p = 0.021$) but not C ($p = 0.822$). In addition, C > W ($p = 0.004$) but not RW ($p = 0.186$). No significant difference was found between RW and W ($p = 0.531$).

T $(0.465)^{a}$ > C $(0.441)^{a,b}$ > RW $(0.386)^{b,c}$ > W $(0.349)^{c}$

EP, IC, IU x Staining Agents - No significant differences of effect of staining agent were found within EP, IC, and IU [Table 5 and Figure 19].

Z x Staining Agents - Within Z specimens immersed in various staining agents, the average surface roughness values compared are as follows: W (0.383), RW (0.344), C (0.311), T (0.250) [Table 2]. It was found that W > T ($p < 0.001$), C ($p = 0.043$) but not RW ($p = 0.482$) [Table 5 and Figure 19]. Further, RW > T ($p = 0.003$) but not C ($p = 0.628$). Lastly, no significant difference was found between C and T ($p = 0.117$).

W $(0.383)^{a}$ > RW $(0.344)^{a,b}$ > C $(0.311)^{b,c}$ > T^{c} (0.250)

Comparison of Materials for Different Staining Agents

Coffee x Materials - Within specimens immersed in coffee, the average surface roughness values compared are as follows: EC (0.441), IC (0.431), Z (0.311), IU (0.231), EP (0.192) [Table 5 and Figure 19]. It was found that EC > EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$) but not IC ($p = 0.996$). In addition, IC > EP ($p < 0.001$), IU ($p < 0.001$) and Z ($p < 0.001$). Further, Z > EP ($p < 0.001$) and IU ($p = 0.029$). Lastly, no significant difference was found between IU and EP ($p = 0.630$).

EC (0.441)^{a} > IC (0.431)^{a} > Z (0.311) > IU (0.231)^{b} > EP (0.192)^{b}

Red wine x Materials - Within specimens immersed in red wine, the average surface roughness values compared are as follows: IC (0.483), EC (0.386), Z (0.344), IU (0.199), EP (0.169) [Table 5 and Figure 19]. It was found that IC > EP ($p < 0.001$), IU ($p < 0.001$), Z ($p < 0.001$) and EC ($p = 0.004$). In addition, EC > EP ($p < 0.001$), IU ($p < 0.001$) but not Z ($p = 0.537$). Further, Z > EP ($p < 0.001$) and IU ($p < 0.001$). Lastly, no significant difference was found between IU and EP ($p = 0.809$).

IC (0.483) > EC (0.386)^{a} > Z (0.344)^{a} > IU (0.199)^{b} > EP (0.169)^{b}

Turmeric x Materials - Within specimens immersed in turmeric, the average surface roughness values compared are as follows: EC (0.465), IC (0.457), Z (0.250), IU (0.241), EP (0.159) [Table 5 and Figure 19]. It was found that EC > EP ($p < 0.001$), IU ($p < 0.001$), Z ($p = 0.001$), but not IC ($p = 0.998$). In addition, IC > EP ($p < 0.001$), IU ($p < 0.001$) and Z ($p < 0.001$). Further, Z > EP ($p = 0.008$) but not IU ($p = 0.998$). Lastly, IU > EP ($p = 0.023$).

EC (0.465)^{a} > IC (0.457)^{a} > Z (0.250) > IU (0.241) > EP (0.159)

Water x Materials - Within specimens immersed in water, the average surface roughness values compared are as follows: IC (0.442), Z (0.383), EC (0.349), IU (0.217), EP (0.170) [Table 5 and Figure 19]. It was found that IC > EP ($p < 0.001$), IU ($p < 0.001$), and EC ($p = 0.006$) but not Z ($p = 0.200$). In addition, Z > EP ($p < 0.001$), IU ($p < 0.001$) but not EC ($p = 0.728$). Further, EC > EP ($p < 0.001$) and IU ($p < 0.001$). Lastly, no significant difference was found between IU and EP ($p = 0.437$).

IC $(0.442)^{a}$ > Z $(0.383)^{a,b}$ > EC $(0.349)^{b}$ > IU $(0.217)^{c}$ > EP $(0.170)^{c}$

Results of a 3-way ANOVA between material, staining agent, and cleaning agent is available in the appendix as it does not provide us with any significant additional information (no main effect of cleaning agent was found) [Table 11].

ΔE (T_2-T_1)

All ΔE_{00} values reported are based on measurements taken against a white tile (refer to methods) as this better simulates the intraoral environment with retainer material against labial surface of teeth.

A 2-way ANOVA found main effects of material and stain as well as an interaction between material and stain.

Across all materials, turmeric resulted in the largest average change in color in terms of ΔE_{00} (19.58), followed by coffee (1.61), red wine (0.56), and water (0.22).

Comparison of Staining Agents for Different Materials

EC x Staining Agents - Within EC specimens immersed in various staining agents, the average ΔE_{00} values compared are as follows: T (8.25), C (2.25), RW (0.62), W (0.25) [Table 6 and

Figure 21]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C >W ($p < 0.001$) and RW ($p < 0.001$). Lastly, RW is not significantly different than W ($p = 0.065$).

T (8.25) > C (2.25) > RW (0.62) > W (0.25)

EP x Staining Agents - Within EP specimens immersed in various staining agents, the average ΔE_{00} values compared are as follows: T (27.63), C (1.59), RW (0.62), W (0.18) Table 6 and Figure 21].. It was found that T>W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C > W ($p < 0.001$) and RW ($p < 0.001$). Lastly, RW > W ($p = 0.023$).

T (27.63) > C (1.59) > RW (0.62) > W (0.18)

IC x Staining Agents - Within IC specimens immersed in various staining agents, the average ΔE_{00} values compared are as follows: T (9.39), C (1.19), RW (0.73), W (0.29) Table 6 and Figure 21].. It was found show that T>W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C > W ($p < 0.001$) and RW ($p = 0.014$). Lastly, RW > W ($p = 0.025$).

T (9.39) > C (1.19) > RW (0.73) > W (0.29)

IU x Staining Agents - Within IU specimens immersed in various staining agents, the average ΔE_{00} values compared are as follows: T (27.68), C (1.23), RW (0.39), W (0.20) Table 6 and Figure 21]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C >W ($p < 0.001$) and RW ($p < 0.001$). Lastly, RW is not significantly different than W ($p = 0.617$).

T (27.68) > C (1.23) > RW (0.39)[a] > W (0.20)[a]

Z x Staining Agents - Within Z specimens immersed in various staining agents, the average ΔE_{00} values compared are as follows: T (24.95), C (1.77), RW (0.44), W (0.19) Table 6 and Figure 21].. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C >W ($p < 0.001$) and RW ($p < 0.001$). Lastly, RW is not significantly different than W ($p = 0.353$).

T (24.95), C (1.77), RW (0.44)[a], W (0.19)[a]

Comparison of Materials for Different Staining Agents

Coffee x Materials -Within specimens immersed in coffee, the average ΔE_{00} values compared are as follows: EC (2.25), Z (1.77), EP (1.59), IU (1.23), IC (1.19) [Table 6 and Figure 20]. It was found that EC > IC ($p < 0.001$), IU ($p < 0.001$), EP ($p < 0.001$) and Z ($p < 0.013$). In addition, Z > IC ($p < 0.002$), IU ($p < 0.005$) but not EP ($p = 0.769$). No other significant difference between the materials were found.

EC (2.25) > Z (1.77)[a] > EP (1.59)[a,b] > IU (1.23)[b] > IC (1.19)[b]

Turmeric x Materials -Within specimens immersed in turmeric, the average ΔE_{00} values compared are as follows: IU (27.68), EP (27.63), Z (24.95), IC (9.39), EC (8.25) [Table 6 and Figure 20]. It was found that IU > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$) but not EP ($p = 0.997$). In addition, EP > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$). Further, Z > EC ($p < 0.001$) and IC ($p < 0.001$). Lastly, IC > EC ($p < 0.001$).

IU (27.68)[a] > EP (27.63)[a] > Z (24.95) > IC (9.39) > EC (8.25)

Red Wine and Water x Materials – Within red wine and water, no significant difference between the materials were found. [Table 6 and Figure 20].

DELTA E (T3-T1)

Comparing Cleaning Agents – staining agent x cleaning agent

Essix C+ (EC)

A 2-way ANOVA of staining agent x cleaning agent found a main effect of staining agent and cleaning agent as well as interaction between staining agent and cleaning agent. Within EC specimens immersed in turmeric, the average ΔE_{00} values compared are as follows: R (5.94), P (5.58), I (4.09) [Table 7 and Figure 22. A Tukey Test found that R > I ($p < 0.001$) but not P ($p = 0.067$). In addition, P > I ($p <0.001$).

R $(5.94)^{a}$ > P $(5.58)^{a}$ > I (4.09)

Essix Plus (EP)

A 2-way ANOVA of staining agent x cleaning agent found a main effect of staining agent, but no main effect of cleaning agent nor interaction between staining agent and cleaning agent. Within EP specimens immersed in red wine, the average ΔE_{00} values compared are as follows: R (0.47), P (0.36), I (0.36) [Table 7 and Figures 23]. It was found that R > I ($p = 0.012$) but not P ($p = 0.512$) In addition, P is not significantly different from I ($p < 0.174$).

R $(0.47)^{a}$ > P $(0.36)^{a,b}$ > I $(0.36)^{b}$

Invisacryl C (IC)

A 2-way ANOVA of staining agent x cleaning agent found main effect of staining agent and cleaning agent as well as interaction between staining agent and cleaning agent. Within IC samples immersed in turmeric, the average ΔE_{00} values compared are as follows: P (6.98), R (6.52), I (6.02) [Table 7 and Figure 24]. It was found that P > I ($p < 0.001$) and R ($p = 0.014$). In addition, R > I ($p = 0.008$)

P (6.98) > R (6.52) > I (6.02)

Invisacryl Ultra (IU)

A 2-way ANOVA of staining agent x cleaning agent found main effect of staining agent. No main effect of cleaning agent was found. No interaction between staining agent and cleaning agent was found. A Tukey Test found no significant differences between cleaning agents [Table 7 and Figure 25].

Zendura (Z)

A 2-way ANOVA of staining agent x cleaning agent found main effect of staining agent and cleaning agent as well as interaction between staining agent and cleaning agent. Within Z samples immersed in turmeric, the average ΔE_{00} values compared are as follows: P (24.96), R (24.90), I (23.38) [Table 7 and Figures 26]. It was found that P > I ($p < 0.001$) but not R ($p = 0.985$). In addition, R > I ($p < 0.001$).

P $(24.96)^a$ > R $(24.90)^a$ > I (23.38).

General Trend

Results of a 3-way ANOVA found the following: main effect of material ($p <0.001$), staining agent ($p < 0.001$), and cleaning ($p < 0.001$). In addition, interaction between all 3 variables were found ($p < 0.001 – 0.008$). On average, I cleaned more than P and R in statistically significant fashion.

Invisalign Cleaning Crystals

Comparing Materials

Comparison of Materials for Different Staining Agents

Coffee x Materials - For all specimens immersed in coffee then cleaned by Invisalign Cleaning Crystals (I), a significant difference between the materials was found. The average ΔE_{00} values compared are as follows: EC (1.00), IC (0.93), EP (0.36), IU (0.30) and Z (0.29) [Table 7 and Figures 22-26]. It was found that EC > Z ($p = 0.005$), IU ($p = 0.006$), EP ($p = 0.017$) but not IC ($p = 0.997$). Also, IC > Z ($p = 0.016$) and IU ($p = 0.016$), and EP ($p = 0.048$). No other significant differences were found. EP similar to IU and IC similar to EC. EP, IU and Z have least delta E

EC (1.00)^a > IC (0.93)^a > EP (0.36)^b > IU (0.30)^b > Z (0.29)^b

Turmeric x Materials - For all specimens immersed in turmeric then cleaned by Invisalign Cleaning Crystals (I), a significant difference between the materials was found. The average ΔE_{00} values compared are as follows: IU (27.81), EP (27.47), Z (23.38), IC (6.02), and EC (4.09) [Table 7 and Figures 22-26]. It was found that IU > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$), but not EP ($p = 0.984$). Also, EP > EC ($p < 0.001$), IC ($p < 0.001$) and Z ($p < 0.001$). Further, Z > EC ($p < 0.001$) and IC ($p < 0.001$). Lastly, IC > EC ($p < 0.001$). IU and EP had the highest delta E while EC had the lowest delta E.

IU (27.81)[a] > EP (27.47)[a] > Z (23.38) > IC (6.02) > EC (4.09)

For all specimen immersed in red wine then cleaned by Invisalign Cleaning Crystals, no significant difference between the materials was found. Similarly, for materials stained by water then cleaned by Invisalign cleaning crystals, no significant difference between the materials was found.

Comparing Staining Agents

<u>Comparison of Staining Agents for Different Materials</u>

<u>EC x Staining Agents</u> - Within EC specimens cleaned by Invisalign cleaning crystals, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (4.09), C (1.00), RW (0.28), and W (0.21) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). Also, C > W ($p < 0.001$) and RW ($p = 0.002$). Lastly, RW is not significantly different than W ($p = 0.991$).

T (4.09) > C (1.00) > RW (0.28)[a] > W (0.21)[a]

<u>EP x Staining Agents</u> - Within EP specimens cleaned by Invisalign cleaning crystals, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (27.36), RW (0.37), C (0.36) and W (0.14) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), C ($p < 0.001$) and RW ($p < 0.001$). No other significant difference between staining agents were found.

T (27.36) > RW (0.37)[a] > C (0.36)[a] > W (0.14)[a]

IC x Staining Agents - Within IC specimens cleaned by Invisalign cleaning crystals, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (6.02), C (0.93), W (0.28) and RW (0.27) [Table 7 and Figures 22-26]. It was found that T > RW ($p < 0.001$), W ($p < 0.001$) and C ($p < 0.001$). Also, C > RW ($p = 0.007$) and W ($p = 0.008$). Lastly, W is not significantly different than RW ($p = 1.000$).

T (6.02) > C (0.93) > W (0.28)[a] >RW (0.27)[a]

IU x Staining Agents - Within IU specimens cleaned by Invisalign cleaning crystals, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (27.47), C (0.30), RW (0.29) and W (0.21) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). No other differences were found.

T (27.47) > C (0.30)[a] > RW (0.29)[a] > W (0.21)[a]

Z x Staining Agents - Within Z specimens cleaned by Invisalign cleaning crystals, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (23.38), W (0.33), RW (0.33) and C (0.29) [Table 7 and Figures 22-26]. It was found that T > C ($p < 0.001$), RW ($p < 0.001$) and W ($p < 0.001$). No other differences were found.

T (23.38) > W (0.33)[a] > RW (0.33)[a] > C (0.29)[a]

Polident (P)

Comparing Materials

Comparison of Materials for Different Staining Agents

Coffee x Materials - For all specimens immersed in coffee then cleaned by Polident (P), a significant difference between the materials was found. The average ΔE_{00} values compared are as follows: EC (1.11), IC (1.02), IU (0.38), Z (0.37), EP (0.36) [Table 7 and Figures 22-26]. It was found that EC > EP (p = 0.003), Z (p = 0.003), IU (p = 0.004) but not and IC (p = 0.994). In addition, IC > EP (p = 0.012), Z (p = 0.013) and IU (p = 0.016). No other differences were found.

EC (1.11)^{a} > IC (1.02)^{a} > IU (0.38)^{b} > Z (0.37)^{b} > EP (0.36)^{b}

Turmeric x Materials - For all specimens immersed in turmeric then cleaned by Polident (P), a significant difference between the materials was found. The average ΔE_{00} values compared are as follows: IU (27.55), EP (27.46), Z (24.96), IC (6.98), EC (5.58) [Table 7 and Figures 22-26]. It was found that IU > EC, IC and Z but not EP. In addition, EP > EC, IC and Z. Further, Z > EC and IC. Lastly, IC > EC.

IU (27.55)^{a} > EP (27.46)^{a} > Z (24.96) > IC (6.98) > EC (5.58)

Red Wine and Water x Materials - For specimens stained by red wine and cleaned by Polident (RW-P), there is not a significant difference between the materials (p = 0.177). Similarly, for samples stained by water and cleaned by the P cleaner (RW-P), there is not a significant difference between the materials (p = 0.830).

Comparing Staining Agents

Comparison of Staining Agents for Different Materials

EC x Staining Agents - Within EC specimens cleaned by Polident, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (5.58), C (1.11), RW (0.37) and W (0.20) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C >W ($p < 0.001$) and RW ($p = 0.002$). Lastly, RW is not significantly different than W ($p = 0.843$).

T (5.58) > C (1.11) > RW (0.37)[a] > W (0.20)[a].

EP x Staining Agents - Within EP specimens cleaned by Polident, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (27.46), RW (0.66), C (0.36) and W (0.15) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). No other differences were found.

T (27.46) > RW (0.66)[a] > C (0.36)[a] > W (0.15)[a]

IC x Staining Agents - Within IC specimens cleaned by Polident, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (6.98), C (1.02), RW (0.58) and W (0.15) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), C ($p < 0.001$) and RW ($p < 0.001$). In addition, C > W ($p < 0.001$) but not RW ($p = 0.138$). Lastly, RW is not significantly different than W ($p = 0.160$).

T (6.98) > C (1.02)[a] > RW (0.58)[a,b] > W (0.15)[b]

IU x Staining Agents - Within IU specimens cleaned by Polident, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T

(27.55), C (0.38), RW (0.31) and W (0.26) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). There are no other significant differences.

T (27.55) > C (0.38)[a] > RW (0.31)[a] > W (0.26)[a]

<u>Z x Staining Agents</u> - Within Z specimens cleaned by Polident, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (24.96), C (0.37), W (0.36) and RW (0.23) [Table 7 and Figures 22-26]. It was found that T > RW ($p < 0.001$), W ($p < 0.001$) and C ($p < 0.001$). There are no other significant differences.

T (24.96), C (0.37)[a], W (0.36)[a] and RW (0.23)[a]

<u>*Retainer Brite (R)*</u>

Comparing Materials

<u>Coffee x Materials</u> - For all specimen immersed in coffee then cleaned by Retainer Brite (R), a significant difference between the materials was found. The average ΔE_{00} values compared are as follows: EC (1.11), IC (0.94), Z (0.74), EP (0.47), IU (0.33) [Table 7 and Figures 22-26]. It was found that EC > IU ($p < 0.002$), EP ($p = 0.018$) but not Z (0.405) nor IC ($p = 0.928$). In addition, IC > IU ($p = 0.027$) but not EP ($p = 0.158$) nor Z ($p = 0.882$). No other differences were found.

EC (1.11)[a] > IC (0.94)[a,b] > Z (0.74)[a,b] > EP (0.47)[b] > IU (0.33)[b]

<u>Turmeric x Materials</u> - For all specimens immersed in turmeric then cleaned by Retainer Brite (R), a significant difference between the materials was found. The average ΔE_{00} values compared are as follows: IU (27.81), EP (27.19), Z (24.90), IC (6.52), EC (5.94) [Table 7 and Figures 22-

26]. It was found that IU > EC ($p < 0.001$), IC ($p < 0.001$), Z ($p < 0.001$) and EP ($p = 0.023$). In addition, EP > EC ($p < 0.001$), IC ($p < 0.001$), and Z ($p < 0.001$). Further, Z > EC ($p < 0.001$) and IC ($p < 0.001$). Lastly, IC > EC ($p = 0.043$).

IU (27.81) > EP (27.19) > Z (24.90) > IC (6.52) > EC (5.94)

Red Wine and Water x Materials - For all specimens immersed in red wine then cleaned by Retainer Brite (RW-R), there is not a significant difference between the materials ($p = 0.040$). Similarly, for all specimen immersed in water and cleaned by Retainer Brite (W-R), there is not a significant difference between the materials ($p = 0.986$).

Comparing Staining Agents

<u>EC x Staining Agents</u> - Within EC specimens cleaned by Retainer Brite, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (5.94), C (1.11), RW (0.35) and W (0.23) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C > W ($p < 0.001$) and RW ($p = 0.002$). Lastly, RW is not significantly different than W ($p = 0.927$).

T (5.94) > C (1.11) > RW (0.35)[a] > W (0.23)[a]

<u>EP x Staining Agents</u> - Within EP specimens cleaned by Retainer Brite, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows d: T (27.19), RW (0.84), C (0.47) and W (0.14) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), C ($p < 0.001$) and RW ($p < 0.001$). In addition, RW > W ($p < 0.004$) but not C ($p = 0.273$). No other differences were found.

T (27.19) > RW (0.84)^{a} > C (0.47)^{a,b} > W^{b} (0.14)

<u>IC x Staining Agents</u> -Within IC specimens cleaned by Retainer Brite, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (6.52), C (0.94), RW (0.40) and W (0.17) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), RW ($p < 0.001$) and C ($p < 0.001$). In addition, C > W ($p < 0.001$) and RW ($p < 0.045$). Lastly, RW is no different than W ($p = 0.692$).

T (6.52) > C (0.94) > RW (0.40)^{a} > W (0.17)^{a}

<u>IU x Staining Agents</u> - Within IU specimens cleaned by Retainer Brite, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (27.81), RW (0.36), C (0.33) and W (0.22) [Table 7 and Figures 22-26]. It was found that T > W ($p < 0.001$), C ($p < 0.001$) and RW ($p < 0.001$). There are no other significant differences.

T (27.81) > RW (0.36)^{a} > C (0.33)^{a} > W (0.22)^{a}

<u>Z x Staining Agents</u> - Within Z specimens cleaned by Retainer Brite, a significant difference between the staining agents was found. The average ΔE_{00} values compared are as follows: T (24.90), C (0.74), W (0.25) and RW (0.25) [Table 7 and Figures 22-26]. It was found that T > RW ($p < 0.001$), W ($p < 0.001$) and C ($p < 0.001$). There are no other significant differences.

T (24.90), C (0.74)^{a}, W (0.25)^{a} > RW (0.25)^{a}

Appendix B

Three Way Analysis of Variance Results for Contrast Ratio, Gloss, and Surface Roughness

<u>**Contrast Ratio**</u> **– Material x Staining Agent x Cleaning Agent**

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Table 9: 3-Way ANOVA for Contrast Ratio - Material x Staining Agent x Cleaning Agent

Source of Variation	**DF**	**SS**	**MS**	**F**	**P-Value**
Material	4	3.528	0.815	1356.683	<0.001
Staining Agent	3	0.00659	0.00220	3.658	0.012
Cleaning Agent	2	0.00192	0.000961	1.601	0.203
Material x Staining Agent	12	0.0140	0.00116	1.940	0.028
Material x Cleaning Agent	8	0.00433	0.000541	0.901	0.516
Staining Agent x Cleaning Agent	6	0.00225	0.000375	0.624	0.711
Material x Staining Agent x Cleaning Agent	24	0.0147	0.000615	1.023	0.433
Residual	540	0.324	0.000600		
Total	599	3.626	0.00605		

Note: no main effect was found for cleaning agent (highlighted in yellow)

<u>Gloss</u> – Material x Staining Agent x Cleaning Agent

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Table 10: 3-Way ANOVA for Gloss - Material x Staining Agent x Cleaning Agent

Source of Variation	DF	SS	MS	F	P-Value
Material	4	107584.597	26896.149	400.282	<0.001
Staining Agent	3	898.960	299.653	4.460	0.004
Cleaning Agent	2	287.110	143.555	2.136	0.119
Material x Staining Agent	12	5740.459	478.372	7.119	<0.001
Material x Cleaning Agent	8	879.920	109.990	1.637	0.111
Staining Agent x Cleaning Agent	6	593.008	98.835	1.471	0.186
Material x Staining Agent x Cleaning Agent	24	1973.870	82.245	1.224	0.213
Residual	540	36284.209	67.193		
Total	599	154242.132	257.499		

Note: no main effect was found for cleaning agent (highlighted in yellow)

Surface Roughness – Material x Staining Agent x Cleaning Agent

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Table 11: 3-Way ANOVA for Surface Roughness - Material x Staining Agent x Cleaning Agent

Source of Variation	DF	SS	MS	F	P-Value
Material	4	7.603	1.766	153.388	<0.001
Staining Agent	3	0.0344	0.0115	0.996	0.394
Cleaning Agent	2	0.00879	0.00440	0.382	0.683
Material x Staining Agent	12	0.519	0.0433	3.761	<0.001
Material x Cleaning Agent	8	0.111	0.0139	1.210	0.291
Staining Agent x Cleaning Agent	6	0.0800	0.0133	1.158	0.327
Material x Staining Agent x Cleaning Agent	24	0.325	0.0136	1.177	0.256
Residual	540	6.216	0.0115		
Total	599	14.358	0.0240		

Note: no main effect was found for cleaning agent (highlighted in yellow)

Appendix C

Three Way Analysis of Variance Results for Color

<u>Color – ΔE*$_{00}$ (T$_3$-T$_1$)</u> – Material x Staining Agent x Cleaning Agent

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Table 12: 3-Way ANOVA for ΔE$_{00}$ (T$_3$-T$_1$) - Material x Staining Agent x Cleaning Agent*

Source of Variation	**DF**	**SS**	**MS**	**F**	**P-Value**
Material	4	3664.138	916.035	4288.024	<0.001
Staining Agent	3	35631.406	11877.135	55597.722	<0.001
Cleaning Agent	2	9.018	4.509	21.107	<0.001
Material x Staining Agent	12	11841.868	986.822	4619.386	<0.001
Material x Cleaning Agent	8	4.236	0.530	2.479	0.012
Staining Agent x Cleaning Agent	6	14.712	2.452	11.478	<0.001
Material x Staining Agent x Cleaning Agent	24	16.040	0.668	3.129	<0.001
Residual	540	115.358	0.214		
Total	599	51296.777	85.637		

Appendix D

Two Way Analysis of Variance Results for Contrast Ratio, Gloss, Surface Roughness, and Color

<u>Contrast Ratio</u> – Material x Staining Agent

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Table 13: 2-Way ANOVA for Contrast Ratio - Material x Staining Agent

Source of Variation	DF	SS	MS	F	P-Value
Material	4	3.718	0.930	1466.734	<0.001
Staining Agent	3	0.0144	0.00479	7.558	<0.001
Material x Staining Agent	12	0.0128	0.00107	1.683	0.067
Residual	580	0.368	0.000634		
Total	599	4.113	0.00687		

<u>Gloss</u> – Material x Staining Agent

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Table 14: 2-Way ANOVA for Gloss - Material x Staining Agent

Source of Variation	DF	SS	MS	F	P-Value
Material	4	99572.887	24893.222	343.207	<0.001
Staining Agent	3	974.640	324.880	4.479	0.004
Material x Staining Agent	12	8200.566	683.380	9.422	<0.001
Residual	580	42068.090	72.531		
Total	599	14.358	0.0240		

Surface Roughness – Material x Staining Agent

Normality Test (Shapiro-Wilk): Failed ($P < 0.050$)

Equal Variance Test (Brown-Forsythe): Failed ($P < 0.050$)

Table 15: 2-Way ANOVA for Surface Roughness - Material x Staining Agent

Source of Variation	DF	SS	MS	F	P-Value
Material	4	6.857	1.714	150.904	<0.001
Staining Agent	3	0.00657	0.00219	0.193	0.901
Material x Staining Agent	12	0.622	0.0518	4.562	<0.001
Residual	580	6.589	0.0114		
Total	599	14.074	0.0235		

Color – ΔE^*_{00} (T_2-T_1) – Material x Staining Agent

Normality Test (Shapiro-Wilk): Failed ($P < 0.050$)

Equal Variance Test (Brown-Forsythe): Failed ($P < 0.050$)

Table 16: 2-Way ANOVA for ΔE^*_{00} (T_2-T_1) - Material x Staining Agent

Source of Variation	DF	SS	MS	F	P-Value
Material	4	2777.182	694.295	1964.726	<0.001
Staining Agent	3	39846.840	13282.280	37586.365	<0.001
Material x Staining Agent	12	8990.525	749.210	2120.125	<0.001
Residual	580	204.961	0.353		
Total	599	51819.507	86.510		

Appendix E

Two Way Analysis of Variance Results for Color

Color – ΔE^*_{00} (T_3-T_1) – Staining Agent x Cleaning Agent; <u>EC Material</u>

Normality Test (Shapiro-Wilk): Failed ($P < 0.050$)

Equal Variance Test (Brown-Forsythe): Failed ($P < 0.050$)

Table 17: 2-Way ANOVA for ΔE^*_{00} (T_3-T_1) - Staining Agent x Cleaning Agent; EC

Source of Variation	DF	SS	MS	F	P-Value
Staining Agent	3	502.587	167.529	1284.194	<0.001
Cleaning Agent	2	5.892	2.946	22.581	<0.001
Staining Agent x Cleaning Agent	6	13.336	2.223	17.038	<0.001
Residual	108	14.089	0.130		
Total	119	535.904	4.503		

Color – ΔE^*_{00} (T_3-T_1) – Staining Agent x Cleaning Agent; <u>EP Material</u>

Normality Test (Shapiro-Wilk): Failed ($P < 0.050$)

Equal Variance Test (Brown-Forsythe): Failed ($P < 0.050$)

Table 18: 2-Way ANOVA for ΔE^*_{00} (T_3-T_1) - Staining Agent x Cleaning Agent; EP

Source of Variation	DF	SS	MS	F	P-Value
Staining Agent	3	16341.613	5447.204	41195.411	<0.001
Cleaning Agent	2	0.288	0.144	1.088	0.341
Staining Agent x Cleaning Agent	6	1.323	0.220	1.668	0.136
Residual	108	14.281	0.132		
Total	119	16357.504	137.458		

Color – ΔE^*_{00} (T_3-T_1) – Staining Agent x Cleaning Agent; <u>IC Material</u>

Normality Test (Shapiro-Wilk): Failed ($P < 0.050$)

Equal Variance Test (Brown-Forsythe): Failed ($P < 0.050$)

Table 19: 2-Way ANOVA for ΔE^*_{00} (T_3-T_1) - Staining Agent x Cleaning Agent; IC

Source of Variation	DF	SS	MS	F	P-Value
Staining Agent	3	813.061	271.020	2068.305	<0.001
Cleaning Agent	2	1.941	0.970	7.405	<0.001
Staining Agent x Cleaning Agent	6	3.302	0.550	4.200	<0.001
Residual	108	14.152	0.131		
Total	119	832.456	6.995		

Color – ΔE^*_{00} (T_3-T_1) – Staining Agent x Cleaning Agent; <u>IU Material</u>

Normality Test (Shapiro-Wilk): Failed ($P < 0.050$)

Equal Variance Test (Brown-Forsythe): Failed ($P < 0.050$)

Table 20: 2-Way ANOVA for ΔE^*_{00} (T_3-T_1) - Staining Agent x Cleaning Agent; IU

Source of Variation	DF	SS	MS	F	P-Value
Staining Agent	3	16787.250	5595.750	53190.884	<0.001
Cleaning Agent	2	0.252	0.126	1.199	0.305
Staining Agent x Cleaning Agent	6	0.454	0.0757	0.719	0.635
Residual	108	11.362	0.105		
Total	119	16799.318	141.171		

Color – ΔE^*_{00} (T_3-T_1) – Staining Agent x Cleaning Agent; Z Material

Normality Test (Shapiro-Wilk): Failed (P < 0.050)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Table 21: 2-Way ANOVA for ΔE^*_{00} (T_3-T_1) - Staining Agent x Cleaning Agent; Z

Source of Variation	DF	SS	MS	F	P-Value
Staining Agent	3	13028.763	4342.921	7629.706	<0.001
Cleaning Agent	2	4.882	2.441	4.288	0.016
Staining Agent x Cleaning Agent	6	12.337	2.056	3.612	0.003
Residual	108	61.475	0.569		
Total	119	13107.457	110.147		