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In-vitro beverage discoloration, stain removal and tooth-brushing abrasion of crown and bridge provisional materials

BOSTON UNIVERSITY

HENRY M. GOLDMAN SCHOOL OF DENTAL MEDICINE

THESIS

IN-VITRO BEVERAGE DISCOLORATION, STAIN REMOVAL AND TOOTH-BRUSHING ABRASION OF CROWN AND BRIDGE PROVISIONAL MATERIALS.

by

JUSTINE OLIVEROS SOLES

DDS, Universidad Santa Maria, 2011.

Submitted in partial fulfillment of the requirements for the degree of

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In the Department of Restorative Sciences and Biomaterials

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Approved by:

First Reader: ______________________________

Dr. Dan Nathanson, DMD, MSD. Professor and Chair, Department of Restorative Sciences and Biomaterials.

Second Reader: ______________________________

Dr. Yuwei Fan, MSc, PhD. Research Assistant Professor, Department of Restorative Sciences and Biomaterials.

Third Reader: ______________________________

Dr. Mohammad Hossein Dashti, DMD. Clinical Associate Professor, Department of Restorative Sciences and Biomaterials.

Approved by:

Department Chairman:

____

Dan Nathanson, D.M.D., M.S.D.

Professor and Chairman

Department of Restorative Sciences and Biomaterials

DEDICATION

Dedicated to my parents,

Emma Soles and Gabriel Oliveros.

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JUSTINE OLIVEROS SOLES

Boston University, Goldman School of Dental Medicine, 2017

Major Professor: Dr. Dan Nathanson, Professor and Chair, Department of Restorative Sciences and Biomaterials

ABSTRACT

Purpose

To determine the potential discoloration of provisional materials by exposure to beverages and evaluate the efficacy of simulated tooth-brushing on stain removal and the effect on surface roughness.

Methods

Materials included: Jet Set-4 (Lang), Protemp Plus (3M ESPE), Luxatemp (DMG), Artbloc (Merz), Telio-CAD (Ivoclar), and Vita-CAD (Vita).
Specimens (n=10/group) were immersed in: distilled water, coffee, red wine, tea, coke and cranberry juice. Color measurements were taken (Xritei5 spectrophotometer) at: baseline, 24 hours, 4 and 8 weeks. Specimens in coffee and red wine for 8 weeks were exposed to tooth-brushing for three minutes and color

measurements were taken subsequently. Color differences (ΔE) after treatments were calculated using one-way ANOVA, MANOVA and post hoc Tukey test.
Another group of specimens (n=10/group) were exposed to tooth-brushing under a 1.91N load using toothpaste slurry (Crest® Cavity Protection) for 20,000 cycles in two modes: soft and medium bristles (both Oral B Indicator®). Surface roughness was measured using a Mitutoyo SJ201 profilometer before and after brushing. Surface roughness R_a values were compared using one-way ANOVA and post hoc Tukey test.

Results

Coke and water had no significant discoloration effect ($p>0.05$). Red wine and coffee exhibited the highest discoloration effect. CAD-CAM blocks showed significantly lower color change, at all durations, and after brushing ($p<0.001$).

Tooth-brushing had a significant effect (R_a) on Telio-CAD, Artbloc, Jet Set-4 and Vita-CAD. Protemp-Plus and Luxatemp groups showed no significant difference.

Conclusions

Traditional materials showed less color stability when compared to CAD/CAM blocks. Tooth-brushing effect varies depending on bristle and material type.

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LIST OF ABBREVIATIONS

Δ: Greek letter delta representing "difference of"

ADA: American Dental Association

ANOVA: Analysis of variance

CAD/CAM: Computer-aided design and computer-aided manufacturing

CIELAB: International Commission on Illumination color space

HSD: Honest significant difference

ISO: International organization for standardization

LS mean: Least square means

MANOVA: Multivariate analysis of variance

SD: Standard deviation

CHAPTER 1: INTRODUCTION

Provisional fixed restorations play an important role in the prosthetic treatment. They are useful for diagnostic purposes, protect the remaining teeth, prevent leakage (Sham et al. 2004), provide comfort and function, maintain positional stability and occlusion (Akova, Ozkomur, and Uysal 2006), improve esthetics and help identify a positive outcome or limitations before the completion of the treatment (Bayindir, Kürklü, and Yanikoğlu 2012).

There are several materials available for fabricating provisional restorations, polyethyl methacrylate, polymethyl methacrylate, urethane methacrylate, polyvinil methacrylate, bis-GMA and bis-acrylics. (Christensen 2003) (Bayindir, Kürklü, and Yanikoğlu 2012).

Occasionally, depending on the complexity of the treatment plan, provisional restorations are necessary for long periods of time and they have to provide protection and stability for extended intervals while the final treatment is accomplished. That is why is so important to maintain color stability over the course of provisional treatment, discoloration can produce serious esthetic complications (Yannikakis et al. 1998), that may lead to patient discomfort and replacement of the material (Burns, Beck, and Nelson 2003). Hence, stainability is a very important criterion to evaluate on provisional materials for esthetic areas and choosing the right restorative material is crucial for the success of the treatment (Bayindir, Kürklü, and Yanikoğlu 2012).

When a material is exposed for a long term in the oral cavity, the esthetical and functional aspect is subjected inevitably to degradation, accumulation of plaque which leads to gingival inflammation, loss of gloss, staining and discoloration and ultimately fracture. Another important factor is that restorative materials are exposed to thermal stress, due to eating and drinking (Hiroyuki et al. 2007).

Discoloration of a restorative material may occur by intrinsic and extrinsic factors (Ren et al. 2012). The intrinsic factors affect the discoloration of the material itself, by degrading the bonding between the fillers and the resin matrix or by altering the matrix, meaning that the resin matrix plays an important role on the material stainability. Extrinsic factors may occur by a contamination of exogenous sources, such as dietary common colorants (Hiroyuki et al. 2007)(Ren et al. 2012), that cause staining by adsorption and absorption of liquids that is relative to the environmental conditions (Burns, Beck, and Nelson 2003). Several studies had found that the staining susceptibility of different materials are affected depending of the composition of the resin, type of colorants or the polishing methods used after fabrication of the restoration (Hiroyuki et al. 2007). Other factors also can affect the degree of discoloration, such as, chemical reactivity, oral hygiene (Asmussen and Hansen 1986), pH variations, surface smoothness (Bayindir, Kürklü, and Yanikoğlu 2012a), water sorption (Cho, Yi, and Heo 2002), incomplete polymerization and thermal stress (Ren et al. 2012).

Another important aspect that needs to be taken into consideration is that the surfaces of these materials are also exposed to different factors that may affect their quality. Oral hygiene is one of the main factors, daily tooth brushing with dentifrice may negatively affect the surface roughness of these restorative materials (Heintze et al. 2010), meaning that even polished restorative materials may be affected by subsequent home care (Goldstein and Lerner 1991). The presence of irregularities is of serious concern, it is undesirable due to esthetic and biologic disadvantages, because it influences directly on the appearance, leading to surface discoloration, attracting dental plaque causing gingival inflammation, hence, increasing the risk of decay and periodontal disease (Lepri and Palma-Dibb 2012). *In vitro* studies have been used to simulate the effects of some of these factors on provisional restorative materials and even though they do not replicate exactly the oral cavity conditions it allows us to understand the response of the materials exposed to these conditions (Doray, Li, and Powers 2001).

1.1 COLOR DIFFERENCES:

The capacity of a material to be color stable is a desirable characteristic to be able to determine success of a restoration on the long term. Being able to maintain the color throughout the course of the treatment is key, and this characteristic varies among different restorative materials.

The perception of color has been subject of intense debate for years, and it could be considered a subjective analysis when it relies on human evaluation. However, with the advances in technology regarding computerized color systems and with the control of artificial light sources, an objective way to analyze color is now available. With the aid of software packages, color can be interpreted into formulas with mathematical calculations.

The spectrophotometers provide readings from Commission Internationale de l'Eclairage (CIE) L*, a*, b* color space, where L* ranges between one hundred and zero, representing the amount of white and black within a color), a* and b* do not have any numerical value, positive a* represents red and negative a* represents green, as for positive b* represents yellow and negative b* represents blue (Johnston 2009) (Figure 1). This color system is widely used in research involving *in vivo* and *in vitro* experiments. (Schanda, 2007) (CIE Central Bureau , 2004).

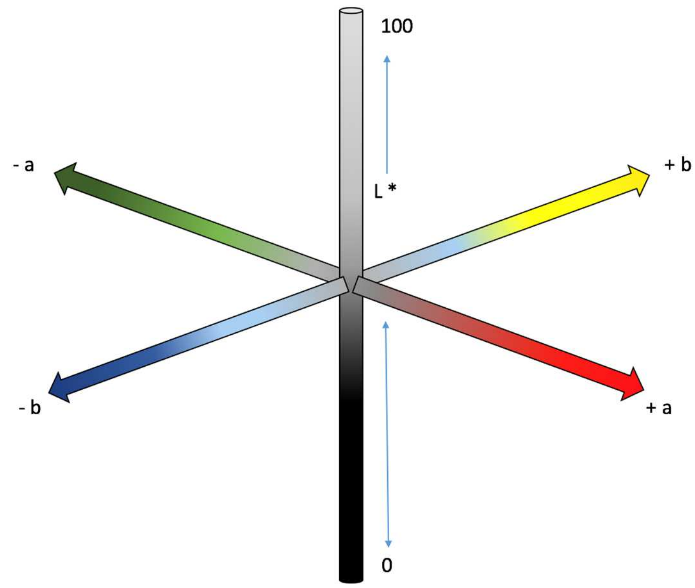


Figure 1: CIELAB color space diagram.

The L* axis goes from top to bottom. The maximum of L* is 100, re9presenting white and the minimum is 0, representing black. The a* positive represents red and a* negative represents green. The positive b* represents blue and the b* negative represents yellow.

One of the most important parameters taken into consideration in color research is the Eucledian distance between two color points (ΔE), used to determine color differences (Vichi et al. 2012). In dentistry the Delta E* is used widely to establish clinical perceptibility thresholds (Johnston 2009) by comparing the capacity of the human eye to perceive the difference in color present and when is it really clinically relevant.

The first color difference formula that related a measurement to a known set of CIE L* a* b* coordinates was the 1976 formula:

$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$

The human eye has a limited perception when it comes to color differences.

There has been a debate in dental literature about acceptability and perceptibility thresholds of color differences. Ruyter el at established that the clinical acceptability threshold is 3.3 (1987), this means that any color difference below 3.3 is clinically acceptable and it cannot be perceived by the human eye. For example, 50% of the observers will accept a restoration with a Delta E* value lower than 3.3 as compared to the adjacent tooth and reject it when the Delta E* is higher than 3.4 (Ragain and Johnston 2000).

1.2 DISCOLORATION OF DENTAL MATERIALS:

Several studies have used different solutions such as beverages, oral rinses, cleaning products, to examine the color stability of different provisional restorative materials. The solution employed is an important criterion to take into consideration because they contain different types of dyes (natural or artificial), pH, different consistencies and depending on the characteristics that they present, one solution could be more prone to discolor a surface more than others.

In the present study there is a comparison between the staining effect of conventional materials and CAD/CAM blocks, because there is not enough information found in the literature regarding these blocks intended for provisional restorations. From the conventional materials, the most common ones are the acrylic-based and bis-acrylics. The composition and fabrication method may vary, hence, the staining characteristic on each material is different as well. There is a major difference in composition between acrylic-based and bis-acrylics, the latter being more heterogeneous as compared to the the acrylic based that are known to be more homogeneous. Meaning that acrylic-based have exhibited less discoloration than bis-acrylics, due to the oxidation of unreacted double bonds present on these (Turgut et al. 2013).

Besides the composition of the materials another important factor is the fabrication method, the downside of the powder-liquid presentation is that the mixing technique may affect the integrity of the restoration because if a poor technique is employed it may cause a more porous restoration. Factor that is eliminated on the injectable bis-acrylics with a controlled dispensing syringe, providing the accurate ratio of activator and catalyst.

1.3 SURFACE ROUGHNESS:

The parameters that are most commonly used to evaluate the surface characteristics of dental materials are gloss, scanning electron microscope images and surface roughness (R_a). The latter is based on the depth of the scratches present of a surface, it involves the arithmetic average in a two dimensional measurement by providing a general description of the height variations in surface, to quantitatively describe the surface roughness but it does not describe the appearance of the surface (Kamonkhantikul et al. 2016).

The instrument used in several *in vitro* studies to measure the surface abrasivity is the profilometer. It is a device to measure fluctuation of surface profile, by providing roughness average (R_a) values for each profile. The profilometer uses a digital and analogue hardware by producing a tracing and it calculates the roughness values for the resultant tracing (Kumar et al. 2015). According to Kamonkhantikul et al the R_a value of surface roughness of 0.2 μm was claimed to be threshold to prevent plaque accumulation and staining (2016) and the average surface roughness of enamel is 0.64 μm (Bollen, Lambrechts, and Quirynen 1997).

1.4 TOOTHPASTE AND TOOTH BRUSHING:

In order to achieve optimum conditions in the oral cavity, to reduce plaque, caries and periodontal disease, good oral hygiene must be performed. Tooth pastes aid to improve oral hygiene by abrasive and antiseptic action, depending on the active ingredients they contain, mainly to reduce caries by fluoride action. It replaces the hydroxyapatite of tooth with fluoroapatite which is not only much stronger but also resistant to caries.

Components of toothpaste:

- *Abrasives:*

Abrasives have the main purpose of removing the stained pellicle from teeth and removal of plaque, they work in combination with the toothbrush and do not have the capability to cause an effect on their own. Some abrasives used in toothpastes are hydrated alumina, silica compounds, calcium carbonate and dicalcium phosphates. It is very important that these abrasives do not damage the tooth structure while executing their function, they should be nearly inert chemically. Few factors that influence the abrasivity are the particle size and shape, hydration, purity, source and physical or chemical treatment.

- *Detergents:*

These are added for cleaning and the mechanism of action is by lowering the surface tension to solubilize substances, to allow penetration and to loosen the surface deposits, causing foaming action. It should be mild so that the soft mucosa is not irritated. The most common detergent used in dentifrices is sodium lauryl sulfate.

- *Humectant systems:*

Humectants are key to maintain the the consistency of the tooth paste, so that other ingredients can be incorporated, keeping the moisture and to prevent microbial growth. The most common humectants used are polyols.

- *Flavoring agents:*

Natural or artificial flavoring agents are added to tooth paste, they do not contain sugar but some contain artificial sweeteners.

1.4.1 Effects of tooth brushing:

The surface of a restoration should be polished and smooth to prevent, plaque accumulation, bacterial growth and staining. However, the polished surfaces get deteriorated in the oral cavity by the effect of different factors, which include tooth brushing since it is an example of a three body wear (tooth brush bristles being the antagonist and the tooth paste the medium) (Kamonkhantikul et al. 2016). Several studies have shown the effect of tooth brushing on the gloss and surface roughness of composite resins, different variables were tested such as brushing force, number of cycles, and abrasivity of the particles contained in tooth paste. However, there is not enough information regarding the effect of tooth brushing on conventional and CAD/CAM provisional crown and bridge restorations.

Once the surface of the material gets rough after tooth brushing, it may lead to discoloration of the material by retention of the stains, hence, the quality of the restoration is affected.

An important variable to take into consideration is the design of the tooth brush and its bristle types. These are categorized in three different types according to the diameter of the bristles: soft (0.2mm), medium (0.3mm) and hard (0.4mm). Also, the bristles can be fabricated of different materials, such as nylon or polyester. They have different lengths, compactness and the geometry of the tip can vary depending on its main indication.

According to Carvalho et al the misuse of the tooth brush may lead to harmful effects on dentition, it has been proven that hard bristles tooth brush heads cause more abrasion that soft bristles (2007). On the contrary, other studies have shown that soft bristles tooth brush heads lead to more abrasion than hard bristles (Dyer et al, 2000). The reason behind this rationale is that due to the flexibility of the soft bristles, they retain more tooth paste than hard bristles and they cover a larger surface area, causing more abrasive effect on the dentition. Even though this is known, it is still unclear how abrasion varies depending on the bristle type and the role of tooth paste in this whole process, and which combination it is the ideal to cause less harm.

A way of simulating a normal oral condition is by mechanical tooth brushing, by providing a stable applied force, same stroke distance and frequency of the specimens. One effect that has been also noticed is the polishing effect of tooth brushing, which is the opposite of surface roughening, it all depends on the interaction between the type of surface that is abraded, the medium and the characteristics of the tooth brush head employed.

In this study, tooth brushing was simulated for up to 2 years, with two different types of tooth brush bristles (soft and medium). In normal clinical conditions, 30 brushing cycles happen per day approximately, meaning that 10,000 cycles per year are applied. Several studies have shown that simulated tooth brushing is an adequate method to evaluate the effects of different types of dentifrices and tooth brush heads, and that the results vary also depending on the type of material tested.

1.5 STATEMENT OF THE PROBLEM:

Other investigators have used different types of solutions over several time intervals to compare the effects on color stability of provisional and resin composites (Bayindir et al, 2012; Guler et al, 2005; Jalali et al, 2012) but there is not enough literature about the effect of simulated tooth brushing after the exposure to these solutions in terms of stain removal.

Also even though many other comparative studies exist showing tooth brushing abrasion of different types of restorative materials, such as composites and ceramics (Flury et al, 2017; Jain et al, 2013), limited information is available concerning the effect on surface roughness of contemporary provisional restorative materials.

1.6 PURPOSE:

The purpose of this study is to determine the effect of beverage discoloration on color stability, to evaluate the stain removal performance and abrasion of tooth brushing of provisional restorative materials for crowns and bridges.

1.7 OBJECTIVES:

- Evaluate the potential staining of provisional restorative materials for crowns and bridges upon exposure to various beverages.
- Evaluate the effect of tooth brushing on stain removal after the immersion in beverages of the provisional restorative materials for crowns and bridges.
- Evaluate the effect of tooth brushing abrasion on the surface roughness of the provisional restorative materials for crowns and bridges.

CHAPTER 2: MATERIALS AND METHODS

2. 1 MATERIALS USED IN THIS STUDY:

2.1.1 Provisional materials used in this study:

Six provisional crowns and bridges restorative materials of different chemistries were included: a conventional powder-liquid self-curing acrylic: Jet Set-4 (Lang); two injectable bis-acrylic materials: Protemp Plus (3M ESPE), and Luxatemp (DMG); three CAD/CAM blocks: Artbloc Temp (Merz), Telio-CAD (Ivoclar), and Vita-CAD Temp (Vita) (Figure 2). The clinical advantages and disadvantages and chemical compositions are shown in Tables 1 and 2 below.

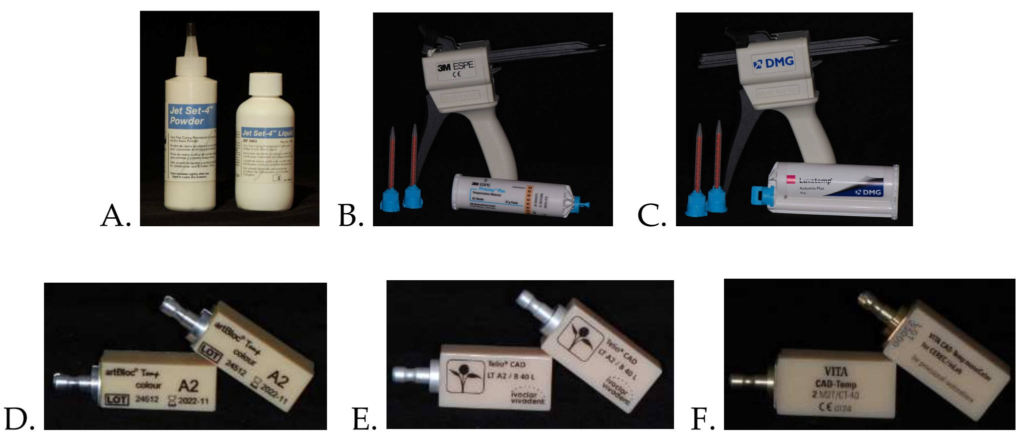


Figure 2: Provisional materials used in this study.

A. Jet Set-4 (Lang); B. Protemp Plus (3M ESPE); C. Luxatemp (DMG);
D. Artbloc Temp (Merz); E. Telio CAD (Ivoclar); F. Vita CAD-Temp (Vita).

Table 1: Types of provisional materials, advantages and disadvantages.

Material Type	Material Tested	Clinical Advantages	Clinical Disadvantages
Methyl Methacrylate	Jet Set 4 (Lang) Lot numbers: 3820 (Powder) 3803 (Liquid)	Color stability and esthetics. Good marginal adaptation. Capable of high polish. Relatively inexpensive. Easily repaired.	Exothermic polymerization. Polymerization shrinkage. Poor wear resistance. Pulpal irritation associated with excess monomer.
Bis-acrylic	Protemp Plus (3M ESPE) Lot number: 542731 Luxatemp (DMG) Lot number: 709066	Good surface hardness. Good transverse strength. Easy to use. Low exothermic reaction. Low polymerization shrinkage. Good marginal fit. Good abrasion resistance. Minimal pulpal irritation.	Limited shades. Expensive. Brittle. Alteration and repair are difficult. Poor stain resistance. Poor color stability.

Material Type	Material Tested	Clinical Advantages	Clinical Disadvantages
Cross-linked PMMA	Artbloc Temp (Merz) Lot numbers: 24512 50914 Telio CAD (Ivoclar) Lot number: 538569 Vita-CAD temp (Vita) Lot numbers: 33000 38180	Process reliability. Reduce mixing errors and air entrapments. Homogenity. No MMA irritation. No thermal irritation and marginal gingiva. No polymerization shrinkage. No undercuts.	Limited Shades. Expensive.

Table 2: Materials tested and their composition.

Resin Group	Type	Materials tested	Composition
Methyl Methacrylate	Chemically activated (Powder/liquid)	Jet Set-4 (Lang)	Powder: Polymer < 99% Diethyl Phthalate <22% Liquid: Methyl Methacrylate > 95% N-dimethyl-p-toluidine.

Resin Group	Type	Materials tested	Composition
Bis-acryl	Chemically activated (Injectable)	Protemp Plus (3M ESPE)	Base paste: Dimethacrylate 50-60% Silane treated Amorphous silica 20-30% Polyurethane methacrylate 10-20% Silane treated silica 5-10. Catalyst paste: Ethanol 2,2 70-80% Diacetate Benzyl-phenyl-Barbituric acid <10% Silane- treated silica < 10%
Bis-acryl	Dual Cure (Injectable)	Luxatemp (DMG)	Base Paste: Acrylic resin glass powder silica. Catalyst paste: Urethane dimethacrylate Aromatic dimethacrylate Glycol methacrylate.
Cross-linked PMMA	CAD/CAM block	Artbloc (Merz)	Polymethylmethacrylate
Cross-linked PMMA	CAD/CAM block	Telio CAD (Ivoclar)	Polymethylmethacrylate > 98%
Cross-linked PMMA	CAD/CAM block	Vita CAD Temp (Vita)	Modified polymethylmethacrylates, SiO2 and pigments.

2.1.2 Beverages used in this study:

Six different beverages were used in this study: coffee (Nescafé Clásico®, dark roast), iced tea (Lipton® Peach iced tea), cranberry juice (Ocean Spray® 100% Juice), soda (Coca-Cola®), red wine (Frontera® Chile, Merlot) and distilled water as control.

2.1.3 Tooth brushes used in this study:

Soft bristles and medium bristles tooth brushes were selected for this study (both Oral B Indicator®).

2.1.4 Toothpaste used in this study:

One toothpaste was selected for this study: Crest® Cavity Protection. Toothpaste slurry was fabricated 1:1 ratio using a magnetic stir. Chemical composition shown on Table 3 below.

Table 3: Chemical composition of Crest® Cavity Protection toothpaste.

Chemical name	Weight %
Silica gel, precipitated, cryst.-free	15-20
Sulfuric acid monododecyl ester sodium salt (1:1)	1-5
Sodium fluoride	0.1-1.0

2.2 METHODOLOGY:

2.2.1 Part I: Beverage discoloration and stain removal performance of tooth brushing

Specimen Preparation

Ten specimens of each provisional restorative material were fabricated for each beverage group in two different ways. Injected and powder-liquid materials were fabricated using custom-made silicon molds and CAD/CAM blocks were sectioned into tiles using the Isomet 2000 Precision Diamond Saw (Figure 3). All specimens measured 15.5mm x 19 mm x 4mm. For the purpose of standardization, after the specimens were cut, they were polished starting with graded diamond grits 45 μm and 15 μm in size with water and further polishing with 6 μm and 1 μm polycrystalline diamond suspensions applied to special pads using the Buehler grinding-polishing system (Ecomet 250, Buehler Ltd, IL, USA). The polished specimens were stored in distilled water at 37 ^{o}C for 24 hours.

Figure 3: Precision saw used to cut CAD/CAM blocks into identical sized tiles.

Baseline Color Measurements

Color of the polished specimens was assessed using a spectrophotometer (Color i5, GretagMacbeth, Regansdorf, Switzerland) before immersion, using CIE L*a*b* values, against a 50 % gray background.

The measurement mode properties were the following:

- R/T mode: Reflectance.
- Specular condition: Included.
- Extended measurements: normal (single mode).
- UV filter Pos/UV energy: UV D65 Cal/UV Cal

- Port plate aperture/lens: Port MAV (10mm).
- Lens: Lens=Port

After selecting the reflectance mode, the spectrophotometer was calibrated following the manufacturer instructions. First the calibration process was launched from the software interface. The calibration tile was presented and removed to prepare for the black trap. Once the calibration process was completed, the calibrated LED became lit, indicating a green light for calibrated and a red light when it needs calibration.

Preparation of the beverages and immersion of the specimens

Ten specimens of each provisional material were immersed on each of six different beverages: Distilled water, coffee, red wine, tea, coke and cranberry juice. Distilled water was used as control group.

To prepare the coffee solution, 20 g of coffee was poured in 1000 mL of boiled distilled water and filtered through a filter paper. The other solutions were red wine, tea, coke and cranberry juice whereby 1000ml of each was poured into a jar. A rubber base was fabricated in a way to place the specimens inside the jar and to be exposed equally to the beverage on both sides (Figure 4).

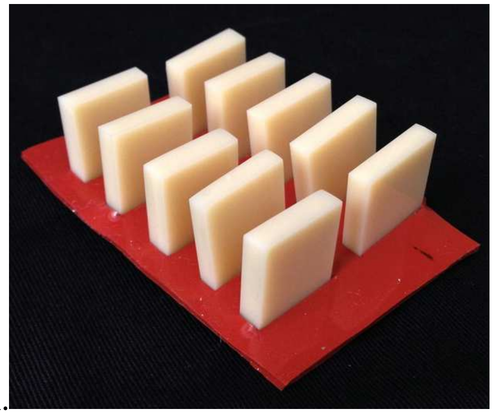
A.  B. 

Figure 4: Specimens holder and container.
A. Rubber base specimen holder. B. Specimens inside the jar before the beverage was poured.

All the specimens were placed into the sealed jars with the beverages and kept in the incubator (Precision Economy Incubator, Precision Scientific) at 37 ^{o}C. (Figure 5).

Figure 5: Specimens immersed in different beverages in sealed jars at the incubator at 37 ^{o}C.

Solutions were refreshed once every three days and were stirred once a day using a magnetic stirrer to reduce the precipitation of particles in solutions.

Color measurements after immersion

Color measurements were taken after 24 hours, 4 weeks and 8 weeks. Before each measurement, specimens were removed from the beverage and rinsed in distilled water, excess water was removed with tissue paper and specimens were allowed to dry. Color measurements were assessed using the spectrophotometer (Color i5, GretagMacbeth, Regansdorf, Switzerland) using CIE L*a*b* values, against a 50% gray background.

Simulated tooth brushing

After the color measurements on week 8 were done, the specimens that presented the highest level of discoloration (Coffee and Red wine groups) were thoroughly cleaned for three minutes in an automatic tooth brushing machine (Figure 6) using toothbrush heads with medium-grade bristles (Oral B Indicator®) and a slurry composed of a 1:1 ratio of deionized water and dentifrice (Crest® Cavity Protection).

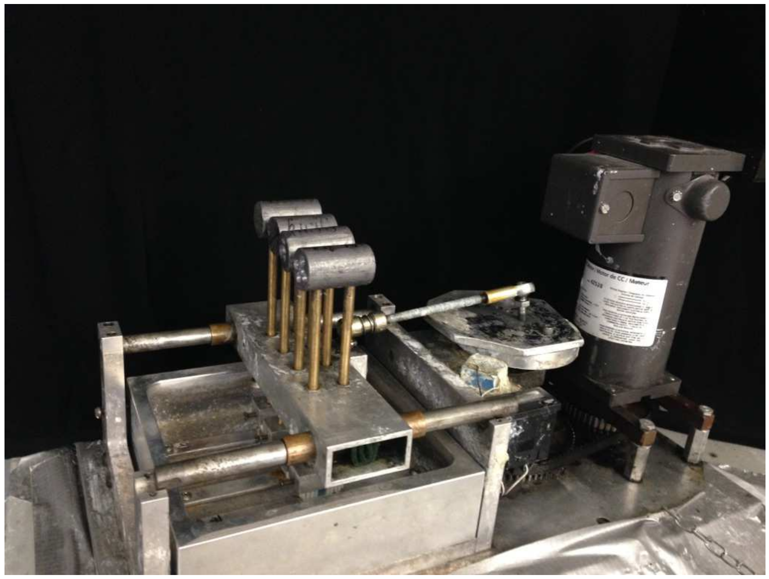


Figure 6: Simulated tooth brushing machine.

The tooth brushing machine used in this study simulates a horizontal linear movement. This machine is comprised of two main parts: upper and lower.

The upper compartment is responsible for holding the toothbrush heads. The toothbrushes were cut 1 cm from the head and were mounted parallel to each other into metal rods held by this compartment and loaded 194 gram weight to represent the average *in vivo* brushing force of 1.9 N (Figure 7). The rods carrying the toothbrush heads were joined to the motor by an arm and a wheel designed to provide controlled-amplitude horizontal cyclic movements, allowing the brushes to move linearly to provide a 100 mm span at a speed of approximately 120 brush strokes per minute for three minutes in total.

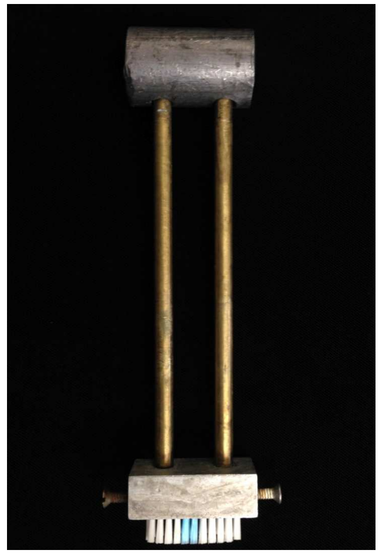


Figure 7: Toothbrush head mounted into metal rods with a load of 194 grams.

The lower compartment was designed to hold the metal plates carrying the different materials (Figure 8). This lower compartment was designed to contain toothpaste slurry 1:1 ratio covering all the specimens at all times during the test.

Figure 8: Lower compartment of simulated tooth brushing machine, holding the specimens in bars attached with sticky wax.

Color measurements after tooth brushing

The cleaned specimens after simulated tooth brushing were rinsed with distilled water, blotted dry and color measurements were taken again using the spectrophotometer (Color i5, GretagMacbeth, Regansdorf, Switzerland) using CIE L*a*b* values, against a gray background.

Data Analysis

All data was exported and compiled in a Microsoft Excel file and the following equation was used to determine changes in shade color of the specimens at the different timelines (baseline, 24 hours, 4 weeks, 8 weeks and after tooth brushing):

$$\Delta E^*_{ab}=\sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

One-way analysis of variance (ANOVA) and multivariate analysis (MANOVA) was performed on the data set as a whole. Tukey Kramer-honest significant difference (HSD) was also performed for comparisons for all pairs using JMP Statistical Discovery from SAS software, version 13.0.0.

Summary of the methodology is shown in Figure 9 below.

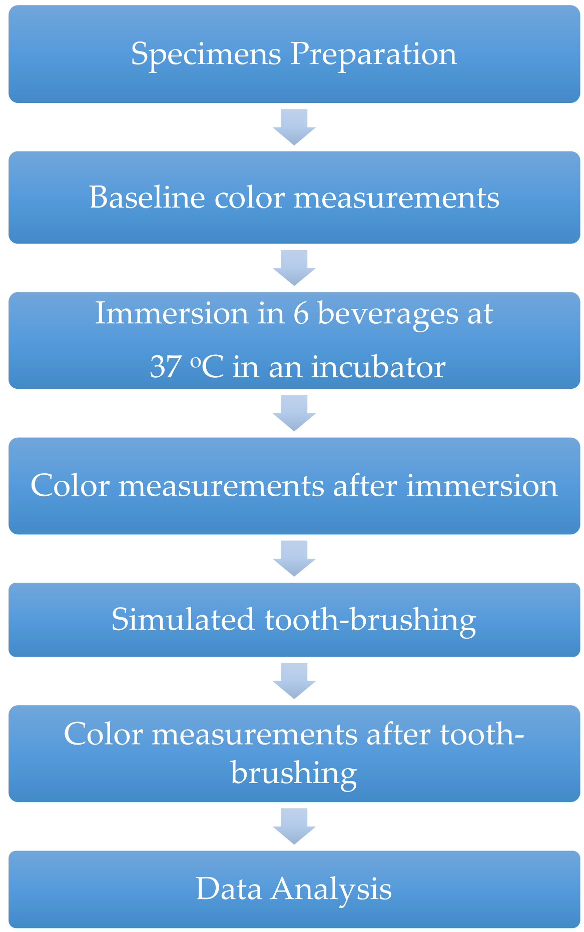


Figure 9: Part I methodology in summary.

Specimens were prepared, sectioned and polished, then they were cleaned and dried before taking baseline measurements using the Xrite i5 Spectrophotometer and CIE L*a*b* values against a gray background. Specimens were then immersed in six different beverages (water, coffee, red wine, cranberry juice and iced tea) for 8 weeks at 37 ^{0}C in an incubator. Color measurements were taken at 24 hours, 4 weeks and 8 weeks. Specimens that presented the highest level of discoloration (Coffee and Red Wine groups) were subjected to simulated tooth brushing for three minutes and color measurements were taken again subsequently. Data was then analyzed for statistical significance.

2.2.2 *Part II: Simulated tooth brushing and surface roughness.*

Specimen preparation

Ten specimens of each provisional restorative material were fabricated for each group (Soft bristles and Medium Bristles), in two different ways. Injected and powder-liquid materials were fabricated using custom-made silicon molds and CAD/CAM blocks were sectioned into tiles using the Isomet 2000 Precision Diamond Saw. All specimens measured 15.5mm x 19 mm x 2mm. (Figure 10).

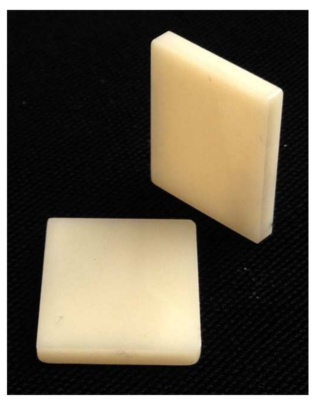


Figure 10: Telio CAD specimens after sectioning and polishing.

For the purpose of standardization, after the specimens were cut, they were polished starting with diamond grinding disc grits 45 μm and 15 μm in size with water and further polishing with 6 μm and 1 μm polycrystalline diamond suspensions applied to special pads using the Buehler grinding-polishing system

(Ecomet 250, Buehler Ltd, IL, USA). The polished specimens were stored in distilled water at 37 ^{0}C for 24 hours.

Baseline surface roughness measurements

Surface roughness (R_a) of each material was measured using a Mitutoyo SJ201 profilometer (Figure 11). The specimens were placed with the stylus contacting the polished surfaces of the specimens with the detector parallel to each polished surface. The measurements were made using a cut-off length of 0.8 mm. Tip radius 5μm, measuring force: 0.75 mN.

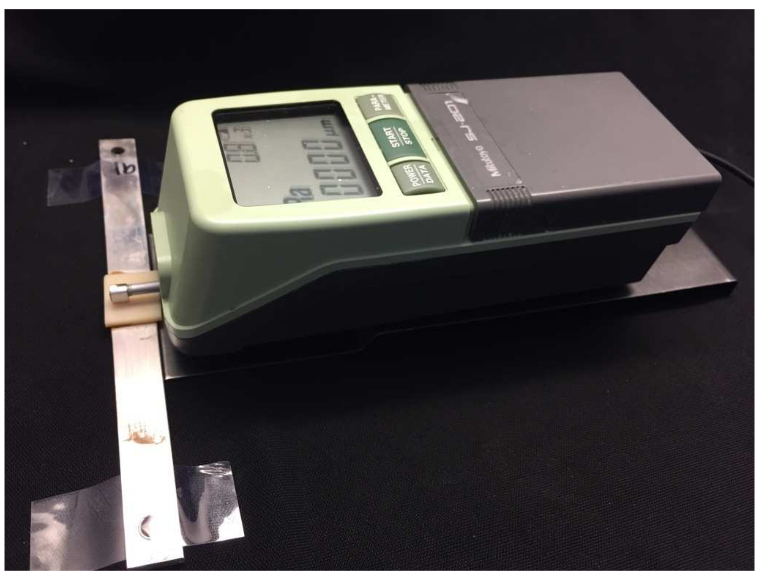


Figure 11: Mitutoyo SJ201 profilometer.

Simulated tooth brushing

Specimens were exposed to mechanical tooth brushing under a 1.91N load using toothpaste slurry using a 1:1 ratio of tooth paste and water (Crest® Cavity Protection) for 20,000 cycles in two different modes: soft bristles and medium bristles (both Oral B Indicator®). Using a custom made tooth brushing simulator machine.

Surface roughness measurements after tooth brushing

The tooth brushing simulator was run for 20,000 cycles, representing 2 years of tooth brushing. According to Roselino et al 10,000 simulated brushing cycles are equivalent to 1 year of brushing under clinical conditions (2015). The specimens were removed from the brushing machine and cleaned. Surface roughness was measured 10 times randomly across the groove pattern. Each material was tested using a portable profilometer.

Data Analysis

Surface roughness R_a values were compared using one-way ANOVA and post hoc Tukey test for multiple comparisons between the different test groups using JMP Statistical Discovery from SAS software, version 13.0.0. Summary of the methodology is shown in Figure 12 below.

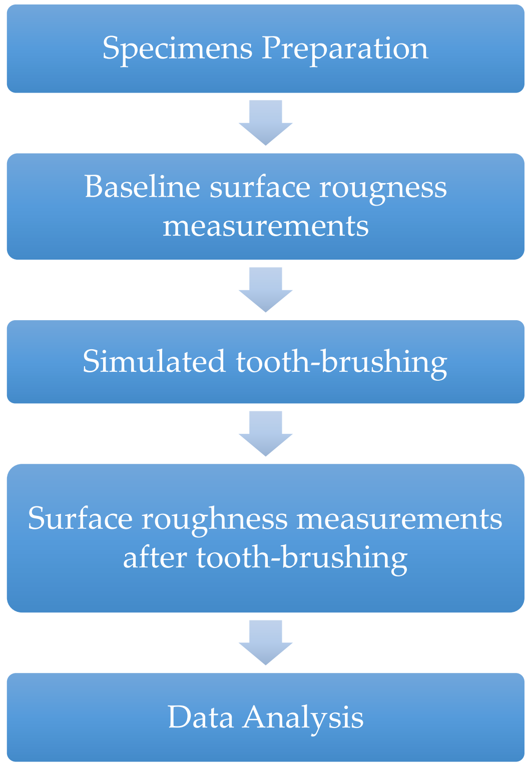


Figure 12: Part II methodology in summary.

Specimens were prepared, sectioned and polished, then they were cleaned and dried before taking baseline measurements using a profilometer (SJ-201, Mitutoyo Corp, Japan). Specimens were divided into two groups: Soft Bristles and Medium Bristles and were subsequently exposed to mechanical tooth brushing under a 1.91N load using toothpaste slurry (Crest® Cavity Protection) for 20,000 cycles. Using a custom made tooth brushing simulator machine. Surface roughness measurements were taken after simulated tooth brushing and data was then analyzed for statistical significance.

CHAPTER 3: RESULTS

3.1 Beverage discoloration and stain removal performance of tooth brushing:

Results were calculated to show the differences in color measurements of the specimens before and after immersion in six different beverages (coffee, tea, water, soda, red wine, cranberry juice) and after simulated tooth brushing to remove the stains of the two groups that presented the highest levels of discoloration (Coffee and Red Wine groups).

The equation of differences in color measurements,

$$\Delta E^*_{ab}=\sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

hereto referred to as ΔE^*_{ab} refers to the amount of color change that occurs from the immersion in different beverages and after simulated tooth brushing and is calculated by subtracting the color measurements of the specimens immersion in beverages and after brushing from the baseline color measurements of the tiles. Our first aim was to evaluate the level of discoloration and the stain removal performance of tooth brushing.

3.1.1 Descriptive results of color changes:

Table 5 shows the ΔE* values and their standard deviations after immersion in six different beverages for one day, four weeks, eight weeks and after simulated tooth brushing for the groups immersed in coffee and red wine.

Table 4: Mean (SD) Delta E* of Provisional Restorative Materials Immersed in Different Beverages.

MATERIAL	BEVERAGE	TIME OF IMMERSION			AFTER ABRASION
		24 HOURS (ΔE*)	4 WEEKS (ΔE*)	8 WEEKS (ΔE*)	
VITA CAD	COFFEE	2.6(1.26)	4.53(1.53)	7.14(2.58)	0.3(0.27)
	RED WINE	0.65(0.2)	4.29(0.85)	30.67(1.4)	0.41(0.34)
	CRANBERRY	0.72(0.44)	7.1(1.78)	8.36(2.25)	-
	TEA	0.39(0.19)	0.67(0.3)	0.76(0.52)	-
	COCA COLA	0.24(0.12)	0.34(0.21)	0.25(0.11)	-
	WATER	0.32(0.19)	0.3(0.15)	0.22(0.18)	-
TELIO CAD	COFFEE	2.62(1.31)	4.58(1.58)	6.17(2.55)	0.37(0.48)
	RED WINE	0.53(0.16)	4.24(1.12)	23.71(4.74)	0.43(0.45)
	CRANBERRY	1.22(0.51)	6.84(2.39)	9.15(2.17)	-
	TEA	0.69(0.57)	0.81(0.62)	1.07(0.86)	-
	COCA COLA	0.28(0.15)	0.36(0.17)	0.31(0.15)	-
	WATER	0.51(0.52)	0.56(0.72)	0.63(0.84)	
ARTBLOC	COFFEE	3.53(1.24)	6.25(1.90)	7.21(2.00)	1.33(0.70)
	RED WINE	0.57(0.13)	2.91(1.49)	31.09(4.46)	0.27(0.21)
	CRANBERRY	0.42(0.24)	3.99(2.22)	5.31(2.58)	-
	TEA	0.80(0.54)	0.95(0.92)	0.97 (0.56)	-
	COCA COLA	0.22(0.11)	0.31(0.13)	0.38(0.11)	-
	WATER	0.14(0.8)	0.16(0.08)	0.22(0.1)	-
PROTEMP PLUS	COFFEE	8.34(2.51)	17.11(3.78)	17.65(3.96)	13.42(6.05)
	RED WINE	6.25(3.12)	13.03(3.12)	42.10(2.25)	12.60(4.00)
	CRANBERRY	3.24(1.38)	9.60(2.92)	9.88(2.81)	-
	TEA	1.64(0.62)	4.39(2.26)	5.68(0.85)	-
	COCA COLA	0.84(0.43)	2.10(0.40)	2.69(0.48)	-

MATERIAL	**BEVERAGE**	**TIME OF IMMERSION**			**AFTER ABRASION**
		24 HOURS (ΔE^*)	4 WEEKS (ΔE^*)	8 WEEKS (ΔE^*)	
	WATER	0.52(0.29)	1.88(0.34)	3.03(0.76)	-
LUXATEMP	COFFEE	8.17(2.26)	13.44(2.19)	15.59(4.57)	13.98(4.74)
	RED WINE	5.36(2.85)	13.17(4.75)	26.67(5.12)	14.22(4.57)
	CRANBERRY	1.70(0.64)	8.21(1.66)	8.75(1.93)	-
	TEA	1.77(0.90)	3.96(1.32)	4.73(1.76)	-
	COCA COLA	0.42(0.30)	1.27(0.28)	1.17(0.31)	-
	WATER	0.54(0.47)	0.61(0.38)	0.92(0.70)	-
JET SET 4	COFFEE	3.86(1.50)	10.45(1.83)	11.06(2.29)	7.41(1.05)
	RED WINE	1.14(0.41)	5.67(1.64)	27.42(6.94)	5.76(1.08)
	CRANBERRY	2.32(0.85)	8.89(2.85)	9.89(2.08)	-
	TEA	1.34(0.86)	3.83(1.77)	5.54(1.18)	-
	COCA COLA	0.44(0.31)	2.31(0.76)	2.68(0.97)	-
	WATER	0.49(0.54)	2.17(0.68)	3.50(0.89)	-

Mean ΔE^* values of each material immersed in six different beverages at different intervals of 24 hours, 4 weeks and 8 weeks are depicted in Figures 13-18 below. The ΔE^* values in different beverages after 8 weeks of immersion were significantly higher than those after one day and 4 weeks of immersion. After immersion in coffee, red wine, tea and cranberry juice, the specimens generally became darker in color as the immersion period increased. Color changes of the specimens after water and Coca-Cola immersion were found not significant.

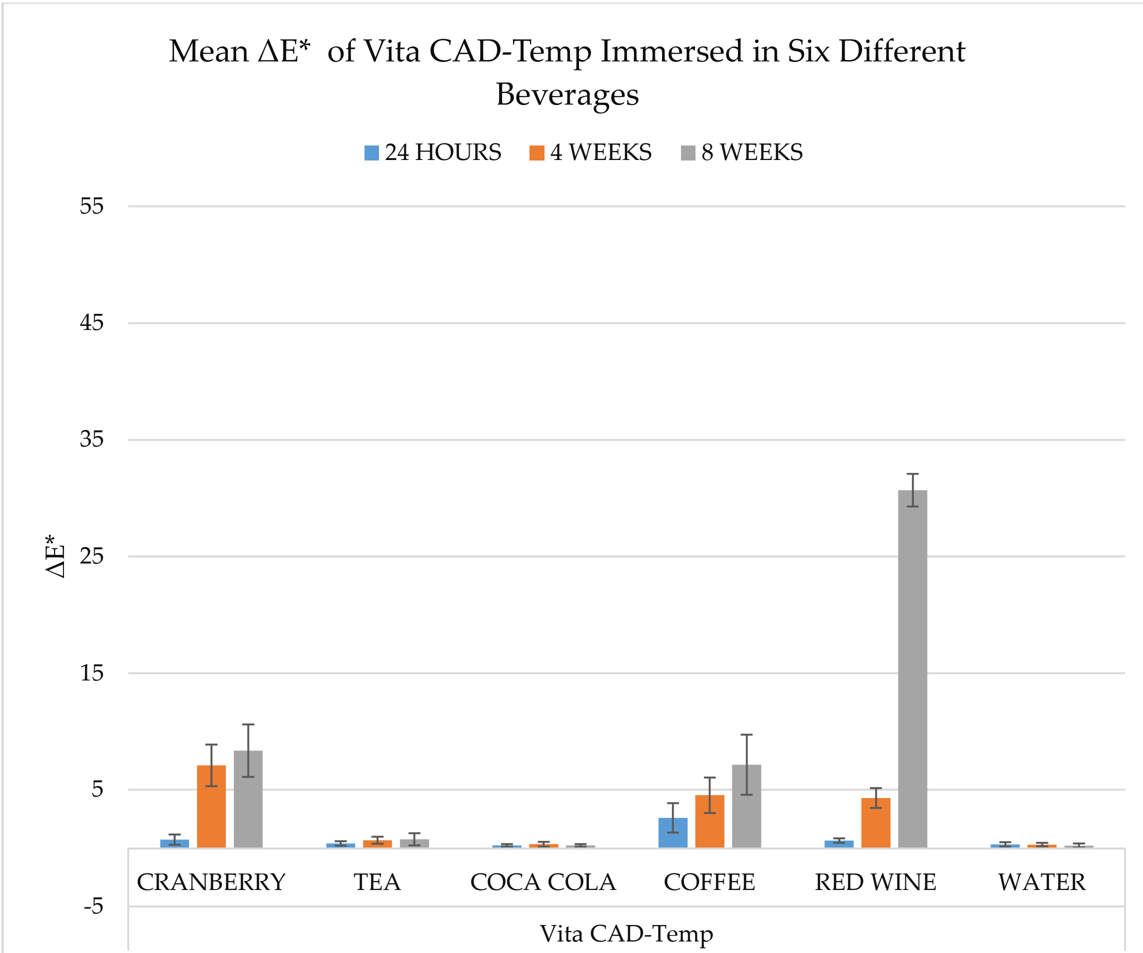


Figure 13: Mean (SD) ΔE^* values of Vita CAD-Temp at different intervals after immersion in six different beverages.

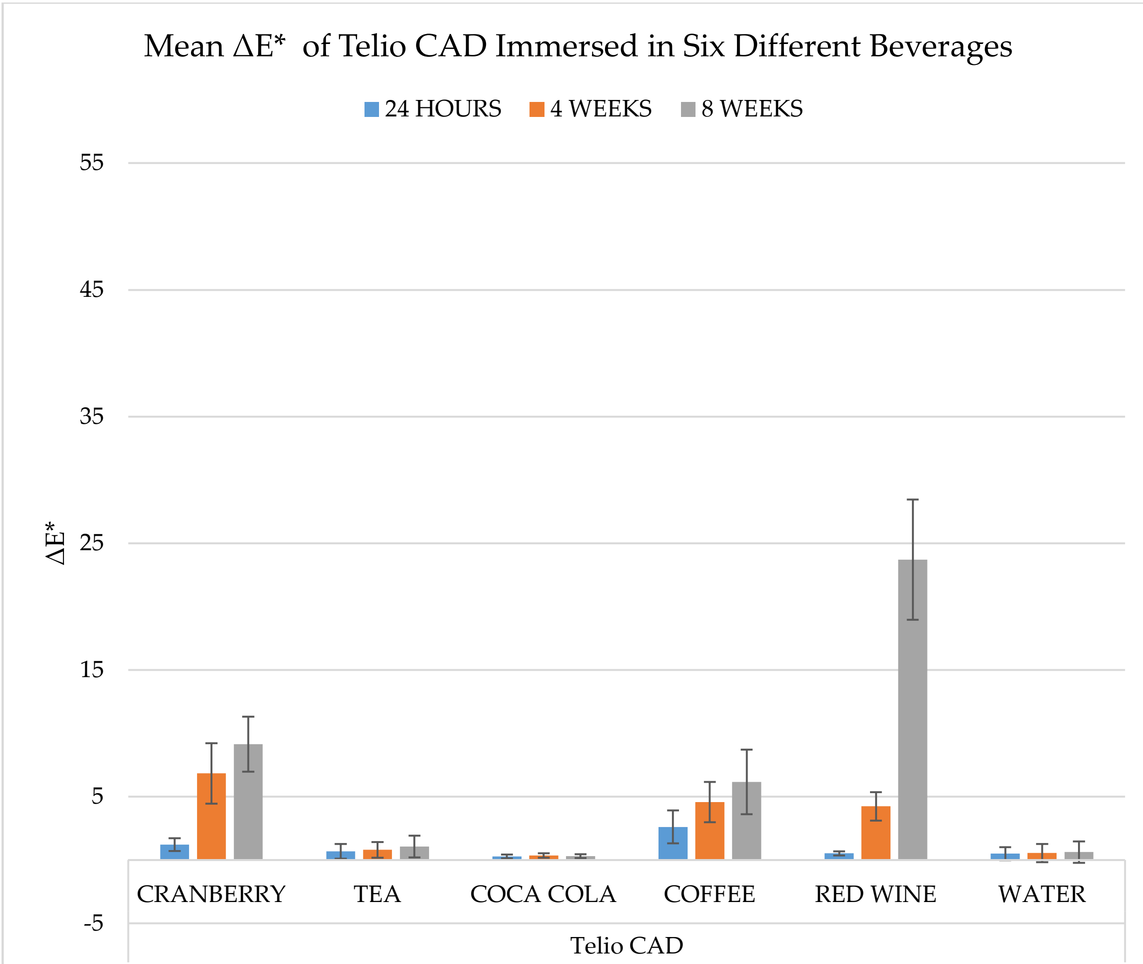


Figure 14: Mean (SD) ΔE* values of Telio CAD at different intervals after immersion in six different beverages.

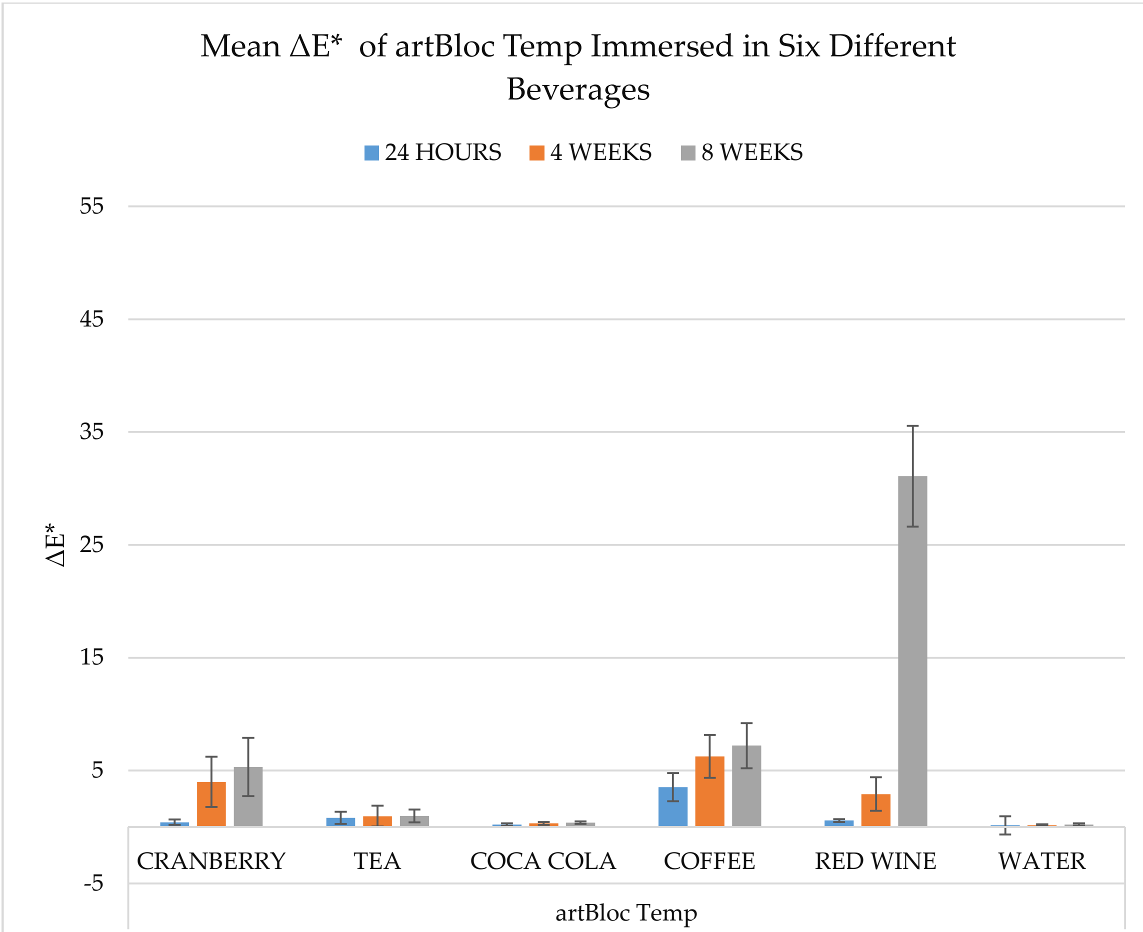


Figure 15: Mean (SD) ΔE* values of artBloc Temp at different intervals after immersion in six different beverages.

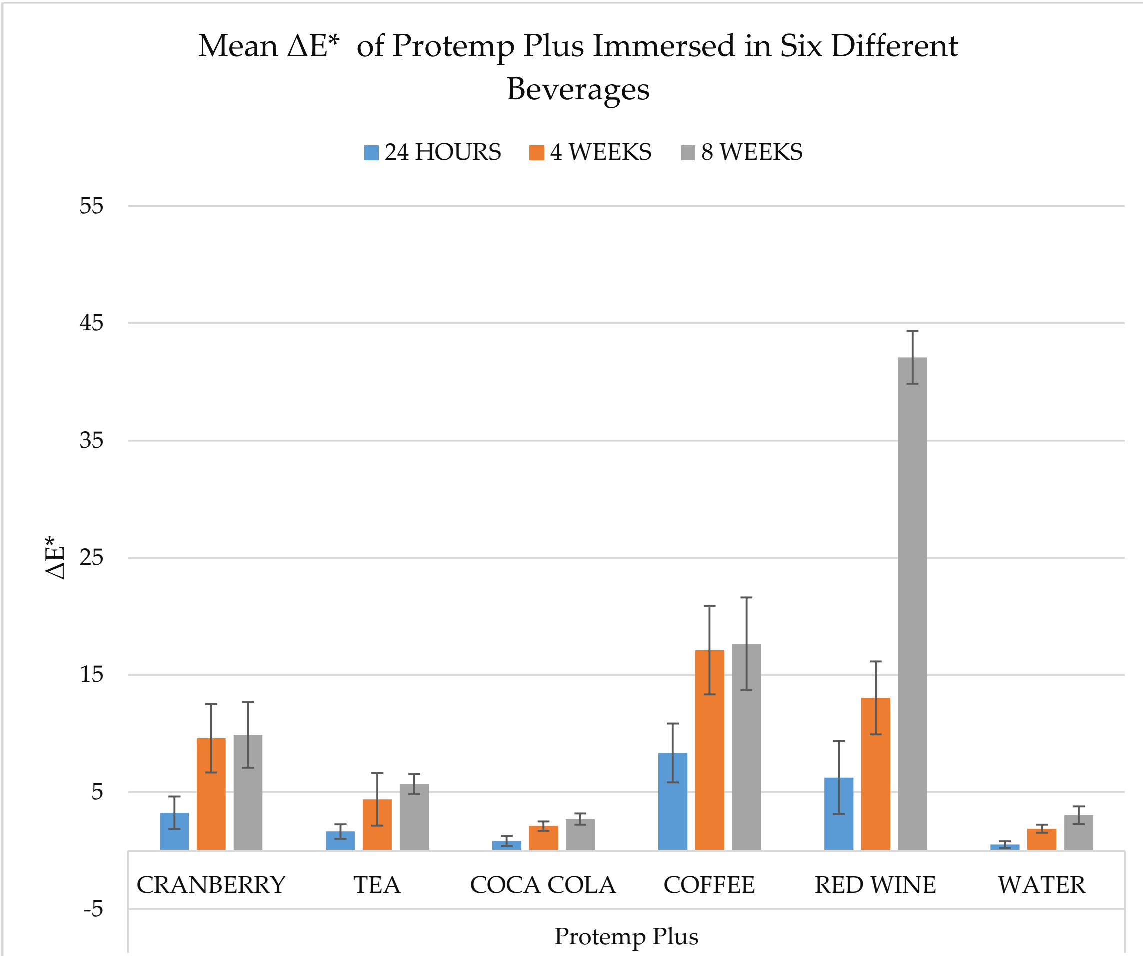


Figure 16: Mean (SD) ΔE* values of Protemp Plus at different intervals after immersion in six different beverages.

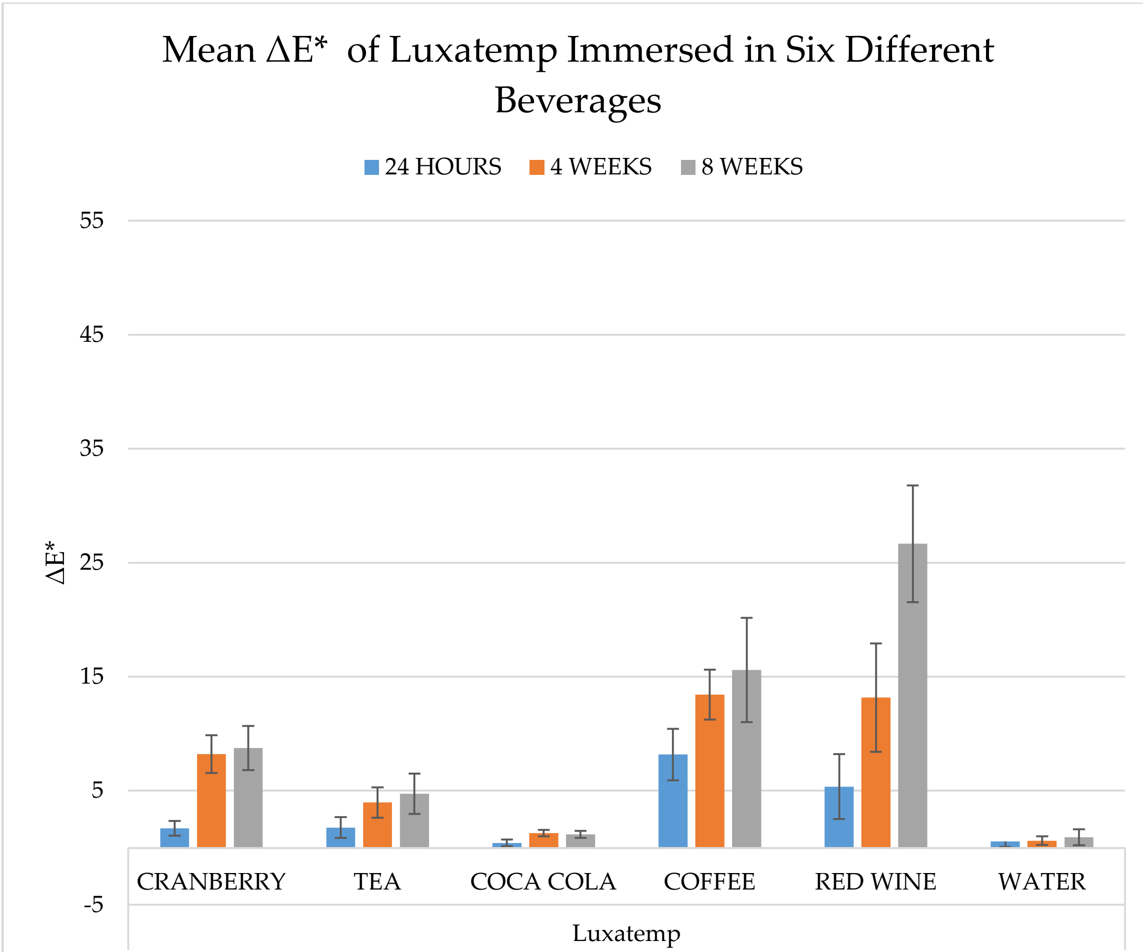


Figure 17: Mean (SD) ΔE* values of Luxatemp at different intervals after immersion in six different beverages.

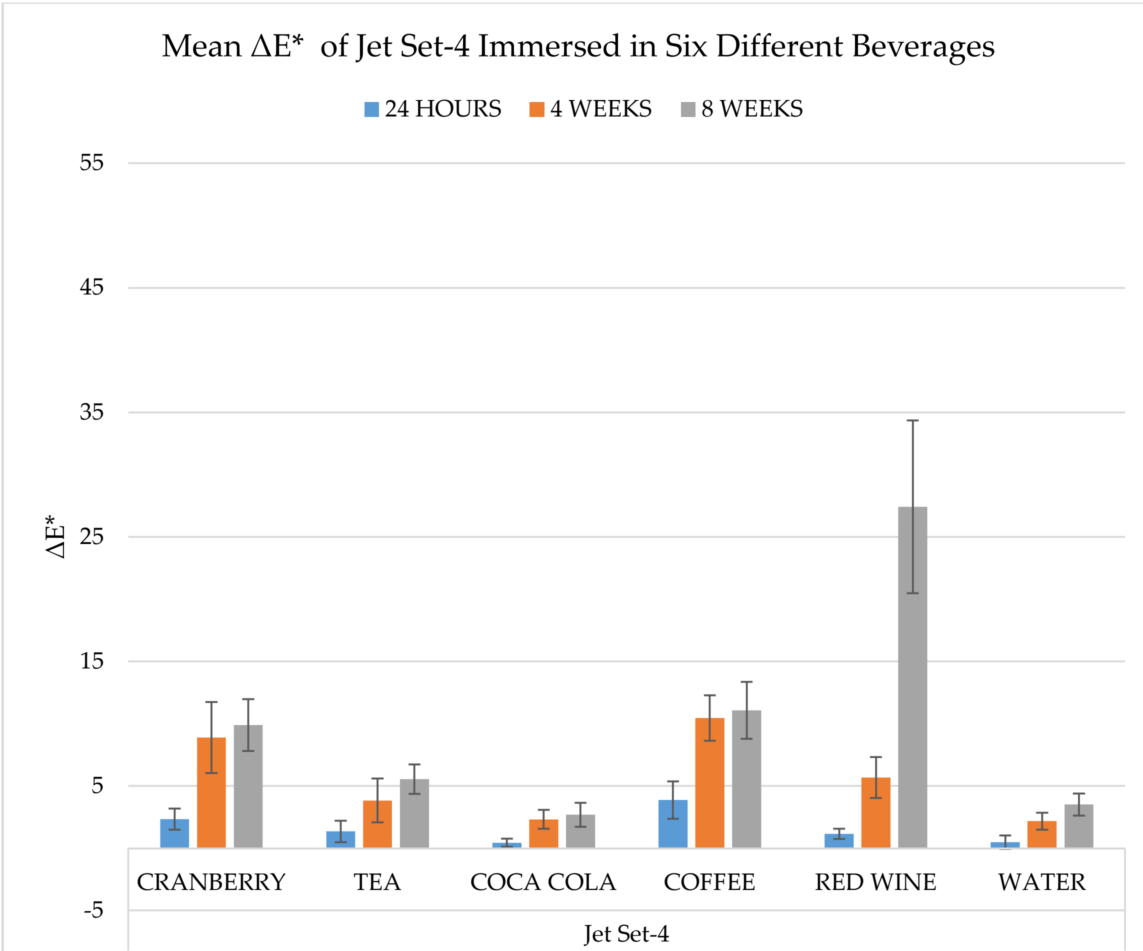


Figure 18: Mean (SD) ΔE* values of Jet Set-4 at different intervals after immersion in six different beverages.

After the immersion in beverages, the specimens on the two groups that presented the highest level of discoloration (red wine and coffee) were subjected to three minutes of simulated tooth brushing. The mean ΔE* of immersion in beverages and after tooth brushing are depicted on the graphs below (Figures 19-21).

Tooth brushing reversed the coffee discoloration to an acceptable ΔE* value for CAD/CAM blocks.

Coca-Cola and water had no significant discoloration effect on any of the tested materials ($p<0.05$).

Red wine and coffee caused the highest level of discoloration in all materials at week 8 of immersion.

Regarding staining potential, the solutions were ranked in this order (from lowest to highest level of discoloration): water < Coca-Cola < tea < cranberry juice < coffee < red wine.

After simulated tooth brushing for three minutes, nearly all CAD-CAM materials showed significant reduction in ΔE^* values ($p < 0.001$). Jet Set-4, Protemp Plus and Luxatemp which are conventional restorative provisional materials still demonstrated perceptibly high discoloration (ΔE 5.76-14.22) after polishing.

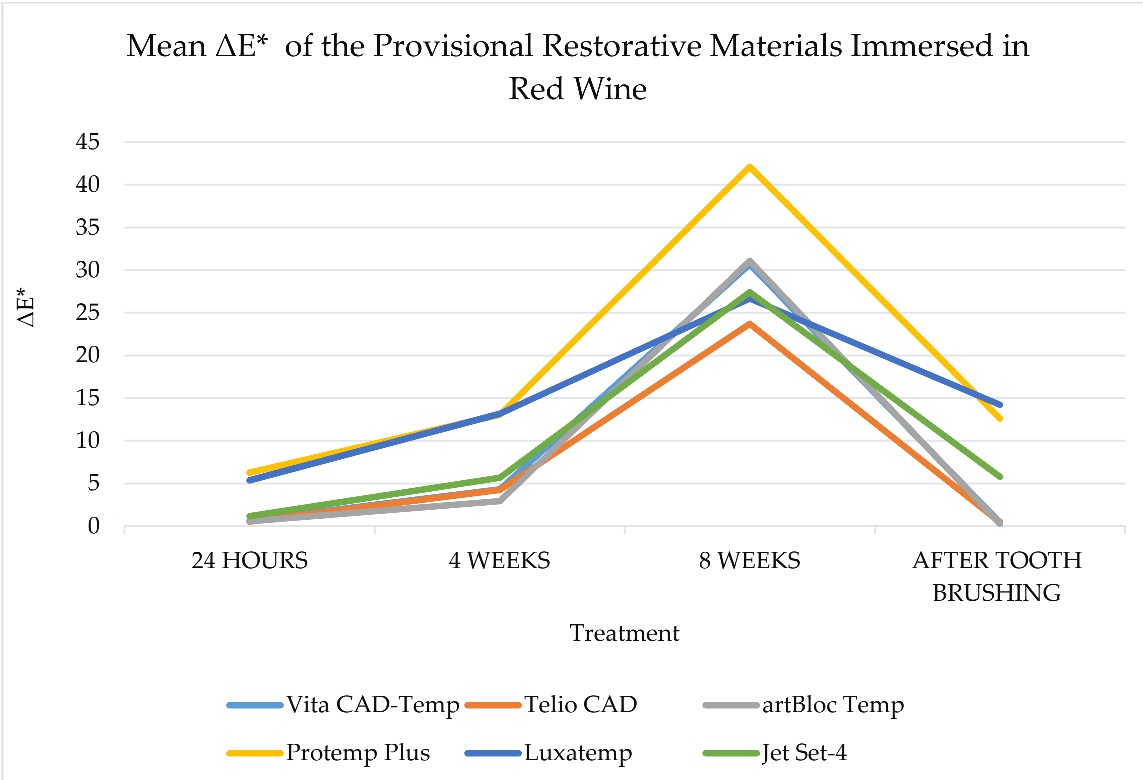


Figure 19: Mean ΔE* of the Provisional Restorative Materials Immersed in Red Wine at different intervals and after tooth brushing.

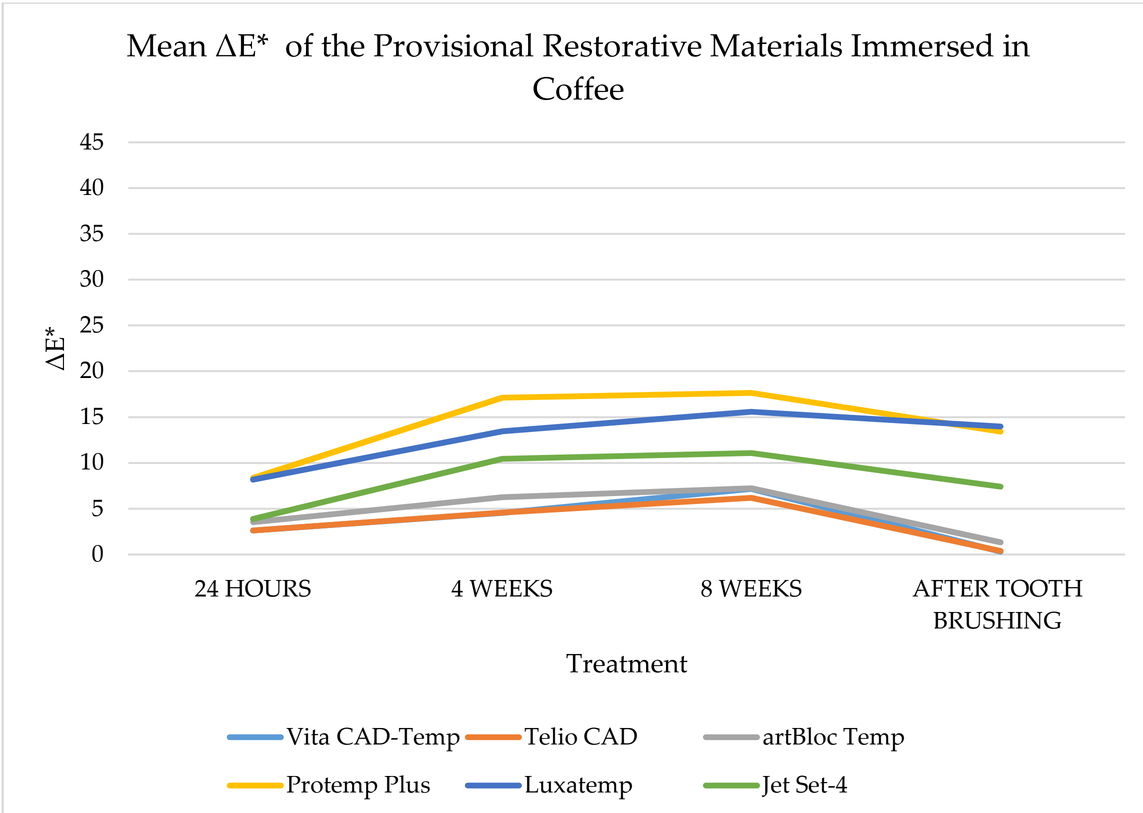


Figure 20: Mean ΔE* of the Provisional Restorative Materials Immersed in Coffee at different intervals and after tooth brushing.

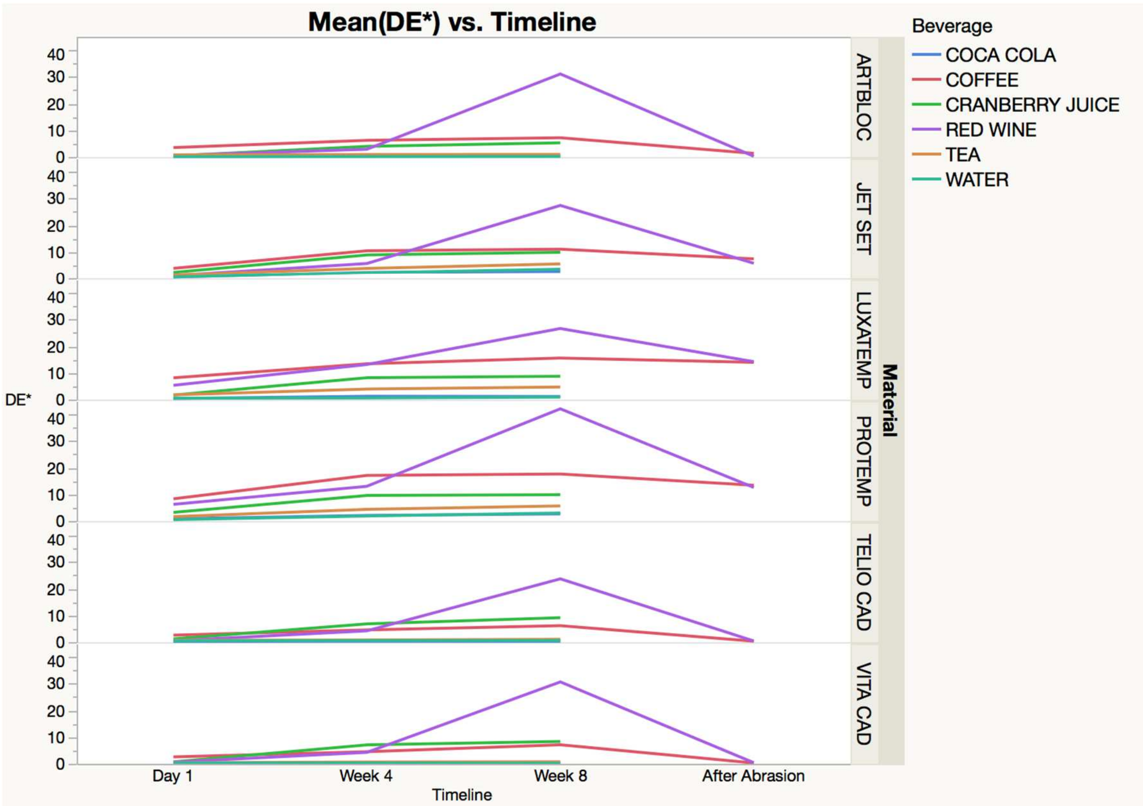


Figure 21: Summary of the effect of six different beverages at different intervals and after tooth brushing.

3.1.2 *Oneway Analysis of ΔE*: Timeline vs Provisional Material*

Oneway Analysis of ΔE* at 24 hours of immersion in six different beverages by Provisional Material

Oneway ANOVA and Tukey-Kramer HSD was performed at 24 hours comparing the timeline versus provisional material. Showing significant difference between injectable bisacrylics and CAD/CAM blocks. There was no significant difference between Jet Set-4 and Telio CAD specimens (Figure 22 and Tables 6 and 7).

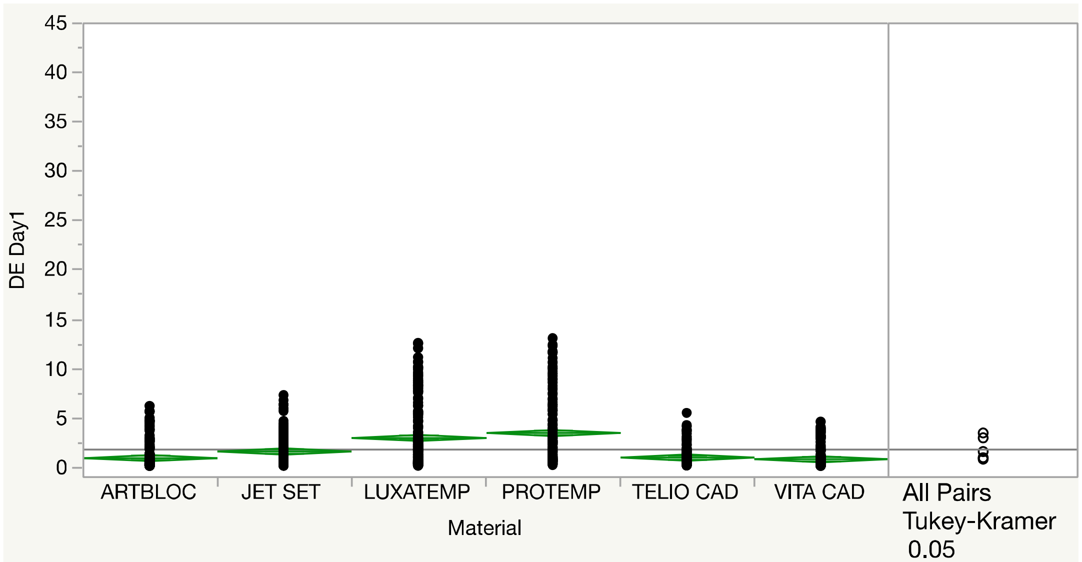


Figure 22: Oneway Analysis of ΔE* of each provisional material at 24 hours.

Table 5: Analysis of Variance of ΔE* of each provisional material at 24 hours.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material	5	1192.4543	238.491	51.5250	<.0001*
Error	1075	4975.7908	4.629		
C. Total	1080	6168.2451			

Table 6: Comparisons using Tukey-Kramer HSD of ΔE* of each provisional material at 24 hours.

Level				Mean
PROTEMP	A			3.47
LUXATEMP	A			2.98
JET SET		B		1.59
TELIO CAD		B	C	0.97
ARTBLOC			C	0.94
VITA CAD			C	0.81

Levels not connected by same letter are significantly different.

Oneway Analysis of ΔE* at 4 weeks of immersion in six different beverages by Provisional Material

Oneway ANOVA and Tukey-Kramer HSD was performed at 4 weeks comparing the timeline versus provisional material. Showing significant difference between injectable bisacrylics and CAD/CAM blocks. There was no significant difference between Jet Set-4 and Luxatemp specimens (Figure 23 and Tables 8 and 9).

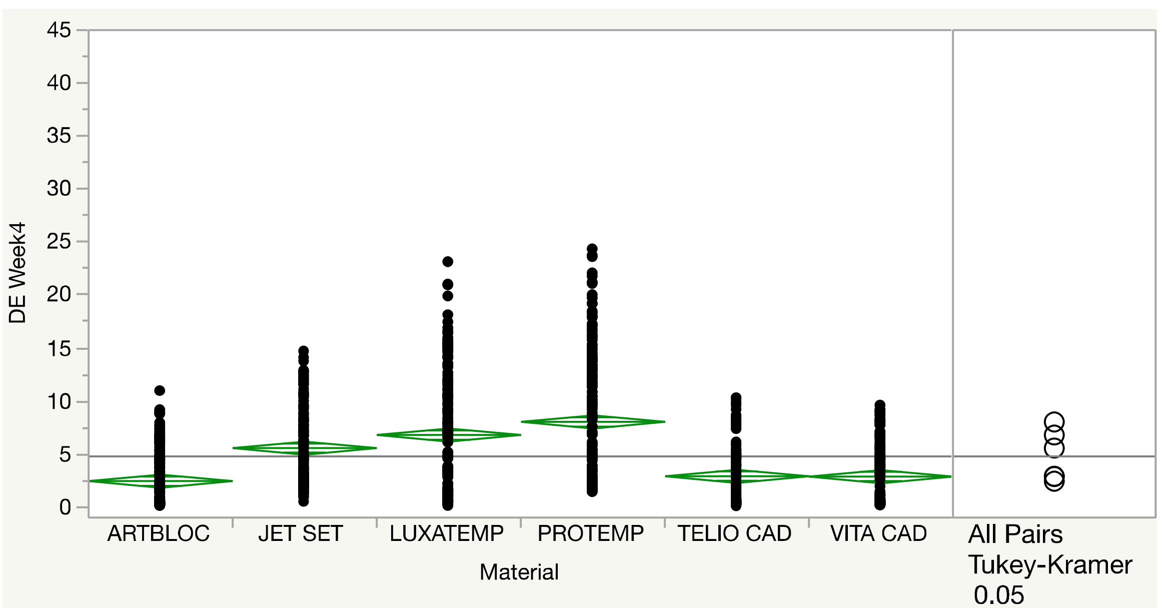


Figure 23: Oneway Analysis of ΔE* of each provisional material at Week 4.

Table 7: Analysis of Variance of ΔE* of each provisional material at Week 4.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material	5	5013.127	1002.63	56.2161	<.0001*
Error	1075	19172.854	17.84		
C. Total	1080	24185.981			

Table 8: Comparisons using Tukey-Kramer HSD of ΔE* of each provisional material at Week 4.

Level				Mean
PROTEMP	A			8.02
LUXATEMP	A	B		6.78
JET SET		B		5.55
TELIO CAD			C	2.89
VITA CAD			C	2.87
ARTBLOC			C	2.42

Levels not connected by same letter are significantly different.

Oneway Analysis of ΔE* at 8 weeks of immersion in six different beverages by Provisional Material

Oneway ANOVA and Tukey-Kramer HSD was performed at 8 weeks comparing the timeline versus provisional material. Showing significant difference between Protemp Plus and the rest of the materials tested (Figure 24 and Tables 10 and 11).

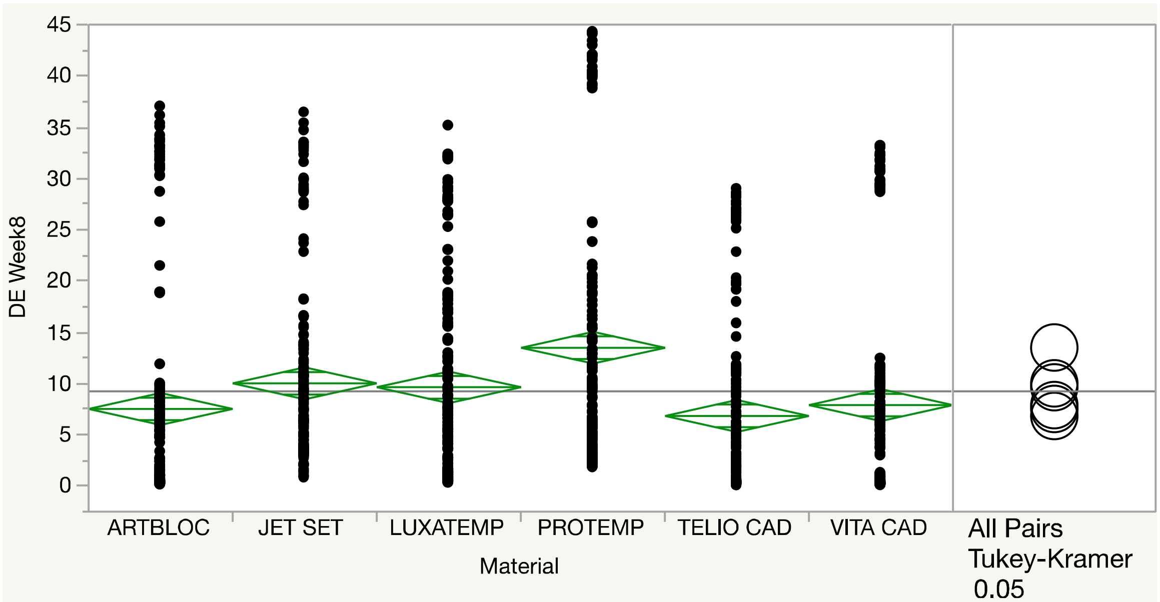


Figure 24: Oneway Analysis of ΔE* of each provisional material at Week 8.

Table 9: Analysis of Variance of ΔE* of each provisional material at Week 8.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material	5	5297.47	1059.49	9.3238	<.0001*
Error	1075	122156.45	113.63		
C. Total	1080	127453.92			

Table 10: Comparisons of ΔE* of each provisional material at Week 8.

Level			Mean
PROTEMP	A		13.5
JET SET		B	10.02
LUXATEMP		B	9.63
VITA CAD		B	7.9
ARTBLOC		B	7.52
TELIO CAD		B	6.84

Levels not connected by same letter are significantly different.

Oneway Analysis of ΔE* of each provisional material after brushing specimens immersed in coffee and red wine

Oneway ANOVA and Tukey-Kramer HSD was performed after simulated tooth brushing of the specimens immersed in red wine and coffee. Showing significant difference between injectable bisacrylics, powder-liquid self-cure acrylic and CAD/CAM blocks. (Figure 25 and Tables 12 and 13).

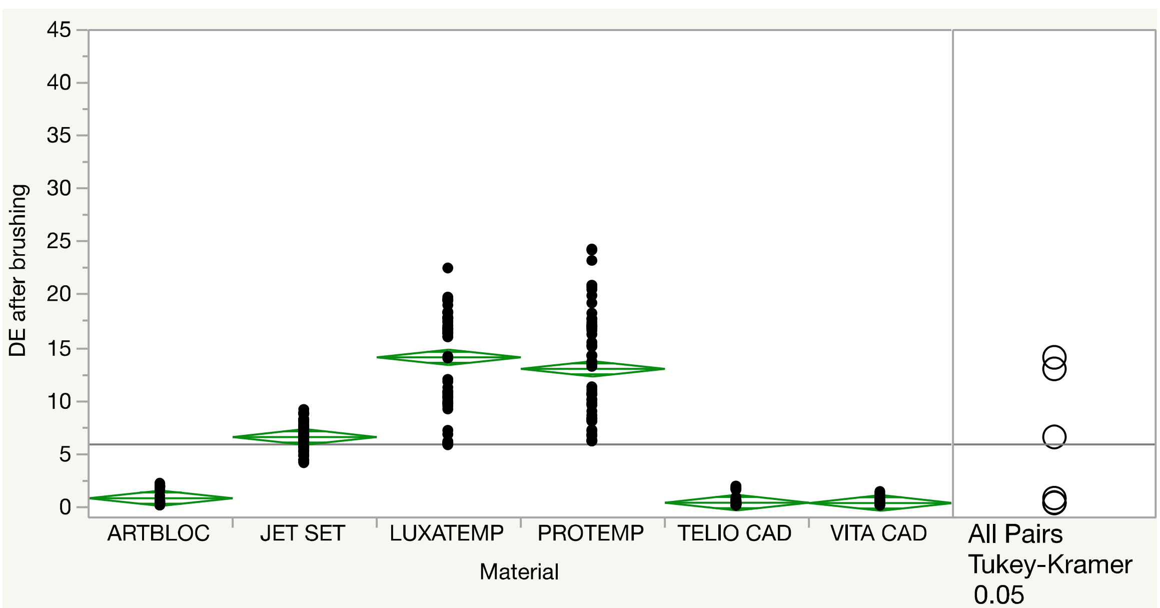


Figure 25: Oneway Analysis of ΔE* after brushing by provisional material.

Table 11: Analysis of Variance of ΔE* after brushing by provisional material.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material	5	12305.790	2461.16	295.0277	<.0001*
Error	354	2953.112	8.34		
C. Total	359	15258.902			

Table 12: Comparisons using Tukey-Kramer HSD of ΔE* after brushing by provisional material.

Level				Mean
LUXATEMP	A			14.1
PROTEMP	A			13
JET SET		B		6.58
ARTBLOC			C	0.81
TELIO CAD			C	0.39
VITA CAD			C	0.35

Levels not connected by same letter are significantly different.

3.1.3 Oneway analysis of ΔE: Timeline vs Beverage*

Oneway Analysis of ΔE* at 24 hours of immersion in six different beverages by Beverage

Oneway ANOVA and Tukey-Kramer HSD was performed at 24 hours comparing the timeline versus beverage. Shown in the graph and tables below (Figure 26, Tables 13 and 14.) Water and Coca-Cola groups presented the lowest values, showing significant difference when compared to the other groups. Cranberry juice and tea are in the same group, and coffee and red wine presented the highest values and significant difference against all the groups and between each other.

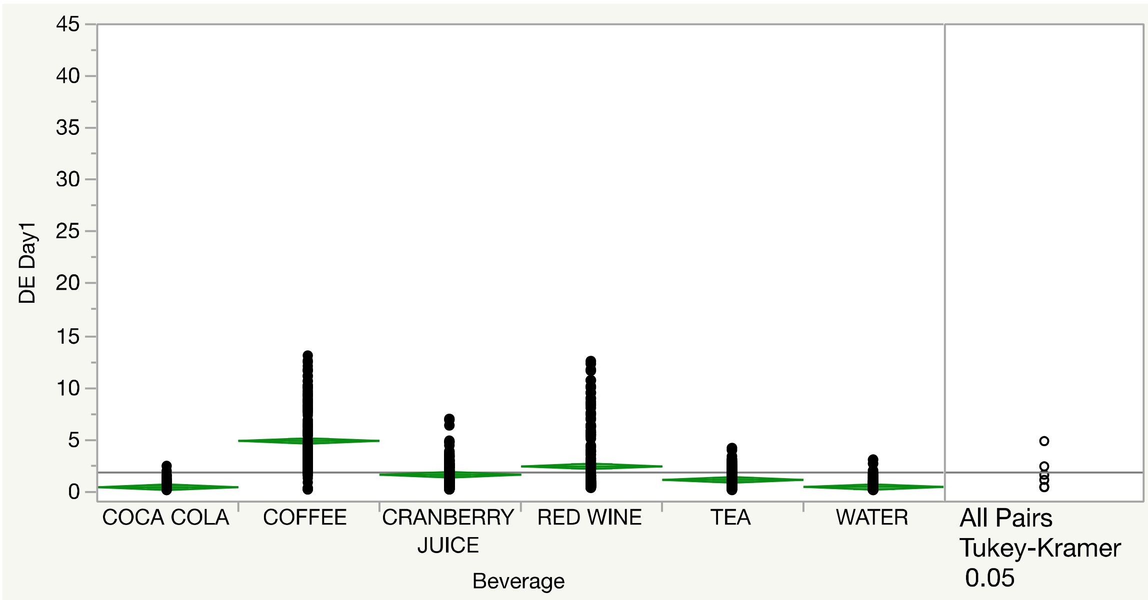


Figure 26: Oneway Analysis of ΔE* at 24 hours by Beverage.

Table 13: Analysis of Variance of ΔE* at 24 hours by beverage.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Beverage	5	2530.0563	506.011	149.5145	<.0001*
Error	1075	3638.1888	3.384		
C. Total	1080	6168.2451			

Table 14: Comparisons using Tukey-Kramer HSD of ΔE* at 24 hours by beverage.

Level					Mean
COFFEE	A				4.85
RED WINE		B			2.41
CRANBERRY JUICE			C		1.6
TEA			C		1.1

WATER				D	0.42
COCA COLA				D	0.4

Levels not connected by same letter are significantly different.

Oneway Analysis of ΔE* at 4 weeks of immersion in six different beverages by Beverage

Oneway ANOVA and Tukey-Kramer HSD was performed at 4 weeks comparing the timeline versus beverage. Shown in the graph and tables below (Figure 27 and Tables 15 and 16).

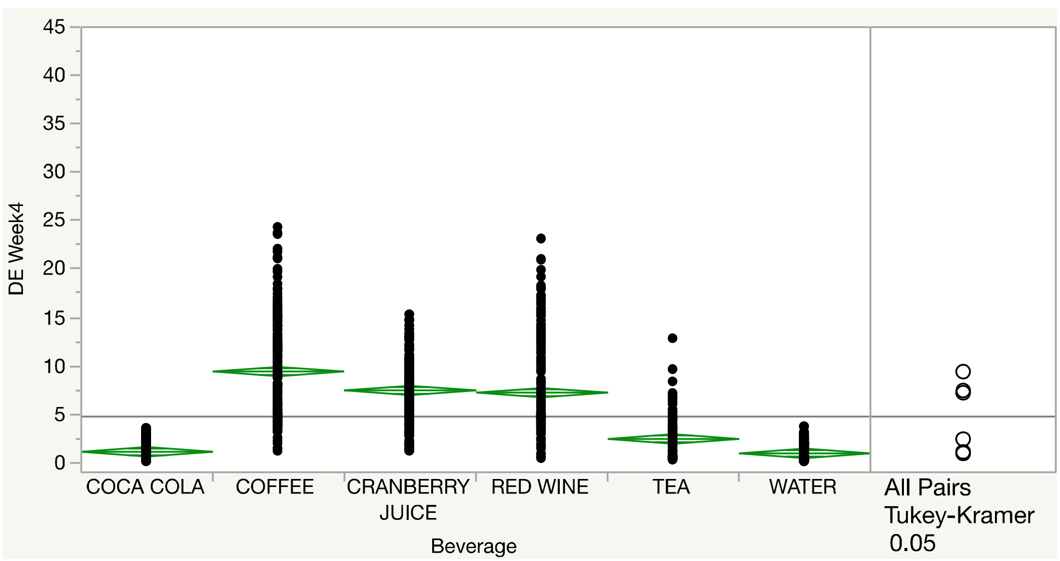


Figure 27: Oneway Analysis of ΔE* at 4 weeks by Beverage.

Table 15: Analysis of Variance of ΔE* at 4 weeks by beverage.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Beverage	5	12241.925	2448.38	220.3618	<.0001*
Error	1075	11944.056	11.11		

C. Total	1080	24185.981			

Table 16: Comparisons using Tukey-Kramer HSD of ΔE* at 4 weeks by beverage.

Level					Mean
COFFEE	A				9.39
CRANBERRY JUICE		B			7.44
RED WINE		B			7.21
TEA			C		2.43
COCA COLA				D	1.11
WATER				D	0.94

Levels not connected by same letter are significantly different.

Oneway Analysis of ΔE* at 8 weeks of immersion in six different beverages by Beverage

Oneway ANOVA and Tukey-Kramer HSD was performed at 8 weeks comparing the timeline versus beverage. Shown in the graph and tables below (Figure 28 and Tables 17 and 18). Red wine and coffee presented the highest values after 8 weeks of immersion, 30.27 ΔE* and 10.80 ΔE* respectively. Water and Coca-Cola presented the lowest values after 8 weeks of immersion 1.43 and 1.24 ΔE* respectively and also they did not show significant difference between each other.

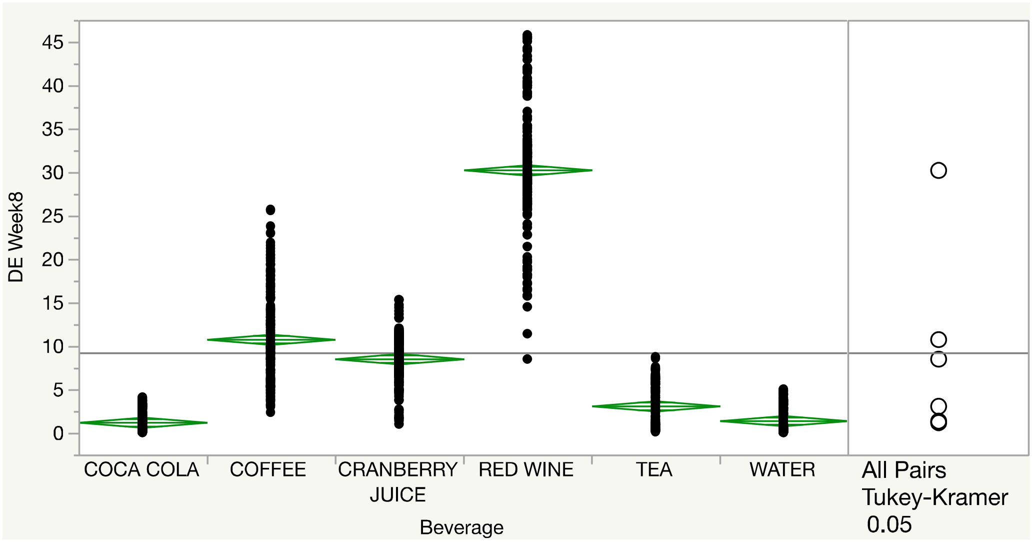


Figure 28: Oneway Analysis of ΔE* at 8 weeks by Beverage.

Table 17: Analysis of Variance of ΔE* at 8 weeks by beverage.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Beverage	5	109388.63	21877.7	1301.864	<.0001*
Error	1075	18065.29	16.8		
C. Total	1080	127453.92			

Table 18: Comparisons using Tukey-Kramer HSD of ΔE* at 8 weeks by beverage.

Level						Mean
RED WINE	A					30.27
COFFEE		B				10.80
CRANBERRY JUICE			C			8.56
TEA				D		3.12
WATER					E	1.43
COCA COLA					E	1.24

Levels not connected by same letter are significantly different.

Oneway Analysis of ΔE* after tooth brushing specimens immersed in coffee and red wine by Beverage

Oneway ANOVA and Tukey-Kramer HSD was performed after simulated tooth brushing comparing the timeline versus beverage. Shown in the graph and tables below (Figure 29 and Table 19).

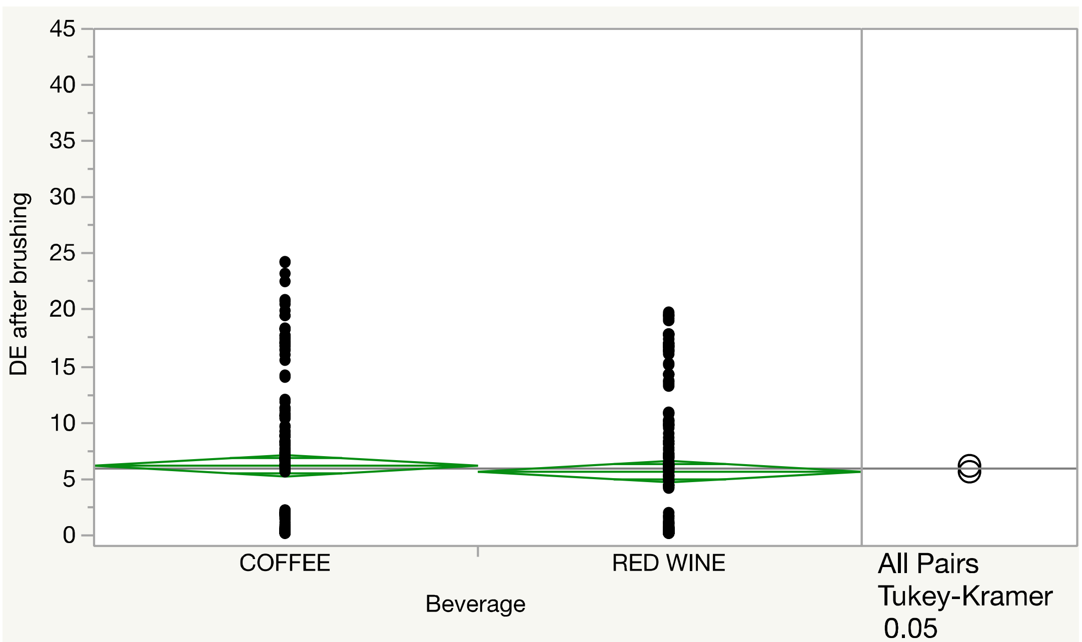


Figure 29: Oneway Analysis of ΔE* after brushing by Beverage.

Table 19: Analysis of Variance of ΔE* after brushing by beverage.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Beverage	1	24.748	24.7481	0.5816	0.4462
Error	358	15234.154	42.5535		
C. Total	359	15258.902			

3.1.4 Oneway analysis of ΔE: Timeline vs Material Category*

Provisional materials were divided into two different categories to be analyzed: CAD CAM materials (artBloc Temp, Vita CAD temp and Telio CAD) and traditional materials (Jet Set-4, Protemp Plus and Luxatemp).

The ΔE* after immersion in beverages were significantly different ($p < 0.001$) for the levels of types of materials and their rank follows: PMMA blocks < bis-acrylics < powder-liquid self-cure acrylic.

Oneway Analysis of ΔE* at 24 hours of immersion in six different beverages By Material Category

Oneway ANOVA and Tukey-Kramer HSD was performed at 24 hours comparing the timeline versus material category. Showing significant difference between traditional and CAD/CAM blocks ($p<.0001$*). (Figure 30 and Table 20).

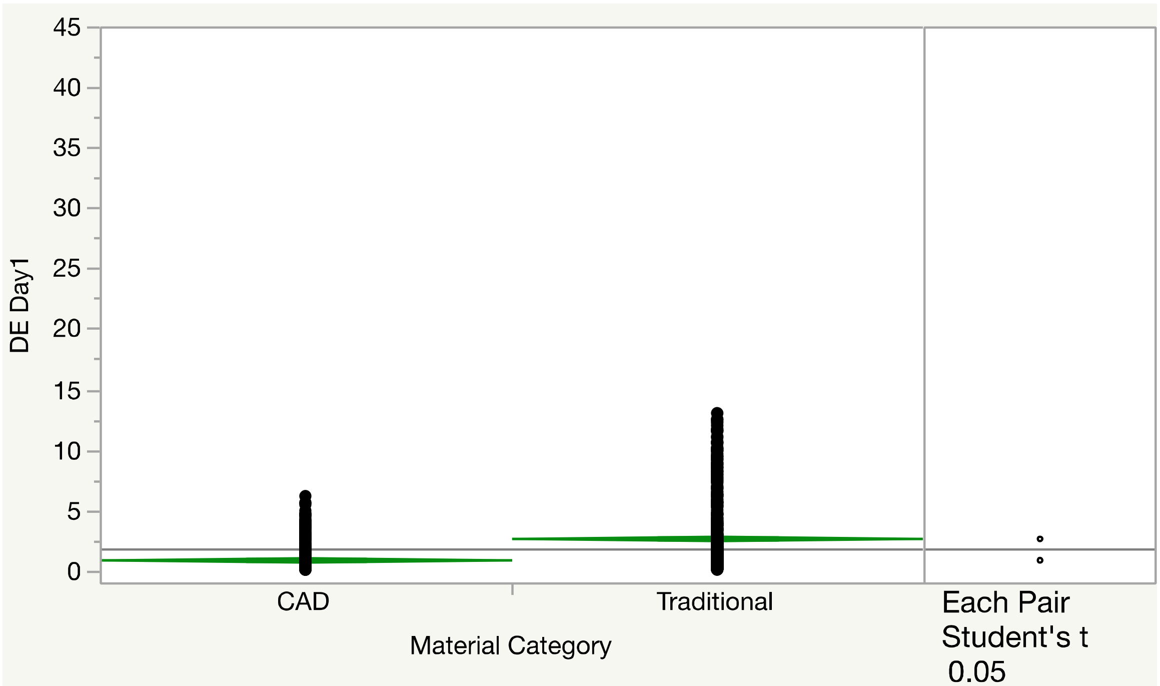


Figure 30: Oneway Analysis of ΔE* at 24 hours By Material Category.

Table 20: Analysis of Variance of ΔE* at 24 hours By Material Category.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material Category	1	849.0910	849.091	172.2396	<.0001*
Error	1079	5319.1541	4.930		
C. Total	1080	6168.2451			

Oneway Analysis of ΔE* at 4 weeks of immersion in six different beverages By Material Category

Oneway ANOVA and Tukey-Kramer HSD was performed at 4 weeks comparing the timeline versus material category. Showing significant difference between traditional and CAD/CAM blocks (p<.0001*). (Figure 31 and Table 21).

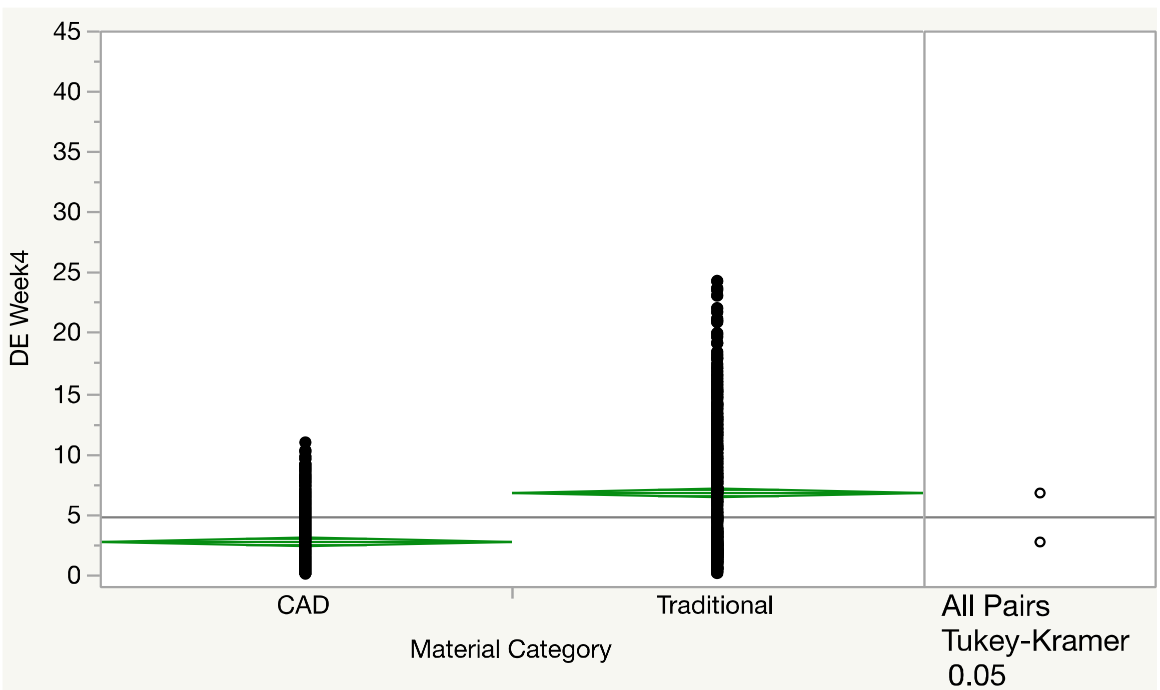


Figure 31: Oneway Analysis of ΔE* at Week 4 By Material Category.

Table 21: Analysis of Variance of ΔE* at Week 4 By Material Category.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material Category	1	4440.092	4440.09	242.6257	<.0001*
Error	1079	19745.889	18.30		
C. Total	1080	24185.981			

Oneway Analysis of ΔE* at 8 weeks of immersion in six different beverages By Material Category

Oneway ANOVA and Tukey-Kramer HSD was performed at 8 weeks comparing the timeline versus material category. Showing significant difference between traditional and CAD/CAM blocks ($p<.0001$*). (Figure 32 and Table 22).

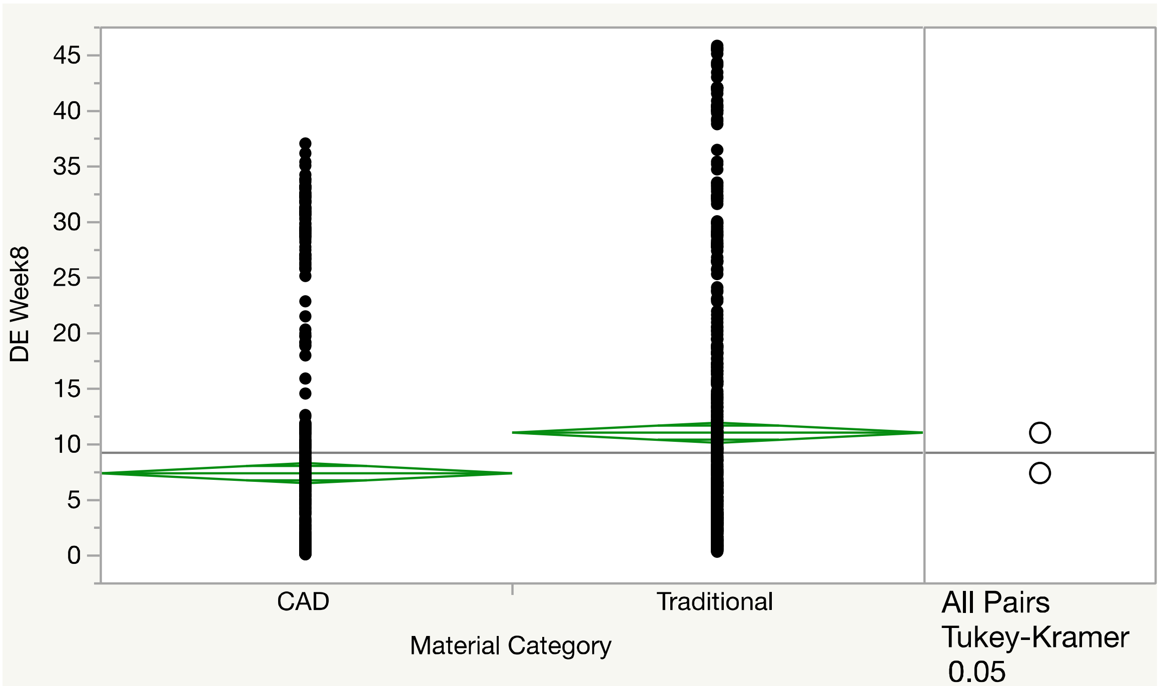


Figure 32: Oneway Analysis of ΔE* at Week8 By Material Category.

Table 22: Analysis of Variance of ΔE* at Week8 By Material Category.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material Category	1	3556.59	3556.59	30.9737	<.0001*
Error	1079	123897.33	114.83		
C. Total	1080	127453.92			

Oneway Analysis of ΔE* after brushing specimens immersed in coffee and red wine by Material Category

Oneway ANOVA and Tukey-Kramer HSD was performed after simulated tooth brushing in specimens immersed in red wine and coffee, to compare the difference between materials' category. Showing significant difference between traditional and CAD/CAM blocks ($p<.0001^*$). (Figure 33 and Table 23).

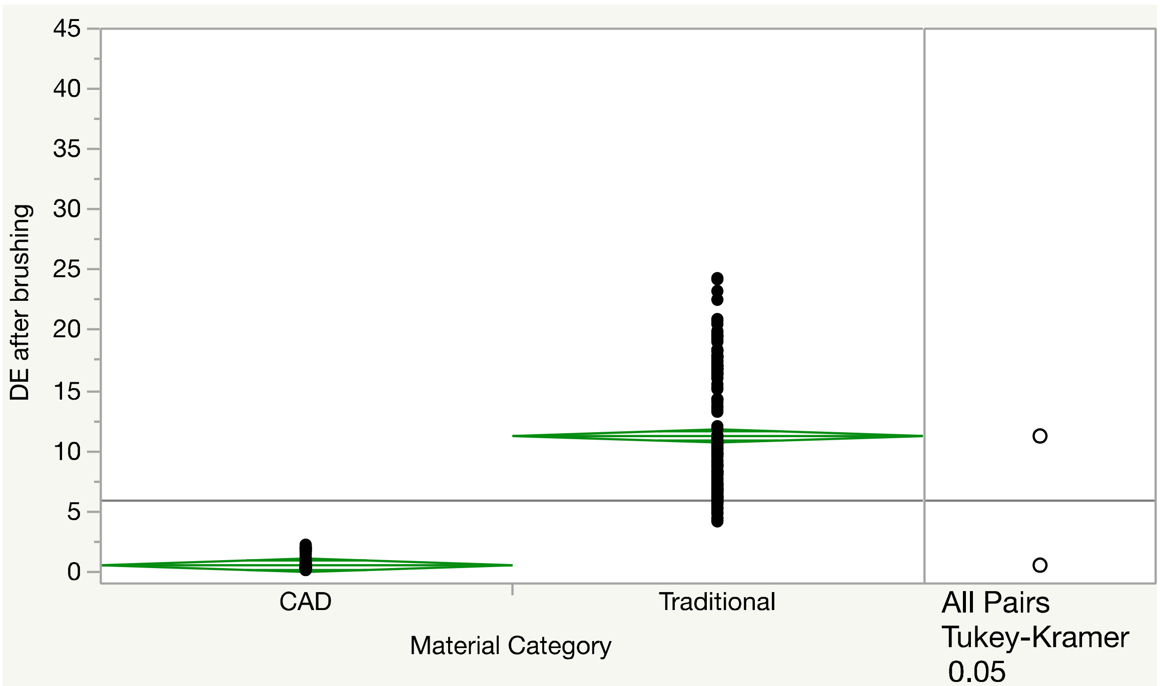


Figure 33: Oneway Analysis of ΔE* after brushing By Material Category.

Table 23: Analysis of Variance of ΔE* after brushing By Material Category.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Material Category	1	10319.661	10319.7	747.9771	<.0001*
Error	358	4939.240	13.8		
C. Total	359	15258.902			

3.1.5 Manova:

Multivariate analysis was performed on the data set as a whole, between subjects, per material, beverage and comparing material versus beverage. Also within subjects, per time and time versus material, time versus beverage, and time versus material and versus beverage (Table 24).

Table 24: Manova between and within subjects.

	Factor	Test	Value	Exact F	NumDF	DenDF	Prob>F
Between subjects	All Between	F Test	15.861784	473.5875	35	1045	<.0001*
	Material	F Test	1.9121323	399.6357	5	1045	<.0001*
	Beverage	F Test	12.502034	2612.9251	5	1045	<.0001*
	Material* Beverage	F Test	1.4485152	60.5479	25	1045	<.0001*
Within Subjects	All within interactions	Wilks' Lambda	0.0201409	180.3522	70	2088	<.0001*
	Time	F Test	11.573438	6041.3346	2	1044	<.0001*
	Time*Material	Wilks' Lambda	0.5296155	78.1129	10	2088	<.0001*
	Time*Beverage	Wilks' Lambda	0.0291854	1013.4147	10	2088	<.0001*
	Time*Material*Beverage	Wilks' Lambda	0.4510409	20.4203	50	2088	<.0001*

Overall Least Square Means are depicted on the graph below (Figure 34), at 24 hours Delta E* value is 1.8, at 4 weeks 4.76 and at 8 weeks 9.24.

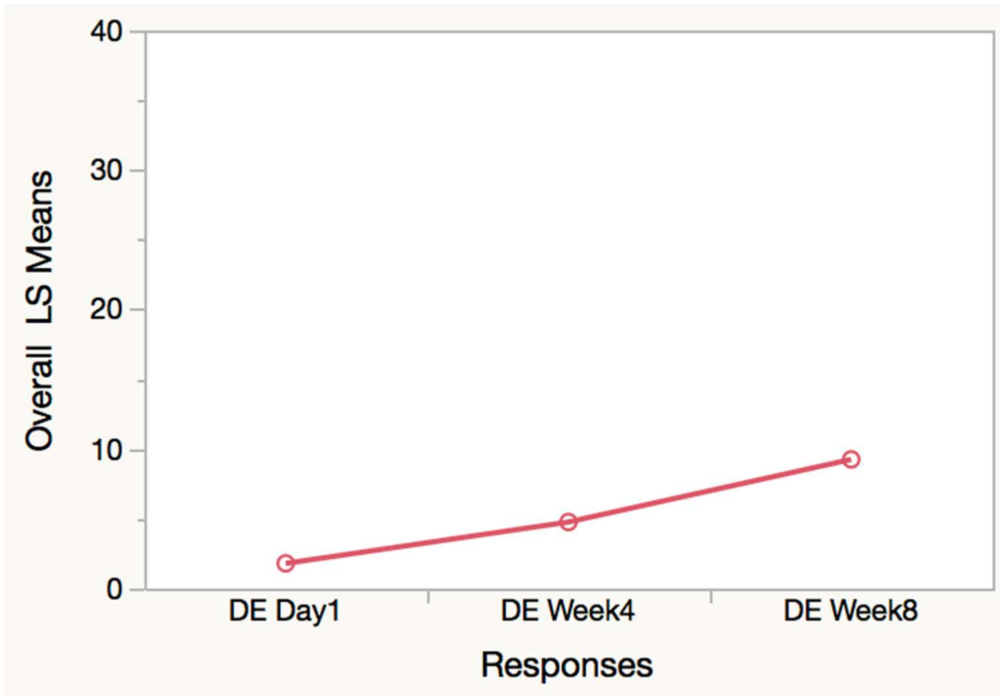


Figure 34: Overall Least Square Means

Least Square Means by materials at different timelines are shown in Figure 35 and Table 25.

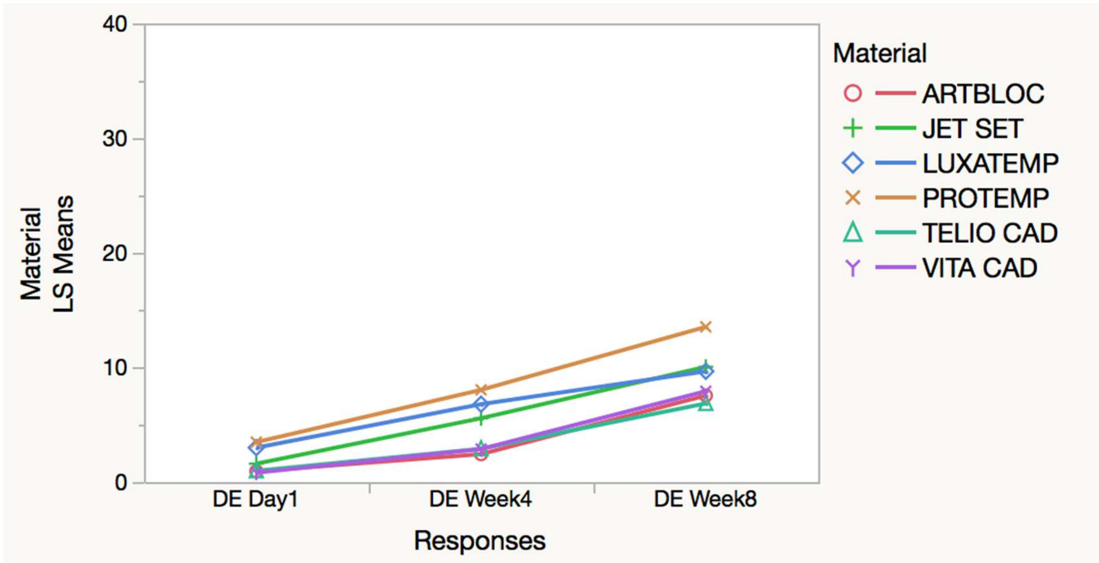


Figure 35: Least Square Means by Material.

Table 25: Least Square Means by Material.

Level	DE Day1	DE Week4	DE Week8
ARTBLOC	0.94	2.42	7.52
JET SET	1.59	5.55	10.02
LUXATEMP	2.99	6.77	9.63
PROTEMP	3.47	8.02	13.5
TELIO CAD	0.97	2.89	6.84
VITA CAD	0.81	2.87	7.9

Least Square Means by beverage at different timelines are shown in Figure 36 and Table 26.

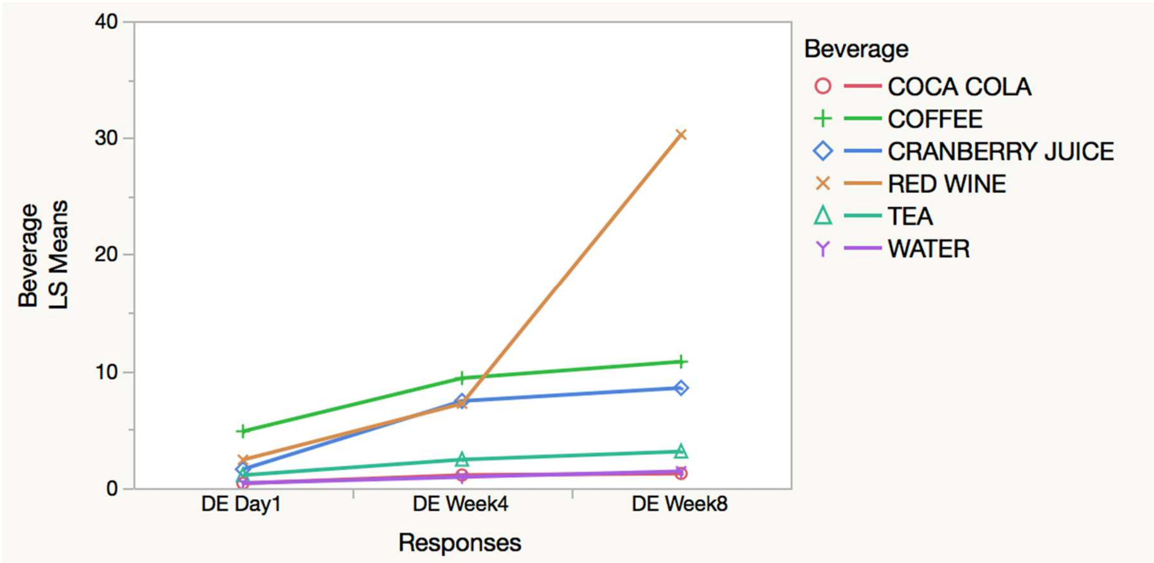


Figure 36: Least Square Means by Beverage at different timelines.

Table 26: Least Square Means By beverage at different timelines.

Beverage Level	DE* Day1	DE* Week4	DE* Week8
COCA COLA	0.4	1.11	1.24
COFFEE	4.85	9.39	10.8
CRANBERRY JUICE	1.6	7.44	8.55
RED WINE	2.41	7.21	30.27
TEA	1.1	2.43	3.12
WATER	0.42	0.94	1.43

3.2 Simulated tooth brushing and surface roughness.

3.2.1 Mean (SD) R_a values:

Tooth-brushing had a significant effect (R_a) on the surface of artBloc Temp, Telio CAD, and Jet Set-4. Protemp Plus and Luxatemp groups showed no significant difference after tooth-brushing. Vita CAD-Temp showed a significant difference with “medium” toothbrushes. Medium bristles caused significantly higher R_a than soft bristles. Mean (SD) R_a values are depicted below in Figure 37 and Table 27.

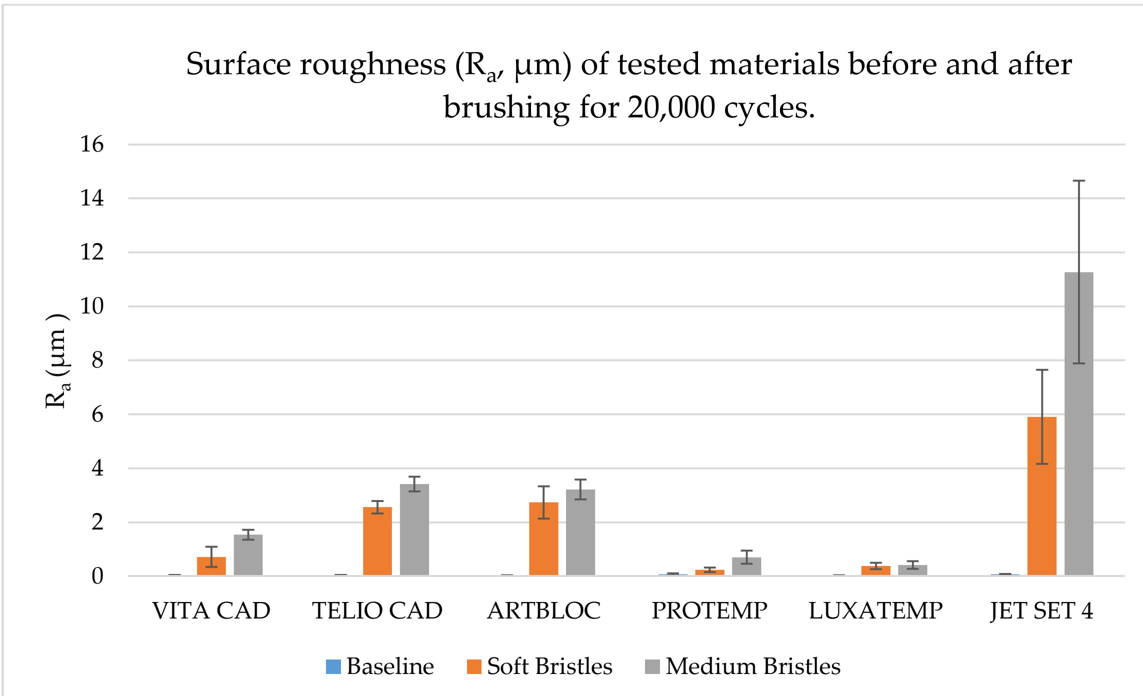


Figure 37: Surface roughness (R_a, µm) of tested materials before and after brushing for 20,000 cycles.

Table 27: Surface roughness (R_a, µm) of tested materials before and after brushing for 20,000 cycles.

MATERIAL	**BASELINE (R_a, µm)**	**AFTER ABRASION (R_a, µm)**	
		SOFT BRISTLES	MEDIUM BRISTLES
VITA CAD	0.04(0.01)F	0.72(0.37)EF	1.54(0.18)DE
TELIO CAD	0.04(0.01)F	2.56(0.23)CD	3.42(0.27)C
ARTBLOC	0.03(0.01)F	2.74(0.6)CD	3.22(0.37)C
PROTEMP	0.07(0.04)F	0.24(0.08)F	0.71(0.24)EF
LUXATEMP	0.03(0.01)F	0.38(0.12)EF	0.42(0.14)EF
JET SET 4	0.08(0.01)F	5.91(1.74)B	11.27(3.38)A

Levels not connected by same letter are significantly different.

3.2.2 *Square mean linear regression:*

Analysis of variance was performed on the data set as a whole. (Tables 28 and 29).

Table 28: Analysis of Variance of whole model of surface roughness.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	17	1548.2058	91.0709	146.6105
Error	222	137.9011	0.6212	Prob > F
C. Total	239	1686.1069		<.0001*

Table 29: Whole model effect tests of surface roughness.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	2	2	496.83627	399.9158	<.0001*
Material	5	5	764.50473	246.1475	<.0001*
Material*Treatment	10	10	571.81655	92.0539	<.0001*

Least Squares means differences Tukey HSD

Treatment

Least squares means differences per type of tooth brush bristle employed are depicted on Table 30, showing significant differences between baseline, soft and medium bristles

Table 30: LS means differences Tukey HSD test per Treatment.

Level				Least Sq Mean
Hard bristles	A			3.44
Soft bristles		B		2.09
Baseline			C	0.05

Levels not connected by same letter are significantly different.

Material

Least squares means differences per material are depicted on Table 31, showing significant differences between Jet Set-4 and rest of the materials. No significant difference was evident between Telio CAD and artBloc, and between Vita CAD temp, Protemp Plus and Luxatemp.

Table 31: LS means differences Tukey HSD test per Material.

Level				Least Sq Mean
JET SET	A			5.75
TELIO CAD		B		2.02
ARTBLOC		B		2
VITA CAD			C	0.76
PROTEMP			C	0.34
LUXATEMP			C	0.27

Levels not connected by same letter are significantly different.

Material vs Treatment

Least Square Means differences are shown on Table 32, comparing the material and its effect by each type of treatment (soft bristles and medium bristles). We can appreciate that there is no significant difference of all the materials at baseline. Jet Set-4 had significant difference after treatments compared to the rest of the materials, presenting the highest values of 5.91 μm and 11.27 μm for soft and medium bristles respectively.

There was no significant difference between Telio CAD and artBloc Temp after treatment on both modalities, also no significant difference was found between Vita CAD, Luxatemp and Protemp Plus (with soft bristles only).

An important finding was that there was no significant difference compared to the baseline after treatments of Protemp Plus, Luxatemp and Vita CAD (with soft bristles only).

Table 32: LS means differences Tukey HSD test Material*Treatment

Level							Least Sq Mean
JET SET,Hard bristles	A						11.27
JET SET,Soft bristles		B					5.91
TELIO CAD,Hard bristles			C				3.47
ARTBLOC,Hard bristles			C				3.22
ARTBLOC,Soft bristles			C	D			2.74
TELIO CAD,Soft bristles			C	D			2.56
VITA CAD,Hard bristles				D	E		1.53
VITA CAD,Soft bristles					E	F	0.72
PROTEMP,Hard bristles					E	F	0.71
LUXATEMP,Hard bristles					E	F	0.41
LUXATEMP,Soft bristles					E	F	0.38
PROTEMP,Soft bristles						F	0.23
JET SET,Baseline						F	0.08
PROTEMP,Baseline						F	0.07
TELIO CAD,Baseline						F	0.05
VITA CAD,Baseline						F	0.03
LUXATEMP,Baseline						F	0.03
ARTBLOC,Baseline						F	0.03

Levels not connected by same letter are significantly different.

CHAPTER 4: DISCUSSION

This study assessed the effect of beverage discoloration on color stability, evaluated the stain removal performance and abrasion of tooth brushing of provisional restorative materials for crowns and bridges.

The color stability was evaluated by using beverages that are frequently ingested in our daily diet and some of them are known to have the potential to stain restorative materials.

These beverages have different types of food pigments, that could be either natural or artificial. Some of the most common ones are: chlorophyll, anthocyanin, turmeric, tannins and carotenoids. Anthocyanin is present in blue and purple foods such as grapes, blueberries and cranberries. Tannin is the pigment responsible for the brown color when coffee is roasted. (The Chemistry of Food Colorings 2017). The main two pigments present in tea are chlorophyll and carotenoids (Gebely, 2015).

In the present study, the three beverages that presented the highest levels of discoloration were: red wine, coffee and cranberry juice. Red wine being the highest, could be attributed to the fact that alcohol content increases staining potential by making the resin matrix softer (Ardu et al. 2010). However, it is not clear whether staining by red wine was due to the alcohol or by the presence of pigments in wine, such as anthocyanin. In the case of coffee, this beverage contains tannic acid and pigments with low polarity that seems to be main cause of the staining due to their affinity to the polymer network. Cranberry juice also contains the same pigment as red wine, anthocyanin. It could be assumed that the contents of anthocyanin and tannic acids may increase the staining effect, reflecting the importance of the chemical composition of beverages, thus, if a material is capable of absorbing water, it is also more likely to absorb water-soluble pigments, resulting in discoloration. (Nasim et al. 2010)

The staining ability of tea may be attributed to the presence of chlorophyll and carotenoids.

Coca-Cola presented Delta E* values similar to the group immersed in distilled water. None of the groups showed significant discoloration effect on any of the materials tested ($p<0.05$). Probably because of the low staining effect of the solutions' components. According to Ruyter et al, Coca-Cola has an acidic pH and this might cause damage to the surface of the material, but the stains contained in it have low polarity with few yellow stains, resulting in low staining performance (1987).

Time of immersion is also an important factor to take into consideration, the longest the material were immersed, the higher the level of discoloration. Several studies suggested that immersion in beverages for one week was equivalent to seven months of beverage intake. Therefore, eight weeks of immersion of specimens in beverages for evaluation of the resulting staining effect might be an exaggeration of the reality, but it could represent the effect of these on the long term (Lauvahutanon et al. 2017).

The type of the material was also an important variable that was observed.

Injectable bisacrylics presented significant levels of discoloration even after simulated tooth-brushing. These materials are susceptible to staining maybe because of their high degree of water absorption, which is affected by the nature of the resin matrix, resulting in intrinsic stains.

CAD-CAM blocks showed significantly lower color change as compared to traditional materials, at all durations, and after brushing ($p<0.001$), which is most likely related to the higher residual monomer ratio, difference in porosity, filler content, polymerization and fabrication method. CAD/CAM blocks are industrially fabricated under optimum manufacturing conditions. Such conditions provide better mechanical properties than those that are fabricated manually. Tooth brushing also reversed the coffee and red wine discoloration to an acceptable Delta E* value for CAD/CAM blocks, indicating that discolorations were extrinsic on these materials. It is assumed that regular tooth brushing can eliminate or reduce surface stains effectively.

Protemp Plus and Luxatemp groups showed no significant difference after tooth-brushing. The R_a values of these materials using soft bristles ranged between 0.24 and 0.38 μm, almost at the limit of bacterial adhesion (R_a: 0.2 μm) (Kamonkhantikul et al. 2016) and below the average surface roughness of enamel (R_a: 0.64 μm) (Bollen, Lambrechts, and Quirynen 1997). This may lead to the belief that the effect of tooth brushing could prevent plaque accumulation on the surfaces of the bisacrylics tested. Moreover, the visual perception of a surface roughness value lower than 1 μm is considered to be smooth, and patients can differentiate roughness when the difference is over 0.5 μm R_a (Jones et al, 2014). Nevertheless, the R_a values of the CAD/CAM blocks and powder-liquid self-cure acrylic after brushing for 20,000 cycles exceeded these threshold limits. Bisacrylic resins are chemically formed by two or more types of material, it is a hydrophobic material similar to bis-GMA, so when this resin is mixed with inorganic fillers it combines to create a provisional restorative material similar to composite resins (Burns et al. 2003). The fact that injectable bisacrylics showed better tooth brushing wear resistance could be attributed to the filler size and content. Based on the findings by Turssi et al, which stated that the small-sized filler particles and higher load content contributes to better wear resistance of composite resins (2005). This means that due to the higher quantity of particles subjected to abrasion, there is more surface area that contacts the antagonist, hence, better wear resistance is observed. Regarding the filler particle size, it could be explained by the fact that the mean distance between these small particles is less than bigger or coarse particles, making it easier to prevent wear (Jørgensen andAsmussen 1978).

In the present study the material that presented the highest level of surface roughness after tooth brushing was Jet Set-4, this is a self-cure powder-liquid acrylic, and could result in a porous surface after fabrication. Porosity is a negative consequence of polymerization of the acrylic resin and it may occur due to various reasons: lack of adequate pressure at the time of fabrication, vaporization of the monomer or lack of uniformity of the acrylic mixture. According to Hiramatsu, powder-liquid self-cure acrylics fabricated using a direct technique presented the highest porosity values, as compared to provisionals fabricated in a more accurate way, where the pressure and temperature conditions are more controlled (2011). There could be a positive correlation between roughness and porosity.

CAD/CAM blocks exhibited a significant increase in surface roughness, artBloc Temp and Telio CAD with soft and medium bristles and Vita CAD only with medium bristles. According to Kamonkhantikul Telio CAD does not have filler content, meaning that simulated tooth brushing could create scratches on an easier way in the same direction of the strokes for 20,000 cycles (2016). So one could assume that the amount of filler content and porosity could justify the deep scratches along the brushing direction of the CAD/CAM blocks. The exact amount of filler content, particle sizes and components are not disclosed by the manufacturers of any of the materials.

CHAPTER 5: CONCLUSION

The effect of beverages on color stability of provisional materials depends on type of beverage and composition of the material used. Coffee and red wine exhibited the highest discoloration effect at 8 weeks of immersion. Coca-Cola and water had no significant discoloration effect on any of the tested materials. CAD-CAM blocks presented the highest levels of color stability at all time points as compared to traditional materials.

Simulated tooth brushing after 8 weeks of immersion in beverages reduced the effect of discoloration on CAD-CAM blocks to near baseline values. In terms of effect of tooth brushing on surface roughness it can be concluded that medium bristles caused significantly higher R_a than soft bristles and injectable bisacrylics presented the lowest levels of surface roughness after 20,000 cycles of tooth-brushing.

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