Staining and Cleaning Effects of Copolyester and Copolymer Retainer Materials

BY

LAURIE SUSARCHICK
B.A., Rowan University, 2009
D.D.S., Columbia University College of Dental Medicine, 2018

THESIS

Submitted as partial fulfillment of the requirements for
the degree of Master of Science in Oral Sciences in the
Graduate College of the University of Illinois at Chicago, 2021

Chicago, Illinois

Defense Committee:

Phimon Atsawasuwan, Chair and Advisor, Orthodontics
Veerasathpurush Allareddy, Orthodontics
Spiro Megremis, American Dental Association
Grace Viana, University of Illinois at Chicago

This thesis is dedicated to my family and friends. A special feeling of gratitude to my mother, Shirley, my brother, Thomas, and my husband, Rutger, whose encouragement, confidence, love, and support pushed me to succeed. I also dedicate this thesis to Arshad Mohammad, who was always too good for this world.

ACKNOWLEDGMENTS

Thank you to my thesis committee, Dr. Phimon Atsawasuwan, Dr. Veerasathpurush Allareddy, Dr. Spiro Megremis, Dr. Henry Lukic, and Grace Viana. You were all more than generous with your time.

A special thanks to Dr. Phimon, my thesis advisor for his knowledge and encouragement throughout the process. Thank you, Dr. Lukic and Dr. Megremis, for allowing us to continue at the ADA, especially throughout this time of COVID uncertainty, and for always being available to answer questions. Thank you, Dr. Allareddy, you always prioritize the residents and have an open door. We appreciate you. Thank you, Grace Viana, you seemed to have limitless patience with us and our data. We appreciate you.

Lastly, I would like to thank all the donors that made this research possible.

LS

TABLE OF CONTENTS

CHAPTER PAGE

I. INTRODUCTION 1
A. Background 1
B. Objective 1
C. Hypotheses 2
D. Eligibility 2

II. RELATED LITERATURE 4
A. Orthodontic Relapse 4
1. Treatment of Orthodontic Relapse and Prevention 5
2. Orthodontic Retainer Options 6
3. Clear Retainer Materials and Fabrication 8
4. Clear Retainer Staining and Cleaning Methods 10
B. Managing Relapse 19

III. METHODOLOGY 21
A. Specimen Fabrication 21
1. Staining Experiments 25
2. Destaining Experiments 30
B. Colorimetry Study using a Spectrophotometer 34
C. Light Transmittance Study using a Spectrometer 37
D. Material Composition Study using Raman Spectrometry 39

IV. RESULTS 42
A. Statistical Analysis 42
B. Comparison between Essix ACE® and Essix C+® 42
C. Essix ACE® Staining Results 43
1. Differences of Textured vs. Smooth Surface Textures at Each Timepoint 43
2. Differences among Staining Solutions at Each Timepoint 47
3. Differences between Surfaces at Specific Timepoints among Staining Solutions 51
D. Essix ACE® Destaining Results 57
1. Textured vs. Smooth Surface 57
2. Mechanical Cleaning 59
3. Cleaning Solution 62
4. Staining 67
E. Essix C+® Staining Results 82
1. Differences of Textured vs. Smooth Surface Textures at Each Timepoint 82
2. Differences among Staining Solutions at Each Timepoint 86
3. Differences between Surfaces at Specific Timepoints among Staining Solutions 90
F. Essix C+® Destaining Results 97
1. Textured vs. Smooth Surface 97

TABLE OF CONTENTS (continued)

CHAPTER PAGE

2. Mechanical Cleaning 99
3. Cleaning Solution 102
4. Staining 106

G. Raman Spectrometer Baseline and Staining 121
1. Baseline 121
2. Raman Staining Day 28 122
3. Raman Destaining 125

V. DISCUSSION 130

VI. STUDY LIMITATIONS AND FUTURE CONSIDERATIONS 136

VII. CONCLUSIONS 137

CITED LITERATURE 138

APPENDICES 143
Appendix A 143
Appendix B 148

VITA 153

LIST OF TABLES

TABLE PAGE

I. MATERIAL COMPOSITION DATA 10

II. BIOSTAR® THERMOFORMING PARAMETERS FOR SPECIMEN FABRICATION ... 24

III. COLORIMETRY RANGE 34

IV. LABELS OF COLOR CHANGE, ACCORDING TO THE NATIONAL BUREAU OF STANDARDS (NBS) 36

V. THE P-VALUES OF ESSIX ACE® AT DAY 7 IN STAINING EXPERIMENTS 45

VI. THE P-VALUES OF ESSIX ACE® AT DAY 14 IN STAINING EXPERIMENTS 46

VII. THE P-VALUES OF ESSIX ACE® AT DAY 28 IN STAINING EXPERIMENTS 47

VIII. THE P-VALUES OF ESSIX ACE® IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS. . 49

IX. THE P-VALUES OF ESSIX ACE® IN THE BLACK TEA STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS. 49

X. THE P-VALUES OF ESSIX ACE® IN THE DISTILLED WATER STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS. 50

XI. THE P-VALUES OF ESSIX ACE® IN THE RED WINE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS. . 50

XII. THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS. 51

XIII. THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS. 52

XIV. THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE DISTILLED WATER STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS. 52

LIST OF TABLES (continued)

TABLE PAGE

XV. THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS. 53

XVI. ESSIX ACE® ΔE NBS VALUE (ΔE X 0.92)..... 55

XVII. ESSIX ACE® MEDIAN VALUES FOR ΔT BETWEEN SURFACES AMONG STAINING SAMPLES..... 56

XVIII. THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT 58

XIX. THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT 58

XX. THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT 59

XXI. THE P-VALUES OF ESSIX ACE® AFTER DESTAINING 60

XXII. THE P-VALUES OF ESSIX ACE® STAINED WITH COFFEE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING..... 60

XXIII. THE P-VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING..... 61

XXIV. THE P-VALUES OF ESSIX ACE® STAINED WITH RED WINE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING..... 61

XXV. THE P-VALUES OF ESSIX ACE® STAINED WITH COFFEE AFTER DESTAINING.. 64

XXVI. THE P-VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AFTER DESTAINING 65

XXVII. THE P-VALUES OF ESSIX ACE® STAINED WITH RED WINE AFTER DESTAINING. 66

LIST OF TABLES (continued)

TABLE		PAGE
XXVIII.	MEDIAN VALUES OF ESSIX ACE® STAINED WITH COFFEE AND DESTAINED – ULTRASONIC VS NON-ULTRASONIC.	68
XXIX.	MEDIAN VALUES OF ESSIX ACE® STAINED WITH COFFEE AND DESTAINED – SURFACE TEXTURES.	69
XXX.	MEDIAN VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AND DESTAINED – ULTRASONIC VS NON-ULTRASONIC	69
XXXI.	MEDIAN VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AND DESTAINED – SURFACE TEXTURES	70
XXXII.	MEDIAN VALUES OF ESSIX ACE® STAINED WITH RED WINE AND DESTAINED – ULTRASONIC VS NON-ULTRASONIC	70
XXXIII.	MEDIAN VALUES OF ESSIX ACE® STAINED WITH RED WINE AND DESTAINED – SURFACE TEXTURES	71
XXXIV.	THE P-VALUES OF ESSIX C+® AT DAY 7 IN THE STAINING EXPERIMENTS	84
XXXV.	THE P-VALUES OF ESSIX C+® AT DAY 14 IN THE STAINING EXPERIMENTS	85
XXXVI.	THE P-VALUES OF ESSIX C+® AT DAY 28 IN THE STAINING EXPERIMENTS	86
XXXVII.	THE P-VALUES OF ESSIX C+® IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.	88
XXXVIII.	THE P-VALUES OF ESSIX C+® IN THE TEA STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.	88
XXXIX.	THE P-VALUES OF ESSIX C+® IN THE DISTILLED WATER STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.	89
XL.	THE P-VALUES OF ESSIX C+® IN THE WINE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.	89
XLI.	THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.	90
XLII.	THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.	91

LIST OF TABLES (continued)

TABLE PAGE

XLIII. THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE WATER STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS. 91

XLIV. THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS. 92

XLV. ESSIX C+® MEDIAN VALUES FOR ΔE NBS VALUE (ΔE X 0.92) 95

XLVI. ESSIX C+® MEDIAN VALUES FOR ΔT BETWEEN SURFACES AMONG STAINING SAMPLES..... 96

XLVII. THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT 98

XLVIII. THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT 98

XLIX. THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT 99

L. THE P-VALUES OF ESSIX C+® AFTER DESTAINING 100

LI. THE P-VALUES OF ESSIX C+® STAINED WITH COFFEE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING. 100

LII. THE P-VALUES OF ESSIX C+® STAINED WITH BLACK TEA AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING. 101

LIII. THE P-VALUES OF ESSIX C+® STAINED WITH RED WINE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING. 101

LIV. THE P-VALUES OF ESSIX C+® STAINED WITH COFFEE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING 104

LV. THE P-VALUES OF ESSIX C+® STAINED WITH BLACK TEA AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING 105

LVI. THE P-VALUES OF ESSIX C+® STAINED WITH RED WINE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING 106

LIST OF TABLES (continued)

TABLE		PAGE
LVII.	MEDIAN VALUES OF ESSIX C+® STAINED WITH COFFEE AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC	108
LVIII.	MEDIAN VALUES OF ESSIX C+® STAINED WITH COFFEE AND DESTAINED – TEXTURED VS. SMOOTH	108
LIX.	MEDIAN VALUES OF ESSIX C+® STAINED WITH BLACK TEA AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC	109
LX.	MEDIAN VALUES OF ESSIX C+® STAINED WITH BLACK TEA AND DESTAINED – TEXTURED VS. SMOOTH	109
LXI.	MEDIAN VALUES OF ESSIX C+® STAINED WITH RED WINE AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC	110
LXII.	MEDIAN VALUES OF ESSIX C+® STAINED WITH RED WINE AND DESTAINED – TEXTURED VS. SMOOTH	110

LIST OF FIGURES

FIGURE PAGE

1. Biostar® Scan pressure machine 22
2. Stainless steel block mold with two surface textures..... 23
3. Thermoformed material over custom stainless-steel block. 23
4. Specimen staining preparation. 26
5. Essix C+® stained with coffee solution..... 28
6. Essix C+® stained with black tea solution 28
7. Essix C+® stained with red wine solution..... 28
8. Essix ACE® stained with coffee solution 29
9. Essix ACE® stained with black tea solution 29
10. Essix ACE® stained with red wine solution..... 29
11. Specimen destaining preparation 31
12. Destaining process. 31
13. Flowchart 33
14. Spectrophotometer. 37
15. Spectrometer Integrating sphere. 39
16. Raman spectrometer diagram 41
17. Raman spectrometer..... 41
18. Mean value of ΔE in Essix ACE®..... 53
19. Mean value of ΔT in Essix ACE®..... 54
20. Comparison of Essix ACE® ΔT for textured surfaces 72
21. Comparison of Essix ACE® ΔT for smooth surfaces 73
22. Essix ACE® stained with coffee and destained with the non-ultrasonic. 74

LIST OF FIGURES (continued)

FIGURE PAGE

23. Essix ACE® stained with black tea and destained with the non-ultrasonic. 75

24. Essix ACE® stained with distilled water and destained with the non-ultrasonic. 76

25. Essix ACE® stained with red wine and destained with the non-ultrasonic. 77

26. Essix ACE® stained with coffee and destained with the ultrasonic. 78

27. Essix ACE® stained with black tea and destained with the ultrasonic. 79

28. Essix ACE® stained with distilled water and destained with the ultrasonic. 80

29. Essix ACE® stained with red wine and destained with the ultrasonic. 81

30. Mean value of ΔE in Essix C+® 93

31. Mean value of ΔT in Essix C+® 94

32. Comparison of Essix C+® ΔT among destaining solutions for textured surface 111

33. Comparison of Essix C+® ΔT among destaining solutions for smooth surface 112

34. Essix C+® stained with coffee and destained with the non-ultrasonic. 113

35. Essix C+® stained with black tea and destained with the non-ultrasonic. 114

36. Essix C+® stained with distilled water and destained with the non-ultrasonic. 115

37. Essix C+® stained with red wine and destained with the non-ultrasonic. 116

38. Essix C+® stained with coffee and destained with the ultrasonic. 117

39. Essix C+® stained with black tea and destained with the ultrasonic. 118

40. Essix C+® stained with distilled water and destained with the ultrasonic. 119

41. Essix C+® stained with red wine and destained with the ultrasonic. 120

42. Raman spectrometer at baseline 122

43. Raman spectrometer at day 28 after staining with coffee 124

44. Raman spectrometer at day 28 after staining with distilled water 125

LIST OF FIGURES (continued)

FIGURE PAGE

45. Raman spectrometer after destaining - Essix ACE® stained with coffee 126

46. Raman spectrometer after destaining - Essix ACE® stained with distilled water 127

47. Raman spectrometer after destaining - Essix C+® stained with coffee 128

48. Raman spectrometer after destaining - Essix C+® stained with distilled water 129

LIST OF ABBREVIATIONS

Δ	Delta
Δa*	Change in Red/Green Hue
Δb*	Change in Yellow/Blue Hue
ΔL*	Change in Lightness
ΔT	Change in Translucency
ABS	Acrylonitrile Butadiene Styrene
ADA	American Dental Association
ASTM	American Society for Testing Materials
CAT	Clear Aligner Therapy
CIE	Commission Internationale de I'Eclairage
CNC	Computer Numerical Control
CSF	Circumferential Supracrestal Fiberotomy
FDM	Fused Deposition Modeling
H_2O_2	Hydrogen Peroxide
H(1)	Hypothesis 1
H(2)	Hypothesis 2
H(3)	Hypothesis 3
H(4)	Hypothesis 4
IMF	Intermaxillary Fixation
IPR	Interproximal Reduction
MTM	Minor Tooth Movement
NBS	National Bureau of Standards
PDL	Periodontal Ligament
PETG	Polyethylene Terephthalate Glycol
PU	Polyurethane
RCT	Randomized Control Trials
SD	Standard Deviation

LIST OF ABBREVIATIONS (continued)

SDS	Standardized Data Sheet
SEM	Scanning Electron Microscope
T0	Timepoint 0
T1	Timepoint 1
T2	Timepoint 2
T3	Timepoint 3
T4	Timepoint 4
T5	Timepoint 5
TAD	Temporary Anchorage Device
TOA	Transparent Orthodontic Aligners
TP	Translucency Parameter
UIC	University of Illinois at Chicago

SUMMARY

The increasing demand for aesthetics by all patients makes clear aligner therapy (CAT) and clear retainers in orthodontic treatment popular. The removable nature of clear retainers is often a benefit, allowing patients to remove the retainers to maintain good oral hygiene. However, long-term compliance is required of all post-orthodontic treatment patients and when patients become non-compliant, relapse can occur. Relapse is complicated and unpredictable. To prevent relapse, all patients should be treated as if they are at high-risk for relapse. Often, patients become non-compliant as the clear retainers become less clear, and therefore, less aesthetic. Thus, the need for research on how to maintain clear aligner translucency and color, while also maintaining the retainer properties and integrity, is needed. The objective of this study is to investigate the staining ability of different staining solutions on retainer materials and the effect of destaining agents on the light transmittance and color changes of two retainer materials, *in vitro*.

This research focuses on the study of two retainer materials, copolyester (Essix ACE®) and copolymer (Essix C+®), stained with red wine, coffee, black tea, and distilled water followed by destaining with five cleaning materials, namely, hydrogen peroxide (H_2O_2), Invisalign® Cleaning Crystals, Retainer Brite®, Polident® denture cleaner, and Listerine® mouthwash. The retainer materials were thermoformed over a custom stainless-steel block with disposable polymer molds rendering two surfaces, smooth and textured. The smooth surface mimics retainers made from plaster models and the textured surface mimics retainers made from 3D-printed models. The translucency and color changes of retainer material specimens were analyzed using specific spectrophotometers, and composition changes were evaluated by Raman Spectrometry before and after the 28-day staining and one-session destaining processes. During the destaining, one group of stained specimens were

subjected to either ultrasonic or non-ultrasonic cleaning methods. The Δ values were used to evaluate the change of color and translucency in each material from baseline to the end of the destaining process.

Due to the non-normal distribution of the data analyzed by the Shapiro-Wilk test, the data was analyzed with non-parametric statistics, namely, Kruskal Wallis statistical and Mann-Whitney tests. Due to the differences in the innate characteristics of the individual materials, the outcome data were not compared between copolymer and copolyester. The results showed staining in both materials increased with exposure time. The most staining occurred at the end of staining (T3, 28 days) and the specimens were more susceptible to coffee and black tea staining. At the end of the staining period, the coffee and black tea staining solutions resulted in the most color change.

After immersion in the destaining solutions, no statistically significant differences for light transmittance or color change were found between ultrasonic and non-ultrasonic cleaning groups. No major changes were noted among all staining solutions or destaining means. All cleaning solutions showed improved light transmittance. Both materials had statistically significant changes in translucency and color change on the textured surface. Qualitatively, no composition changes were observed in any groups at the end of destaining.

The results from this study will contribute to an evidence-based approach for clinical guidance for long-term maintenance of clear retainer.

I. INTRODUCTION

A. Background

In orthodontics, one of the most common complications and frustrations for patients and providers is relapse. Relapse occurs when teeth return to their original position after orthodontic treatment.[1] Relapse is most commonly found in the lower anterior dentition, from canine to canine[1]. To avoid wasting time, money, and resources, as well as to maintain the treatment outcomes, research is needed to identify the most effective and safe method to manage relapse.[1] Retainers are the only effective approach to prevent relapse. Clear retainers have gained in popularity due to their aesthetic aspect as a clear appearance; however, the maintenance of their clear appearance needs an effective cleaning agent for long-term maintenance. Until now, only a few studies have investigated the effectiveness of cleaning solutions for clear retainers. Their ability to be stained and destained must be investigated in further detail.

B. Objective

This study aims to investigate the ability of two retainer materials, copolyester (Essix ACE®) and copolymer (Essix C+®), with two different surfaces, smooth and textured, to be stained with four different staining solutions by evaluation of their translucency and color changes overtime. This study also aims to investigate the ability of five destaining solutions with, and without, an ultrasonic cleansing mean by evaluation of their translucency and color change after one session of destaining.

C. Hypotheses

H(1) – There is no difference of light transmittance and color change at different time points after staining with four staining solutions on copolyester (Essix ACE®) and copolymer (Essix C+®) material.

H(2) There is no difference of light transmittance and color change at different time points after staining with four staining solutions on the different surfaces of copolyester (Essix ACE®) and copolymer (Essix C+®) material.

H(3) – There is no difference in light transmittance and color change among the destaining agents on copolyester (Essix ACE®) and copolymer (Essix C+®) material.

H(4) – There is no difference in light transmittance and color change between ultrasonic and non-ultrasonic means on copolyester (Essix ACE®) and copolymer (Essix C+®) material.

D. Eligibility

1. Inclusion Criteria

Retainer materials – 0.040” thickness

- Essix ACE® - Dentsply® International Inc.
- Essix C+® - Dentsply® International Inc.

4 Staining methods

- Coffee - Nescafe® Original, Nestle, Vevey, Vaud, Switzerland
- Black tea - Lipton® Unsweetened Black Iced Tea Mix

- Red wine - Paint Box® Cabernet, Cabernet Sauvignon, CA, California Misc, 27.0 proof
- Distilled water

5 cleaning methods and a storage solution

- Invisalign® Cleaning Crystals
- Polident® denture cleaner
- Listerine® mouthwash
- Retainer Brite®
- 3% hydrogen peroxide (H_2O_2)
- Artificial saliva (storage solution for specimens when not in staining/destaining solution)

Ultrasonic cleaning method - BioSonic® UC300R, Coltene/Whaledent

Non-ultrasonic cleaning method

2. Exclusion Criteria

- Non-Essix ACE® or non-Essix C+® retainer materials
- Any cleaning method not listed above

II. Related Literature

A. Orthodontic Relapse

Studies ranging from two to over ten years post-orthodontic treatment, demonstrate that up to 90% of patients have unacceptable alignment ten years after completing treatment.[1,2] Relapse may occur within the first few hours of orthodontic appliance removal thus, the retention phase can be considered one of the most complex and crucial phases for successful orthodontic treatment.[2] Many times, relapses occurs from a lack of removable retainer compliance.[3] Retainer compliance can rely on various variables such as age, gender, amount of time since braces removal, and type of retainer.[45]

Orthodontic relapse is different for each individual and is difficult to predict. Relapse can occur from slower bone deposition on the compression side of tooth movement or from slower turnover rate of gingival collagen fibers.[2] It is not yet possible to predict which patients will relapse since there are so many complex factors such as bone composition, gingiva type, periodontal ligament (PDL), types of movement, occlusal stability, and blood and lymphatic circulation involved.[2] Other factors such as over-expansion of the arches and eruption of the third mandibular molars may also affect relapse.[1] Patient age, growth potential, length of retention, third molars, as well as doctor experience may also influence a cause-and-effect role in relapse.[2] Over time, maxillary and mandibular dental arch forms have become narrower and shorter with age, which may explain crowding in adulthood where no crowding was previously observed.[2] Hence, the problem is multifactorial and many of the relapse observations cannot be distinguished from the normal aging process.[2]

1. Treatment of Orthodontic Relapse and Prevention

There is considerable variation regarding relapse and individual patients. When trying to predict whether a patient will have long-term relapse, practitioners often analyze pre-treatment records looking for severity of anterior crowding and initial Angle classification or other variables such as patient age, third molars, oral habits, and length of retention.[6] In the past, it was thought that post-orthodontic stability could be achieved after one year. This was then extended to two years, five years, and then ten years.[6] Orthodontists in the past used to believe that stability came with appropriate treatment and that when relapse occurred, it was a sign of inappropriate treatment, misdiagnosis, or incorrect mechanics.[6] However, no one variable can be used to predict relapse. Little et al. found initial crowding a very poor predictor of relapse, though most cases with severe initial crowding likely relapse in some capacity. In addition, it was found that arch width and length typically decrease in retention, regardless of expansion or constriction treatment and patients with minimal crowding initially tended to become more crowded while patients with severe initial crowding leveled off.[6] Lastly, it was found that satisfactory alignment is maintained in less than thirty percent of patients.[6]

Circumferential supracrestal fiberotomy (CSF) is a procedure that is occasionally recommended in teeth that were initially severely rotated and are considered high-risk for relapse. The soft tissue surgical procedure severs the dento-givingal and interdental gingival fiber attachments surrounding the tooth. There is weak evidence of success with CSF and Little et al. found that even with CSF, relapse can still occur.[7] The only way to prevent relapse is the use of fixed or removable retention for life.[7] Instability should be assumed by Orthodontist, educated to the patient, and planned for.

Re-treatment due to relapse can occur various ways. However, there has been no evidence that any one treatment is superior to another in terms of managing relapse.[1] According to Yu et al., there

is no evidence from randomized controlled trials (RCTs) to guide orthodontists in selecting an effective method for treatment of relapse of alignment of lower front teeth following initial orthodontic treatment.[1]

Treating relapse can occur through, most extensively, complete re-treatment with fixed appliances or with removable aligners. Alternatively, some orthodontists treat relapse patients with a Hawley spring aligner or with several active clear aligners, depending on the severity. Both options, Hawley spring aligner and clear aligners, often involve some interproximal reduction (IPR) to reduce the width of the teeth to alleviate the crowding. Both types must be worn full time, approximately 22 hours per day, and therefore require patient motivation and compliance. In addition, both involve active treatment and must be supervised, therefore, the patient must come to the office or be virtually monitored.

2. Orthodontic Retainer Options

The only effective approach to prevent orthodontic relapse and achieve a stable result is long-term retainer wear.[2] Patients must be informed, before initiation of treatment, that retention is not optional, but a strongly advised continuation of their orthodontic treatment.[2] The two types of retainers used to stabilize the teeth and prevent relapse post-orthodontic treatment, are fixed and removable.[1]

Fixed retainers are bonded to the lingual side of the anterior teeth and can be used on the upper arch, lower arch, or both. It is generally thought that fixed retainers may produce more gingival inflammation, plaque, and calculus buildup, however, Heier et al. found there were comparable amounts of gingival inflammation with both types of retainers.[8] Fixed retainers require long-term responsibility and must be examined regularly to check the bonding and confirm no breakage or

damage causing unwanted tooth movement or iatrogenic damage. Fixed retainers are typically placed in higher relapse risk cases such as patients with periodontitis or with diastemas. Fixed retainers are commonly hand-shaped by an orthodontist and bonded from canine to canine, with variations that may include only the central incisors or from premolar to premolar. Memotain®, a robotically bent custom-made retainer, is also an option for fixed retainers.

For removable retainers, the two most common are "clear" retainers made of various plastics and Hawley retainers made from a combination of metal and acrylic.[1] Clear retainers are more aesthetic but are rigid and cannot be adjusted for minor tooth movements. Hawley retainers are durable and adjustable, allowing for many adjustments for minor tooth movements. The major disadvantage to removable retainers is compliance and prolonged use required by patients. However, the advantage of the removable design includes the ability to brush and floss, whereas the fixed requires interproximal brushes or specially designed floss.

Clear retainers revealed better compliance than Hawley retainers in the first two years after orthodontic treatment.[45] However, after two years, compliance increased, and over time, long-term compliance was greater with Hawley retainers.[5] The author speculates the Hawley durability makes it less vulnerable to discoloration and that the initial increase of compliance with clear retainers for the first two years may be due to its aesthetic nature.[5] According to Rowland et al., patients prefer clear aligners and wear them more consistently than Hawley retainers.[9] In addition, clear retainers, presumably due to better compliance, were more effective at maintaining the correction of the mandibular labial segments.[9]

With advancements of orthodontic techniques, clear retainers have increased in popularity due to their aesthetic nature and comparable treatment times.[1] Clear retainers can also be used for

comprehensive care, and are then referred to as aligners, not retainers. These clear retainers were created to increase patient acceptance and are presented to the general public as aesthetic, comfortable, economical, as well as quickly fabricated, and replaced, as needed.[10] Clear retainers can also be used for minor tooth movements and also as whitening trays.[11,12] One obstacle to clear aligners are studies which have reported clear retainers to make patients more susceptible to caries as saliva flow over the tooth surface is blocked and therefore, provides no protective shield to bacteria.[13,14]

Initially, clear aligners were only used treat mild malocclusions. However, with advancing techniques, clear aligner therapy (CAT) can now be used for more complicated treatment plans including extraction cases and orthognathic surgery via clear splints.[15] By using clear aligners, the dentition can be engaged in full cuspal coverage and allows for intermaxillary fixation. Often, the techniques are used with adjunctive devices such as temporary anchorage devices (TADs), intermaxillary fixation (IMF) screws, buttons, and brackets.[15] Thus, the indications for CAT are expanding as research and technology progress forward.

At the end of orthodontic treatment, orthodontists will often give a combination retention protocol involving a fixed retainer with a removable fitted on top. However, in terms of evidence-based protocols, a literature review by Alassiry in 2019 found a lack of high quality evidence for retention protocol and a need for further studies.[16]

3. Clear Retainer Materials and Fabrication

Clear retainers, clear aligners, invisible retainers, clear aligner therapy, transparent orthodontic aligners (TOA) all refer to the same product type which has multiple uses and primarily were created to satisfy an aesthetic need in Orthodontics. These clear retainers are composed of

amorphous, partially crystalline polymers, which allow visible light through and give them a clear appearance.[17] Their popularity has led to research investigating tooth movements, activation protocols, forces and moments per aligners, as well as torque and force after insertion.[17]

There are several methods to manufacture thermoplastic retainers. Retainers can be fabricated in-house with plaster models or with a 3D printer often using copolyester or copolymer materials or via larger companies, such as Invisalign®. 3D-printing software uses volumetric data that can replicate patient tooth structure and arch forms, which in turn is used to fabricate accurate retainers and aligners.[18] Plaster models are becoming obsolete as intraoral scanners and 3D printers become more available.[18] A patient digital scan combined with computer software can digitally realign teeth to create a series of 3D printed models.[18] These models can then be used to manufacture aligners in-house. In-house retainers are typically fabricated with Essix ACE®, Essix A+®, or Essix C+® materials.

In-house retainer and aligner fabrication is often delegated to auxiliary staff, who can easily be taught to fabricate these retainers, entirely eliminating the need for lab fees and technical wire bending training.[19] Additional benefits of 3D-printed digital models over the traditional plaster model is the time saved, space saved, and quick and easy ability to archive and find models when needed.[18]

To fabricate a clear retainer, a thermoforming machine heats the plastic material of choice. When heated sufficiently, pressure is used to shape the soft plastic around a model of the patient mouth. The plastic is then cut out from the model and polished and cleaned before delivery. This same method can be used to fabricate in-house aligners.

The most popular materials for clear retainers are polyethylene copolymers, considered to be more durable, and polypropylene polymers, considered to be more transparent and aesthetic, see Table I[13]. The safety data sheets (SDS) report Essix ACE® material is composed of 95% copolyester and

5% trade secret and Essix C+® is composed of 95% polypropylene/ethylene copolymer and 5% trade secret.[20,21] A popular commercially made clear retainer is Vivera® by Invisalign®, a polyurethane blend of methylene diphenyl diisocyanate and 1,6-hexanediol.

TABLE I

MATERIAL COMPOSITION DATA[21,22,20]

	Material Composition	**Brand Names**
Copolyester	Copolyester + Trade secret	Essix ACE®
Copolymer	Polypropylene/ethylene copolymer + stabilizers (trade secret)	Essix C+®
Polyurethane	Polyurethane from methylene diphenyl diisocyanate and 1, 6-hexanediol + additives	Zendura® Vivera® and Invisalign® Invisalign® Templates

4. Clear Retainer Staining and Cleaning Methods

Clear retainers must be maintained as material reactions such as discoloration, plaque and calculus buildup, bacteria buildup and retention, and loss of translucency and material integrity can

occur.[23,24] Color stability can also be affected by ultraviolet radiation, mouthwashes, and various beverages.[25] Staining could occur from food or drinks which may stain the retainer materials as well as allow the stain to accumulate in the retainer. Various studies have reported that polyurethane materials are susceptible to pigment absorption in the oral cavity.[26–28] Changes in durability and wear resistance have been observed within a few months of intraoral wear.[29] Crucial to maintaining the truly clear nature of these retainers is an effective cleaning technique. Only a few scientific studies on the proper maintenance for clear retainers have been performed.[30–34]

Long-term use of clear retainers may come with disadvantages including compromise to the physical and chemical properties of the materials.[35] Ahn et al. demonstrated vacuum-formed thermoplastic retainer materials with long-term intraoral exposure could accelerate changes in surface morphology, tensile strength, and elastic modulus.[36] These materials can be affected by heat, moisture, and oral enzymes.[37] The Essix ACE® and Essix C +® manufacturer reports an average lifespan of only 24 months.[38]

In 2013, Moshiri et al. advised patients to remove aligners before eating and then after eating, to remove any white deposits from the aligners, to brush with a soft toothbrush for two minutes, to floss, and to rinse with a fluoride mouth rinse before replacing the aligner into the mouth.[39] Instructions were also specified on how to clean the aligner, advising an ultrasonic cleaner or the use of Invisalign® Cleaning System detergent.[39] No information was provided regarding cleaning efficacy of the cleaning methods. It seems the curved internal surface of the aligner surfaces may cause stagnation of salivary flow, obstruction of tongue, and buccal soft tissue cleaning mechanical action.[40] This, in combination with a lack of proper oral hygiene, may have detrimental effects on plaque retention leading to the discoloration of Invisalign® appliances.[40]

Ryu et al. evaluated the physical and mechanical properties of TOAs, according to material type and thickness after thermoforming using a Biostar (Scheu Dental) per manufacturer instructions over a model mimicking the average maxillary central incisor in Korean adults.[17] Materials used in the study consisted of Duran (Scheu Dental, Iserlohn, Germany), eCligner (eCligner, Seoul, Korea), Essix A+® (Dentsply Raintree Essix, Sarasota, FL, USA), and Essix ACE® (Dentsply Raintree Essix).[17] Transparency was measured with a spectrophotometer (CM-3500D; Konica Minolta, Tokyo, Japan) to investigate the aesthetic aspect of the materials. Tests for water absorption and solubility were used to evaluate how materials react when placed into the oral cavity with saliva. Additionally, surface hardness tests using Knoop hardness tester (DMH-2; Mastsuzawa Siki Co. Ltd, Akita, Japan) to determine material rigidity, and three-point bending tests and tensile tests using a universal testing machine (Model 5942, Instron®) to assess tooth movement effectiveness and durability were performed.

Ryokawa et al. studied the mechanical properties of thermoplastic materials in a simulated oral environment. The materials included "ethylene-vinyl acetate copolymer (Bioplast®), polyethylene (Copyplast®), polyethylene terephthalate glycol (PETG) (Duran®), polypropylene (Hardcast®), polycarbonate (Imprelon® "S"), copolyester (Essix A+®), polypropylene/ethylene copolymer (>95%) (Essix C+®) and polyurethane from methylene diphenyl diisocyanate and 1,6-hexanedial (Invisalign®). Each polymer was categorized into amorphous or partially crystalline based on the material melting point.[41] Essix C+® material is crystalline; an opaque material due to its mix of crystalline and amorphous polymers.[41] Crystalline materials have a lower water absorption rate.[41] Three tests were performed on the materials, 1. after two weeks for water absorption, 2. for thickness change after thermoforming and water absorption, and 3. tested for tensile strength under room temperature and a simulated oral environment.[41] Results showed water absorption, via air humidity or

immersion, increased with time and that the materials are affected by their amorphous/crystalline structure as well as by temperature, humidity, and pressure.[41]

There are very few literatures which report on the translucency stability of stained retainer materials. A study by Zafeiriadis et al. studied *in vivo* discoloration and consisted of two groups of post-orthodontic treatment patients, one that received Vivera® retainers, polyurethane polymer, and one group that received Essix C+® retainers, copolymer.[42] Duplicates were made and served as controls. Measurements were taken intraorally after insertion, after 15 days, 30 days, and three months and then compared to the control retainers. In this study, both Vivera® retainers and Essix C+® retainers exhibited greater color change that increased with wear time.[42] However, the color changes were considered clinically acceptable, with a ΔE less than 3.7, which is clinically perceptible at a glance.[42]

Zafeiriadis et al. studied the *in vitro* effect of staining solutions on Vivera® retainer materials, primarily made of polyurethane (PU). Thirty flat specimens were thermoformed and immersed in either Group A: distilled water, Group B: coffee (Nescafe Classic®, Nestle, Switzerland), Group C: tea (Yellow Label® tea, Lipton, Kenya), Group D: red wine (Rapsani®, Tsantali Vineyards & Wineries, Greece), or Group E: Coca-Cola® (Coca-Cola®, Coca-Cola Hellenic, Greece).[23] After each immersion, the specimens were rinsed with tap water and blot dried before each color measurement.[23] Measurements with a spectrophotometer were used at T0, before immersion, T1, after 12 hours of immersion, T2, after three days of immersion, and T3, after seven days of immersion.[23] Results showed Group B, coffee, to have the most prominent staining followed by Group C, tea, and Group D, red wine. Coffee caused a significant decrease in the mean L* (lightness) and mean a* (red/green) values but an increase in the mean b* (yellow/blue) values.[23] Tea caused a significant increase in the mean

a* and b* values.[23] Red wine increased the mean a* value.[23] When using delta (Δ) values, the coffee and tea changes were found to be visible but the changes from red wine were found to be invisible.[23]

Liu et al studied sixty clear aligners by Invisalign® (Align Technology®, Santa Clara, CA, USA), Angelalign® (EA Medical Instruments, Shanghai, China), and Smartee® (Smartee Denti-Technology, Shanghai, China). Invisalign® was shown to be polyurethane-based, Angelalign® is polycarbonate-based, and Smartee® is PETG-based.[43] The aligners were exposed to coffee (G7 Pure Black Instant Coffee®, Trung Nguyen, Bac giang, Vietman), tea (Yellow Label® tea, Lipton, Hefei, China), and red wine (Cabernet Sauvignon red wine; Saflam, Yantai, China), with distilled water as the control.[43] Twenty aligners in the four groups were immersed for 12 hours or 7 days. Using a standard VITA Easyshade Compact colourimeter (Vita Zahnfabrik, Bad Sackingen, Germany), color analysis was performed before staining (T0), after 12 hours (T1), and after 7 days (T2).[43] The specimens were washed in an ultrasonic for 5 minutes and dried with tissue paper before measurements were taken. Coffee was found to stain more heavily and was additional examined with Fourier transformation infrared spectroscopy and a scanning electron microscope (SEM).[43] After 12 hours, all materials had slight color changes.[43] After 7 days, Invisalign® showed marked color change when immersed in coffee.[43] After 7 days, all others showed only slight color change.[43] After infrared spectroscopy, all materials showed no significant chemical change after coffee immersion.[43] SEM results showed that all materials exhibited rough surfaces after coffee immersion for 7 days, with Invisalign® showing the most roughness, pores, and peeling.[43]

In previous studies by Wible and Agarwal et al., long-term effects of different cleaning methods on light transmittance, surface roughness, and flexibility of polyurethane, copolyester, and copolymer retainer materials were tested using Invisalign® Cleaning Crystal, Retainer Brite®, Polident® denture cleaner, Listerine® mouthwash, vinegar, sodium hypochlorite, 3% hydrogen

peroxide, and toothbrushing.[32–34] After exposure to those eight cleaning methods for 6 months *in vitro*, light transmittance was the only tested property of the clear thermoformed specimens that significantly and consistently, changed from baseline to the 6-month timepoint in all materials.[32–34] The result showed that the effect of cleaning methods on the amount of intrinsic change in light transmittance values for the thermoformed retainer materials depends on the types of polymer, and the cleaning methods used had no effect on surface roughness with some degrees of flexural changes.[32–34]

In one study, it was reported that for copolyester retainer materials, Invisalign® Cleaning Crystals and Retainer Brite® could be used twice a week without adverse effects.[34] However, toothbrushing and Listerine were not recommended.[34] In another study, it was found that the copolymer specimens in all groups demonstrated aging in the appearance of decreasing translucency over time after exposure to cleaning methods.[33] The results reported no ideal cleaning method for polypropylene/ethylene copolymer retainer materials.[33]

In the copolyester group, all cleaning methods showed varying degrees of a decrease in translucency after 6 months.[34] Compared among the tested cleaning methods, Listerine® mouthwash affected the long-term translucency followed by the toothbrushing method, while other methods had comparable changes among one another.[34] It is suggested that the 21.6% of ethanol found in Listerine® mouthwash could affect the copolyester properties.[44] For the copolymer group, all cleaning methods caused a significant decrease in translucency after 6 months.[34] All cleaning methods exhibited comparable changes among one another.[34]

Pascual et al. investigated thermoformed Essix C+® and PETG thermoplastic polymer retainer materials and subjected them to several cleaning methods, namely, continuous exposure to air control, distilled water, Listerine® mouthwash, mint Crest® ProHealth, Polident® denture cleaner,

and 3% hydrogen peroxide for two years. The results of this study found that all tested cleansers can be used to clean thermoplastic retainers without increasing the risk of fracture.[29] The Essix C+® material increased resistance to fracture with Crest® ProHealth and hydrogen peroxide.[29] However, hydrogen peroxide was also shown to decrease the Essix C+® material resistance to plastic fracture growth.[29]

In a study by Bernard et al., 300 thermoplastic aligners from Invisalign®, Clear Correct®, and Minor Tooth Movement® (MTM), were exposed to coffee, tea, cola, red wine, and a saliva replacement gel mixed with distilled water and then subjected to destaining. Minor Tooth Movement material is made of a PETG-based polyester, similar to Essix ACE®.[45] The specimens were immersed in the staining solutions for either 12 hours or 7 days and then divided and destained with either Invisalign® Cleaning Crystals or the Cordless Sonic Cleaner combined with a Retainer Brite® tablet.[46] After both 12 hours and 7 days of immersion, there was a significant difference in mean colorimetry values for Invisalign® stained with coffee and red wine.[46] From the initial stain to the final destain, the Retainer Brite® tablet combined with the sonic bath showed more destaining than the Invisalign® Cleaning Crystals for the Invisalign® and Minor Tooth Movement® materials stained with wine.[46] They also found that both destaining methods brought all the black-tea materials almost back to their original color indicating that both present good black-tea stain-removal potential.[46]

Papadopoulou et al. investigated the surface roughness and mechanical properties of Invisalign® aligners after exposure to one or two weeks of clinical oral use. The specimens underwent cleaning with ultrasonic and non-ultrasonic chemical cleaning to remove plaque and calculus. The results of this study showed that clinical use may lead to a decrease in the materials coefficient of friction and may explain the material deterioration with time.[40]

Research by Porojan et al. found a very weak relationship between microroughness of removable thermoplastic aligners and color change after seven days of immersion in coffee, tea, and water.[47] There are also studies that suggest it is the water absorption properties of thermoplastic materials that affect its composition as well as thermoforming and temperature changes.[41] However, in contrast, Poroian et al. showed an insignificant increase in roughness after thermoforming.[47] After having a hot beverage, the oral cavity increases temperature, thus the molecular and crystal structures within the plastic may change.

Ahn et al. investigated intraoral exposures on clear retainers and found to prevent plaque accumulation, surface roughness should be below 0.2 μm.[36] This was based on 48 patients over a two week and six month time period with either vacuum forming aging or intraoral exposure.[36] Raman spectrometer showed that the retainers had a significant change in the molecular composition, specifically a decrease in the composition rate of carbon, the presence of silicon, phosphorus, and calcium.[36] In this study, thermoforming and intraoral exposure led to molecular, morphological, and mechanical changes in the retainers.[36]

Gracco et al. investigated the clinical, chemical, and morphological changes for Invisalign® aligners.[35] For this study, one 'as-received' Invisalign® aligner, one 'as-received' Invisalign® aligner immersed in artificial saliva (Biotene Oral Balance, Biopharm Sas. Peschiera Borromeo, Italy), and 10 Invisalign aligners worn by ten randomly selected patients for 14 days.[35] The aligner immersed in artificial saliva was kept at 37 ºC for 22 hours per day, for 14 days.[35] Twice a day, the aligners were cleaned with a toothbrush and dried with air to simulate the removal of the aligners at mealtimes.[35] The aligners worn by patients were removed twice a day, cleaned with a toothbrush, and immersed in disinfectant Amuchina (Gruppo Angelini, Italy) for 15 minutes.[35]

Fourier transformation infra-red analysis resulted in major changes for all specimens, indicating molecular change on the specimen surfaces.[35] This technique provides information about the chemical bonding and molecular structure of materials.[35] The intensity, or height of the peaks, and the width of the bases, the stretching, increased with the aging aligners.[35] This was attributed to the formation of a carbon coating.[35] The changes in shape and intensity were thought to be a decrease of the isocyanate group following hydrolysis reaction by the ambient medium.[35] These results agreed that the artificially aged samples underwent chemical modification.[35] For SEM analysis, samples were cut into 5x5mm specimens from the central incisor, canine, first premolar, and third molar areas.[35] Results revealed no surface damage to the 'as received' aligners and significant damages to the aligners worn by patients.[35] The used aligners showed separation of the polyurethane material in the interproximal areas and calcified deposits in the occlusal surfaces.[35] No damages were seen to the aligners in artificial saliva; however, some deposits could be seen which were probably from the artificial saliva constituents.[35] A Varian Cary UV spectrophotometer was used to analyze the color and transparency changes.[35] Results showed the as-received aligner were more transparent than the artificially aged and patient used aligners, which were noticeably more opaque.[35]

Lastly, Schuster et al. investigated the effects of Invisalign® aligners intraorally and reported no substantial alterations in the composition using bright-field optical reflection microscopy, Fourier infrared microspectroscopy, SEM, and Vickers hardness testing.[48] The control aligners were subjected to artificial aging for two weeks. The retrieved aligners from this study resulted in substantial morphological variation relative to the baseline specimens, which involved abrasion at the cusp tips and localized calcification of the precipitated biofilm at stagnation sites.[48]

B. Managing Relapse

Managing relapse is often associated with substantial cost, time, and practice management. There is a strong need for an evidence-based retention and maintenance protocol for clear aligners. If there is an ideal retainer material that stains less and an ideal cleaning method that can destain the stained retainers effectively, as well as stabilize the clear nature of the clear retainers, without compromising the properties of the materials, the compliance for retainer wear would increase and the post-treatment relapse rate would decrease. Survey results have shown that brushing and soaking retainers in chemical agents are the most popular ways to clean retainers.[49] However, there is no evidence-based research to support these options. As a result of the shortage of studies on cleaning methods of clear retainers, this study aims to investigate the ability of retainer materials stained by different staining solutions and cleaned with several solutions with, and without, ultrasonic cleansing units.

To our knowledge, until now, there is no study on the ability of cleaning methods to destain stained retainer materials with a two-surface texture model. The effect of different surface textures of the retainer materials on their ability to be stained has not been reported. To establish an evidence-based method for the cleaning of these clear retainers, we investigated the ability of different cleaning methods to restore the light transmittance of stained clear thermoformed retainer materials and to evaluate the ability of these materials to be stained with different surface textures.

The process of wear is complex and involves many factors. Certain microorganisms can degrade polymers, water can cause filler leaching, and alcohol, found in some mouthwashes, can plasticize certain polymers.[50] Thus, day-to-day retainer wear exposes the retainers to chemicals common to the diet, which may cause corrosive effects when worn full time.[19] Therefore, we investigated changes in composition using a Raman spectroscopy.

The results from this study will provide scientific evidence of the selection of clear retainer cleaning methods. Ultimately, orthodontists will be able to provide patients with an evidence-based recommendation of how to maintain clear retainers properly and promote patients' oral hygiene and retainer compliance for successful orthodontic treatment.

III. METHODOLOGY

A. Specimen Fabrication

Eight hundred standardized retainer specimens were used for testing: 400 Essix ACE® copolyester (polyethylene-tetraphthalate-glycol (PETG)) and 400 Essix C+® polyethylene/polypropylene copolymer. Both retainer materials were generously provided by Dentsply Sirona Inc., York, PA, USA and all materials were 0.040" (1mm) round.

The retainer materials were vacuum-formed using the Biostar® Scan pressure machine (Great Lakes Orthodontics, LTD, Tonawanda, NY, USA). All specimens were identical in shape after thermoforming, as all materials were thermoformed over a stainless-steel mold, see Figure 2. Each mold used one material sheet, which rendered 3 identical specimens for further tests (Figure 3). Each specimen was a standard dimension of 50.8mm x 12.7mm x 1.0mm. This dimension is recommended by the American Society for Testing Materials (ASTM D 790) "Standard Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials", which provides for alternative test specimen sizes for materials that are less than 1.6mm thick.[14] This ASTM standard was used instead of ANSI/ADA Standard No. 139 "Dental Base polymers" because the sheets used to prepare the specimens were less than the standard thickness specified in Standard No. 139.[15]

The Biostar® Scan pressure machine, see Figure 1, was set for each material as shown in Table II, according to the manufacturers' instructions. In this study, the stainless-steel template mold was fabricated with a slot for disposable, removable inserts to generate two-surfaces, textured and smooth. Based on the same authors' previous publications[32–34] with an effect size equal to 1, an alpha-level of 5% and statistical power of 80%, a sample of five units of analysis, per study group, is required. One type of surface roughness, the textured side, imitates the internal surface texture of 3D-printed,

commercially available retainers while the other imitates the smooth surface of in-house plaster models.

We performed a preliminary study at the ADA to determine the average depth of surface roughness of an Invisalign® retainers (Vivera®, Align Technology, CA), which was found to vary depending on tooth position, from around 4 µm to 42 µm. However, the average overall surface roughness is approximately 10.6 µm with a variation of 86% for all retainer positions. To determine the roughness of the custom block, four measurements were taken for each area on the thermoformed specimen using an interferometer and profilometer (NewView™ 8300 Optical Surface Profiler, Zygo Corporation). The average roughness of the custom block is approximately 16.5 with a 6% variation. (data not shown)

Figure 1: Biostar® Scan pressure machine (Great Lakes Orthodontics, Ltd.)

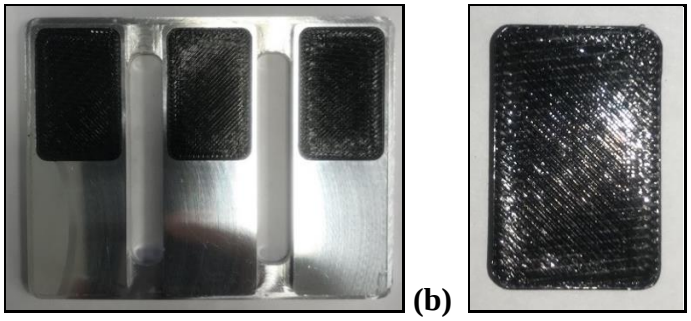


Figure 2: (a) Stainless steel block mold with two surface textures. One surface imitates a smooth surface of plaster model (bottom) and the other surface imitates the internal texture commercial models (top – black), (b) Removable polymer insert mimicking the textured surface.

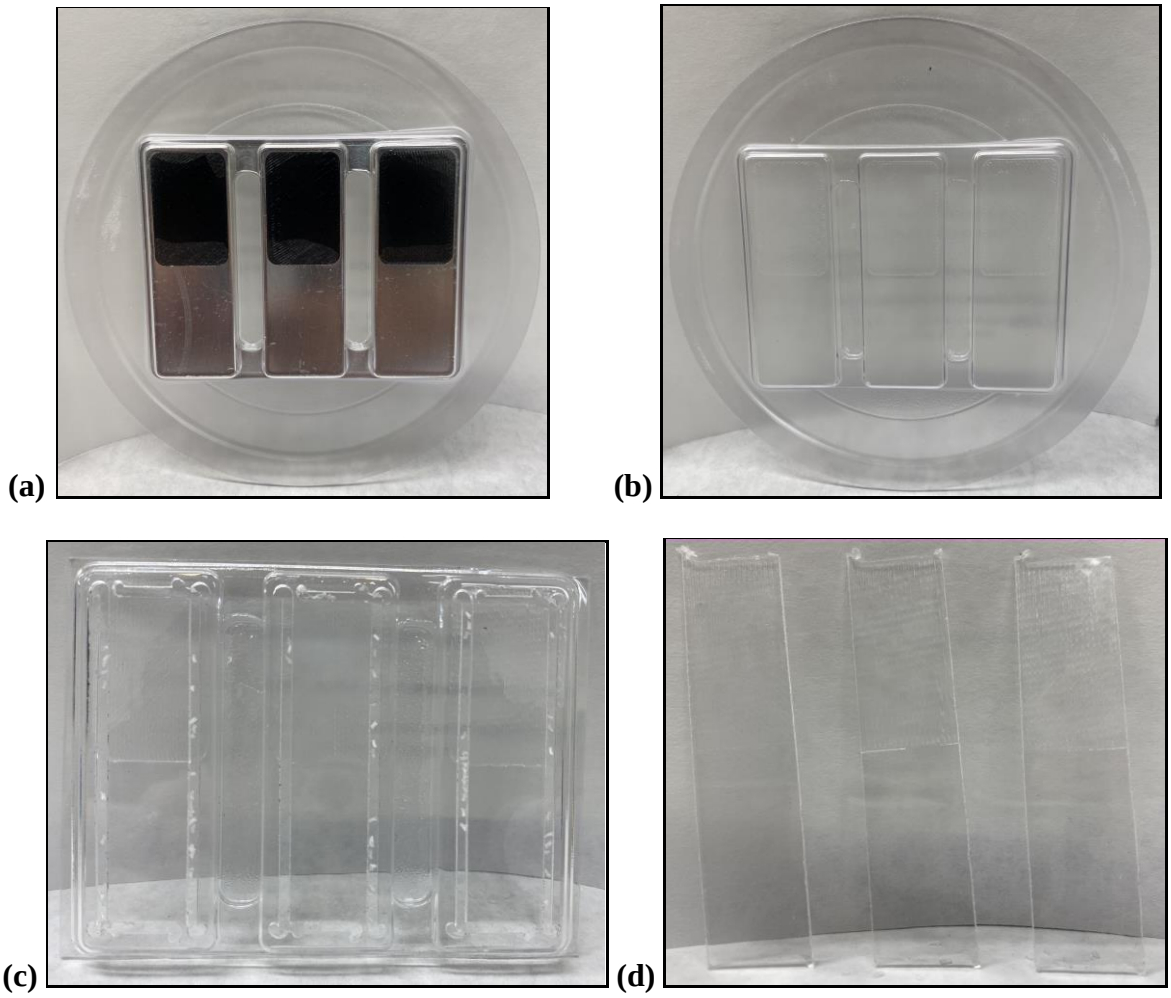


Figure 3: (a) Thermoformed material over custom stainless-steel block, (b) Thermoformed material, (c) Diamond saw cut material, (d) Specimens ready to be stained.

The smooth surface of the specimens was created from the surface of the stainless-steel block. The textured surface was created by the surface of the removable, disposable insert. The 3D printed inserts were made from acrylonitrile butadiene styrene (ABS) material (ABSplusP430, Stratasys Ltd.) at the Standard laboratory, American Dental Association (ADA) (Figure 2, right). Each individual 3D printed insert with dimension of 0.5x1.0 inch was replaced after every five thermoplastic molds to account for plastic heat deformation, if it occurred. Specimens were randomly sampled to confirm homogeneity of the textured surfaces (data not shown). The samples were then cut into the standard dimensions using a diamond saw and automated Computer Numerical Control (CNC) milling machine at the Standard laboratory, ADA.

TABLE II

BIOSTAR® THERMOFORMING PARAMETERS FOR SPECIMEN FABRICATION

Brand (manufacturer)	**Component**	**Thickness (mm)**	**Heating time (s)**	**Cooling time (s)**
Essix ACE® (Dentsply Raintree Essix)	Copolyester	0.40	45	120
Essix C+® (Dentsply Raintree Essix)	Copolymer	0.40	90	240

1. Staining Experiments

Four different staining solutions were used. The staining solutions were chosen as coffee, black tea, and red wine based on their capability to stain clear retainers in the previous literatures.[23,43,46,47] Specimens were placed on a stainless-steel rack and placed in a 10”x13.75” container with sealed lid. The staining solutions were prepared with a volume of 2.6 liters, enough to completely submerge all specimens, and were maintained in a 37 ºC incubator (Figure 4, right) at the University of Illinois at Chicago (UIC) to simulate body temperature. Both materials, Essix ACE® and Essix C+®, were stained with coffee, black tea, red wine, and distilled water, in each container for 28 days. Solutions were freshly prepared on day 0, day 7, day 14, and day 28 of the staining immersion period. The specimens were gently pat-dried before subjected to the spectrophotometers for light transmittance and color change evaluation. Note that on day 28, the specimens were air-dried, rather than pat-dried with paper towels. Specimens were transported for analysis in labelled zip lock bags.

The 400 specimens per retainer material was divided into four groups of 100 specimens, each subgroup being exposed to a different staining solution (coffee, black tea, red wine, distilled water), see Figure 4, center. The specimens in each staining solution were immersed in the staining solution for 7 days, 14 days, and 28 days. Per each staining method, per container, specimens were placed side by side on metal racks and secured at the edges to keep the specimens submerged in the staining solutions while the areas for testing were exposed to the staining solutions, see Figure 4, left.

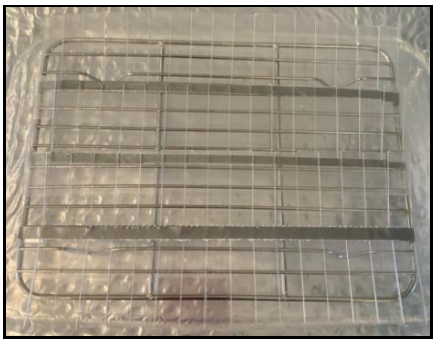
(a)  **(b)**  **(c)** 

Figure 4: (a) Sixty specimens secured to on metal rack, ready for staining solutions, (b) Containers with specimens in the staining solution in the incubator, (c) 37 ºC incubator.

The instant coffee solution was prepared by mixing 688 grams of instant coffee powder (Nescafe® Original, Nestle, Vevey, Vaud, Switzerland) with 8 liters of distilled water in an autoclave, as per the manufacturer's instruction and the previous study by Bernard et al.[46] After the coffee solution was autoclaved and cooled down to room temperature, it was poured over the specimens and placed into an incubator maintained at 37 $^{+}$/$_{-}$ 1 ºC.

The black tea solution was prepared by mixing 150 grams of instant tea powder (Lipton® Unsweetened Black Iced Tea Mix) with 8 liters distilled water in an autoclave according to the manufacturer's instruction. After the tea solution autoclaved and cooled down to room temperature, it was poured over the specimens and placed into an incubator maintained at 37 $^{+}$/$_{-}$ 1 ºC.

The red wine (Paint Box® Cabernet, Cabernet Sauvignon, California, California Misc, 27.0 proof) was used as supplied and poured directly in the staining container and placed into an incubator maintained at 37 $^{+}$/$_{-}$ 1 ºC. The distilled water was used as supplied and poured directly in the staining container and placed into an incubator maintained at 37 $^{+}$/$_{-}$ 1 ºC.

At day 0, before staining, the specimens were analyzed for their light transmittance values with a spectrometer (Flame, Ocean Optics Inc.), for their color values with a spectrophotometer (CM-2600D Spectrophotometer, Konica Minolta), and for their composition profiles with Raman Spectrometry (XplorRA Plus, Horiba Scientific). The actual values obtained from each device were recorded in Excel spreadsheets. On staining day 7, day 14, and day 28, the specimens were analyzed for light transmittance and color values. Before each analysis, the specimens were rinsed with distilled water and gently pat-dried with a soft paper towel (Henry Schein 570-0704, Melville, NY). After the analysis, the specimens were placed back into the containers with freshly prepared staining solutions and incubated in the 37°C $^{+}$/$_{-}$ 1 ºC incubator.

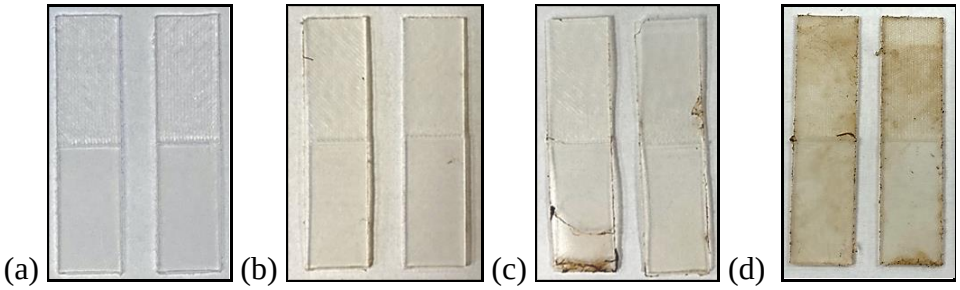


Figure 5: Essix C+® stained with coffee solution; Top section: textured, Bottom section: smooth (a) day 0, (b) day 7, (c) day 14, (d) day 28

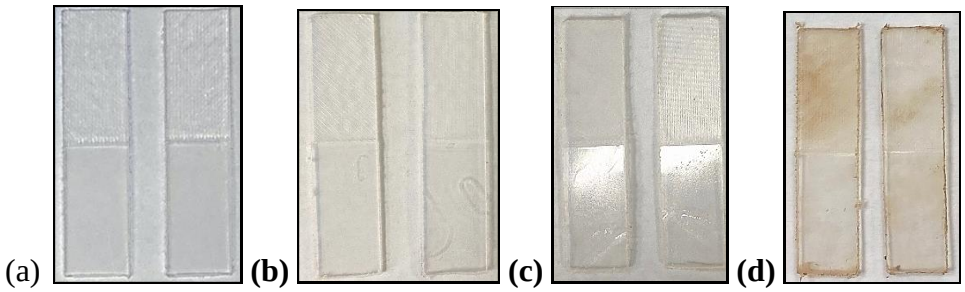


Figure 6: Essix C+® stained with black tea solution; Top section: textured, Bottom section: smooth (a) day 0, (b) day 7, (c) day 14, (d) day 28

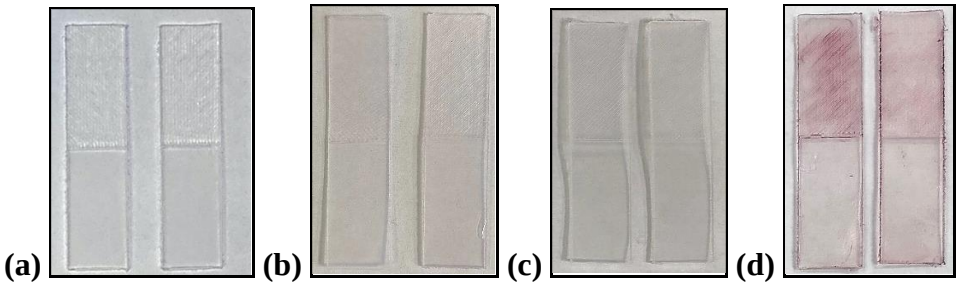


Figure 7: Essix C+® stained with red wine solution; Top section: textured, Bottom section: smooth (a) day 0, (b) day 7, (c) day 14, (d) day 28

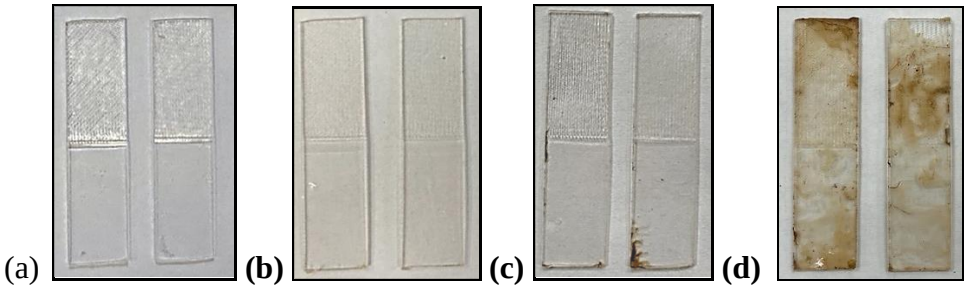


Figure 8: Essix ACE® stained with coffee solution; Top section: textured, Bottom section: smooth (a) day 0, (b) day 7, (c) day 14, (d) day 28

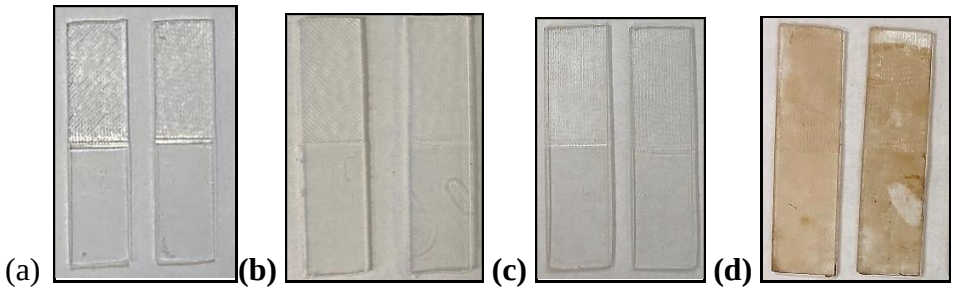


Figure 9: Essix ACE® stained with black tea solution; Top section: textured, Bottom section: smooth (a) day 0, (b) day 7, (c) day 14, (d) day 28

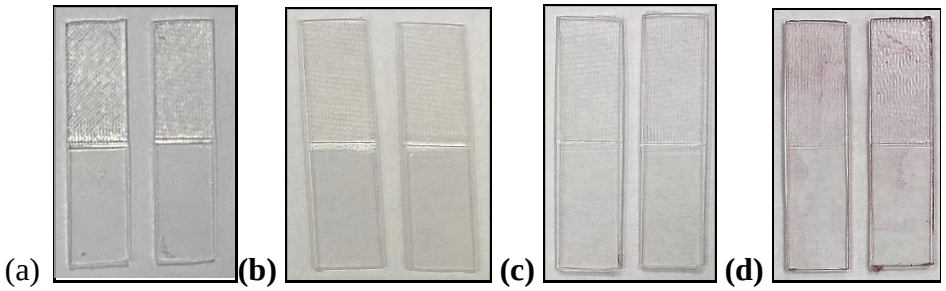


Figure 10: Essix ACE® stained with red wine solution; Top section: textured, Bottom section: smooth (a) day 0, (b) day 7, (c) day 14, (d) day 28

2. Destaining Experiments

After staining for 28 days, each group of stained specimens, per staining solution, were further divided into 2 subgroups and 5 smaller subgroups for destaining experiments. Fifty specimens were subjected to five cleaning solutions (n=10) in an ultrasonic cleaner unit (BioSonic® UC300R, Coltene/Whaledent) at 42,000 Hz vibrating frequency for 15 minutes, while the other 50 specimens were subjected to the various cleaning solutions without the ultrasonic mean; stirred with a magnetic stirrer (non-ultrasonic). The specimens were kept in artificial saliva at 37°C between the testing sessions. The artificial saliva was prepared based on the Nakagawa publications, using 0.4g NaCl, 0.4g KCl, 0.795g $CaCl_2$-$2H_20$, 0.78g NaH_2PO_4-$2H_20$, 0.005g Na_2S-$9H_20$, 1.0g NH_2CONH_2, and 1000 mL of distilled and autoclaved water.[51]

Fifty stained specimens from each group (coffee, black tea, red wine, and distilled water) stated above (n=10) were destained in five different cleaning solutions for 15 minutes, except for Polident® denture cleaner. Polident® denture cleaner was soaked for 3 minutes, per manufacturers' instructions.

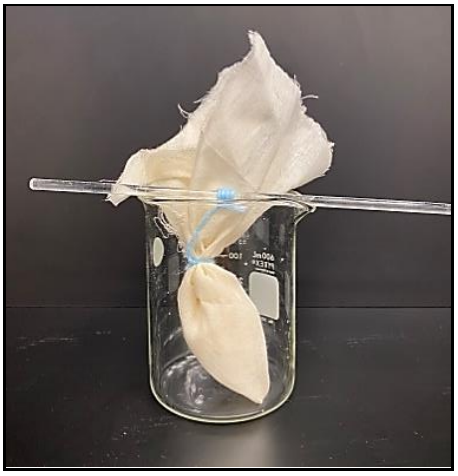
(a)  **(b)** 

Figure 11: (a) Demonstration of five stained specimens prepared for destaining in cheesecloth and separated with glass beads to ensure homogeneous exposure of the destaining solution, (b) Each beaker contained 10 stained specimens of each material wrapped in cheesecloth, separated by glass beads and secured with a glass rod in a destaining container.

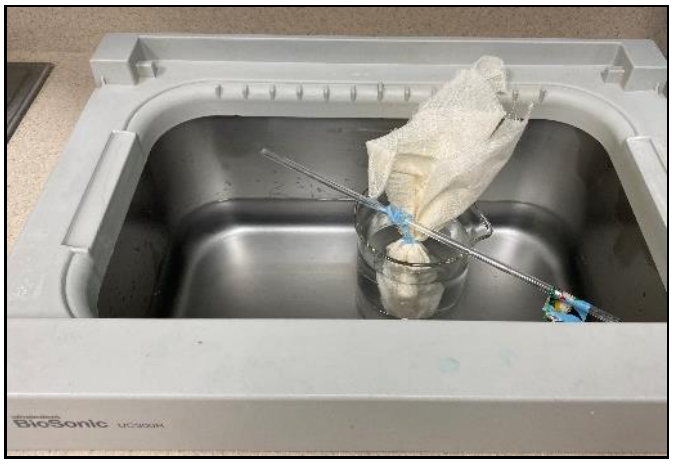
(a)  **(b)** 

Figure 12: Destaining process settings (a) Ultrasonic cleaner unit, (b) Non-ultrasonic cleaning mean (soaked in destaining solution with a magnetic stirrer).

The selected cleaning solutions in this study were chosen based on previous studies[32–34] that showed the least amount of change of light transmittance values of the studied retainer materials after 6 months of exposure. The cleaning solutions included Invisalign® Cleaning Crystals (sodium carbonate and sodium dichloro-1,3,5-trianzinetrione) (Align Technology Inc., San Jose, CA, USA), Retainer Brite® (sodium carbonate and sodium perborate) (Dentsply Sirona Inc., York, PA, USA), Polident® denture cleaner (sodium carbonate and sodium perborate monohydrate), Listerine® mouthwash (ethyl alcohol and mineral oil), and 3% hydrogen peroxide (H_2O_2). The stained specimens were destained in the cleaning solutions in groups of 10 for 15 minutes, except Polident®. Polident® denture cleaner was soaked for 3 minutes, per manufacturers' instructions. One package of Invisalign® Cleaning Crystals were diluted in 300 mL of distilled water at room temperature (22 ºC) immediately before specimen immersion according to the manufacturer's instruction. Two Retainer Brite® tablets were placed in 400 mL of distilled water at room temperature immediately before specimen immersion. Two Polident® tablets were placed in 400 mL of distilled water at room temperature immediately before specimen immersion according to the manufacturer's instruction. 3% H_2O_2 was prepared by diluting 30 ml of 30% H_2O_2 in distilled water to make 300 ml of solution. Listerine mouthwash was used as supplied.

Ten specimens were placed in cheesecloth (Regency Natural Ultra Fine 100% cotton cheesecloth) and suspended by glass rods atop a round beaker (Figure 11, right). Specimens were separated from each other by glass beads to allow the material to be completely immersed (Figure 11, left). The bundle of cheesecloth was tied tightly with yarn. The light transmittance, color change, and composition change of the specimens were analyzed as explained in the staining experiment section. Specimens were evaluated on day 0 of the destaining experiment (day 28 of staining) and at the end of destaining. Raman Spectrometry (XploRA Plus, Horiba Scientific) was used on three randomly

selected specimens from each solution. Before light transmittance and color analysis, samples were rinsed with distilled water and pat-dried gently with a paper towel. After analysis, at day 0 of the destaining experiment, the specimens were placed into the freshly prepared cleaning solutions. An additional flow chart schematic illustrates the sample distribution (see Figure 13).

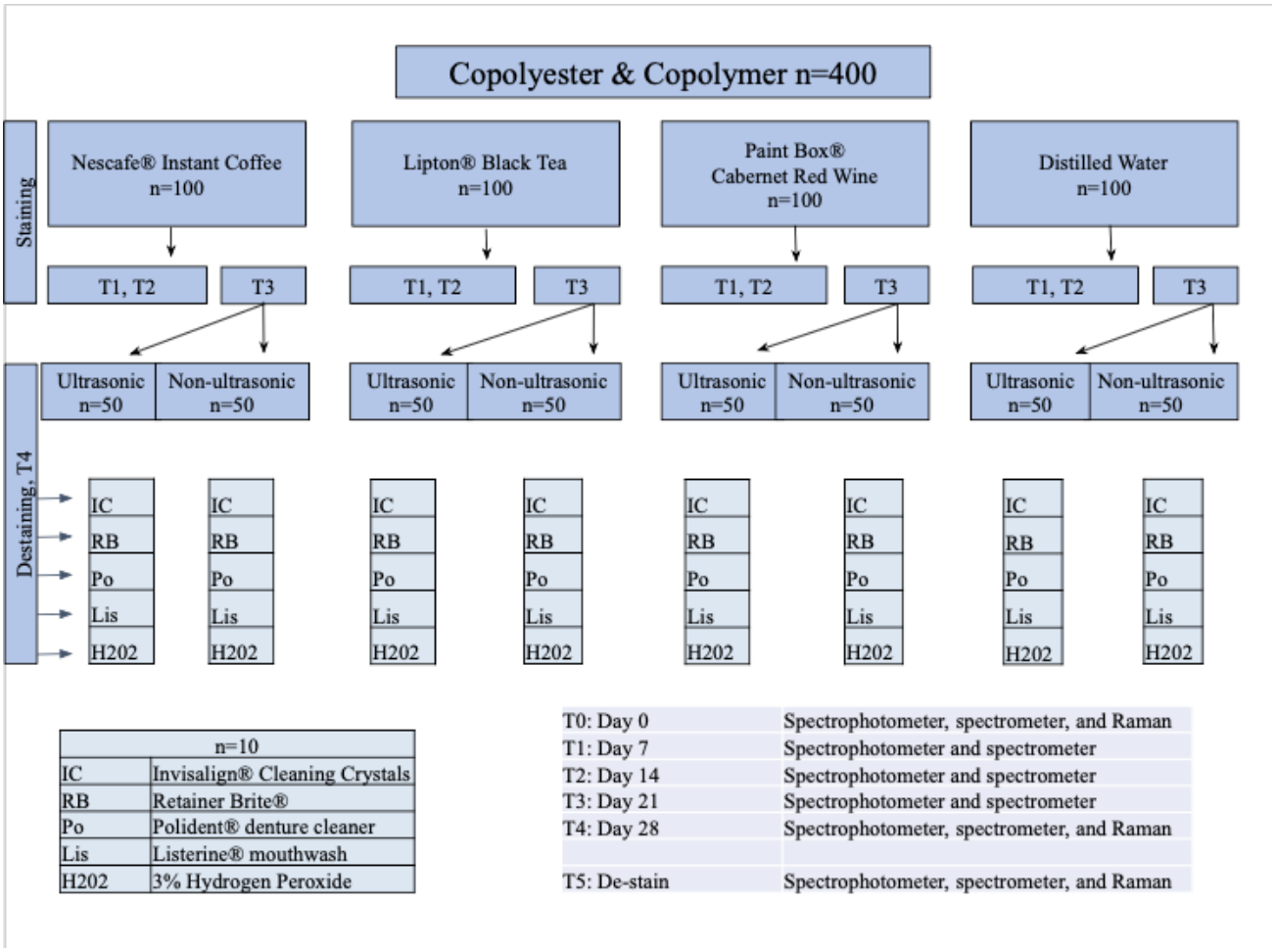


Figure 13: Flowchart

B. Colorimetry Study using a Spectrophotometer

The color change of the samples was recorded using the Commission Internationale de I'Eclairage (CIE) L*a*b* color system values. The L* value is a measure of lightness, from darkness to lightness, and ranges from 0, representing the color black, to 100, representing the color white.[42] The a* value represents positions on a red/green axis and the b* value represents positions on a yellow/blue axis. A positive a* value corresponds to red and a negative a* value corresponds to green. A positive b* value corresponds to yellow and a negative b* value corresponds to blue, see Table III.

TABLE III

COLORIMETRY RANGE

	Measure	**Range**	
L*	Lightness	 0 (black)	 100 (white)
a*	Red/green axis	 (negative value)	 (positive value)
b*	Blue/yellow axis	 (negative value)	 (positive value)

Staining experiment timepoints included, T0: thermoformed and standardized cut specimens before immersion, T1: after 7-day exposure to a staining solution, T2: after 14 days exposure to a staining solution, T3: after 28 days exposure to a staining solution or day 0 of destaining experiment, and T4: after exposure to destaining solution for 15 minutes*.

Five randomly selected specimens were tested for each material group and were scanned at each time point, T0-T4. Both the smooth and textured sides were measured at each time point of staining and destaining using a Spectrophotometer (CM-2600d Spectrophotometer, Konica Minolta) with a custom custom-fabricated specimen holder (Figure 14). Before each measurement, the Konica spectrophotometer was calibrated, with no specimen, using a transparent and white target. Each specimen was measured in triplicate, and the averages were calculated automatically and recorded. The baseline color values were measured before staining was initiated. After T0, the specimens were placed into the staining solutions, coffee, black tea, red wine, and distilled water. Replicating the Bernard et al. study, measurements were taken 0.5" from the vertical dimension of the specimen to obtain a mean as reproducible as possible.[46]

Replicating the Liu et al. study, the values from each measurement were averaged for each material and the color difference (ΔE) values were calculated according to the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

A value of ΔE* above 3.3 can be detected by a non-skilled person and is considered unacceptable for aesthetic aligners.[52,53] ΔE describes and quantifies the difference in color change using the CIE values, as viewed from the human eye.[54] When using ΔE, a scale from 0-100 is used, where 0 indicates less color change and 100 indicates complete color change.[54] A ΔE* value below 1 is considered undetectable and a value between 1 and 3.3 is considered acceptable.[52,53] Other tables note that any value above 2 is perceptible at a glance.[54] For this study, ΔE values less than 3 were

considered clinically acceptable. All measurements were conducted in the same room with standardized lighting. Descriptive statistics that included mean, standard deviation, median, and minimum and maximum values were calculated for each CIE L*a*b* parameter. The national bureau of standards (NBS) system was used to describe visual color change inspection, see Table IV. Color change values (ΔE) of all materials were multiplied by a factor of 0.92 to obtain the NBS values.[55]

TABLE IV

LABELS OF COLOR CHANGE, ACCORDING TO THE NATIONAL BUREAU OF STANDARDS (NBS)[55]

NBS unit	Critical remarks of color differences	
0.0–0.5	Trace	Extremely slight change
0.5–1.5	Slight	Slight change
1.5–3.0	Noticeable	Perceivable
3.0–6.0	Appreciable	Marked change
6.0–12.0	Much	Extremely marked change
12.0 or more	Very much	Change to other color

Figure 14: Spectrophotometer (CM-2600d Spectrophotometer, Konica Minolta) for color parameter change.

C. Light Transmittance Study using a Spectrometer

Translucency is the state between complete transparency and complete opacity. Translucency parameter (TP), defined as color difference over a white and black background, is determined based on material thickness and scattering and absorption coefficients.[56] A completely opaque material will have a TP value of zero. The greater the TP value, the more translucent the material. The measurement of TP was performed as follows.

The individual specimens were positioned in custom-fabricated holders and light transmittance was determined according to the method previously published by Spink et al. for measuring translucency of dental ceramics.[57] Briefly, this method quantifies the percent of light transmittance through the retainer material into a spectrometer with an integrating sphere system, see Figure 14, left, consisting of the following components: a miniature spectrometer (Flame-S-VIS-NIR, Ocean Optics, Largo, FL); a tungsten halogen lamp (Nikon MK II illuminator, Tokyo, Japan) with a flexible light guide (0.25 in x 0.312 in x 72 in, Dolan-Jenner, Boxborough, MA); an integrating sphere (Labsphere,

North Sutton, NH); a fiber optic cable (QP100-2-UV-VIS, Ocean Optics); and custom-fabricated specimen holders, see Figure 15, right.

For our study, five randomly selected specimens from each test group were analyzed via the Ocean Optics spectrophotometer to measure the TP values of the smooth and textured surfaces of the specimens. During the procedure, light energy readings were taken with the tungsten halogen light source connected to the spectrometer/integrating sphere system through the custom-fabricated specimen holder, attached to a port in the integrating sphere. First, the light energy readings were taken without a specimen to produce a baseline measurement. Afterward, the specimens were positioned for a second light energy reading. The two light energy readings were then used to find the percent of light transmittance through the specimen between 380 nm and 740 nm.

Replicating the Bernard study, measurements were taken 0.5" from the vertical dimension of the specimen to obtain a mean as repeatable as possible.[46] The measurements were conducted in the same room, with the same investigator, with standardized illumination.[43]

For translucency analysis, ΔT values were used. ΔT describes TP differences between the baseline and result. During the staining experiment, the twenty measurements of transmission values at baseline (T0) of each material (Essix ACE® and Essix C+®) were averaged and the translucency change parameter (ΔT) values were calculated by subtracting the average value at the baseline ($T0_{avg}$) with the transmission value of each timepoint (T1, T2, T3); ($\Delta T_i = T0_{avg} - T_i$). During the destaining experiment, the five measurements from each group, at timepoint 3 (the end of staining), were averaged ($T3_{avg}$). The T3 average values were then subtracted from timepoint T4 (the end of destaining) to determine the color change parameter difference (ΔTP).

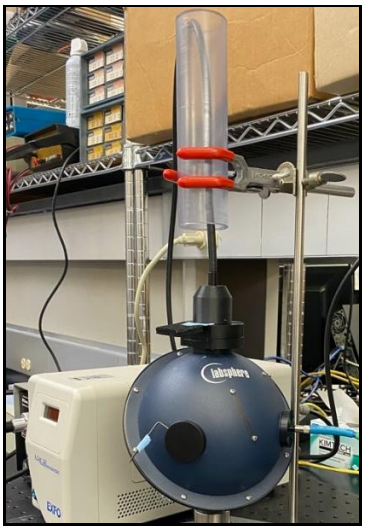
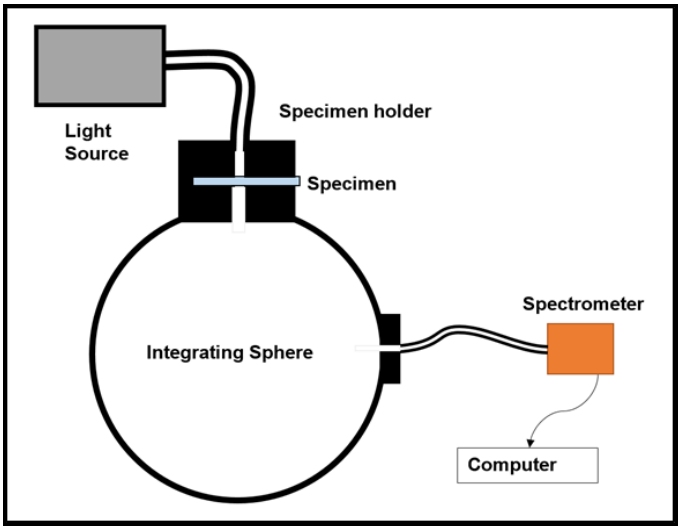
(a)  **(b)** 

Figure 15: (a) Spectrometer Integrating sphere for evaluation of light transmittance, (b) Diagram of light transmission measurement system (Diagram courtesy of Henry Lukic, ADA Science Institute, Research and Standards).

D. Material Composition Study using Raman Spectrometry

Raman spectroscopy is a process in which a photon of light interacts with a specimen to produce radiation of different wavelengths, which can be used to characterize the molecular structure of each material (Figure 16).[58] When radiation hits the specimen, the light can be reflected, absorbed, or scattered.[58] The scattered radiation can be divided into the incident radiation wavelength, known as Rayleigh scattering, which has no change in frequency, and scattered radiation, called Stokes and Anti-Stokes Raman scattering, which does have a change in frequency.[58]

For Raman spectroscopy, Raman scattering refers to the changes in frequency (wavelength)[58] and the Raman effect is very small, about 1 in 100,000 of the incident beam.[59] The Strokes scattering

occurs at lower energy, toward the red-end of the color spectrum, and the Anti-Stokes occurs are much higher energy, toward the blue-end of the color spectrum.[58] Molecular bonds from different materials will require a different amount of energy from the incident photon.[58] Thus, as the two materials are stained and destained, if the composition of the material is altered, the Raman spectrometer will show a change in the molecular structure of the material. The pattern on the Raman graph is characteristic to its specimens molecular species and the intensity is proportional to the scattered molecules in its path of light.[59] The corresponding energy is the Raman frequency shift associated with the transitions between rotational and vibrational states, at high and low energies of the molecule as it scatters.[59]

For T0, five randomly selected specimens from each test group were qualitatively analyzed in a Raman Spectroscopy (XploRA Plus, Horiba Scientific), see Figure 17, before staining (T0), after staining (T3), and after destaining (T4). For T3 and T4, three randomly picked specimens from each test group were analyzed. Before analysis, the specimens were patted dry and measurements were taken approximately 0.5 inches from the vertical axis for accuracy. Measurements were taken at T0: after thermoforming and before staining, at T3: after 28-day exposure to the staining solution: the end of staining experiment, and at T4: the end of destaining experiment.

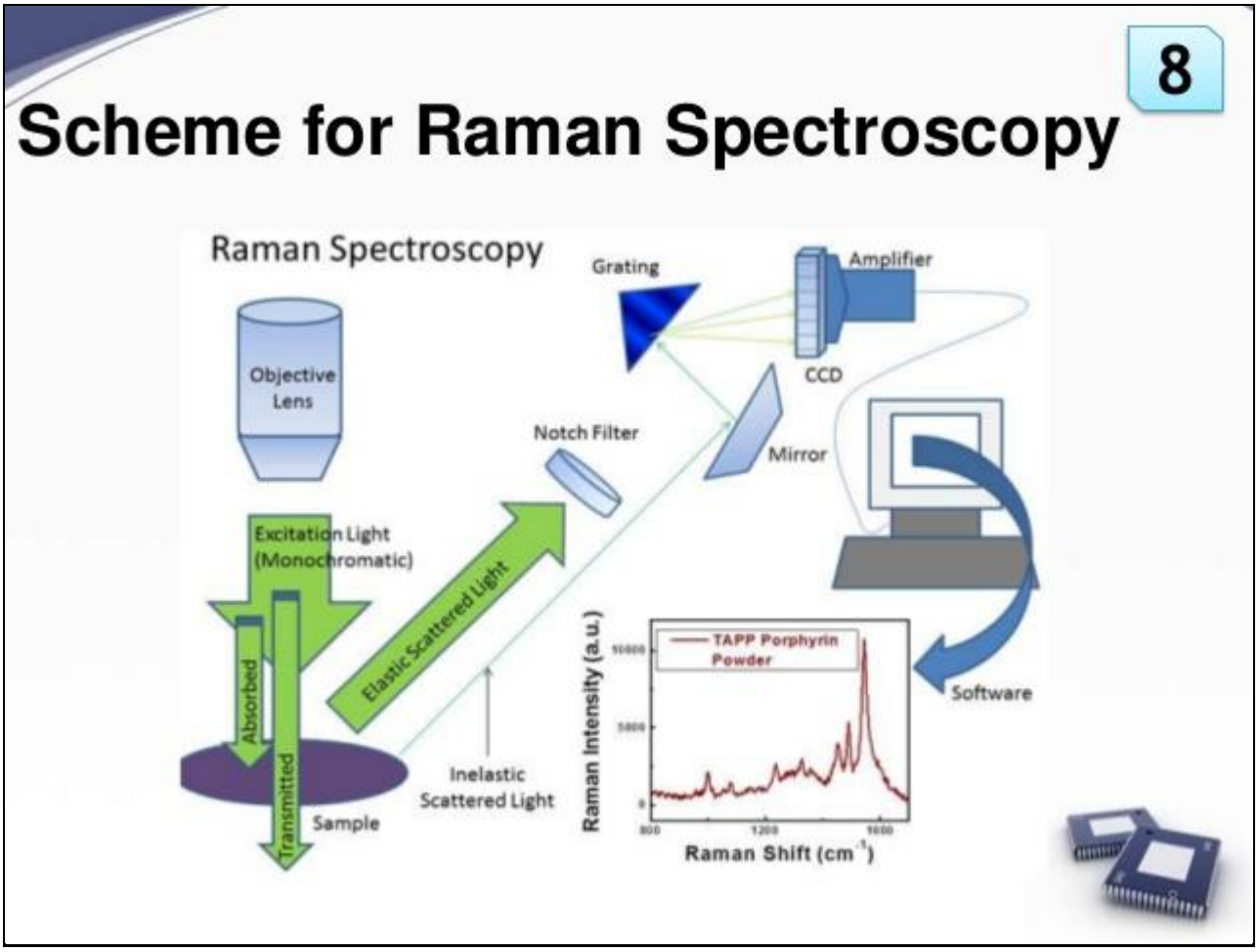


Figure 16: Raman spectrometer diagram

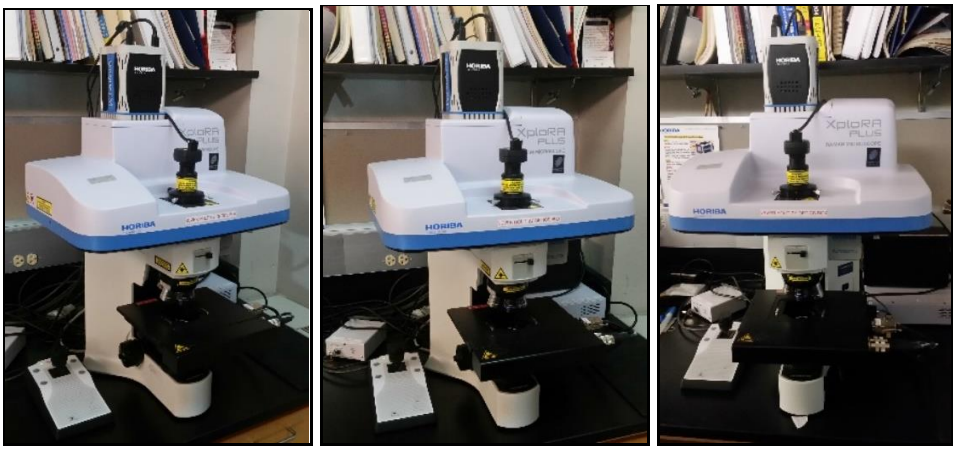


Figure 17: Raman spectrometer (XploRA Plus, Horiba Scientific)

IV. RESULTS

A. Statistical Analysis

All raw data were recorded in Microsoft Excel spreadsheets. The distribution of the raw data values was tested using the Shapiro-Wilk test for pattern of data distribution. The data distribution was non-normal pattern and therefore, non-parametric statistical analyses were performed. The level of significance was set at 0.05. In the case of comparison of differences between a pair, Mann-Whitney testing was used for inter-group comparisons of material while Kruskal-Wallis was used for analysis among groups more than two in the staining and destaining experiments. Post-hoc multiple comparison was used to determine the level of significance of pair comparison using Mann-Whitney test. All numerical data were presented as median values. All calculations and tests were performed with IBM SPSS Statistics for Windows (version 22.0, IBM Corp., Armonk NY).

For this study, ΔT and ΔE were used to evaluate the most severe staining agents for each material, for each surface texture, as well as the best destaining solution for each material, for each surface texture. Median values were used throughout as the data values were non-normally distributed.

B. Comparison between Essix ACE® and Essix C+®

The two materials studied were innately different as analyzed by Mann-Whiney tests, which indicated that there were statistically significant differences between their translucency values at the baseline before staining, p-values<0.001. At baseline for light transmission value, Essix ACE® material displayed greater translucency than Essix C+®. At baseline for colorimetry, Essix ACE® displayed greater lightness (L*), and increased tendency to green (a*) and blue (b*) hues than Essix C+® material.

C. Essix ACE® Staining Results

1. Differences of Textured vs. Smooth Surface Textures at Each Timepoint

Colorimetry and light transmittance results are summarized in Tables V-VII. Kruskal Wallis and Mann-Whitney tests indicated significant differences among the various staining agents.

COLORIMETRY RESULTS (ΔE)

For all timepoints, for colorimetry, all staining agents showed statistically significant differences from one another for the textured and smooth surfaces.

T1 (day 7): For the textured surfaces, there were statistically significant differences between coffee and distilled water, black tea and distilled water, and distilled water and red wine. For the smooth surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, black tea and distilled water, and black tea and red wine, see Table V.

T2 (day 14): For the textured surfaces, there were statistically significant differences between black tea and distilled water and distilled water and red wine. For the smooth surfaces, there were statistically significant differences between coffee and black tea, coffee and distilled water, coffee and red wine, black tea and distilled water, and black tea and red wine, see Table VI.

T3 (day 28): For both the textured and smooth surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, black tea and distilled water, black tea and red wine, and distilled water and red wine, see Table VII. There both surface textures, there was no statistically significant differences between coffee and black tea.

LIGHT TRANSMITTANCE RESULTS (ΔT)

At day 7 and day 28, for light transmittance, all staining agents showed statistically significant differences from one another for the textured and smooth surfaces.

T1 (day 7): For the textured surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, black tea and distilled water, and black tea and red wine. For the smooth surfaces, there were statistically significant differences between coffee and black tea, coffee and distilled water, coffee and red wine, and black tea and distilled water, see Table V.

T2 (day 14): For the textured surfaces, there were statistically significant differences between distilled water and red wine. For the smooth surfaces, there were statistically significant differences between coffee and black tea, coffee and distilled water, coffee and red wine, and black tea and distilled water, see Table VI.

T3 (day 28): For the textured and smooth surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, black tea and distilled water, black tea and red wine, and distilled water and red wine, see Table VII. For both surface textures, there was no statistically significant differences between coffee and black tea.

TABLE V

THE P-VALUES OF ESSIX ACE® AT DAY 7 IN STAINING EXPERIMENTS

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Coffee, Black Tea, Distilled Water, Red Wine	0.002	0.002	0.019	0.002
Coffee – Black Tea	0.690	0.008	1.000	0.151
Coffee – Distilled Water	0.008	0.008	0.016	0.008
Coffee – Red Wine	0.008	0.008	0.548	0.016
Black Tea – Distilled Water	0.008	0.032	0.016	0.008
Black Tea – Red Wine	0.008	0.421	0.841	0.008
Distilled Water – Red Wine	0.690	0.056	0.008	0.095

*significant p-values are highlighted.

TABLE VI

THE P-VALUES OF ESSIX ACE® AT DAY 14 IN STAINING EXPERIMENTS

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Coffee, Black Tea, Distilled Water, Red Wine	0.090	0.003	0.011	0.001
Coffee – Black tea	0.548	0.008	0.151	0.008
Coffee – Distilled Water	0.151	0.008	0.056	0.008
Coffee – Red Wine	0.690	0.008	0.690	0.008
Black Tea – Distilled Water	0.056	0.032	0.008	0.008
Black Tea – Red Wine	0.421	0.421	0.856	0.008
Distilled Water – Red Wine	0.032	0.151	0.032	0.548

*significant p-values are highlighted.

TABLE VII

THE P-VALUES OF ESSIX ACE® AT DAY 28 IN STAINING EXPERIMENTS

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Coffee, Black Tea, Distilled Water, Red Wine	0.001	0.001	0.001	0.001
Coffee – Black tea	1.000	0.841	0.841	0.841
Coffee – Distilled Water	0.008	0.008	0.008	0.008
Coffee – Red Wine	0.008	0.008	0.008	0.008
Black Tea – Distilled Water	0.008	0.008	0.008	0.008
Black Tea – Red Wine	0.008	0.008	0.008	0.008
Distilled Water – Red Wine	0.008	0.008	0.008	0.008

*significant p-values are highlighted.

2. Differences among Staining Solutions at Each Timepoint

Colorimetry and light transmittance results are summarized in Tables VIII-XI. Kruskal Wallis and Mann-Whitney tests indicated significant differences between the textured and smooth surfaces.

COLORIMETRY RESULTS (ΔE)

At all timepoints, coffee and black tea showed statistically significant differences between textured and smooth surfaces, see Table VIII and Table IX. In the distilled water group, the smooth surfaces showed statistically significant differences between the textured and smooth surfaces between

all timepoints, see Table X. In the red wine group, there were no statistically significant differences between textured and smooth surfaces for all timepoints, see Table XI.

In the coffee group for both textured and smooth surfaces, there were statistically significant differences between T1 and T3 and T2 and T3. In the black tea group, both textured and smooth surfaces groups showed statistically significant differences between T1 and T2, T1 and T3, and T2 and T3. In the distilled water group, the smooth surface showed statistically significant differences between T1 and T3, T2 and T3. In the red wine group, there were no statistical differences between the textured and smooth surfaces at any staining timepoint.

LIGHT TRANSMITTANCE RESULTS (ΔT)

At all timepoints, the coffee, black tea, and red wine showed statistically significant differences between textured and smooth surfaces, see Table VIII and Table IX. In the distilled water group, there were no statistical differences between the textured and smooth surfaces between all timepoints, see Table X.

In the coffee and black tea staining groups, for both the textured and smooth surfaces, there were statistically significant differences between T1 and T3 and T2 and T3. There were no statistical differences between timepoints for distilled water for either smooth or textured surfaces. In the red wine group, the textured surface showed statistically significant differences between T1 and T2, T1 and T3, and T2 and T3. In the red wine group, the smooth surface showed statistically significant differences between T1 and T3, and T2 and T3.

TABLE VIII

THE P-VALUES OF ESSIX ACE® IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.009	0.007	0.009	0.005
T1 - T2	0.841	0.310	0.841	0.151
T1 - T3	0.008	0.008	0.008	0.008
T2 - T3	0.008	0.008	0.008	0.008

*significant p-values are highlighted.

TABLE IX

THE P-VALUES OF ESSIX ACE® IN THE BLACK TEA STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.006	0.007	0.002	0.002
T1 - T2	0.222	0.310	0.008	0.008
T1 - T3	0.008	0.008	0.008	0.008
T2 - T3	0.008	0.008	0.008	0.008

*significant p-values are highlighted.

TABLE X

THE P-VALUES OF ESSIX ACE® IN THE DISTILLED WATER STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.147	0.075	0.210	0.018
T1 - T2	N/A	0.151	N/A	1.0
T1 - T3	N/A	0.056	N/A	0.008
T2 - T3	N/A	0.310	N/A	0.032

*significant p-values are highlighted.

TABLE XI

THE P-VALUES OF ESSIX ACE® IN THE RED WINE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.007	0.009	0.085	0.080
T1 - T2	0.008	0.151	0.841	0.690
T1 - T3	0.008	0.008	0.056	0.095
T2 - T3	0.548	0.032	0.095	0.056

*significant p-values are highlighted.

3. Differences between Surfaces at Specific Timepoints among Staining Solutions

Colorimetry and light transmittance results are summarized in Tables XII-XV. Kruskal Wallis and Mann-Whitney found significant differences among the different timepoints.

COLORIMETRY RESULTS (ΔE): For coffee and black tea staining, day 14 showed statistically significant differences between the textured and smooth surfaces, see Table XII and Table XIII. For red wine staining, day 7 showed statistically significant differences between textured and smooth surfaces, see Table XV.

LIGHT TRANSMITTANCE (ΔT): For coffee, distilled water, and red wine, day 7 showed statistically significant differences between the textured and smooth surfaces.

TABLE XII

THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.008	0.151
Day 14	0.151	0.032
Day 28	0.222	0.548

*significant p-values are highlighted.

TABLE XIII

THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.841	0.222
Day 14	0.222	0.016
Day 28	0.095	0.690

*significant p-values are highlighted.

TABLE XIV

THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE DISTILLED WATER STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.008	0.151
Day 14	0.421	0.690
Day 28	0.056	0.222

*significant p-values are highlighted.

TABLE XV

THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.008	0.008
Day 14	0.690	0.095
Day 28	1.000	0.151

*significant p-values are highlighted.

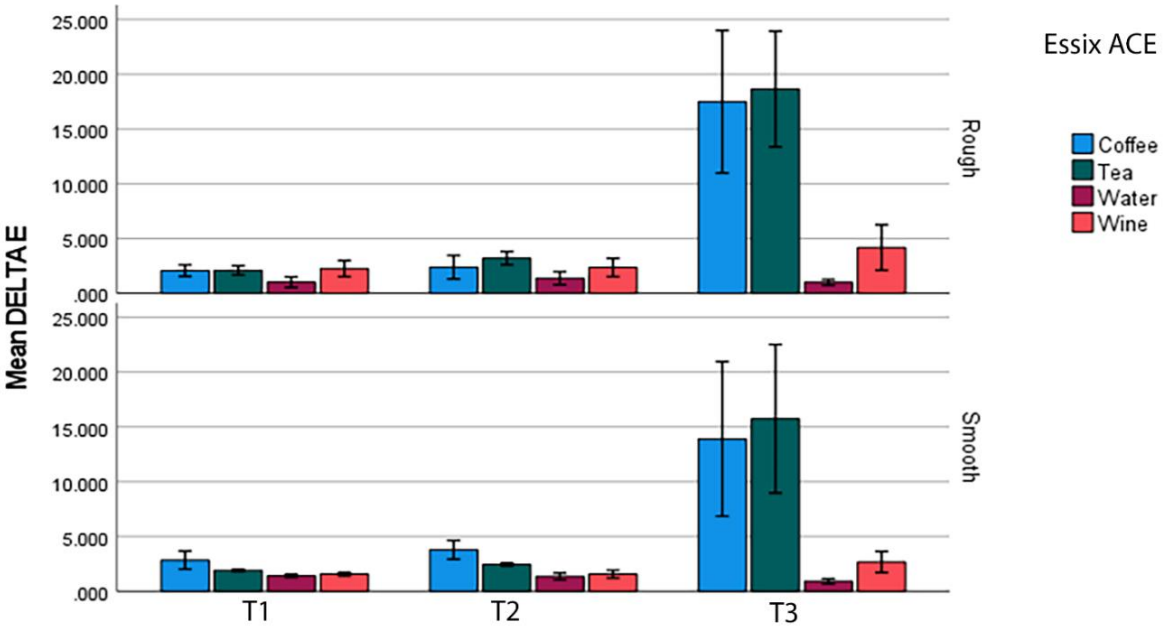


Figure 18: Mean value of ΔE in Essix ACE®

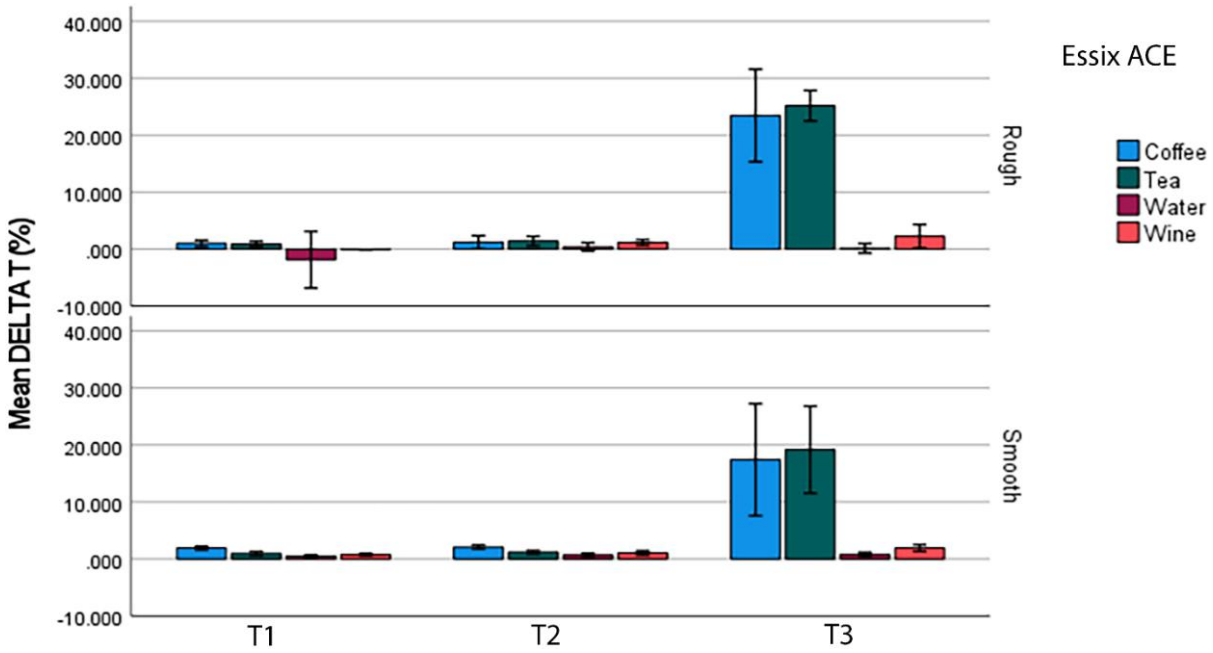


Figure 19: Mean value of ΔT in Essix ACE®

4. NBS Values

NBS ΔE values were calculated using the equation ΔE x 0.92.[55] Values above NBS unit 3.0 are considered to have marked change in color, see Table IV, which, for this study, was considered clinically unacceptable. The mean values for ΔE and ΔT, divided by textured (rough) and smooth surfaces, at T1, T2, and T3 can be viewed in Figure 18 and Figure 19, respectively.

T1 (day 7): Statistically significant differences were found between red wine textured and smooth surfaces. There was no marked color change, see Table XVI.

T2 (day 14): Statistically significant differences were found between coffee and black tea staining solutions on the textured and smooth surfaces. Marked color change was found for coffee on the smooth surfaces and black tea on the textured surfaces, see Table XVI.

T3 (day 28): No statistically significant differences were found between staining solutions for the textured and smooth surfaces. There was no marked color change, see Table XVI.

TABLE XVI

ESSIX ACE® ΔE NBS VALUE (ΔE X 0.92)

	Day 7		**Day 14**		**Day 28**	
	Textured	**Smooth**	**Textured**	**Smooth**	**Textured**	**Smooth**
Coffee	1.69	2.85	1.87	**3.68**	15.58	11.39
Black Tea	1.99	0.85	**3.14**	2.26	16.79	17.22
Distilled Water	0.85	1.33	1.11	1.21	1.00	0.89
Red Wine	0.90	1.48	2.21	1.42	3.56	2.55

*significant values are highlighted, and significant color change values are bolded.

5. Median ΔT

A larger ΔT represents more staining on either the textured specimen surface or the smooth specimen surface. At T1, day 7, coffee, distilled water, and red wine had statistically significant differences. For coffee, distilled water, and red wine, the smooth surfaces exhibited more staining, see Table XVII. For all solutions, no statistically significant difference in surface texture staining was found for T2 and T3, see Table XVII.

TABLE XVII

ESSIX ACE® MEDIAN VALUES FOR ΔT BETWEEN SURFACES AMONG STAINING SAMPLES

	Day 7		**Day 14**		**Day 28**	
	Textured	**Smooth**	**Textured**	**Smooth**	**Textured**	**Smooth**
Coffee	1.0720	1.8790	0.9150	2.1500	26.1610	17.5390
Black Tea	0.8880	0.9612	1.6528	1.1142	24.5548	21.7902
Distilled Water	0.1218	0.5254	0.3552	0.6914	0.4282	0.7654
Red Wine	-0.0666	0.7558	1.3124	1.0518	1.6164	2.1048

*significant values are highlighted.

D. Essix ACE® Destaining Results

Essix ACE® destaining for ΔT and ΔE were studied at each level on four factors, namely, surface texture differences, cleaning methods, cleaning solutions, and staining solution. The raw data distribution was investigated by the Shapiro-Wilk test and statistical significance cut off was at 5%. The distribution of the raw data showed to be non-normally distributed. Therefore, descriptive statistics were calculated. Non-parametric analysis for independent samples were done by Kruskal-Wallis and when statistically significant differences were found, Mann-Whitney test between two independent samples was used.

1. Textured vs. Smooth Surface

COLORIMETRY (ΔE)

For coffee, black tea, and red wine staining, all staining solutions showed statistically significant differences between the textured and smooth surfaces, see Tables XVIII-XX.

LIGHT TRANSMITTANCE (ΔT)

For coffee, all staining solutions showed statistically significant differences between the textured and smooth surfaces, see Table XVIII. For red wine staining, H_2O_2, Polident® Denture Cleaner, and Retainer Brite® solution showed statistically significant differences between the textured and smooth surfaces, see Table XX.

TABLE XVIII

THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.000	0.000
H_2O_2	0.000	0.000
Listerine® mouthwash	0.000	0.000
Polident® Denture Cleaner	0.000	0.000
Retainer Brite®	0.000	0.000

*significant p-values are highlighted.

TABLE XIX

THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.739	0.000
H_2O_2	0.739	0.000
Listerine® mouthwash	0.353	0.000
Polident® Denture Cleaner	1.000	0.000
Retainer Brite®	0.739	0.000

*significant p-values are highlighted.

TABLE XX

THE P-VALUES OF ESSIX ACE® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.739	0.000
H_2O_2	0.009	0.000
Listerine® mouthwash	0.043	0.000
Polident® Denture Cleaner	0.043	0.000
Retainer Brite®	0.009	0.000

*significant p-values are highlighted.

2. Mechanical Cleaning

Testing indicated no statistically significant differences between all cleaning solutions and non-ultrasonic and ultrasonic cleaning methods, see Table XXI. There were no statistically significant differences for coffee staining after non-ultrasonic and ultrasonic cleaning methods, see Table XXII.

LIGHT TRANSMITTANCE (ΔT)

Black tea showed statistically significant differences between non-ultrasonic and ultrasonic cleaning with all cleaning solutions, see Table XXIII. Red wine showed statistically significant differences between non-ultrasonic and ultrasonic cleaning with Retainer Brite®, see Table XXIV.

TABLE XXI

THE P-VALUES OF ESSIX ACE® AFTER DESTAINING

		Δ Transmission (%)		Δ E	
		Non-Ultrasonic	**Ultrasonic**	**Non-Ultrasonic**	**Ultrasonic**
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	**Coffee**	0.405	0.533	0.421	0.270
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	**Black Tea**	0.739	0.232	0.192	0.084
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	**Red Wine**	0.107	0.129	0.655	0.952

TABLE XXII

THE P-VALUES OF ESSIX ACE® STAINED WITH COFFEE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING

	Δ Transmission (%)	Δ E
Invisalign® Cleaning Crystals	0.739	0.796
H_2O_2	0.247	0.912
Listerine® mouthwash	0.796	0.481
Polident® Denture Cleaner	0.579	0.796
Retainer Brite®	0.971	0.123

TABLE XXIII

THE P-VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.029	0.631
H_2O_2	0.019	0.529
Listerine® mouthwash	0.005	0.912
Polident® Denture Cleaner	0.029	0.912
Retainer Brite®	0.004	0.353

*significant p-values are highlighted.

TABLE XXIV: THE P-VALUES OF ESSIX ACE® STAINED WITH RED WINE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.190	0.684
H_2O_2	0.393	0.315
Listerine® mouthwash	0.023	0.393
Polident® Denture Cleaner	0.063	0.739
Retainer Brite®	0.043	0.529

*significant p-values are highlighted.

3. Cleaning Solution

COLORIMETRY (ΔE)

For specimens stained with coffee on the textured surfaces, testing indicated statistically significant differences between solutions: Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Listerine® mouthwash, Invisalign® Cleaning Crystals and Polident® Denture Cleaner, H_2O_2 and Polident® Denture Cleaner, H_2O_2 and Retainer Brite®, and Listerine® mouthwash and Retainer Brite®. For specimens stained with coffee on the smooth surfaces, testing indicated statistically significant differences between solutions: Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Polident® Denture Cleaner, and H_2O_2 and Polident® Denture Cleaner, see Table XXV.

For specimens stained with black tea on the textured surfaces, testing indicated statistically significant differences between solutions: Invisalign® Cleaning Crystals and H_2O_2, H_2O_2 and Listerine® mouthwash, H_2O_2 and Polident® Denture Cleaner, and H_2O_2 and Retainer Brite®. For specimens stained with black tea on the smooth surfaces, testing indicated statistically significant differences between solutions: Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Listerine® mouthwash, Invisalign® Cleaning Crystals and Retainer Brite®, H_2O_2 and Listerine® mouthwash, H_2O_2 and Polident® Denture Cleaner, and Listerine® mouthwash and Polident® Denture Cleaner, see Table XXVI.

For specimens stained with red wine on the smooth surfaces, testing indicated statistically significant differences between solutions: Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Polident® Denture Cleaner, H_2O_2 and Listerine® mouthwash, H_2O_2 and

Retainer Brite®, Listerine® mouthwash and Polident® Denture Cleaner, Listerine® mouthwash and Retainer Brite®, and Polident® Denture Cleaner and Retainer Brite®, see Table XXVII

LIGHT TRANSMITTANCE (ΔT)

For specimens stained with coffee on the textured surfaces, testing indicated statistically significant differences between solutions: Listerine® mouthwash and Retainer Brite®, and Polident® Denture Cleaner and Retainer Brite®. For specimens stained with coffee on the smooth surfaces, testing indicated statistically significant differences between solutions: Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Listerine® mouthwash, and Invisalign® Cleaning Crystals and Polident® Denture Cleaner, see Table XXV.

For specimens stained with black tea on the textured surfaces, testing indicated statistically significant differences between solutions: Invisalign® Cleaning Crystals and H_2O_2, H_2O_2 and Listerine® mouthwash, and H_2O_2 and Retainer Brite®, see Table XXVI.

TABLE XXV

THE P-VALUES OF ESSIX ACE® STAINED WITH COFFEE AFTER DESTAINING.

	Δ Transmission (%)		**Δ E**	
	Textured	**Smooth**	**Textured**	**Smooth**
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	0.028	0.004	0.000	0.015
Invisalign® Cleaning Crystals and H_2O_2	0.280	0.000	0.001	0.000
Invisalign® Cleaning Crystals and Listerine® mouthwash	0.052	0.002	0.011	0.089
Invisalign® Cleaning Crystals and Polident® Denture Cleaner	0.165	0.001	0.015	0.481
H_2O_2 and Polident® Denture Cleaner	0.853	0.853	0.043	0.043
H_2O_2 and Retainer Brite®	0.218	0.105	0.000	0.007
Listerine® mouthwash and Retainer Brite®	0.000	0.123	0.015	0.190
Polident® Denture Cleaner and Retainer Brite®	0.023	0.165	0.105	0.631

*significant p-values are highlighted.

TABLE XXVI

THE P-VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AFTER DESTAINING

	Δ Transmission (%)		**Δ E**	
	Textured	**Smooth**	**Textured**	**Smooth**
Invisalign® Cleaning Crystals, H2O2, Listerine®, Polident®, Retainer Brite®	0.015	0.401	0.001	0.000
Invisalign® Cleaning Crystals and H_2O_2	0.009	0.105	0.000	0.000
Invisalign® Cleaning Crystals and Listerine® mouthwash	0.353	0.218	0.315	0.002
Invisalign® Cleaning Crystals and Retainer Brite®	0.853	0.436	0.912	0.043
H_2O_2 and Listerine® mouthwash	0.019	0.684	0.011	0.035
H_2O_2 and Polident® Denture Cleaner	0.075	0.165	0.000	0.000
H_2O_2 and Retainer Brite®	0.003	0.393	0.000	0.052
Listerine® mouthwash and Polident® Denture Cleaner	0.089	0.315	0.436	0.023

*significant p-values are highlighted

.

TABLE XXVII

THE P-VALUES OF ESSIX ACE® STAINED WITH RED WINE AFTER DESTAINING.

	Δ Transmission (%)		**Δ E**	
	Textured	**Smooth**	**Textured**	**Smooth**
Invisalign® Cleaning Crystals, H2O2, Listerine®, Polident®, Retainer Brite®	0.287	0.186	0.813	0.006
Invisalign® Cleaning Crystals and H_2O_2	N/A	0.218	N/A	0.029
Invisalign® Cleaning Crystals and Polident® Denture Cleaner	N/A	0.579	N/A	0.019
H_2O_2 and Listerine® mouthwash	N/A	0.190	N/A	0.015
H_2O_2 and Retainer Brite®	N/A	0.190	N/A	0.035
Listerine® mouthwash and Polident® Denture Cleaner	N/A	0.089	N/A	0.002
Listerine® mouthwash and Retainer Brite®	N/A	0.035	N/A	0.796
Polident® Denture Cleaner and Retainer Brite®	N/A	0.579	N/A	0.011

*significant p-values are highlighted.

4. Staining

The median data values for ΔT and ΔE are shown in the tables below. For destaining, the higher ΔT and ΔE values indicated the retainer material had a larger change from staining to destaining. Therefore, the higher ΔT and ΔE indicated the better improvement in light transmittance and color change after destaining methods.

COLORIMETRY (ΔE)

All cleaning methods showed a statistically significant difference between both surface textures. For coffee staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table XXVIII.

For black tea staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table XXX. For red wine staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table XXXII.

LIGHT TRANSMITTANCE (ΔT)

For coffee staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table XXVIII. All cleaning methods showed a statistically significant difference between both surface textures, see Table XXIX.

For black tea staining, all cleaning solutions showed a statistically significant difference between ultrasonic vs. non-ultrasonic cleaning units, see Table XXX. There were no statistically significant differences between cleaning solutions and the textured and smooth surfaces, see Table XXXI.

For red wine staining, there were statistically significant differences between ultrasonic vs. non-ultrasonic cleaning units for Listerine® mouthwash and Retainer Brite®, see Table XXXII. All cleaning methods, except Invisalign® Cleaning Crystals, showed a statistically significant difference between both surface textures, see Table XXXIII.

TABLE XXVIII

MEDIAN VALUES OF ESSIX ACE® STAINED WITH COFFEE AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC.

	Δ Transmission (%)		**Δ E**	
	Non-Ultrasonic	Ultrasonic	Non-Ultrasonic	Ultrasonic
Invisalign® Cleaning Crystals	18.496	18.6895	13.972	13.928
H_2O_2	18.790	17.534	13.463	12.9715
Listerine® mouthwash	18.3975	18.8995	13.4125	13.575
Polident® Denture Cleaner	18.7785	18.4165	13.989	14.0085
Retainer Brite®	19.2875	19.826	13.903	14.418

*no significant p-values.

TABLE XXIX

MEDIAN VALUES OF ESSIX ACE® STAINED WITH COFFEE AND DESTAINED – SURFACE TEXTURES.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Invisalign® Cleaning Crystals	23.044	16.246	16.546	11.9935
H_2O_2	22.539	15.747	15.518	11.526
Listerine® mouthwash	21.88	15.6575	15.923	11.6745
Polident® Denture Cleaner	21.951	15.731	15.8525	11.9025
Retainer Brite®	22.9795	16.101	16.284	11.926

*significant p-values are highlighted.

TABLE XXX

MEDIAN VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC.

	Δ Transmission (%)		**Δ E**	
	Non-Ultrasonic	Ultrasonic	Non-Ultrasonic	Ultrasonic
Invisalign® Cleaning Crystals	20.4605	24.7525	15.5555	15.888
H_2O_2	18.6475	23.868	14.367	14.256
Listerine® mouthwash	18.385	24.892	14.928	15.3625
Polident® Denture Cleaner	20.9245	24.5035	15.794	15.682
Retainer Brite®	20.697	24.7435	15.3885	15.9435

*significant p-values are highlighted.

TABLE XXXI

MEDIAN VALUES OF ESSIX ACE® STAINED WITH BLACK TEA AND DESTAINED – SURFACE TEXTURES.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Invisalign® Cleaning Crystals	24.156	21.7805	17.091	14.598
H_2O_2	22.9145	21.1475	16.307	14.0915
Listerine® mouthwash	24.3575	21.222	16.9495	14.306
Polident® Denture Cleaner	23.617	21.668	17.1395	14.5415
Retainer Brite®	24.0795	21.3935	17.15	14.3965

*significant p-values are highlighted.

TABLE XXXII

MEDIAN VALUES OF ESSIX ACE® STAINED WITH RED WINE AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC.

	Δ Transmission (%)		**Δ E**	
	Non-Ultrasonic	Ultrasonic	Non-Ultrasonic	Ultrasonic
Invisalign® Cleaning Crystals	0.0958	1.3945	2.4195	2.1825
H_2O_2	0.946	1.284	2.238	2.472
Listerine® mouthwash	1.436	0.6345	2.3585	2.4455
Polident® Denture Cleaner	1.006	1.4455	2.1295	2.5185
Retainer Brite®	1.7705	1.2155	2.2465	2.305

*significant p-values are highlighted.

TABLE XXXIII

MEDIAN VALUES OF ESSIX ACE® STAINED WITH RED WINE AND DESTAINED – SURFACE TEXTURES.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Invisalign® Cleaning Crystals	1.2955	1.271	3.079	1.905
H_2O_2	1.435	0.936	3.107	1.605
Listerine® mouthwash	1.578	0.682	2.881	1.922
Polident® Denture Cleaner	1.5885	1.006	3.144	1.69
Retainer Brite®	1.728	1.0905	2.9445	1.932

*significant p-values are highlighted.

Estimated means of the destaining solutions at T4 for ΔT rough (textured) and ΔT smooth, grouped by staining solution, can be seen in Figure 20 and Figure 21, respectively. Non-ultrasonic destaining photographs can be seen in Figures 22-25 and ultrasonic destaining photographs can be seen in Figures 26-29.

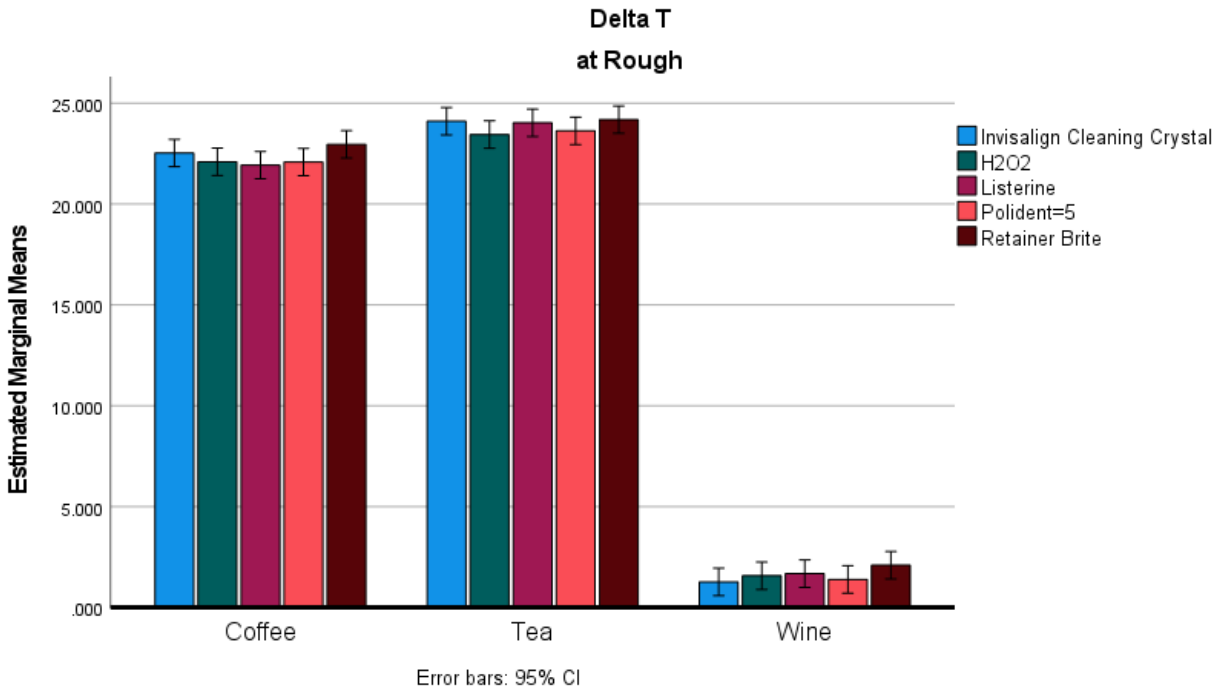


Figure 20: Comparison of Essix ACE® ΔT for textured surfaces among destaining solutions

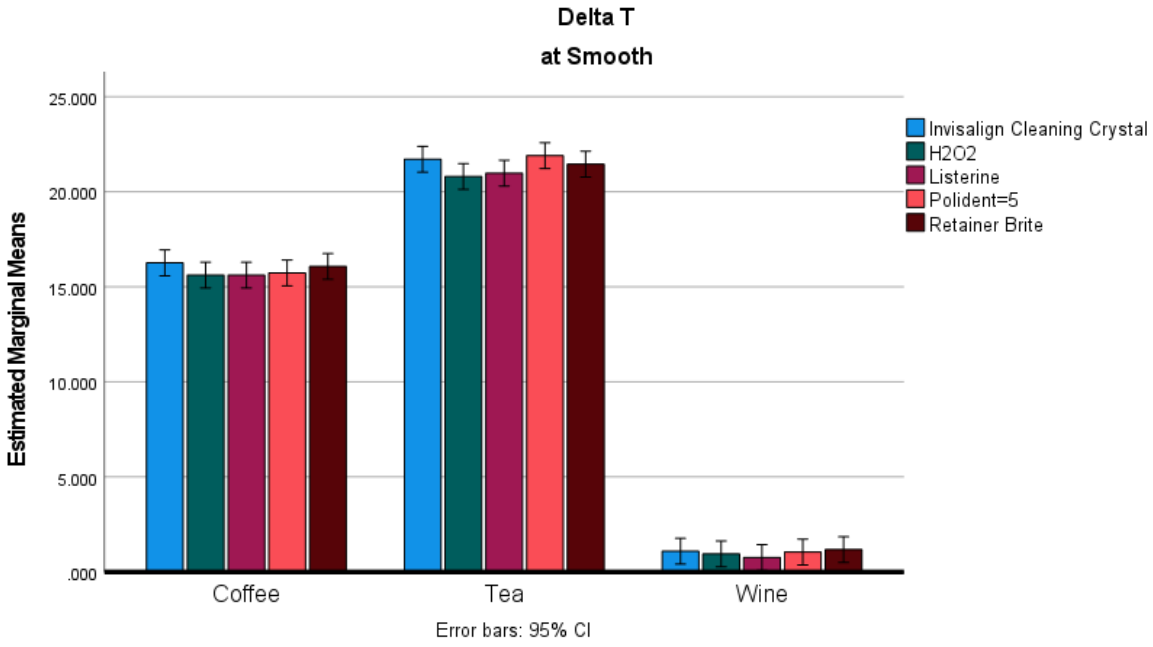


Figure 21: Comparison of Essix ACE® ΔT for smooth surfaces among destaining solutions

5. NON- ULTRASONIC DESTAINING PHOTOS

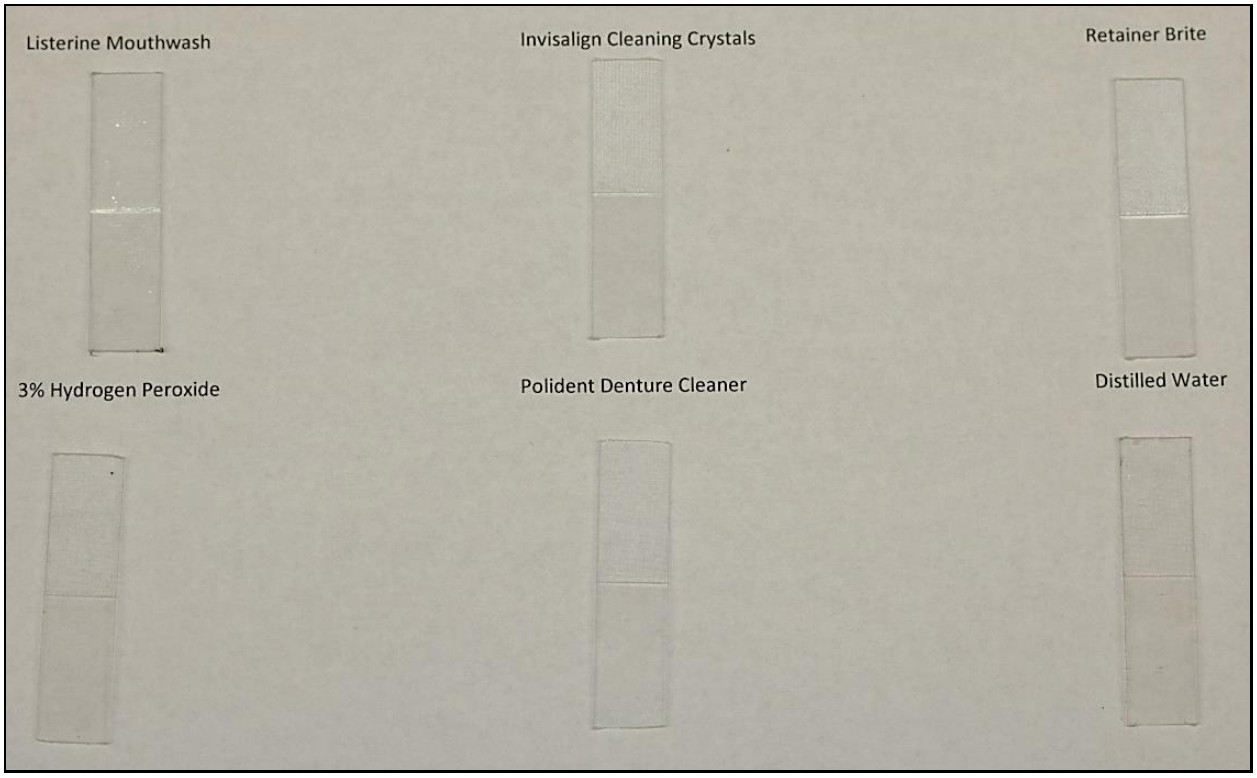


Figure 22: Essix ACE® stained with coffee and destained with the non-ultrasonic.

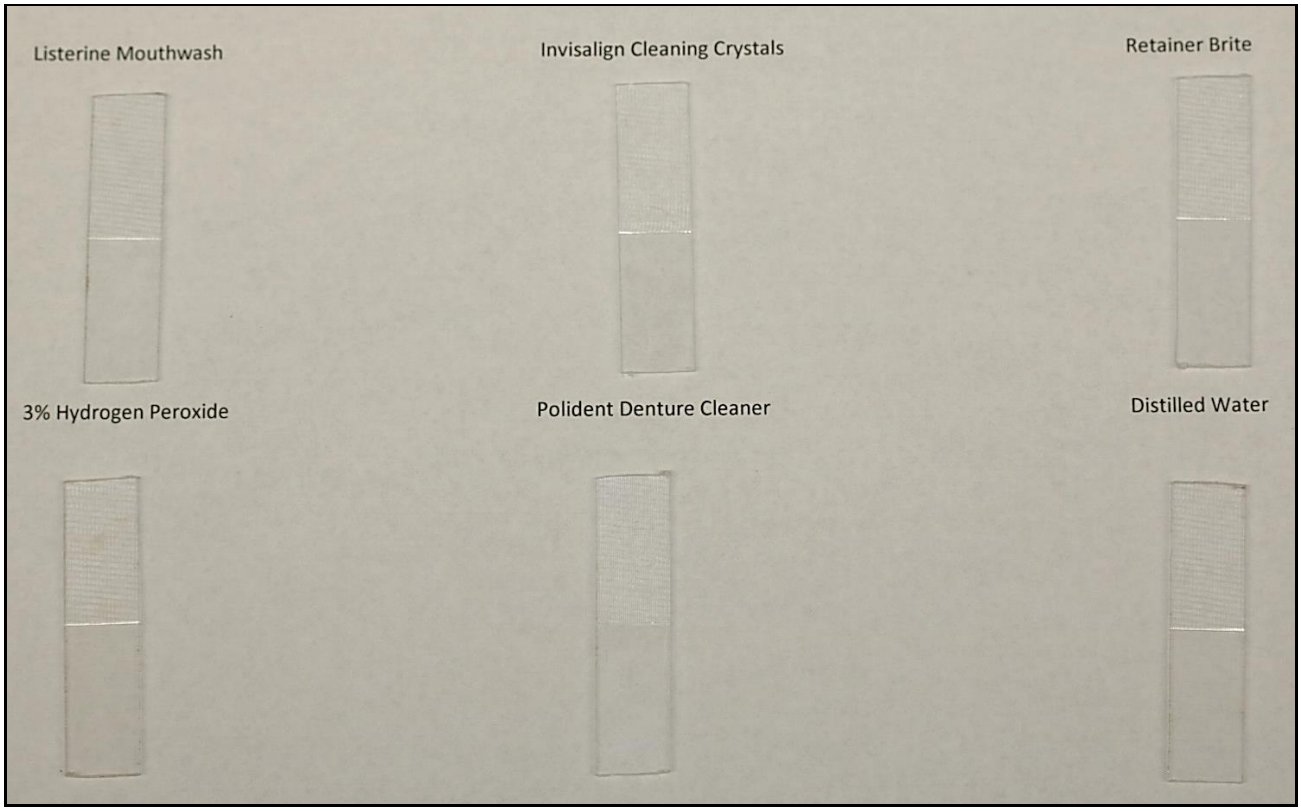


Figure 23: Essix ACE® stained with black tea and destained with the non-ultrasonic.

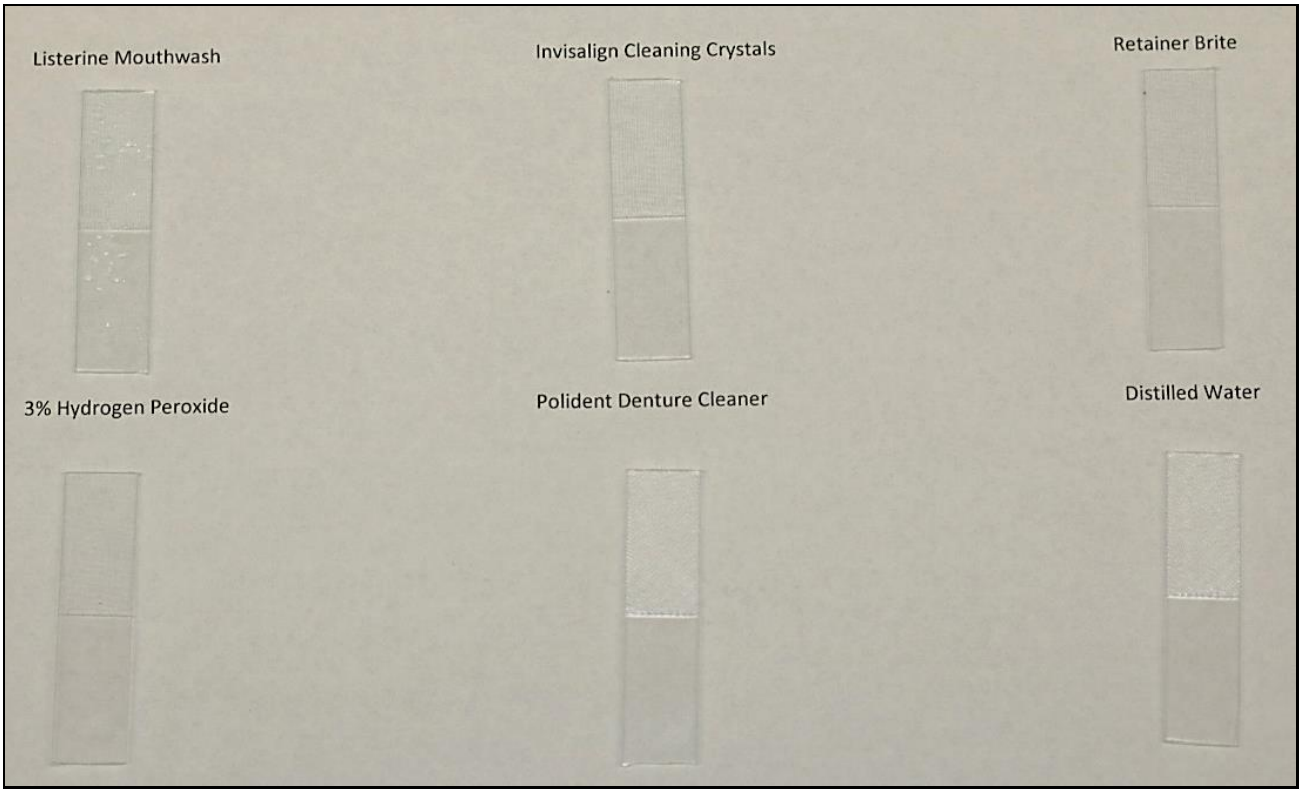


Figure 24: Essix ACE® stained with distilled water and destained with the non-ultrasonic**.**

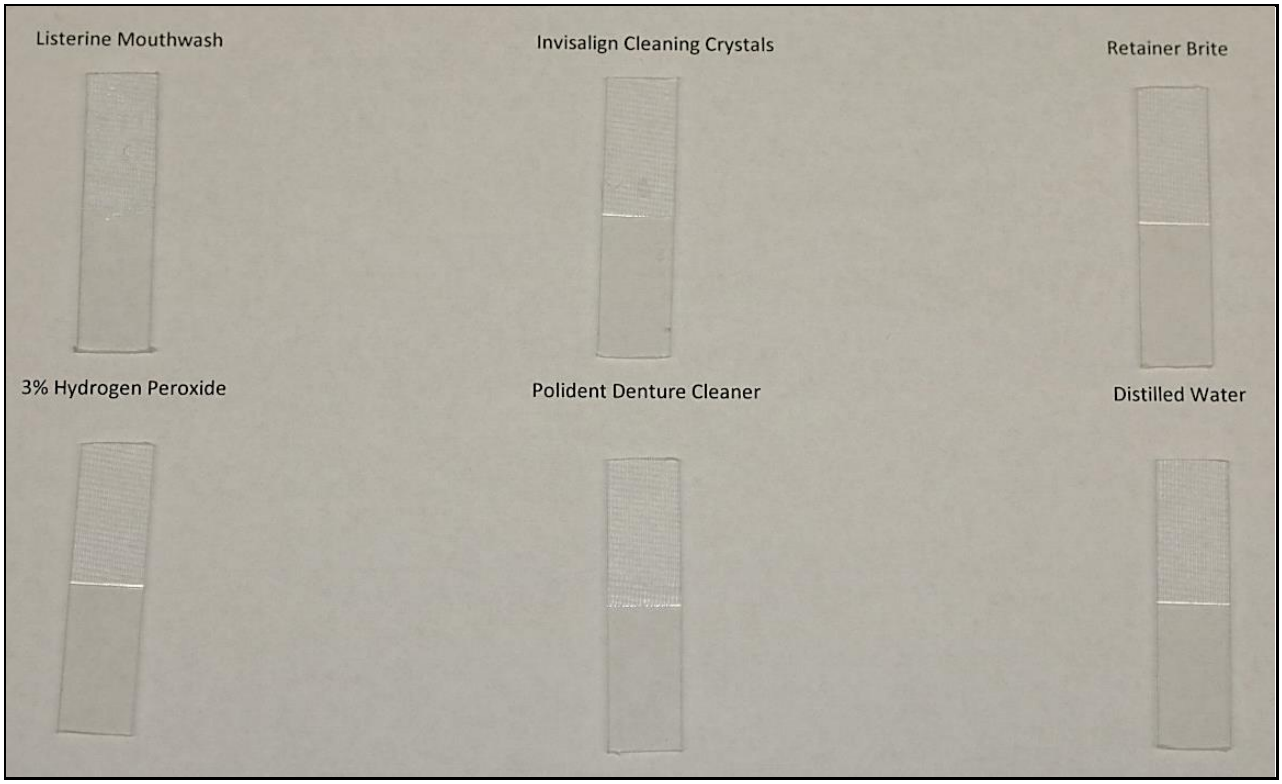


Figure 25: Essix ACE® stained with red wine and destained with the non-ultrasonic**.**

6. ULTRASONIC DESTAINING PHOTOS

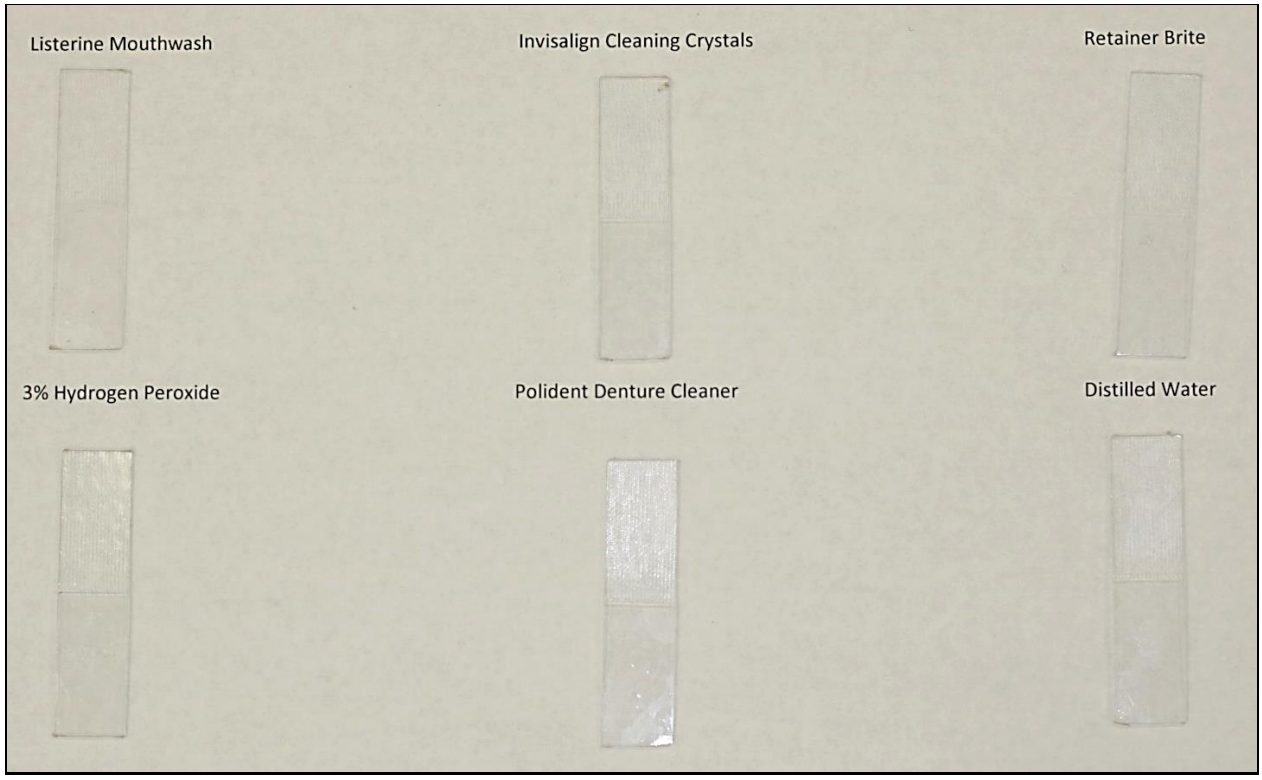


Figure 26: Essix ACE® stained with coffee and destained with the ultrasonic**.**

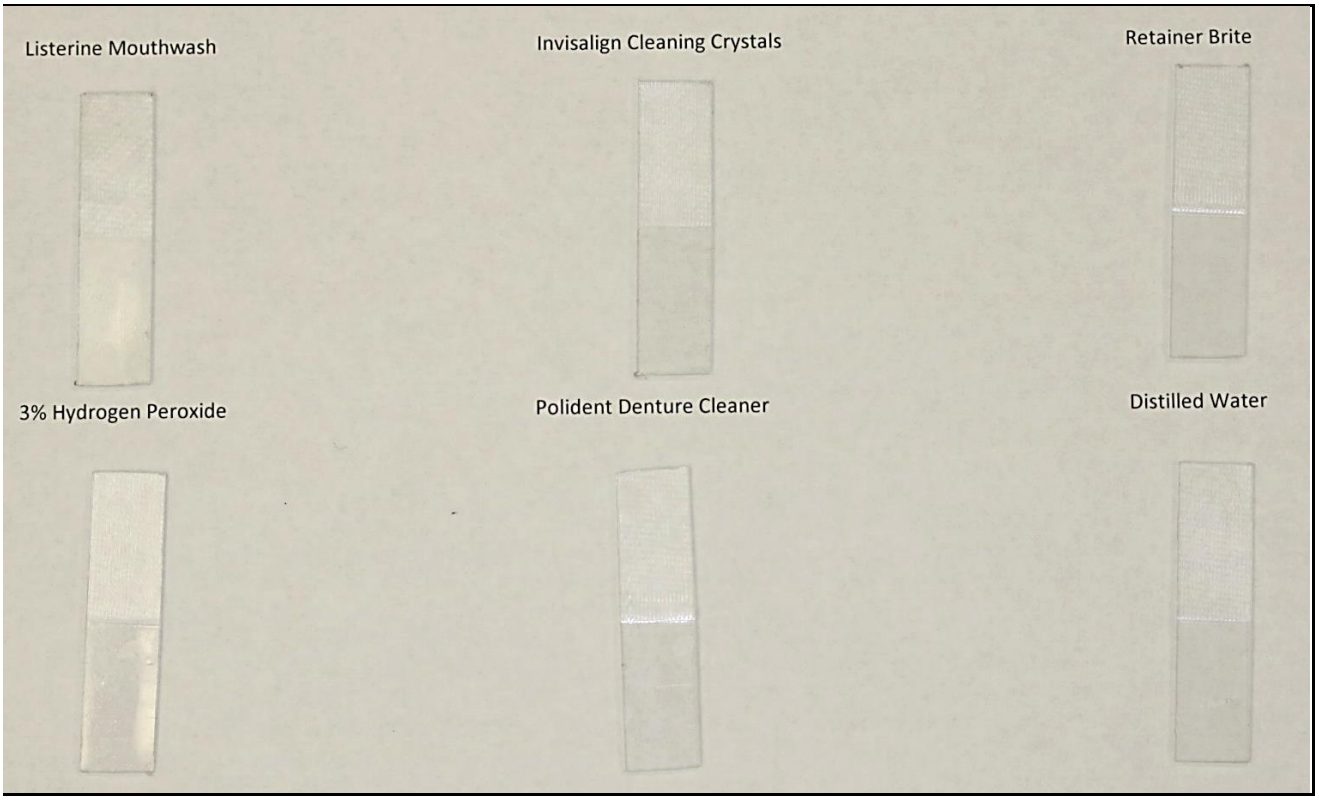


Figure 27: Essix ACE® stained with black tea and destained with the ultrasonic**.**

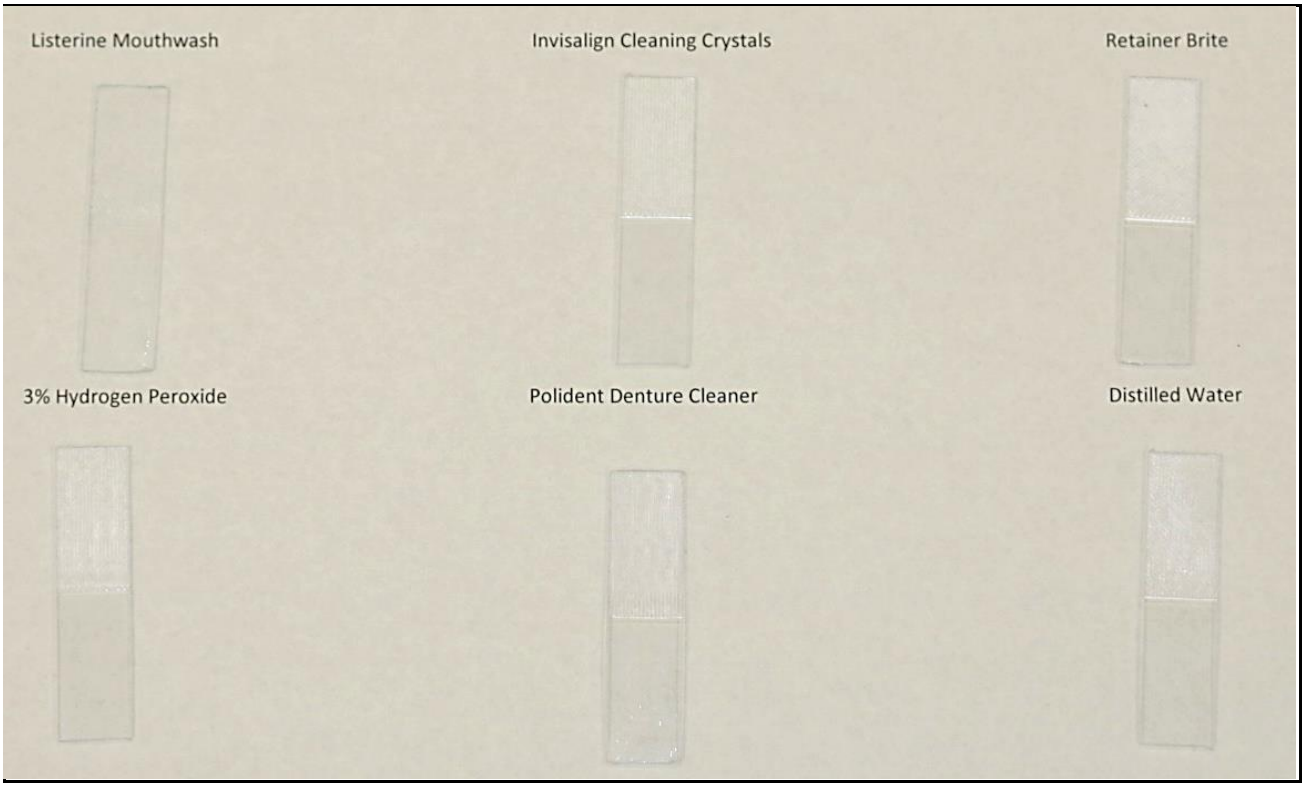


Figure 28: Essix ACE® stained with distilled water and destained with the ultrasonic**.**

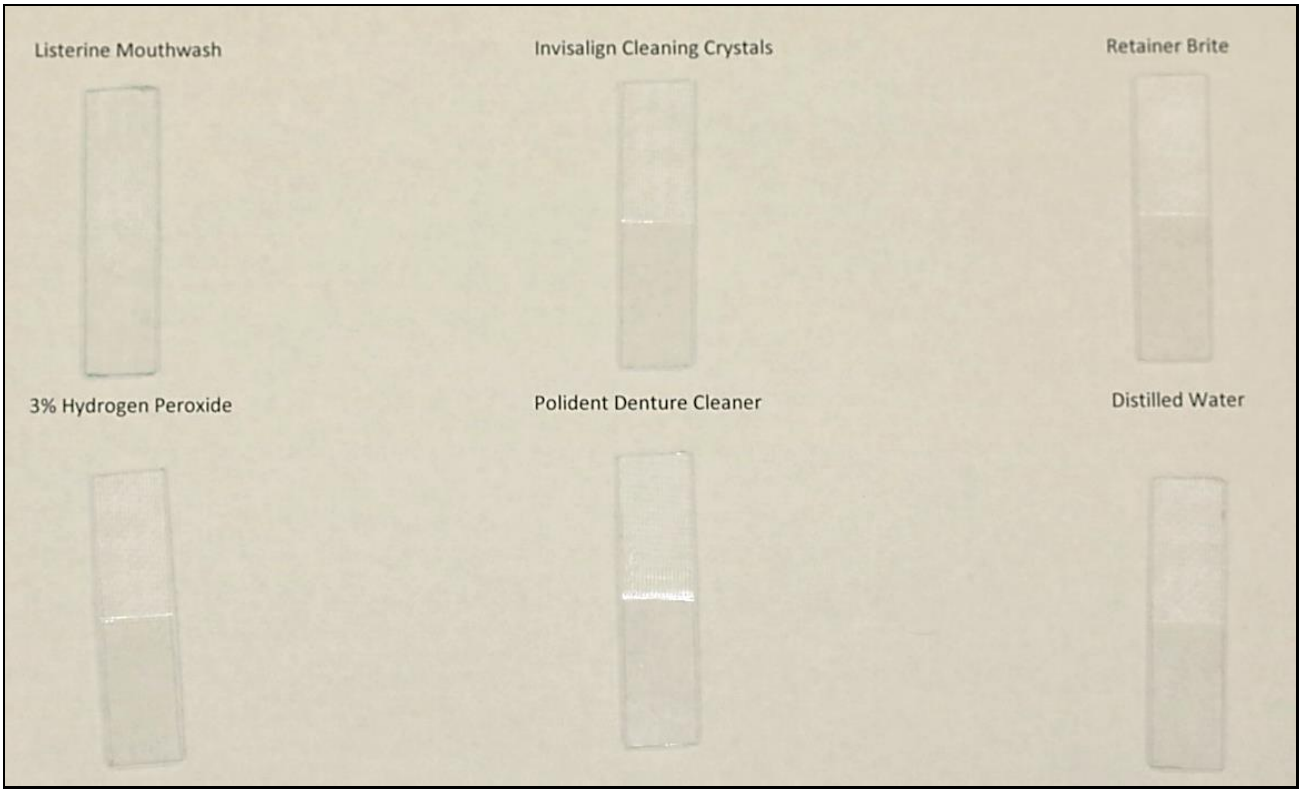


Figure 29: Essix ACE® stained with red wine and destained with the ultrasonic**.**

E. Essix C+® Staining Results

1. Differences of Textured vs. Smooth Surface Textures at Each Timepoint

Colorimetry and light transmittance results were summarized in Tables XXXIV-XXXVI. Kruskal Wallis and Mann-Whitney found significant differences among the various staining agents.

COLORIMETRY RESULTS (ΔE)

For all timepoints, all staining agents showed statistically significant differences from one another for the textured and smooth surfaces.

T1 (Day 7): For the textured surfaces, there were statistically significant differences between coffee and black tea, coffee and distilled water, black tea and distilled water, and distilled water and red wine. For the smooth surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, and black tea and distilled water, see Table XXXIV.

T2 (Day 14): For the textured surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, black tea and distilled water, black tea and red wine, and distilled water and red wine. For the smooth surfaces, there were statistically significant differences between coffee and black tea, coffee and distilled water, coffee and red wine, black tea and distilled water, and distilled water and red wine, see Table XXXV.

T3 (Day 28): For the textured surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, black tea and distilled water, and distilled water and red wine. For the smooth surfaces, there were statistically significant differences between coffee and black tea, coffee and distilled water, coffee and red wine, black tea and distilled water, and distilled water and red wine, see Table XXXVI.

LIGHT TRANSMITTANCE (ΔT)

T1 (Day 7): For both surfaces, there were no statistically significant differences between staining agents.

T2 (Day 14): For the textured and smooth surfaces, there were statistically significant differences between coffee and distilled water. For the smooth surface, there were statistically significant differences between distilled water and red wine. For day 14, all staining agents showed statistically significant differences for the smooth surfaces, see Table XXXV.

T3 (Day 28): For the textured surfaces, there were statistically significant differences between coffee and distilled water, coffee and red wine, black tea and distilled water, and black tea and red wine. For the smooth surfaces, there were statistically significant differences between coffee and distilled water, black tea and distilled water, and distilled water and red wine. For day 28, all staining agents showed statistically significant differences for both surface textures, see Table XXXVI.

TABLE XXXIV

THE P-VALUES OF ESSIX C+® AT DAY 7 IN THE STAINING EXPERIMENTS

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Coffee, Black Tea, Distilled Water, Red Wine	0.722	0.381	0.003	0.006
Coffee – Black tea	0.421	0.548	0.008	0.056
Coffee – Distilled Water	0.421	0.421	0.008	0.008
Coffee – Red Wine	0.548	0.841	0.056	0.032
Black tea – Distilled Water	0.841	0.151	0.032	0.032
Black tea – Red Wine	0.841	0.690	0.421	0.421
Distilled Water – Red Wine	0.841	0.222	0.016	0.056

*significant p-values are highlighted.

TABLE XXXV

THE P-VALUES OF ESSIX C+® AT DAY 14 IN THE STAINING EXPERIMENTS

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Coffee, Black tea, Distilled Water, Red Wine	0.105	0.025	0.001	0.001
Coffee – Black tea	0.690	1.000	0.056	0.008
Coffee – Distilled Water	0.032	0.032	0.008	0.008
Coffee – Red Wine	1.000	0.095	0.008	0.008
Black Tea – Distilled Water	0.095	0.222	0.008	0.008
Black Tea – Red Wine	0.548	0.222	0.016	0.310
Distilled Water – Red Wine	0.095	0.008	0.008	0.008

*significant p-values are highlighted.

TABLE XXXVI

THE P-VALUES OF ESSIX C+® AT DAY 28 IN THE STAINING EXPERIMENTS

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Among staining solutions	0.002	0.021	0.002	0.002
Coffee – Black tea	1.0	1.0	0.095	0.016
Coffee – Distilled Water	0.008	0.032	0.008	0.008
Coffee – Red Wine	0.008	0.222	0.008	0.016
Black Tea – Distilled Water	0.008	0.016	0.016	0.008
Black Tea – Red Wine	0.032	0.310	0.310	0.548
Distilled Water – Red Wine	0.056	0.016	0.008	0.008

*significant p-values are highlighted.

2. Differences among Staining Solutions at Each Timepoint

Colorimetry and light transmittance results were summarized in Tables XXXVII-XL. Kruskal Wallis and Mann-Whitney found significant differences among the textured and smooth surfaces.

COLORIMETRY RESULTS (ΔE)

All timepoints, for all staining agents, showed statistically significant differences between textured and smooth surfaces. For coffee and black tea staining, the textured and smooth surfaces were statistically significant between T1 and T3 and T2 and T3. For distilled water staining, the

textured surfaces were statistically significant between T1 and T2 and T2 and T3. For distilled water staining, the smooth surfaces were statistically significant between T2 and T3. For red wine staining, the textured surfaces were statistically significant between T1 and T2, T1 and T3, and T2 and T3. For red wine staining, the smooth surfaces were statistically significant between T1 and T3 and T2 and T3.

LIGHT TRANSMITTANCE (ΔT)

All timepoints for coffee, black tea, and red wine showed statistically significant differences between textured and smooth surfaces.

For coffee staining, the smooth surfaces were statistically significant between T1 and T2. Additionally, the textured and smooth surfaces were statistically significant between T1 and T3 and T2 and T3. For black tea staining, the textured and smooth surfaces were statistically significant between T1 and T3 and T2 and T3. For red wine staining, the smooth surfaces were statistically significant for T1 and T2. Additionally, the smooth and textured surfaces were statistically significant for T1 and T3.

TABLE XXXVII

THE P-VALUES OF ESSIX C+® IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.006	0.003	0.009	0.005
T1 - T2	0.222	0.016	0.690	0.151
T1 - T3	0.008	0.008	0.008	0.008
T2 - T3	0.008	0.016	0.008	0.008

*significant p-values are highlighted.

TABLE XXXVIII

THE P-VALUES OF ESSIX C+® IN THE TEA STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.004	0.010	0.013	0.009
T1 - T2	0.056	0.421	0.841	1.000
T1 - T3	0.008	0.008	0.008	0.008
T2 - T3	0.008	0.016	0.016	0.008

*significant p-values are highlighted.

TABLE XXXIX

THE P-VALUES OF ESSIX C+® IN THE DISTILLED WATER STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.811	0.160	0.006	0.034
T1 - T2	0.690	0.222	0.016	0.421
T1 - T3	0.690	0.151	0.095	0.095
T2 - T3	0.841	0.310	0.008	0.008

*significant p-values are highlighted.

TABLE XL

THE P-VALUES OF ESSIX C+® IN THE WINE STAINING GROUP BY STUDIED TIMEPOINTS AND SURFACE DIFFERENCES IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
T1 - T2 - T3	0.027	0.004	0.002	0.009
T1 - T2	0.222	0.016	0.008	0.690
T1 - T3	0.008	0.008	0.008	0.008
T2 - T3	0.222	0.032	0.008	0.008

*significant p-values are highlighted.

3. Differences between Surfaces at Specific Timepoints among Staining Solutions

Colorimetry and light transmittance results were summarized in Tables XLI-XLIV. Kruskal Wallis and Mann-Whitney found significant differences among the different timepoints.

COLORIMETRY RESULTS (ΔE)

For coffee staining, day 28 showed statistically significant differences between the textured and smooth surfaces, see Table XLI. For black tea staining, day 7 showed statistically significant differences between the textured and smooth surfaces, see Table XLII. For distilled water and red wine staining, day 14 showed statistically significant differences between the textured and smooth surfaces.

LIGHT TRANSMITTANCE (ΔT)

For coffee staining, day 14 and day 28 showed statistically significant differences between the textured and smooth surfaces, see Table XLI. For black tea staining, day 28 showed statistically significant differences between the textured and smooth surfaces, see Table XLII.

TABLE XLI

THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.056	0.095
Day 14	0.032	0.421
Day 28	0.032	0.008

*significant p-values are highlighted.

TABLE XLII

THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.690	0.008
Day 14	0.222	0.548
Day 28	0.032	0.151

*significant p-values are highlighted.

TABLE XLIII

THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE WATER STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.095	0.222
Day 14	0.056	0.008
Day 28	1.0	0.548

*significant p-values are highlighted.

TABLE XLIV

THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY STUDIED TIMEPOINTS IN THE STAINING EXPERIMENTS.

	Δ Transmission (%)	**Δ E**
Day 7	0.421	1.0
Day 14	0.222	0.008
Day 28	1.000	0.056

*significant p-values are highlighted.

The mean values for ΔE and ΔT, divided by textured (rough) and smooth surfaces, at T1, T2, and T3 can be viewed in Figure 30 and Figure 31, respectively.

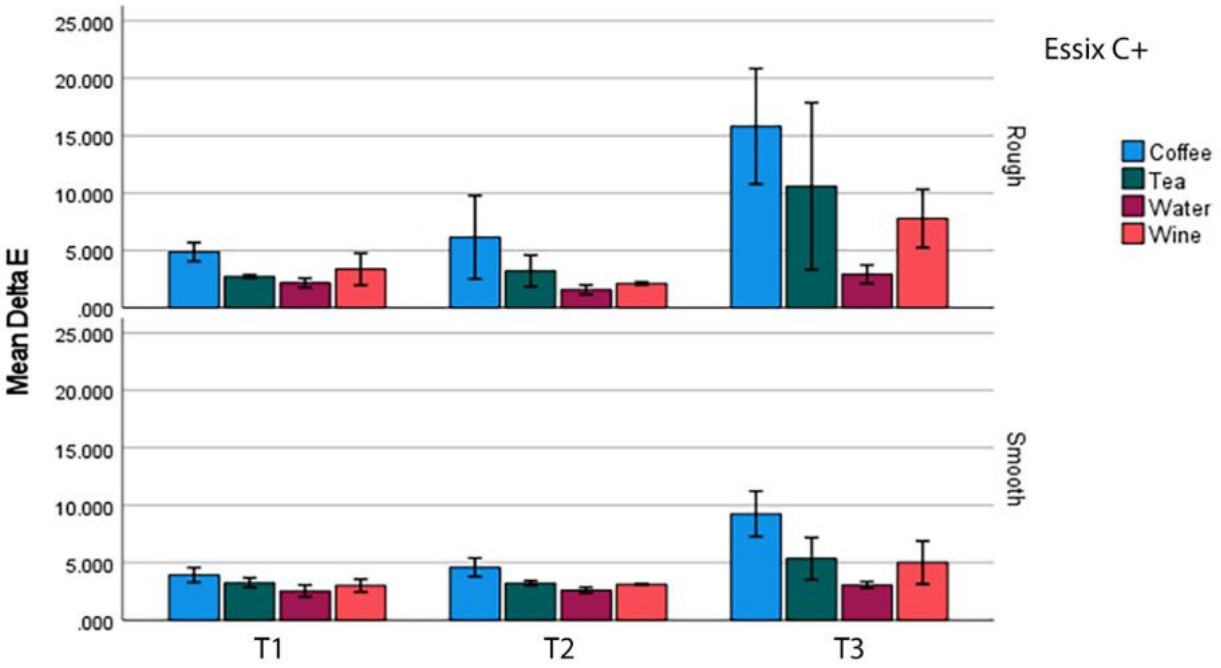


Figure 30: Mean value of ΔE in Essix C+®

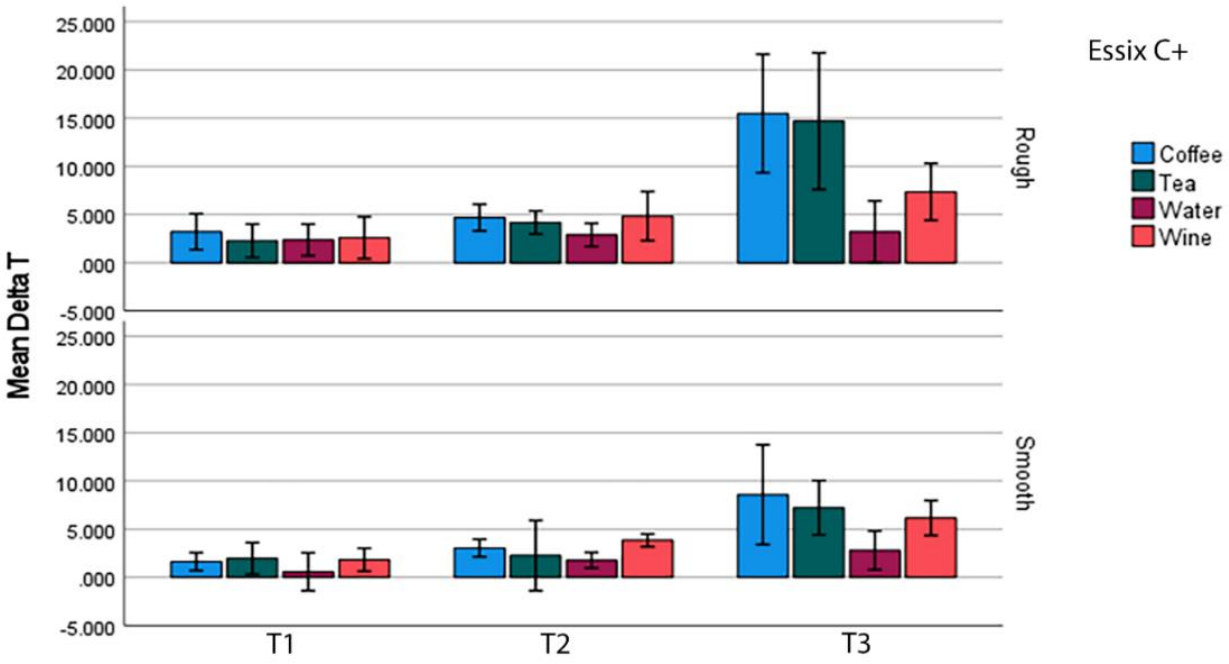


Figure 31: Mean value of ΔT in Essix C+®

4. NBS Values

NBS ΔE values were calculated using the equation ΔE x 0.92.[55] Values above NBS unit 3.0 are considered to have marked change in color, see Table IV, which, for this study, was considered clinically unacceptable. (Table XLV)

T1 (day 7): Statistically significant differences were found between black tea textured and smooth surfaces. There was no marked color change.

T2 (day 14): Statistically significant differences were found for distilled water and red wine staining solutions between the textured and smooth surfaces. There was no marked color change.

T3 (day 28): Statistically significant differences were found between coffee textured and smooth surfaces. There was marked color change.

TABLE XLV

ESSIX C+® MEDIAN VALUES FOR ΔE NBS VALUE (ΔE X 0.92)

	Day 7		**Day 14**		**Day 28**	
	Textured	**Smooth**	**Textured**	**Smooth**	**Textured**	**Smooth**
Coffee	4.54	3.47	4.60	4.59	**13.42**	**8.94**
Black Tea	2.58	2.97	2.53	2.89	9.44	4.62
Distilled Water	2.02	2.34	1.66	2.49	2.43	2.78
Red Wine	2.77	2.68	1.97	2.85	6.45	4.19

* significant values are highlighted, and significant color change values are bolded.

5. Median ΔT

A larger ΔT represents more staining on the either the textured specimen surface or the smooth specimen surface. (Table XLVI)

T1 (day 7): For all solutions, no statistically significant differences were noted.

T2 (day 14): For coffee solution, there were statistically significant differences between the smooth and textured surfaces. The textured surfaces exhibited more staining.

T3 (day 28): For the coffee and black tea staining solutions, there were statistically significant differences between the smooth and textured surfaces. The textured surfaces exhibited more staining.

TABLE XLVI

ESSIX C+® MEDIAN VALUES FOR ΔT BETWEEN SURFACES AMONG STAINING SAMPLES

	Day 7		**Day 14**		**Day 28**	
	Textured	**Smooth**	**Textured**	**Smooth**	**Textured**	**Smooth**
Coffee	3.4250	2.06140	4.5680	2.9894	15.0410	8.8800
Black Tea	2.3298	2.1198	4.3458	3.1338	12.6698	6.7078
Distilled Water	2.7762	0.7986	3.0652	1.5586	3.4012	2.5156
Red Wine	2.8754	1.9406	4.5254	3.6696	5.7674	6.3236

*significant color change values are highlighted.

F. Essix C+® Destaining Results

Essix C+® destaining for ΔT and ΔE were studied at each level on four factors, namely, surface texture differences, cleaning methods, cleaning solutions, and stains. The raw data distribution was investigated by the Shapiro-Wilk test and statistical significance cut off was at 5%. The distribution of the raw data showed to be non-normally distributed. Therefore, descriptive statistics were calculated. Non-parametric analysis for independent samples were done by Kruskal -Wallis and when statistically significant differences were found, Mann-Whitney test between two independent samples were used.

1. Textured vs. Smooth Surface

COLORIMETRY (ΔE)

For coffee, black tea, and red wine staining, all staining solutions showed statistically significant differences between the textured and smooth surfaces, see Tables XLVII-XLIX.

LIGHT TRANSMITTANCE (ΔT)

For coffee and black tea staining, all staining solutions showed statistically significant differences between the textured and smooth surfaces. For red wine staining, Invisalign® Cleaning Crystals solution showed statistically significant differences between the textured and smooth surfaces.

TABLE XLVII

THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE COFFEE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.000	0.000
H_2O_2	0.000	0.000
Listerine® mouthwash	0.000	0.000
Polident® Denture Cleaner	0.000	0.000
Retainer Brite®	0.000	0.000

*significant p-values are highlighted.

TABLE XLVIII

THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE BLACK TEA STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.000	0.000
H_2O_2	0.000	0.000
Listerine® mouthwash	0.000	0.000
Polident® Denture Cleaner	0.000	0.000
Retainer Brite®	0.000	0.000

*significant p-values are highlighted.

TABLE XLIX

THE P-VALUES OF ESSIX C+® BY SURFACE DIFFERENCES IN THE RED WINE STAINING GROUP BY CLEANING METHODS IN THE DESTAINING EXPERIMENT

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.007	0.000
H_2O_2	0.393	0.000
Listerine® mouthwash	0.393	0.000
Polident® Denture Cleaner	0.393	0.000
Retainer Brite®	0.247	0.000

*significant p-values are highlighted.

2. Mechanical Cleaning

Testing indicated no statistically significant differences between all staining solutions and non-ultrasonic and ultrasonic cleaning methods, see Table L.

LIGHT TRANSMITTANCE (ΔT)

For red wine cleaned with H_2O_2, there were statistically significant differences between non-ultrasonic and ultrasonic cleaning methods, see Table LIII.

TABLE L

THE P-VALUES OF ESSIX C+® AFTER DESTAINING

		Δ Transmission (%)		Δ E	
		Non-Ultrasonic	**Ultrasonic**	**Non-Ultrasonic**	**Ultrasonic**
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	**Coffee**	0.323	0.705	0.520	0.686
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	**Black Tea**	0.851	0.753	0.291	0.607
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	**Red Wine**	0.675	0.987	0.364	0.728

TABLE LI

THE P-VALUES OF ESSIX C+® STAINED WITH COFFEE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING.

	Δ Transmission (%)	Δ E
Invisalign® Cleaning Crystals	0.912	0.739
H_2O_2	0.796	0.063
Listerine® mouthwash	0.912	0.739
Polident® Denture Cleaner	0.315	0.971
Retainer Brite®	0.436	0.684

TABLE LII

THE P-VALUES OF ESSIX C+® STAINED WITH BLACK TEA AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING.

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.579	0.190
H_2O_2	0.190	0.393
Listerine® mouthwash	0.796	0.218
Polident® Denture Cleaner	0.796	0.529
Retainer Brite®	0.853	0.190

TABLE LIII

THE P-VALUES OF ESSIX C+® STAINED WITH RED WINE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING.

	Δ Transmission (%)	**Δ E**
Invisalign® Cleaning Crystals	0.315	0.218
H_2O_2	0.035	0.481
Listerine® mouthwash	0.853	0.436
Polident® Denture Cleaner	0.353	0.912
Retainer Brite®	0.218	0.393

*significant p-values are highlighted.

3. Cleaning Solution

COLORIMETRY (ΔE)

For specimens stained with coffee on the textured surfaces, testing indicated statistically significant differences between solutions, Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Listerine® mouthwash, Invisalign® Cleaning Crystals and Retainer Brite®, H_2O_2 and Polident® Denture Cleaner, Listerine® mouthwash and Polident® Denture Cleaner, and Polident® Denture Cleaner and Retainer Brite®. For specimens stained with coffee on the smooth surfaces, testing indicated statistically significant differences between solutions, Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Polident® Denture Cleaner, Invisalign® Cleaning Crystals and Retainer Brite®, H_2O_2 and Retainer Brite®, Listerine® mouthwash and Polident® Denture Cleaner, and Listerine® mouthwash and Retainer Brite®, see Table XIV.

For specimens stained with black tea on the textured surfaces, testing indicated statistically significant differences between solutions, Invisalign® Cleaning Crystals and H_2O_2, Invisalign® Cleaning Crystals and Listerine® mouthwash, Invisalign® Cleaning Crystals and Polident® Denture Cleaner, and Listerine® mouthwash and Retainer Brite®, see Table XV.

For specimens stained with red wine on the textured surfaces, testing indicated statistically significant differences between solutions, Listerine® mouthwash and Polident® Denture Cleaner, and Listerine® mouthwash and Retainer Brite®, see Table XVI.

LIGHT TRANSMITTANCE (ΔT)

For specimens stained with coffee on the textured surfaces, testing indicated statistically significant differences between solutions, Invisalign® Cleaning Crystals and Retainer Brite®, and

Listerine® mouthwash and Retainer Brite®. For specimens stained with coffee on the smooth surfaces, testing indicated statistically significant differences between solutions, Invisalign® Cleaning Crystals and Retainer Brite®, Listerine® mouthwash and Retainer Brite®, and Polident® Denture Cleaner and Retainer Brite®.

TABLE XIV

THE P-VALUES OF ESSIX C+® STAINED WITH COFFEE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING

	Δ Transmission (%)		**Δ E**	
	Textured	**Smooth**	**Textured**	**Smooth**
Invisalign® Cleaning Crystals, H2O_2, Listerine®, Polident®, Retainer Brite®	0.094	0.019	0.005	0.000
Invisalign® Cleaning Crystals and H_2O_2	0.165	0.218	0.019	0.043
Invisalign® Cleaning Crystals and Listerine® mouthwash	0.739	0.853	0.015	0.529
Invisalign® Cleaning Crystals and Polident® Denture Cleaner	0.912	0.315	1.0	0.003
Invisalign® Cleaning Crystals and Retainer Brite	0.019	0.003	0.043	0.000
H_2O_2 and Polident® Denture Cleaner	0.739	0.393	0.002	0.075
H_2O_2 and Retainer Brite®	0.123	0.105	0.631	0.000
Listerine® mouthwash and Polident® Denture Cleaner	0.739	0.684	0.011	0.005
Listerine® mouthwash and Retainer Brite®	0.015	0.015	0.529	0.000
Polident® Denture Cleaner and Retainer Brite®.	0.190	0.011	0.015	0.165

*significant p-values are highlighted.

TABLE LV

THE P-VALUES OF ESSIX C+® STAINED WITH BLACK TEA AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING

	Δ Transmission (%)		**Δ E**	
	Textured	**Smooth**	**Textured**	**Smooth**
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	0.638	0.872	0.004	0.052
Invisalign® Cleaning Crystals and H_2O_2	1.0	N/A	0.023	N/A
Invisalign® Cleaning Crystals and Listerine® mouthwash	0.529	N/A	0.000	N/A
Invisalign® Cleaning Crystals and Polident® Denture Cleaner	0.579	N/A	0.011	N/A
Listerine® mouthwash and Retainer Brite®	0.280	N/A	0.011	N/A

*significant p-values are highlighted.

TABLE LVI

THE P-VALUES OF ESSIX C+® STAINED WITH RED WINE AFTER NON-ULTRASONIC AND ULTRASONIC CLEANING

	Δ Transmission (%)		**Δ E**	
	Textured	**Smooth**	**Textured**	**Smooth**
Invisalign® Cleaning Crystals, H_2O_2, Listerine®, Polident®, Retainer Brite®	0.724	0.733	0.043	0.240
Listerine® mouthwash and Polident® Denture Cleaner	0.684	N/A	0.000	N/A
Listerine® mouthwash and Retainer Brite®	0.971	N/A	0.029	N/A

*significant p-values are highlighted.

4. Staining

The median data values for ΔT and ΔE are shown in the tables below. For destaining, the higher ΔT and ΔE values indicated the retainer material had a larger change from staining to destaining. Therefore, the higher ΔT and ΔE indicated the better improvement in light transmittance and color change after destaining methods.

COLORIMETRY (ΔE)

For coffee staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table LVII. All cleaning methods significantly destained both surface textures, see Table LVIII.

For black tea staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table LIX. All cleaning methods significantly destained both surface textures, see Table LX.

For red wine staining, there were significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table LXI. All cleaning methods significantly destained both surface textures, see Table LXII.

LIGHT TRANSMITTANCE (ΔT)

For coffee staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table LVII. All cleaning methods significantly destained both surface textures, see Table LVIII.

For black tea staining, there were no significant differences between cleaning solutions and ultrasonic vs. non-ultrasonic cleaning units, see Table LIX. All cleaning methods significantly destained both surface textures, see Table LX.

For red wine staining, H_2O_2 cleaning solution had a significant difference between the ultrasonic vs. non-ultrasonic cleaning units, see Table LXI. Only Invisalign® Cleaning Crystals significantly cleaned the retainer materials for both surface textures, see Table LXII.

TABLE LVII

MEDIAN VALUES OF ESSIX C+® STAINED WITH COFFEE AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC.

	Δ Transmission (%)		**Δ E**	
	Non-Ultrasonic	Ultrasonic	Non-Ultrasonic	Ultrasonic
Invisalign® Cleaning Crystals	6.4075	5.9870	7.589	8.8615
H_2O_2	6.4425	5.7760	6.658	8.3055
Listerine® mouthwash	6.9790	6.5550	6.2795	8.1890
Polident® Denture Cleaner	4.3005	5.2940	7.7545	8.0585
Retainer Brite®	4.27750	4.9605	7.5215	6.2950

*significant p-values are highlighted.

TABLE LVIII

MEDIAN VALUES OF ESSIX C+® STAINED WITH COFFEE AND DESTAINED – SURFACE TEXTURES.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Invisalign® Cleaning Crystals	9.1740	4.5165	12.3325	4.917
H_2O_2	7.7520	3.0965	11.272	4.5715
Listerine® mouthwash	8.8255	4.037	11.413	4.9625
Polident® Denture Cleaner	8.6125	4.0720	12.3355	4.0405
Retainer Brite®	6.914	2.1795	11.3985	3.56

*significant p-values are highlighted.

TABLE LIX

MEDIAN VALUES OF ESSIX C+® STAINED WITH BLACK TEA AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC.

	Δ Transmission (%)		**Δ E**	
	Non-Ultrasonic	Ultrasonic	Non-Ultrasonic	Ultrasonic
Invisalign® Cleaning Crystals	5.1375	5.2125	6.1445	5.862
H_2O_2	4.2625	5.513	4.5025	5.1395
Listerine® mouthwash	5.0555	4.5385	4.095	5.692
Polident® Denture Cleaner	6.1605	4.8045	5.8795	5.554
Retainer Brite®	4.7505	5.7715	5.421	5.8705

*significant p-values are highlighted.

TABLE LX

MEDIAN VALUES OF ESSIX C+® STAINED WITH BLACK TEA AND DESTAINED – SURFACE TEXTURES.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Invisalign® Cleaning Crystals	8.861	1.6045	9.4045	2.625
H_2O_2	8.8665	1.7285	8.7565	2.3385
Listerine® mouthwash	8.168	1.492	8.5925	2.5125
Polident® Denture Cleaner	8.5705	1.443	9.047	2.177
Retainer Brite®	8.9455	1.866	9.041	2.5045

*significant p-values are highlighted.

TABLE LXI

MEDIAN VALUES OF ESSIX C+® STAINED WITH RED WINE AND DESTAINED – NON-ULTRASONIC VS. ULTRASONIC.

	Δ Transmission (%)		**Δ E**	
	Non-Ultrasonic	Ultrasonic	Non-Ultrasonic	Ultrasonic
Invisalign® Cleaning Crystals	1.5445	2.05	4.9815	4.5275
H_2O_2	0.887	2.3385	4.394	4.587
Listerine® mouthwash	1.9275	2.1225	4.606	4.681
Polident® Denture Cleaner	1.9265	2.299	4.8115	4.8895
Retainer Brite®	1.616	2.405	3.7335	4.8865

*significant p-values are highlighted.

TABLE LXII

MEDIAN VALUES OF ESSIX C+® STAINED WITH RED WINE AND DESTAINED – SURFACE TEXTURES.

	Δ Transmission (%)		**Δ E**	
	Textured	Smooth	Textured	Smooth
Invisalign® Cleaning Crystals	2.7315	1.465	7.205	2.5065
H_2O_2	2.137	1.522	7.147	2.273
Listerine® mouthwash	2.3975	1.9455	6.700	2.597
Polident® Denture Cleaner	2.3505	2.162	7.0725	2.6015
Retainer Brite®	2.133	1.1995	6.889	2.5305

*significant p-values are highlighted.

Estimated means of the destaining solutions at T4 for ΔT rough (textured) and ΔT smooth, grouped by staining solution, can be seen in Figure 32 and Figure 33, respectively. Destaining experiment photographs for the non-ultrasonic means can be seen in Figures 34-37 and destaining photographs for the ultrasonic means can be seen in Figures 38-41.

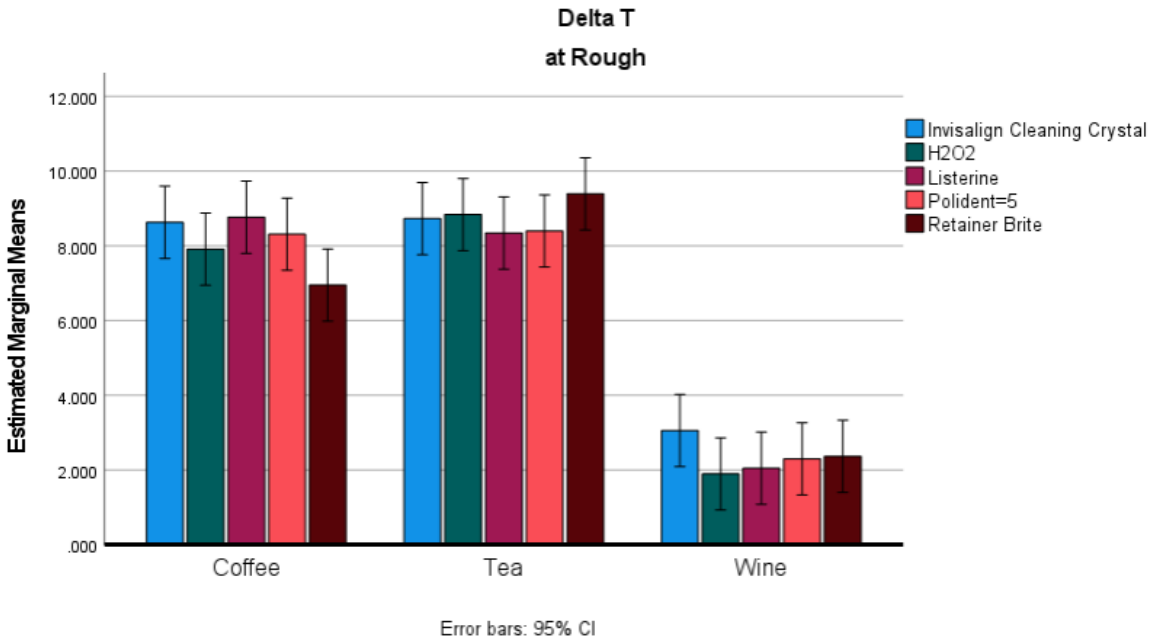


Figure 32: Comparison of Essix C+® ΔT among destaining solutions for textured surface by type of stained specimens

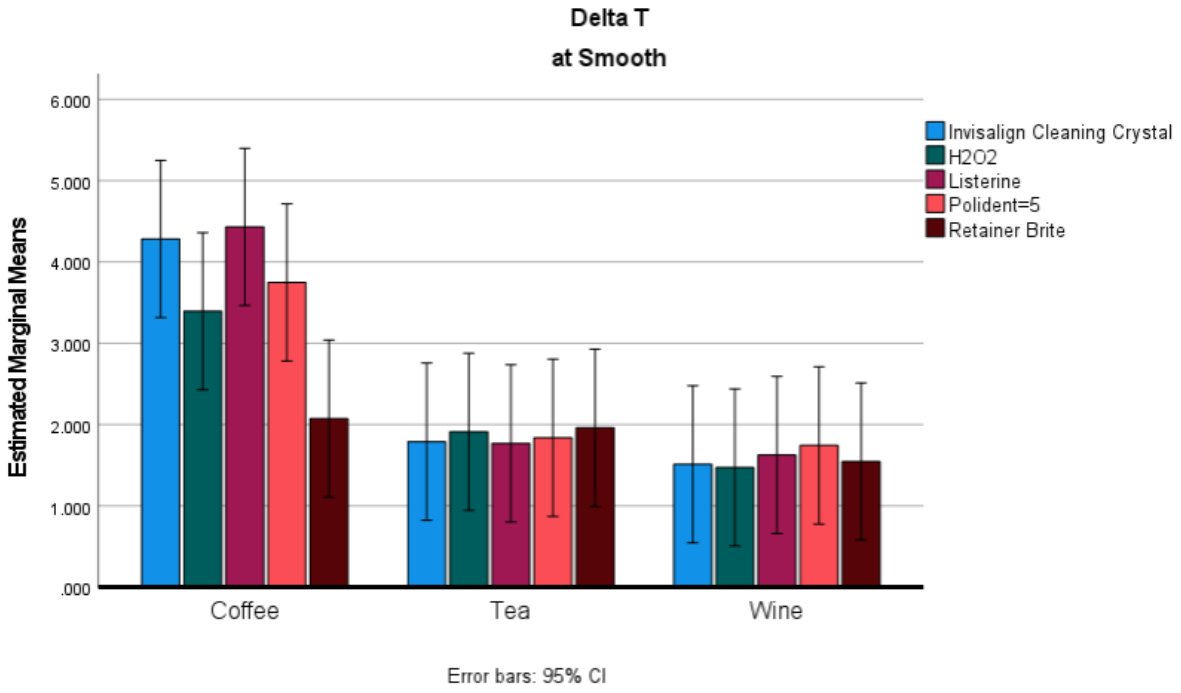


Figure 33: Comparison of Essix C+® ΔT among destaining solutions for smooth surface by type of stained specimens

5. NON-ULTRASONIC DESTAINING PHOTOS

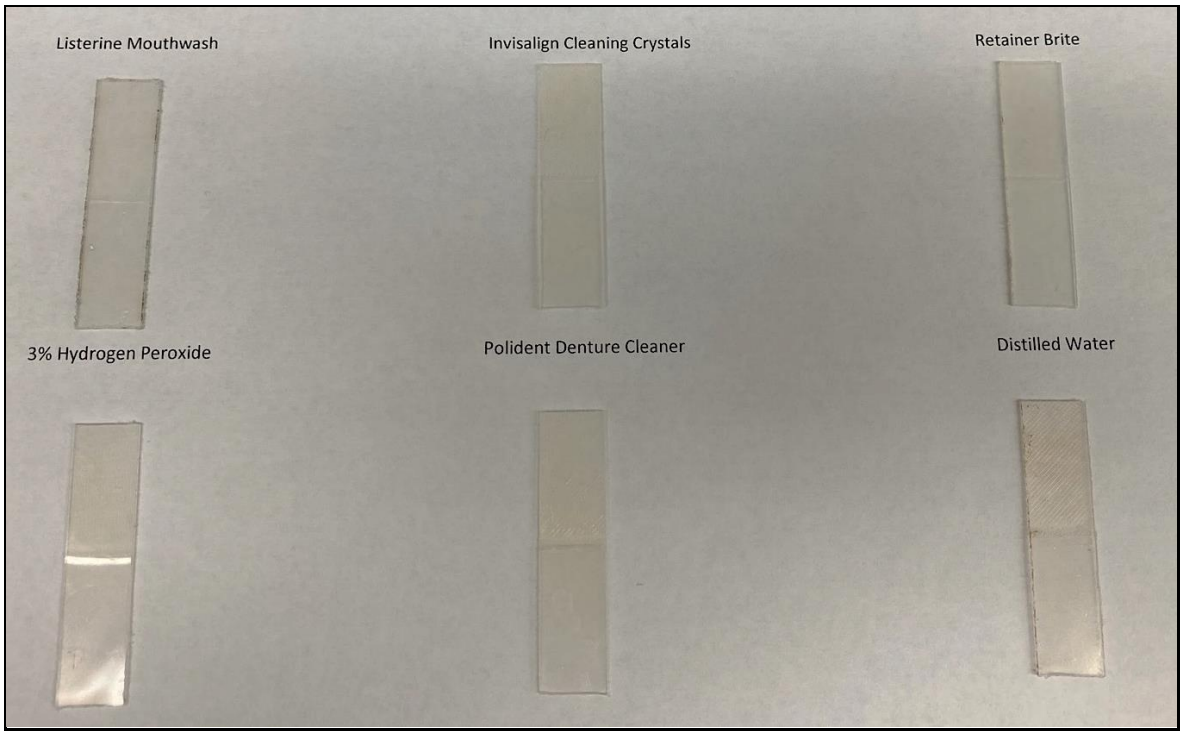


Figure 34: Essix C+® stained with coffee and destained with the non-ultrasonic**.**

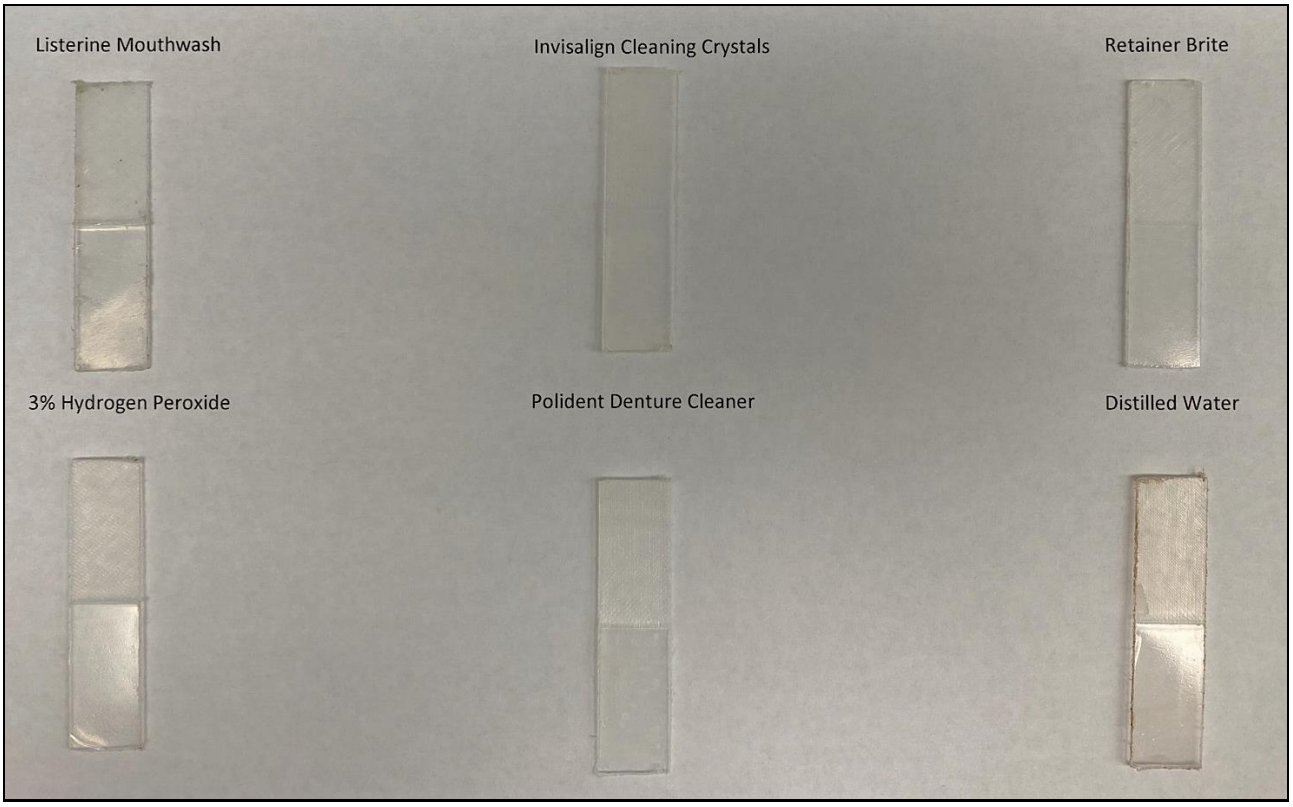


Figure 35: Essix C+® stained with black tea and destained with the non-ultrasonic**.**

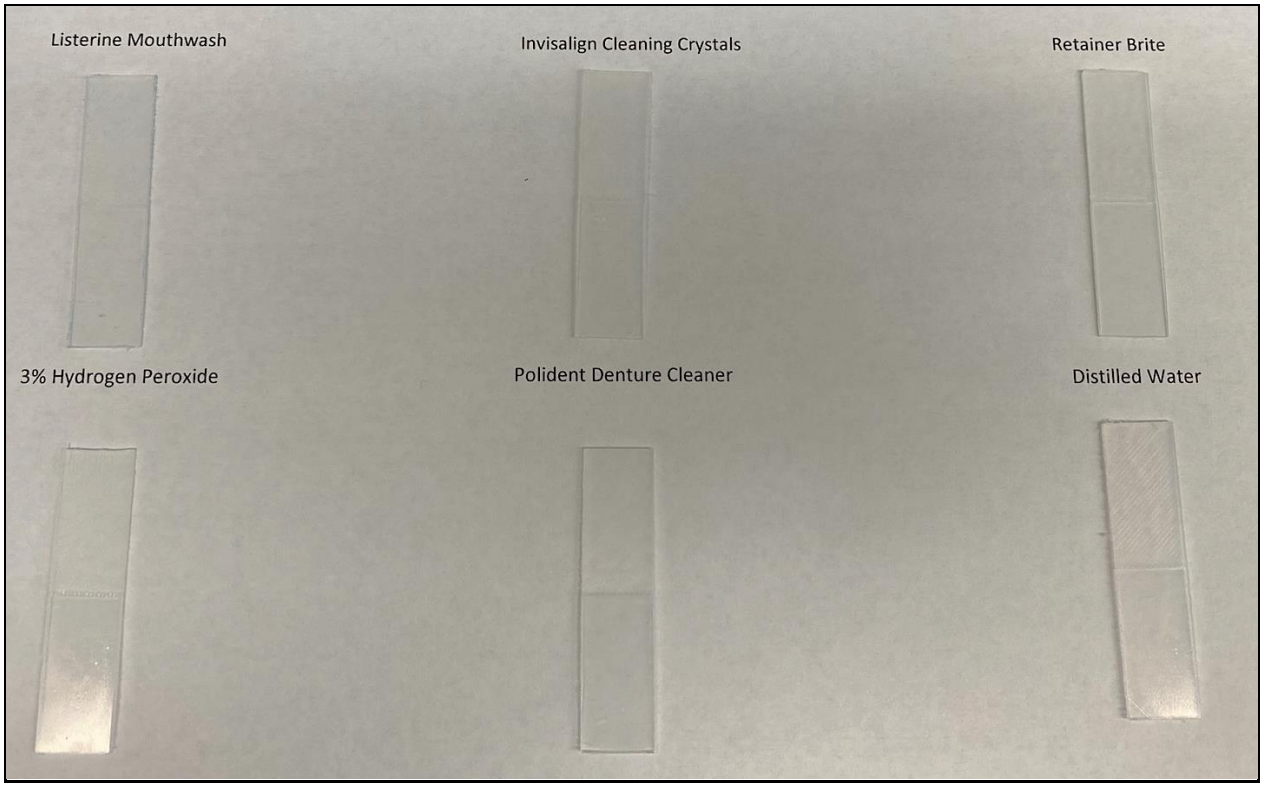


Figure 36: Essix C+® stained with distilled water and destained with the non-ultrasonic**.**

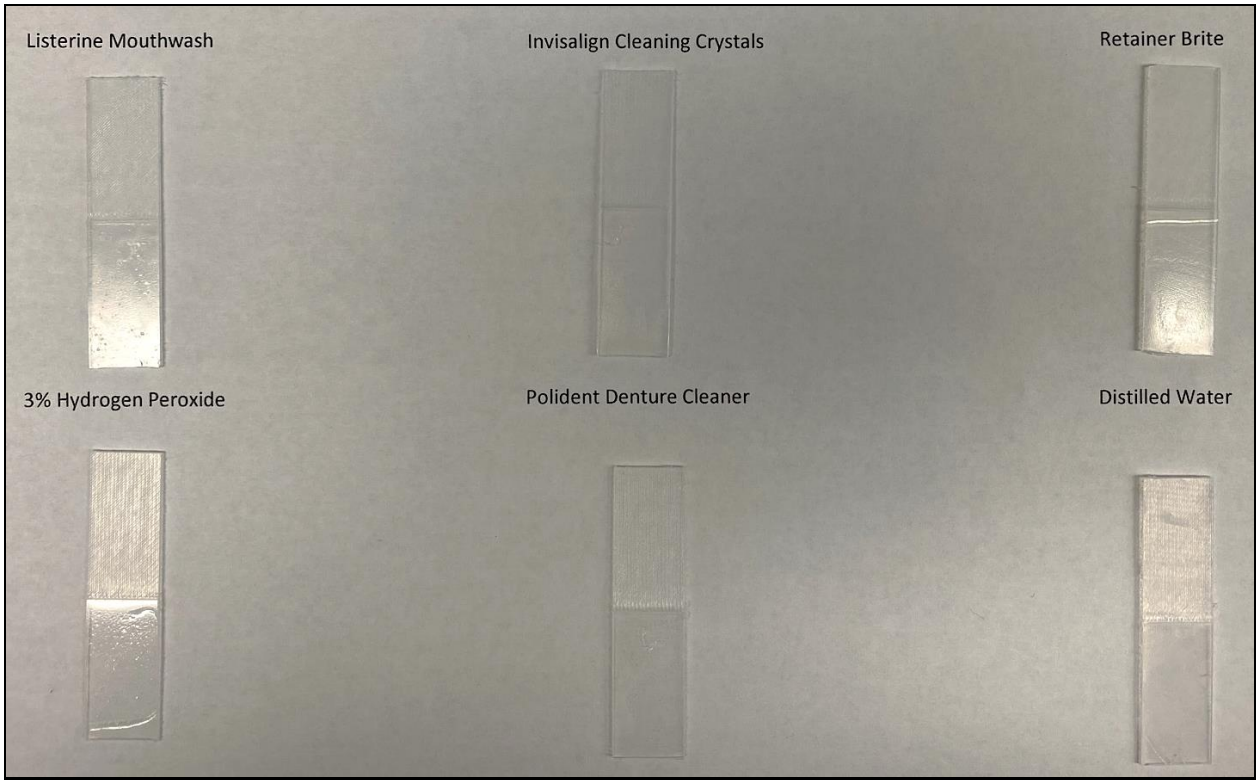


Figure 37: Essix C+® stained with red wine and destained with the non-ultrasonic**.**

6. ULTRASONIC DESTAINING PHOTOS

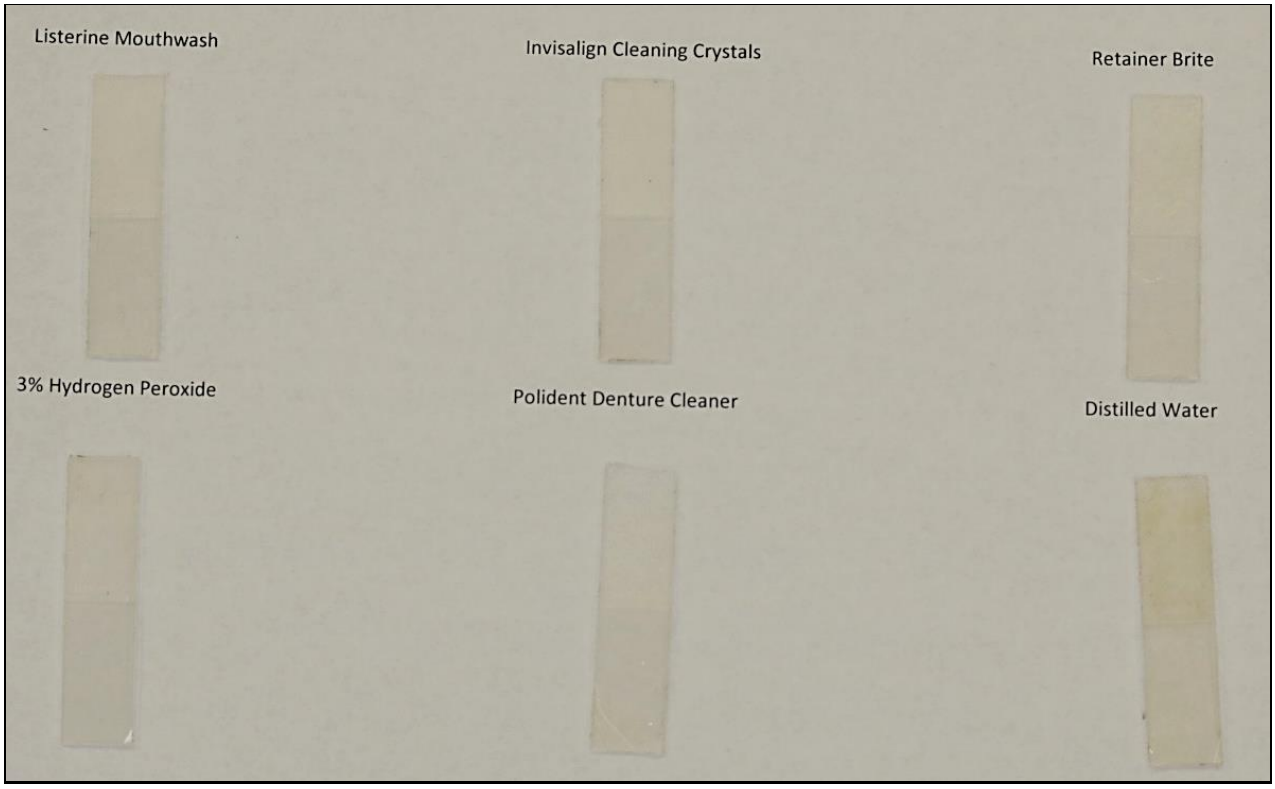


Figure 38: Essix C+® stained with coffee and destained with the ultrasonic**.**

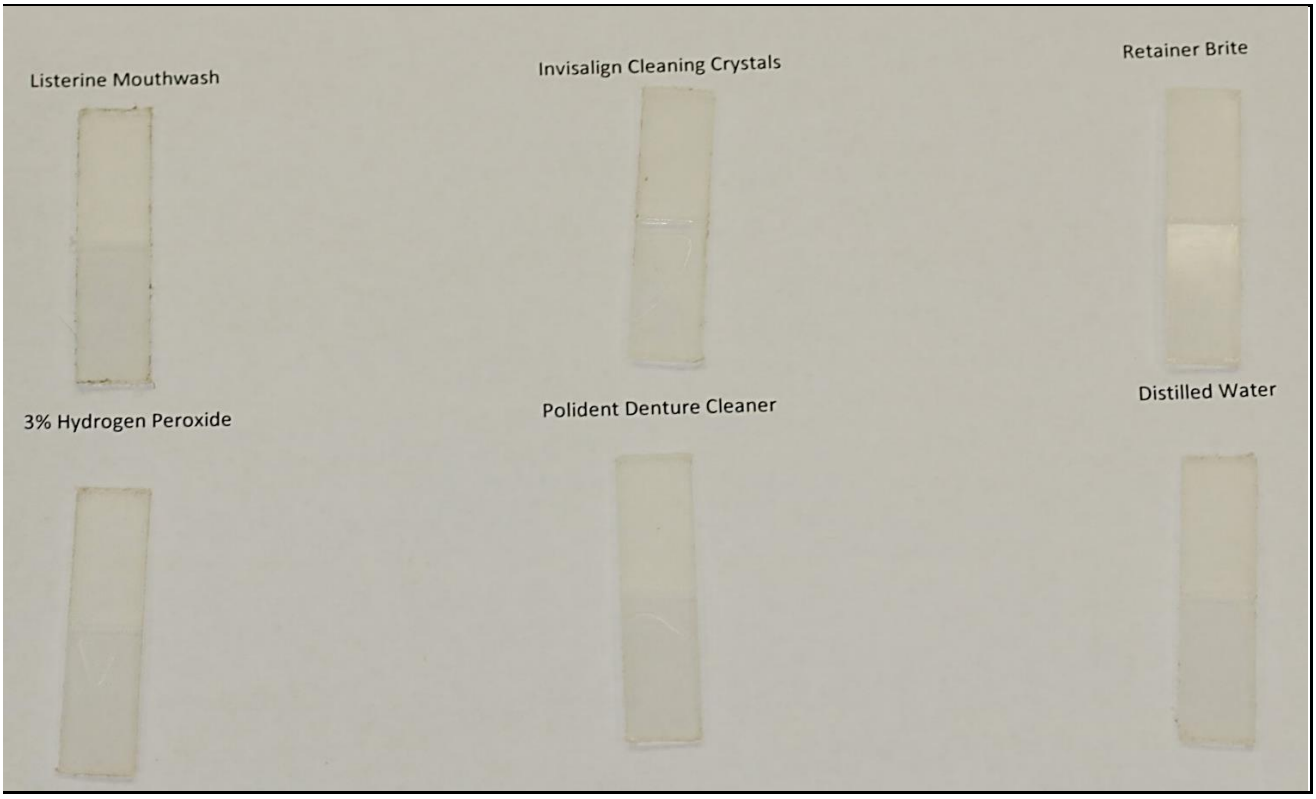


Figure 39: Essix C+® stained with black tea and destained with the ultrasonic**.**

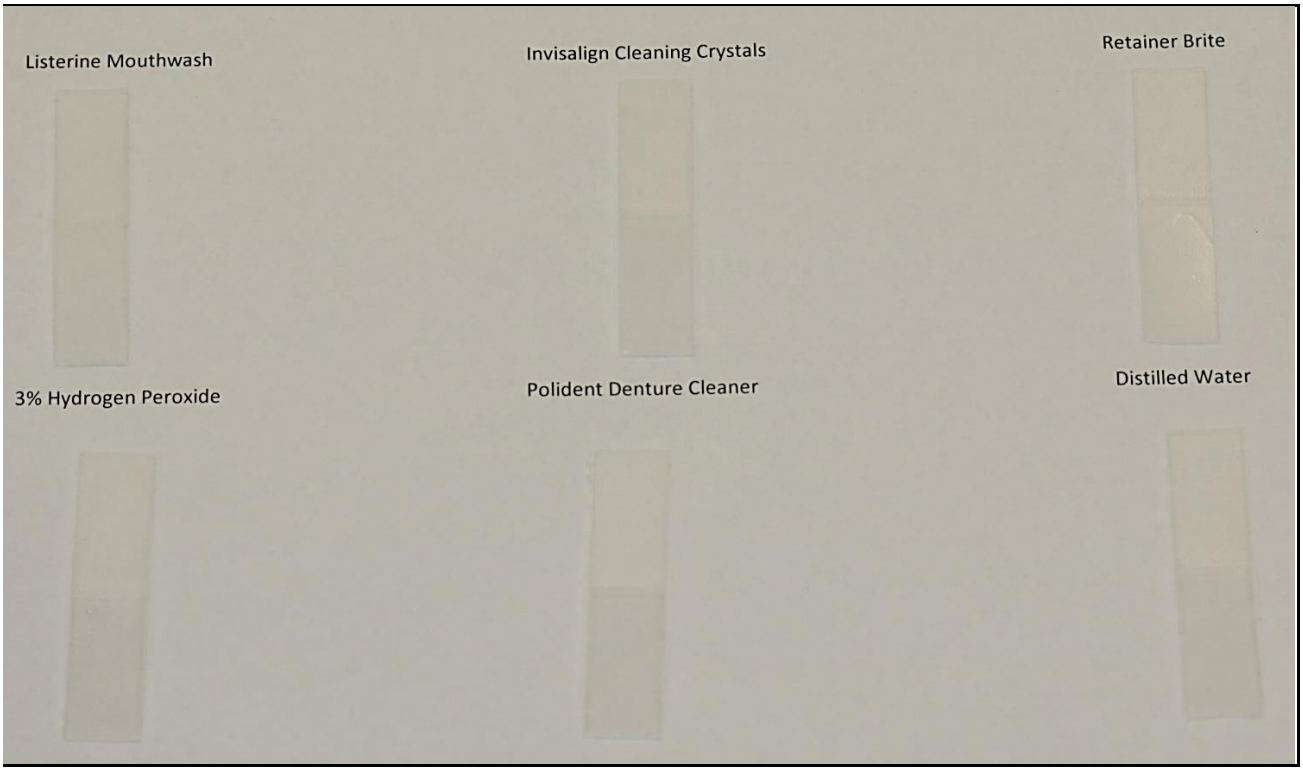


Figure 40: Essix C+® stained with distilled water and destained with the ultrasonic**.**

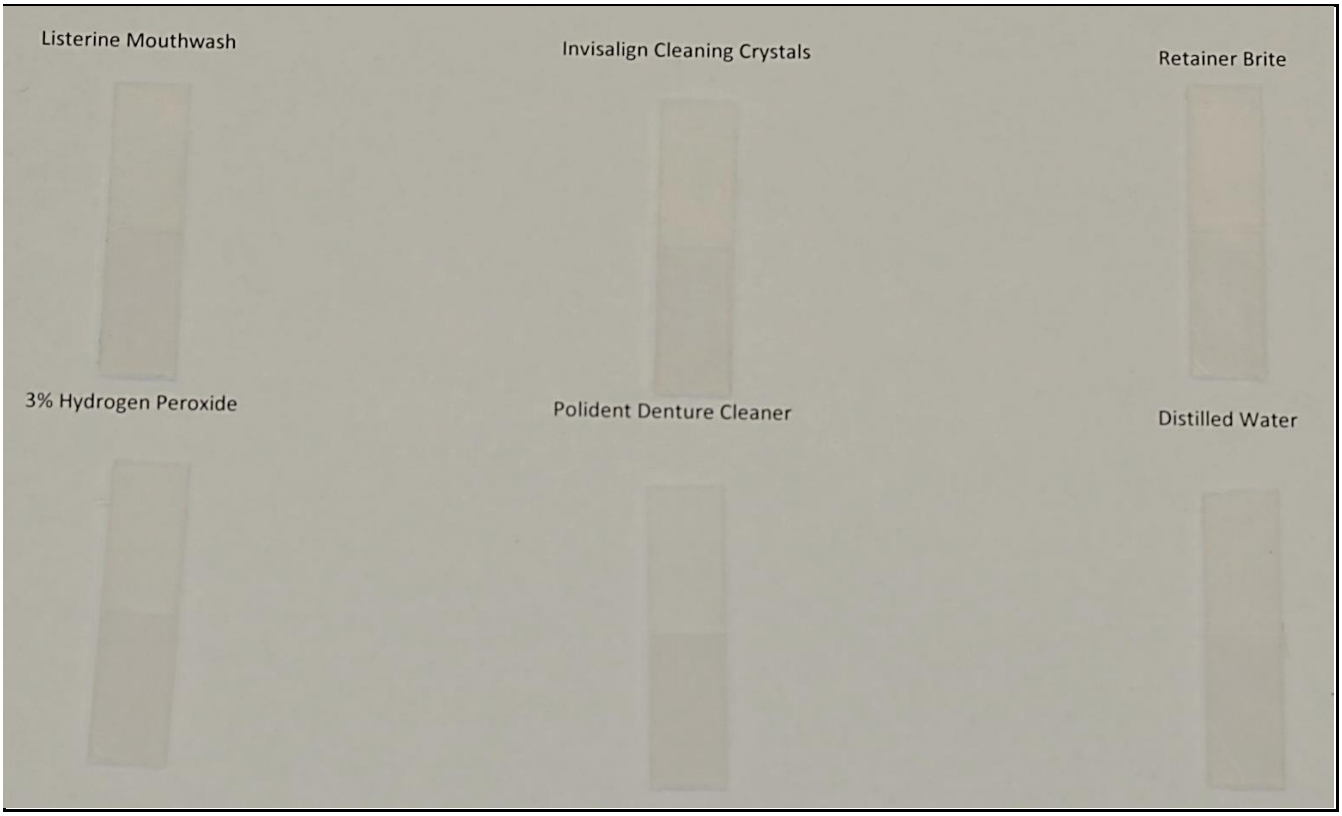


Figure 41: Essix C+® stained with red wine and destained with the ultrasonic**.**

G. Raman Spectrometer Baseline and Staining

1. Baseline

Due to the lack of chemical spectrum library for Raman spectrometer, the analysis of Raman spectrometer will aim on the qualitative analysis and not the quantitative analysis. For Essix ACE® baseline analysis, the textured surface area peak exhibited a higher intensity than the smooth surface texture. However, for Essix C+®, the textured surface area peak exhibited lower intensity than the smooth surface texture. For Essix ACE® material, for all initial measurements, the data collect was incomplete and therefore, only analyzed to a 2500cm^{-1} Raman shift. Visually, there were significant differences of composition between the two materials at baseline, T0.

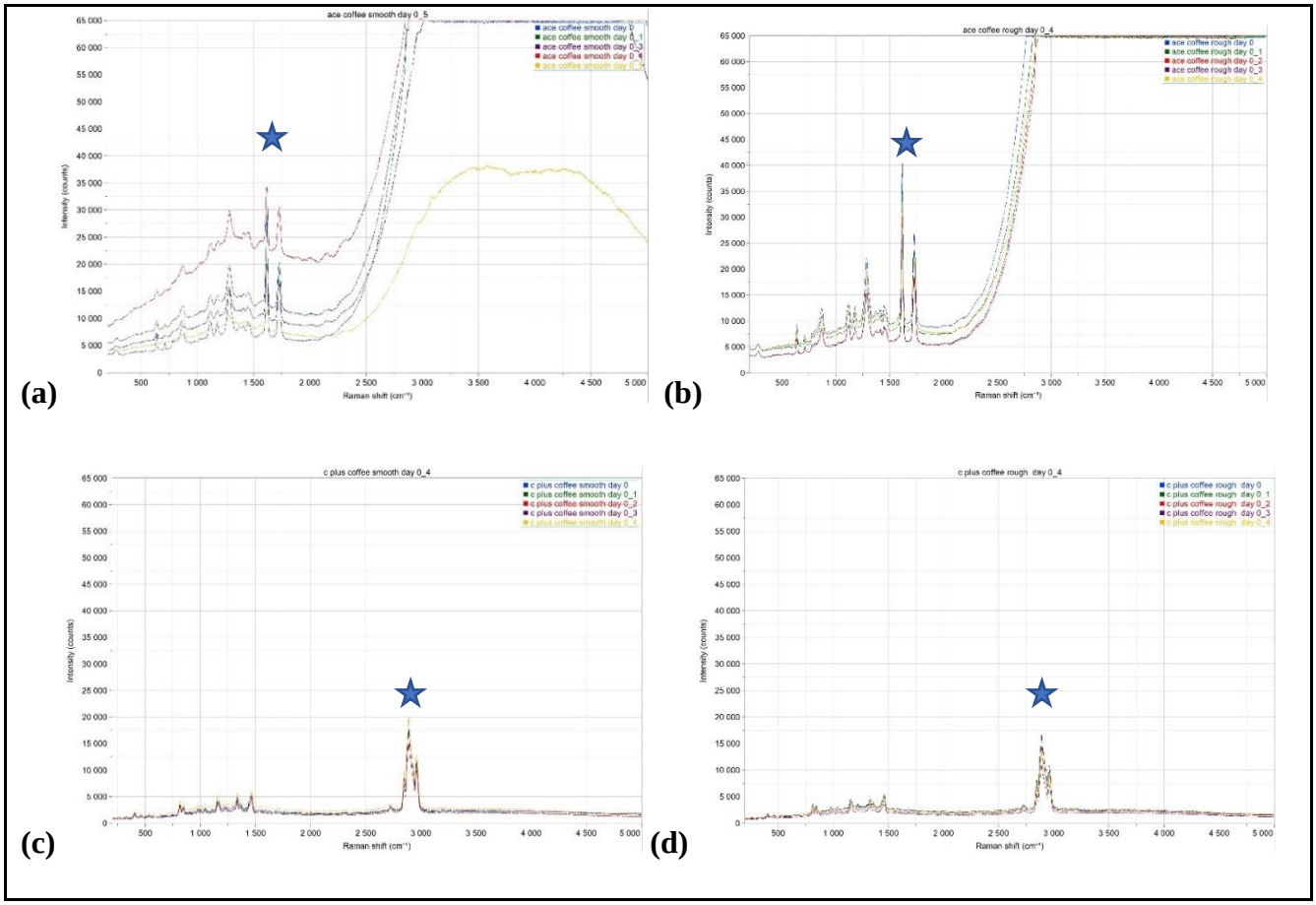


Figure 42: The patterns of composition spectrums of each material from Raman spectrometer at baseline. (a) Essix ACE® smooth, (b) Essix ACE® textured, (c) Essix C+® smooth, (d) Essix C+® textured

2. Raman Staining Day 28

Essix ACE® smooth and textured surfaces exhibited diminished peaks from 0-2500cm^{-1} from baseline to T3, staining day 28, after staining with coffee and distilled water. It is difficult to analyze the data to 5000cm^{-1} due to baseline data error. Qualitatively, there were no differences between smooth and textured surfaces.

Essix C+® smooth and textured surfaces had diminished peaks from baseline to staining day 28 after staining with coffee and distilled water. Qualitatively, for Essix C+® stained with coffee, the textured (rough) surfaces were affected more than the smooth surface as smaller peaks were recorded.

Although distilled water as a staining agent was not expected to diminish the peaks in the materials, it is not surprising. Essix C+® material is crystalline; an opaque material due to its mix of crystalline and amorphous polymers[41], which has a lower water absorption rate.[41] Ryokawa et al. showed water absorption, via air humidity or immersion, increased with time and that the materials are affected by their amorphous/crystalline structure as well as by temperature, humidity, and pressure.[41] In addition, in some materials, water absorption increased after thermoforming and was significantly higher after thermoforming than before.[17] There are also studies that suggest it is the water absorption properties of thermoplastic materials that affect its composition as well as thermoforming and temperature changes.[41] Thus, the materials may have absorbed the distilled water which diminished their baseline peaks.

Additionally, Gracco et al. used Fourier transformation intra-red analysis and found that aligners aged in artificial saliva showed results indicating molecular change on the specimen surfaces.[35] The intensity, or height of the peaks, and the width of the bases, the stretching, changed as the aligners aged.[35] In the Gracco et al. study, this was attributed to the formation of a carbon coating.[35] The changes in shape and intensity were thought to be a decrease of the isocyanate group following hydrolysis reaction by the ambient medium.[35]

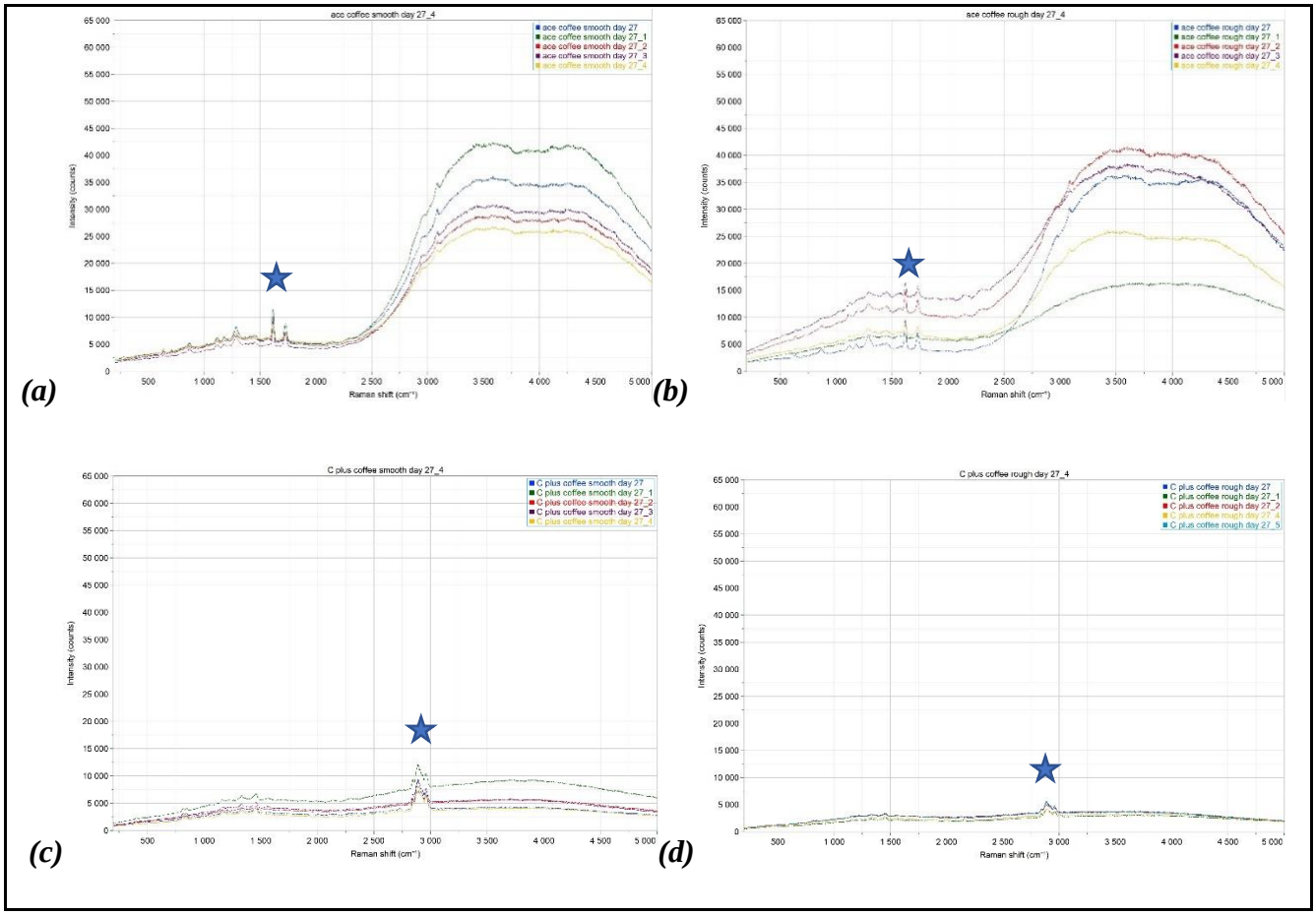


Figure 43: The patterns of composition spectrums of each material from Raman spectrometer at day 28 after staining with coffee (the end of the staining experiment). (a) Essix ACE® smooth, (b) Essix ACE® textured, (c) Essix C+® smooth, (d) Essix C+® textured

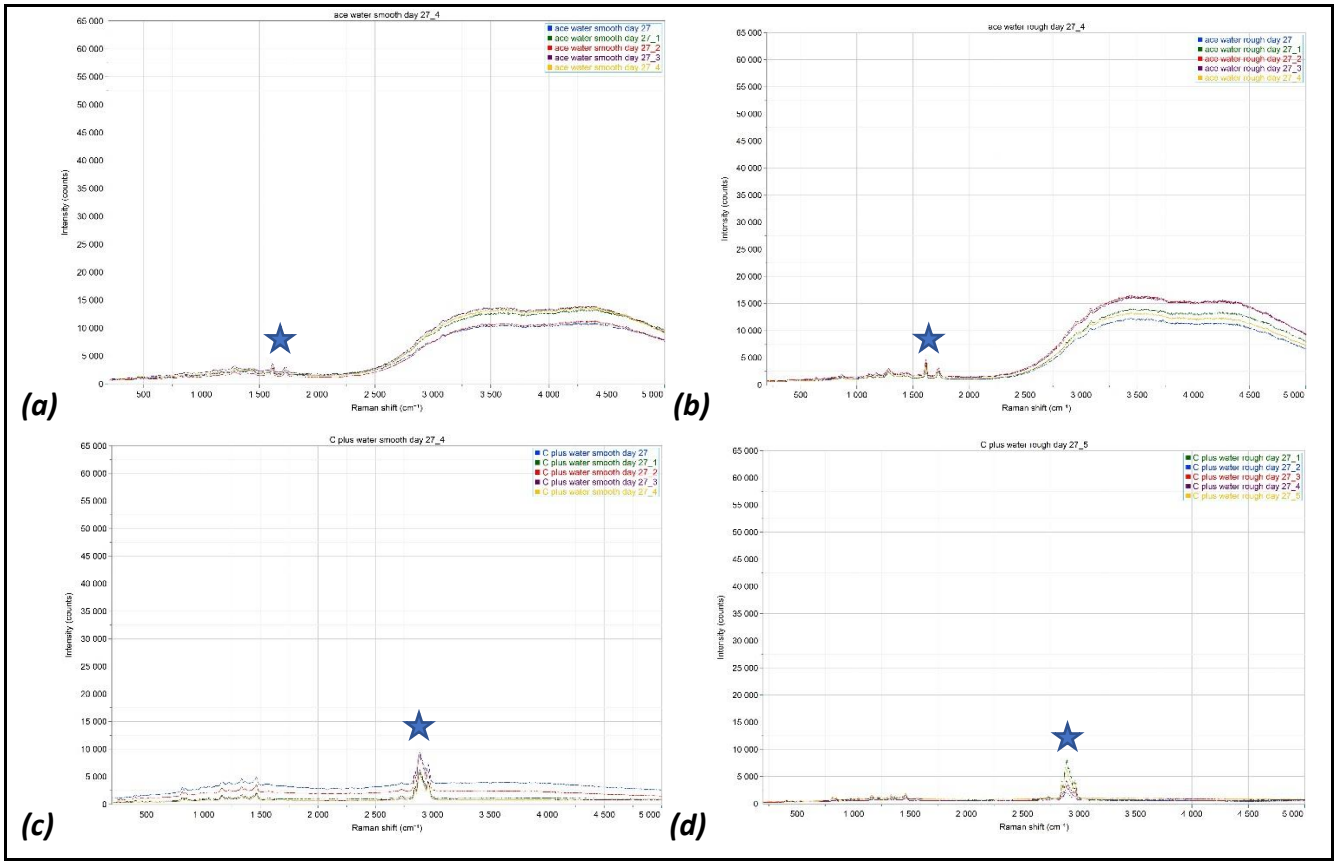


Figure 44: The patterns of composition spectrums of each material from Raman spectrometer at day 28 after staining with distilled water (the end of the staining experiment). (a) Essix ACE® smooth, (b) Essix ACE® textured, (c) Essix C+® smooth, (d) Essix C+® textured

3. Raman Destaining

For Essix ACE® material stained with coffee and destained with Invisalign® Cleaning Crystals in the non-ultrasonic and ultrasonic cleaners, the textured and smooth surfaces remained at lower peaks than baseline after 28 days of staining. Although the peaks were diminished, the pattern remained the same implicating that material composition was not affected by either the destaining solution or the cleaning methods.

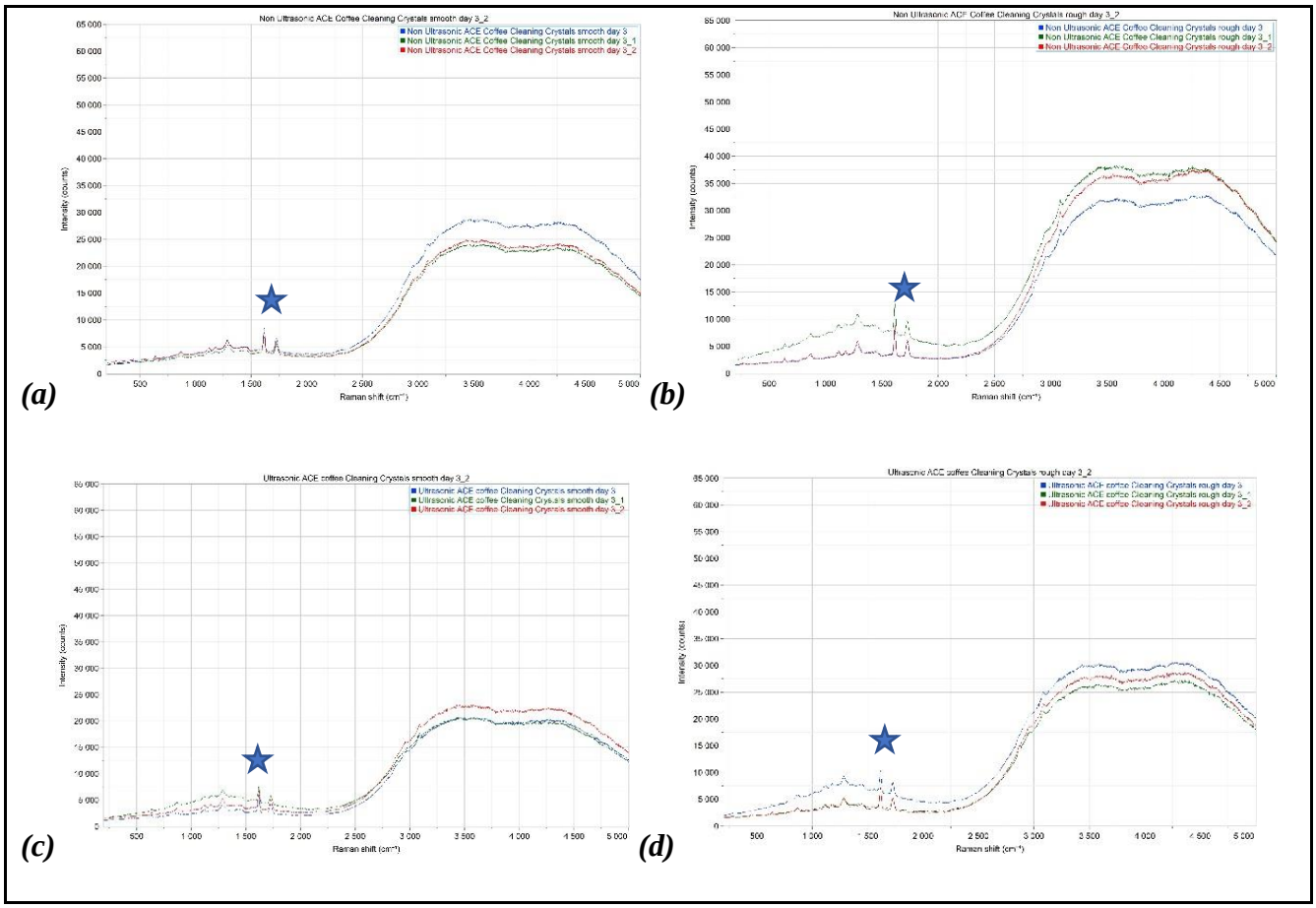


Figure 45: The pattern of spectrum of each material composition from Raman spectrometer after destaining (a) Essix ACE® smooth stained with coffee, cleaned with Invisalign® Cleaning Crystal via non-ultrasonic, (b) Essix ACE® textured stained with coffee, cleaned with Invisalign® Cleaning Crystal via non-ultrasonic points, (c) Essix ACE® smooth stained with coffee, cleaned with Invisalign® Cleaning Crystal via ultrasonic, (d) Essix ACE® textured stained with coffee, cleaned with Invisalign® Cleaning Crystal via ultrasonic

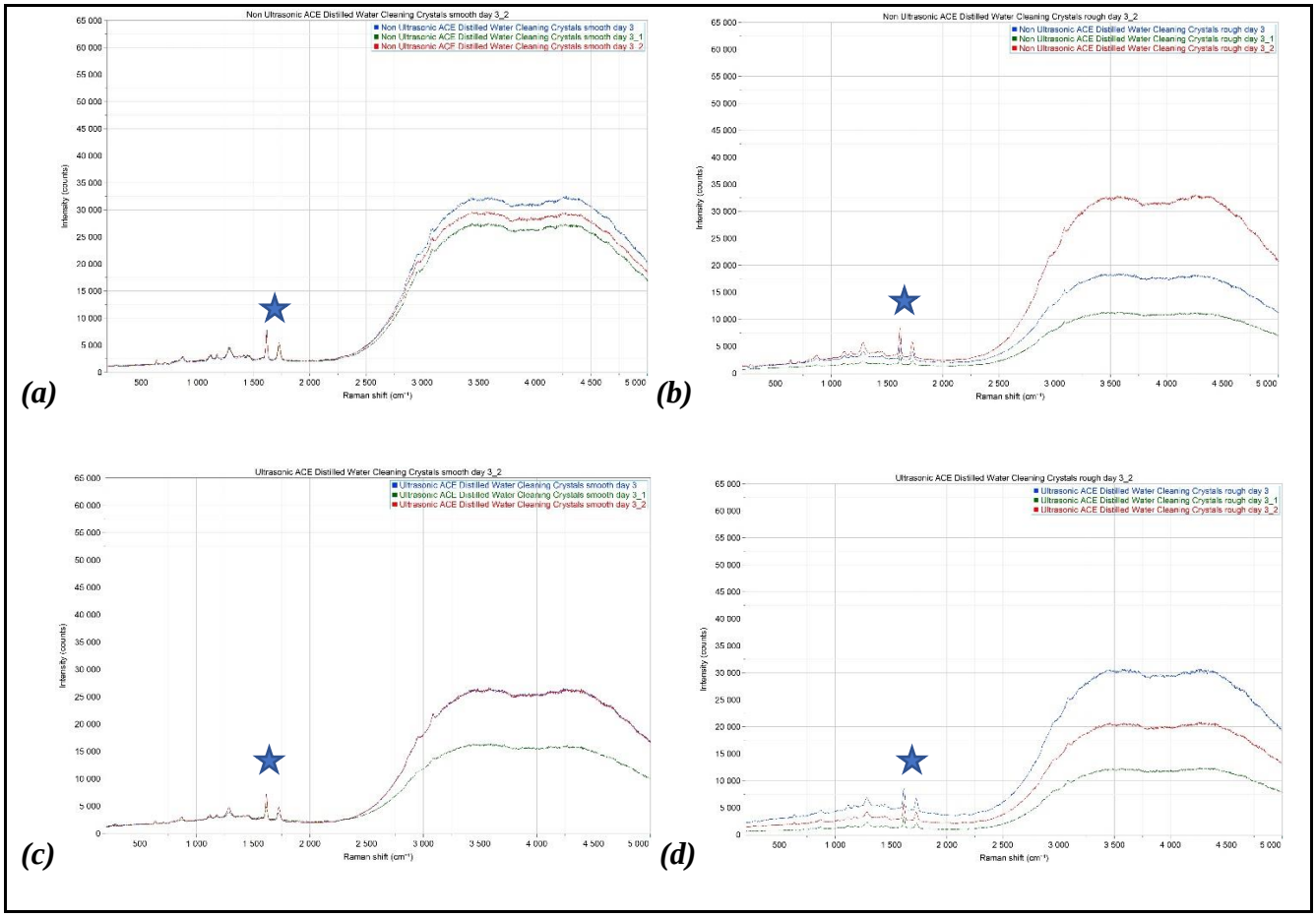


Figure 46: The pattern of spectrum of each material composition from Raman spectrometer after destaining (a) Essix ACE® smooth stained with distilled water, cleaned with Invisalign® Cleaning Crystals via non-ultrasonic, (b) Essix ACE® textured stained with distilled water, cleaned with Invisalign® Cleaning Crystals via non-ultrasonic points, (c) Essix ACE® smooth stained with distilled water, cleaned with Invisalign® Cleaning Crystals via ultrasonic, (d) Essix ACE® textured stained with distilled water, cleaned with Invisalign® Cleaning Crystals via ultrasonic

For Essix C+® material stained with coffee and destained with Invisalign® Cleaning Crystals in the non-ultrasonic and ultrasonic cleaners, the textured and smooth surfaces remained at lower peaks than baseline but higher peaks than after 28 days of staining. Since the material composition pattern was unchanged, this implicated that the material was not affected by either the destaining solution or the cleaning methods.

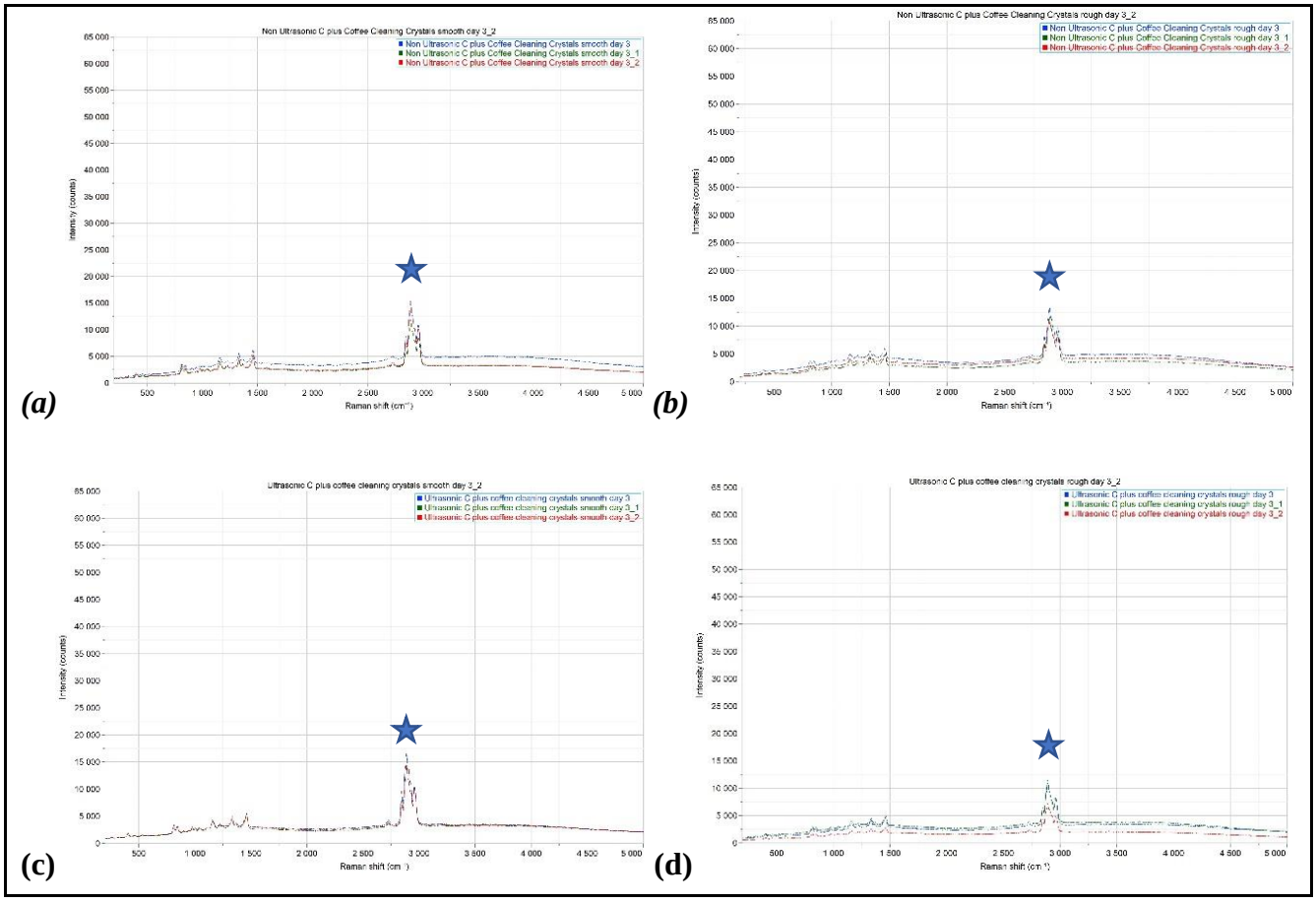


Figure 47: The pattern of spectrum of each material composition from Raman spectrometer after destaining (a) Essix C+® smooth stained with coffee, cleaned with Invisalign® Cleaning Crystals via non-ultrasonic, (b) Essix C+® textured stained with coffee, cleaned with Invisalign® Cleaning Crystals via non-ultrasonic points, (c) Essix C+® smooth stained with coffee, cleaned with Invisalign® Cleaning Crystals via ultrasonic, (d) Essix C+® textured stained with coffee, cleaned with Invisalign® Cleaning Crystals via ultrasonic

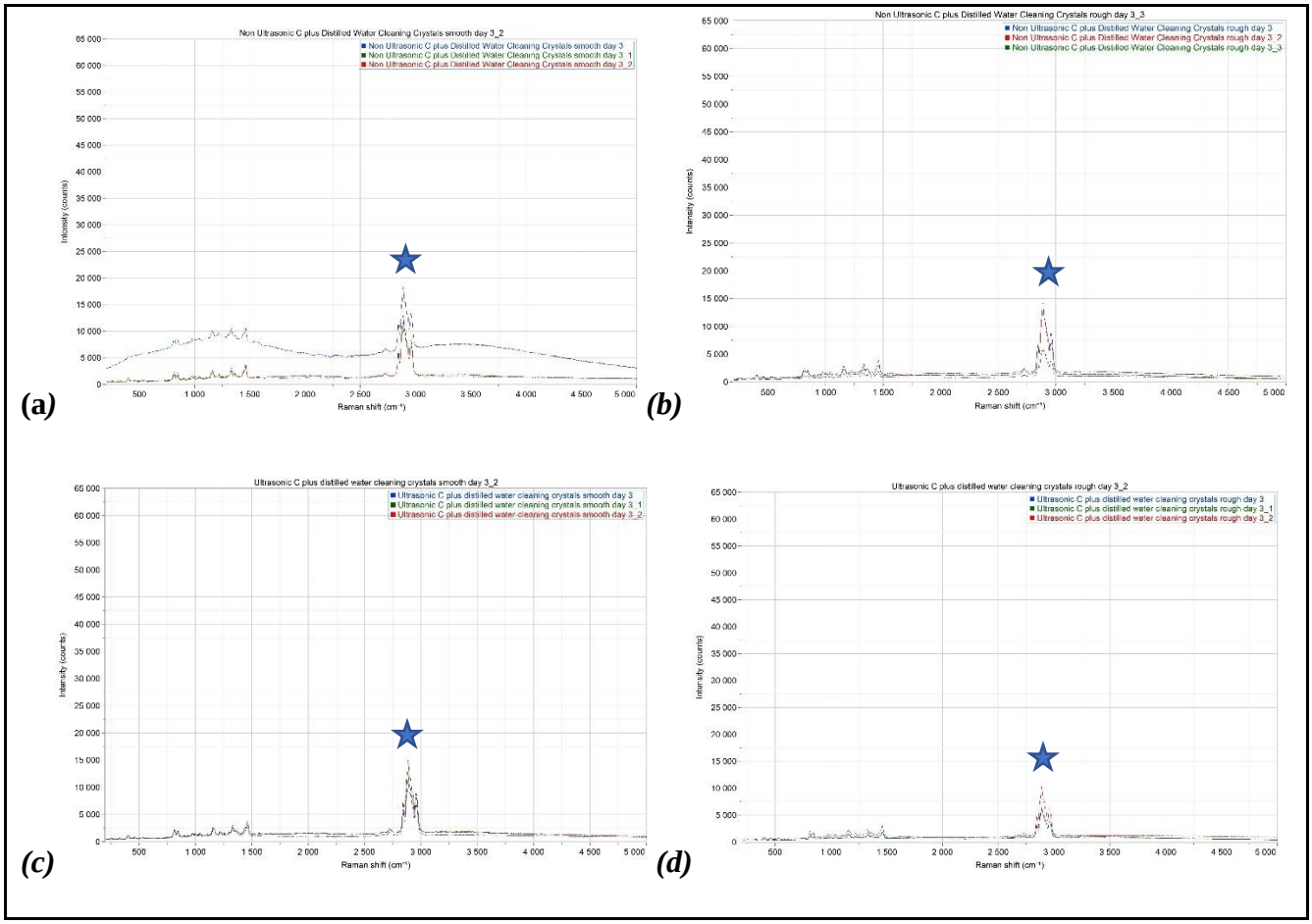


Figure 48: The pattern of spectrum of each material composition from Raman spectrometer after destaining (a) Essix C+® smooth stained with distilled water, cleaned with Invisalign® Cleaning Crystals via non-ultrasonic, (b) Essix C+® textured stained with distilled water, cleaned with Invisalign® Cleaning Crystals via non-ultrasonic points, (c) Essix C+® smooth stained with distilled water, cleaned with Invisalign® Cleaning Crystals via ultrasonic, (d) Essix C+® textured stained with distilled water, cleaned with Invisalign® Cleaning Crystals via ultrasonic

V. Discussion

It has been reported that in the United States, 50% of Americans over 18 years old drink coffee and that coffee drinkers consume an average of 3 cups of coffee per day.[60] When broken down by time of day, 65% of coffee is consumed during breakfast, 30% between meals, and 5% other.[60] According to Oliveria et al., every 24 hours of *in vitro* staining simulates one month of coffee exposure. Thus, 28 days of immersion *in vitro* simulates the susceptibility of thermoplastic material to coffee staining within approximately 2 years of retention.[61] Therefore, providers should clearly inform patients to remove retainers before eating and drinking as staining could occur from food or drinks, which would also allow the stain to accumulate in the retainer.

It is said that the only effective approach to prevent orthodontic relapse and achieve a stable result is long-term retainer wear.[2] With advancements of orthodontic techniques, clear retainers have increased in popularity due to their aesthetic nature and comparable treatment times.[1] Clear retainers must be maintained as material reactions such as discoloration, plaque and calculus buildup, bacteria buildup and retention, and loss of their translucency and material integrity can occur.[23,24] Color stability can also be affected by ultraviolet radiation, mouthwash, and various beverages.[25]

Various studies have reported that polyurethane materials are susceptible to pigment absorption in the oral cavity.[26–28] Changes in durability and wear resistance have been observed within a few months of intraoral wear.[29] Crucial to maintaining the truly clear nature of these retainers is an effective cleaning technique. Only a few scientific studies on the proper maintenance for clear retainers have been performed.[30–34] Chang et al. assessed the removal of a single species biofilm from Essix ACE® orthodontic retainers and found brushing with a toothbrush and toothpaste, brushing with

sterile distilled water, and rinsing with 50ml of sterile distilled water all effectively removed 99% of microorganisms.[62]

It has been suggested that different thermoplastic materials react differently when exposed to staining and destaining solutions.[32,34] The acidic nature of wine and coffee can cause surface roughening conductive to staining. Tannic acid found in coffee is responsible of the yellow-brown color which is reported as the primary staining ingredient causing both absorption and adsorption.[63] Coffee filtering and processing can also affect the staining properties.[64] Coffee has been identified as the strongest staining agent, due to having high chromatic agents, among common beverages due to its tannic acid (pH 6-6.4) which causes its yellow-brown color.[23,61,64,65] Red wine has been reported to cause severe staining on provisional resin materials.[64,66] Bernard et al. found that black tea caused marked extrinsic stains on the surface of aligners but was easily cleaned.[46]

If a material is color stable, there should be no color change detected after staining and destaining. The material composition, thickness, and texture will affect this color change as well. However, discoloration can also occur from incomplete polymerization.[23]

The selected cleaning solutions in this study were chosen based on previous studies[32–34] that showed the least change of light transmittance values of the studied retainer materials after 6-month exposure. In addition, Invisalign® Cleaning Crystals, Retainer Brite®, and Polident® denture cleaners are widely available and commonly used to clean orthodontic retainers. However, there is no study on how the cleanser may alter the physical properties, color, or translucency of the retainer material after prolonged use. Our study is the first to address the comparison of light transmittance and color change among cleaning solutions under well-control experimental designs. To better control this study, one investigator gathered the data measurements for spectrometer and a different investigator gathered the

data measurements for the spectrophotometer and Raman spectrometer. Thus, there were consistent measurements taken for each staining and destaining timepoint.

This study evaluated the effects of staining and destaining methods on a two-surface retainer specimen made of copolyester, Essix ACE®, and copolymer, Essix C+®, and evaluated color change, light transmittance, and material integrity. The smooth surface imitates the surface of a plaster model whereas the textured surface imitates the internal surface of commercial models (see methods). To our knowledge, until now, there is no study on the ability of cleaning methods to destain stained retainer materials with a two-surface texture model. Our study is the first to address the effect of surface on the nature of staining and destaining issues with clear retainer materials. The effect of different surface textures of the retainer materials on their ability to be stained has not been reported.

Similarly to Zafeiriadis et al. who found greater color change of Essix C+® retainers with increased wear time, our study found Essix ACE® and Essix C+® materials displayed marked color change with increased staining days.[42] In addition, supported by another study by Zafeiriadis et al., results showed coffee had the most prominent staining capabilities overall, followed by tea, and red wine. The results of Zafeiriadis et al. found coffee caused a significant decrease in ΔL* (lightness), Δa* (red/green), and an increase in Δb* (yellow/blue) values.[23] Tea caused a significant increase in the Δa* and b* values. Red wine increased the a* values. However, when the Δ values were used in the study by Zafeiriadis et al., the coffee and tea changes were found to be visible but the changes from red wine were invisible.

Likewise, to Liu et al. who found Invisalign® to be stained more heavily with coffee than tea and red wine after seven days of immersion, Essix ACE® and Essix C+® were also found to stain more heavily with coffee after each staining day. In addition, all surface materials used in the Liu et

al. study exhibited rough surface areas. In this study, Raman spectroscopy also found that the material compositions were not affected as the pattern from baseline to staining to destaining maintained the same, only with diminished peaks. Liu et al. did not use destaining methods so we could not address a direct comparison. In contrast to our results, research by Porojan et al. found a very weak relationship between microroughness of removable thermoplastic aligners and color change after seven days of immersion in coffee, tea, and water.[47]

Bernard et al. found after both 12 hours and 7 days of immersion, there were significant differences in mean colorimetry values for Invisalign® stained with coffee and red wine[46], in this study, Essix ACE® and Essix C+® exhibited significant differences in ΔE values when stained with coffee and black tea. In contrast to Bernard et al, the effects of destaining of the cleaning solutions in this study were found to be similar. From the initial staining to the final destaining timepoint, the Retainer Brite® tablet combined with the sonic bath showed more destaining than the Invisalign® Cleaning Crystals for the Invisalign® and Minor Tooth Movement® materials stained with wine.[46] The MTM is made of a PETG-based polyester, similar to Essix ACE®.[45] They also found that both destaining methods brought all the black-tea materials almost back to their original color indicating that both present good black-tea stain-removal potential.[46] This may be due to the differences between innate properties of tested materials and the extent of staining on the tested materials.

Papadopoulou et al. investigated the surface roughness and mechanical properties of Invisalign® aligners after exposure to one or two weeks of clinical oral use. The specimens underwent cleaning with ultrasonic and non-ultrasonic chemical cleaning to remove plaque and calculus. The results of this study showed that clinical use may lead to a decrease in the materials coefficient of friction and may explain the material deterioration with time.[40] Ahn et al. found via Raman spectrometer study that thermoforming and intra oral exposure led to significant molecular,

morphological, and mechanical changes in the retainers.[36] This is not supported with our Raman spectrometer results, in which the graph pattern showed similar patterns of material composition for both materials implicating there were no changes in composition; however, this study could not well-conclude due to lack of chemical identification library of the manufacturer.

Wible et al. found light transmittance significantly and consistently affected by the same cleaning methods used in this study.[33–34] Copolymer and copolyester specimens, in all groups, demonstrated aging in the appearance of decreasing translucency over time after exposure to cleaning methods.[33] The results of the Wible et al. study reported no ideal cleaning method for polypropylene/ethylene copolymer retainer materials and all cleaning methods exhibited comparable changes among one another.[33] This supports our results that the destaining ability of all cleaning solution found to be comparable at the end of destaining experiments. Photographs of the specimens from T0, before staining, to T3, after 28 days of staining immersion in coffee, black tea, red wine, and distilled water are provided in Figures 22-29 and Figures 34-41.

For copolyester, after 14 days of staining immersion, coffee showed marked color change on the smooth surface side of the specimen and black tea showed marked color change on the textured side of the specimen. For copolymer, after 28 days of staining immersion, coffee showed marked color change on both surfaces of the specimen, per the NBS units from Table IV.

To our knowledge, there is no study on the ability of cleaning methods to destain stained retainer materials, with a two-surface texture model, which assess' color change of aligners. Previous research used multiple models and templates and therefore, were inconsistent. In addition, the staining and destaining time intervals were not controlled and therefore, some stains were more difficult to remove. Furthermore, there has been no mechanical property analysis after staining and destaining aligner materials.

All null hypotheses, except H(4), of this study were rejected. Per H(1), the surface textures of the retainer materials influenced the rate and degree of staining and destaining. The textured surfaces showed more staining and improved destaining more than the smooth surfaces. Increased staining on the textured side may be attributed to an accumulation of more pigment which accelerated the staining. Per H(2), the most staining occurred at the end of staining and the specimens were more susceptible to coffee and black tea staining. Per H(3), no major changes were noted among all staining solutions or destaining means. All cleaning reagents showed improved light transmittance. Per H(4), there were no differences between ultrasonic and non-ultrasonic cleaning.

VI. STUDY LIMITATIONS AND FUTURE CONSIDERATIONS

The *in vitro* nature of this study allowed standardization of the staining and destaining conditions. However, the design could not replicate the normal oral environment with the normal compliance, duration of stain exposure, plaque accumulation, wear of retainer due to mastication and composition of human saliva. Future studies should investigate the material staining by studying the changes of light transmittance and color in human oral cavity. When specimens were not destained, they were placed in artificial saliva to imitate the oral environment.

The flat specimens did not reflect the true form of thermoplastic retainers. The standardized flat specimens used were necessary to be able to compare the staining and destaining as well as for the analysis with the spectrometer and spectrophotometer analysis. Future studies could use the actual thermoformed material on plaster or 3D printed models.

Due to the limitation of trade secret composition of the material, the study using Raman spectrometry could not be interpreted comprehensively. In addition, the lack of chemical library from the manufacturer, other approaches for study of composition would be suggested instead of the Raman spectrometer. For future studies, a custom fabricated holder would be useful to ensure the same location for each specimen. In the future, more research should be done with an increased sample size and simulated intraoral conditions should be carried out to increase the validity and relevance of the findings.

VII. Conclusions

The color and light transmittance of copolyester (Essix ACE®) and copolymer (Essix C+®) clear retainers materials appeared to decrease over time when immersed in all staining solutions, namely coffee, black tea, and red wine. Both materials did not exhibit color stability. Coffee and black tea showed a faster rate of staining ability for color and light transmittance than red wine. The textured surfaces exhibited more staining than the smooth surfaces. In addition, the textured surfaces were easier to be destained than the smooth surfaces.

Even though statistically significant differences were found between certain destaining solutions, no ideal cleaning method, for either material, could be determined as ideal for use on Essix ACE® or Essix C+® material. All cleaning reagents showed improved light transmittance and color changes for both copolyester and copolymer retainer materials. Ultrasonic and non-ultrasonic cleaning units appeared to have no effect on cleaning ability of stained studied retainer materials. After 28-days of staining and one session of destaining, under this studied condition, material compositions were not affected.

Cited Literature

1. Yu Y, Sun J, Lai W, Wu T, Koshy S, Shi Z. Interventions for managing relapse of the lower front teeth after orthodontic treatment. *Cochrane Database of Systematic Reviews*. 2013;(9). doi:10.1002/14651858.CD008734.pub2

2. Thilander B. Orthodontic relapse versus natural development. *American Journal of Orthodontics and Dentofacial Orthopedics: Official Publication of the American Association of Orthodontists, Its Constituent Societies, and the American Board of Orthodontics*. 2000;117(5):562-563. doi:10.1016/s0889-5406(00)70200-9

3. Tsomos G, Ludwig B, Grossen J, Pazera P, Gkantidis N. Objective assessment of patient compliance with removable orthodontic appliances: a cross-sectional cohort study. *Angle Orthod*. 2014;84(1):56-61. doi:10.2319/042313-315.1

4. Pratt MC, Kluemper GT, Lindstrom AF. Patient compliance with orthodontic retainers in the postretention phase. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2011;140(2):196-201. doi:10.1016/j.ajodo.2010.02.035

5. Vig KWL. Patient Compliance to Wear Orthodontic Retainers During Postretention may Vary by Age, Gender, and Time Since Braces were Removed. *Journal of Evidence Based Dental Practice*. 2012;12(3, Supplement):202-203. doi:10.1016/S1532-3382(12)70037-4

6. Little RM, Wallen TR, Riedel RA. Stability and relapse of mandibular anterior alignment-first premolar extraction cases treated by traditional edgewise orthodontics. *Am J Orthod*. 1981;80(4):349-365. doi:10.1016/0002-9416(81)90171-8

7. Little RM, Artun J, Riedel RA. An evaluation of changes in mandibular anterior alignment from 10 to 20 years postretention. *Am J Orthod Dentofacial Orthop*. 1988;(5):423-248. doi:10.1016/0889-5406(88)90102-3

8. Heier EE, De Smit AA, Wijgaerts IA, Adriaens PA. Periodontal implications of bonded versus removable retainers. *Am J Orthod Dentofacial Orthop*. 1997;112(6):607-616. doi:10.1016/s0889-5406(97)70225-7

9. Rowland H, Hichens L, Williams A, et al. The effectiveness of Hawley and vacuum-formed retainers: A single-center randomized controlled trial - American Journal of Orthodontics and Dentofacial Orthopedics. Accessed November 13, 2020. https://www.ajodo.org/article/S0889-5406(07)00642-7/fulltext

10. Lindauer SJ, Shoff RC. Comparison of Essix and Hawley retainers. *J Clin Orthod*. 1998;32(2):95-97.

11. Sheridan JJ, Armbruster P. Bleaching teeth during supervised retention. *J Clin Orthod*. 1999;33(6):339-344.

12. Sheridan JJ, Ledoux W, McMinn R. Essix technology for the fabrication of temporary anterior bridges. *J Clin Orthod*. 1994;28(8):482-486.

13. Sheridan JJ, Armbruster P, Moskowitz E, Nguyen P. Avoiding demineralization and bite alteration from full-coverage plastic appliances. *J Clin Orthod*. 2001;35(7):444-448.

14. Thermoplastic retention appliances. A controversial clinical reality. Accessed December 18, 2020. http://journaldatabase.info/articles/thermoplastic_retention_appliances.html

15. Caminiti M, Lou T. Clear Aligner Orthognathic Splints. *J Oral Maxillofac Surg*. 2019;77(5):1071.e1-1071.e8. doi:10.1016/j.joms.2018.12.012

16. Alassiry AM. Orthodontic Retainers: A Contemporary Overview. *J Contemp Dent Pract*. 2019;20(7):857-862.

17. Ryu J-H, Kwon J-S, Jiang HB, Cha J-Y, Kim K-M. Effects of thermoforming on the physical and mechanical properties of thermoplastic materials for transparent orthodontic aligners. *Korean J Orthod*. 2018;48(5):316-325. doi:10.4041/kjod.2018.48.5.316

18. Dawood A, Marti BM, Sauret-Jackson V, Darwood A. 3D printing in dentistry. *British Dental Journal*. 2015;219(11):521-529. doi:10.1038/sj.bdj.2015.914

19. Gardner GD, Dunn WJ, Taloumis L. Wear comparison of thermoplastic materials used for orthodontic retainers. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2003;124(3):294-297. doi:10.1016/S0889-5406(03)00502-X

20. Essix Ace Plastic.pdf. Accessed December 18, 2020. http://site-ir-scdlab.s3.amazonaws.com/MSDS/Essix%20Ace%20Plastic.pdf

21. Essix-C-Plastics.pdf. Accessed December 18, 2020. https://www.dentalbiz.com.au/ESSIX-C-PLASTICS.pdf

22. MSDS_Aligner_Material_EX30_Vivera_Templates.pdf. Accessed July 17, 2020. https://c3-preview.prosites.com/242082/wy/docs/MSDS_Aligner_Material_EX30_Vivera_Templates.pdf

23. Zafeiriadis AA, Karamouzos A, Athanasiou AE, Eliades T, Palaghias G. In vitro spectrophotometric evaluation of Vivera clear thermoplastic retainer discolouration. *Aust Orthod J*. 2014;30(2):192-200.

24. Gardner GD, Dunn WJ, Taloumis L. Wear comparison of thermoplastic materials used for orthodontic retainers. *American Journal of Orthodontics and Dentofacial Orthopedics: Official Publication of the American Association of Orthodontists, Its Constituent Societies, and the American Board of Orthodontics*. 2003;124(3):294-297. doi:10.1016/s0889-5406(03)00502-x

25. Dietschi D, Campanile G, Holz J, Meyer JM. Comparison of the color stability of ten new-generation composites: an in vitro study. *Dent Mater*. 1994;10(6):353-362. doi:10.1016/0109-5641(94)90059-0

26. Ardeshna AP, Vaidyanathan TK. Colour changes of orthodontic elastomeric module materials exposed to in vitro dietary media. *J Orthod*. 2009;36(3):177-185. doi:10.1179/14653120723166

27. Kim S-H, Lee Y-K. Measurement of discolouration of orthodontic elastomeric modules with a digital camera. *Eur J Orthod*. 2009;31(5):556-562. doi:10.1093/ejo/cjp030

28. Fernandes ABN, Ruellas ACO, Araújo MVA, Sant'Anna EF, Elias CN. Assessment of exogenous pigmentation in colourless elastic ligatures. *J Orthod*. 2014;41(2):147-151. doi:10.1179/1465313313Y.0000000083

29. Pascual AL, Beeman CS, Hicks EP, Bush HM, Mitchell RJ. The essential work of fracture of thermoplastic orthodontic retainer materials. *Angle Orthod*. 2010;80(3):554-561. doi:10.2319/042809-232.1

30. Chang CS, Al-Awadi S, Ready D, Noar J. An assessment of the effectiveness of mechanical and chemical cleaning of Essix orthodontic retainer. *J Orthod*. 2014;41(2):110-117. doi:10.1179/1465313313Y.0000000088

31. Pascual AL, Beeman CS, Hicks EP, Bush HM, Mitchell RJ. The essential work of fracture of thermoplastic orthodontic retainer materials. *The Angle Orthodontist*. 2010;80(3):554-561. doi:10.2319/042809-232.1

32. Agarwal M, Wible E, Ramir T, et al. Long-term effects of seven cleaning methods on light transmittance, surface roughness, and flexural modulus of polyurethane retainer material. *Angle Orthod*. 2018;88(3):355-362. doi:10.2319/081517-551.1

33. Wible E, Agarwal M, Altun S, et al. Long-term effects of various cleaning methods on polypropylene/ethylene copolymer retainer material. *Angle Orthod*. 2019;89(3):432-437. doi:10.2319/060818-429.1

34. Wible E, Agarwal M, Altun S, et al. Long-term effects of different cleaning methods on copolyester retainer properties. *Angle Orthod*. 2019;89(2):221-227. doi:10.2319/010218-2.1

35. Gracco A, Mazzoli A, Favoni O, et al. Short-term chemical and physical changes in invisalign appliances. *Aust Orthod J*. 2009;25(1):34-40.

36. 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*. 2015;51:356-366. doi:10.1016/j.jmbbm.2015.07.026

37. Eliades T, Eliades G, Watts DC. Structural conformation of in vitro and in vivo aged orthodontic elastomeric modules. *Eur J Orthod*. 1999;21(6):649-658. doi:10.1093/ejo/21.6.649

38. Plastics | Dentsply Sirona. Accessed December 18, 2020. https://www.dentsplysirona.com/content/dentsply-sirona/en/explore/orthodontics/plastics.html

39. Moshiri M, Eckhart JE, McShane P, German DS. Consequences of poor oral hygiene during aligner therapy. *J Clin Orthod*. 2013;47(8):494-498.

40. Papadopoulou AK, Cantele A, Polychronis G, Zinelis S, Eliades T. Changes in Roughness and Mechanical Properties of Invisalign® Appliances after One- and Two-Weeks Use. *Materials (Basel)*. 2019;12(15). doi:10.3390/ma12152406

41. Ryokawa H, Miyazaki Y, Fujishima A, Miyazaki T, Maki K. The mechanical properties of dental thermoplastic materials in a simulated intraoral environment. *Orthodontic Waves*. 2006;65(2):64-72. doi:10.1016/j.odw.2006.03.003

42. Zafeiriadis AA, Karamouzos A, Athanasiou AE, Eliades T, Palaghias G. An in vivo spectrophotometric evaluation of Vivera® and Essix® clear thermoplastic retainer discolouration. 2018;34(1):14.

43. Liu C-L, Sun W-T, Liao W, et al. Colour stabilities of three types of orthodontic clear aligners exposed to staining agents. *Int J Oral Sci*. 2016;8(4):246-253. doi:10.1038/ijos.2016.25

44. Plastics in Medical Devices - 2nd Edition. Accessed December 18, 2020. https://www.elsevier.com/books/plastics-in-medical-devices/sastri/978-1-4557-3201-2

45. Paszkiewicz S, Szymczyk A, Pawlikowska D, et al. Synthesis and characterization of poly(ethylene terephthalate-co-1,4-cyclohexanedimethylene terephtlatate)-block-poly(tetramethylene oxide) copolymers. *RSC Adv*. 2017;7(66):41745-41754. doi:10.1039/C7RA07172H

46. Bernard G, Rompré P, Tavares JR, Montpetit A. Colorimetric and spectrophotometric measurements of orthodontic thermoplastic aligners exposed to various staining sources and cleaning methods. *Head Face Med*. 2020;16. doi:10.1186/s13005-020-00218-2

47. Porojan L, Vasiliu R-D, Porojan S-D, Bîrdeanu M-I. Surface Quality Evaluation of Removable Thermoplastic Dental Appliances Related to Staining Beverages and Cleaning Agents. *Polymers (Basel)*. 2020;12(8). doi:10.3390/polym12081736

48. Schuster S, Eliades G, Zinelis S, Eliades T, Bradley TG. Structural conformation and leaching from in vitro aged and retrieved Invisalign appliances. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2004;126(6):725-728. doi:10.1016/j.ajodo.2004.04.021

49. Eichenauer J, Serbesis C, Ruf S. Cleaning removable orthodontic appliances: a survey. *J Orofac Orthop*. 2011;72(5):389-395. doi:10.1007/s00056-011-0043-2

50. Knobloch LA, Kerby RE, Seghi R, van Putten M. Two-body wear resistance and degree of conversion of laboratory-processed composite materials. *Int J Prosthodont*. 1999;12(5):432-438.

51. Nakagawa M, Matsuya S, Shiraishi T, Ohta M. Effect of fluoride concentration and pH on corrosion behavior of titanium for dental use. *J Dent Res*. 1999;78(9):1568-1572. doi:10.1177/00220345990780091201

52. Colombo M, Cavallo M, Miegge M, et al. Color stability of CAD/CAM Zirconia ceramics following exposure to acidic and staining drinks. *J Clin Exp Dent*. 2017;9(11):e1297-e1303. doi:10.4317/jced.54404

53. Wieckiewicz M, Opitz V, Richter G, Boening KW. Physical properties of polyamide-12 versus PMMA denture base material. *Biomed Res Int*. 2014;2014:150298. doi:10.1155/2014/150298

54. Daniel. What is Delta E? And Why Is It Important for Color Accuracy? ViewSonic Library. Published January 21, 2020. Accessed February 21, 2021. https://www.viewsonic.com/library/creative-work/what-is-delta-e-and-why-is-it-important-for-color-accuracy/

55. Ergun G, Nagas IC. Color Stability of Silicone or Acrylic Denture Liners: An in Vitro Investigation. *Eur J Dent*. 2007;1(3):144-151.

56. Johnston WM, Ma T, Kienle BH. Translucency parameter of colorants for maxillofacial prostheses. *Int J Prosthodont*. 1995;8(1):79-86.

57. Spink LS, Rungruanganut P, Megremis S, Kelly JR. Comparison of an absolute and surrogate measure of relative translucency in dental ceramics. *Dental Materials*. 2013;29(6):702-707. doi:10.1016/j.dental.2013.03.021

58. Raman Tutorial - HORIBA. Accessed February 10, 2021. https://www.horiba.com/uk/scientific/products/raman-spectroscopy/raman-academy/raman-tutorial/

59. Raman effect | physics. Encyclopedia Britannica. Accessed February 10, 2021. https://www.britannica.com/science/Raman-effect

60. Coffee Statistics. Accessed December 5, 2020. https://www.e-importz.com/coffee-statistics.php

61. de Oliveira CB, Maia LGM, Santos-Pinto A, Gandini Júnior LG. In vitro study of color stability of polycrystalline and monocrystalline ceramic brackets. *Dental Press J Orthod*. 2014;19(4):114-121. doi:10.1590/2176-9451.19.4.114-121.oar

62. Chang CS, Al-Awadi S, Ready D, Noar J. An assessment of the effectiveness of mechanical and chemical cleaning of Essix orthodontic retainer. *Journal of Orthodontics*. 2014;41(2):110-117. doi:10.1179/1465313313Y.0000000088

63. Hollis S, Eisenbeisz E, Versluis A. Color stability of denture resins after staining and exposure to cleansing agents. *J Prosthet Dent*. 2015;114(5):709-714. doi:10.1016/j.prosdent.2015.06.001

64. Guler AU, Yilmaz F, Kulunk T, Guler E, Kurt S. Effects of different drinks on stainability of resin composite provisional restorative materials. *J Prosthet Dent*. 2005;94(2):118-124. doi:10.1016/j.prosdent.2005.05.004

65. Fernandes ABN, Ribeiro AA, de Araujo MVA, Ruellas AC de O. Influence of exogenous pigmentation on the optical properties of orthodontic elastic ligatures. *J Appl Oral Sci*. 2012;20(4):462-466. doi:10.1590/S1678-77572012000400012

66. Rutkunas V, Sabaliauskas V, Mizutani H. Effects of different food colorants and polishing techniques on color stability of provisional prosthetic materials. *Dent Mater J*. 2010;29(2):167-176. doi:10.4012/dmj.2009-075

APPENDICES

Appendix A

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Product Name: Ace Plastic

Part Number(s):
25-025-35,25-420-41,25-450-45,25-325-30,25-020-35,25-050-35,25-478-40,25-425-40,25-320-31,25-350-35,25-347-30,25-347-35

Release Date: December14, 2005

1. Product and Company Identification

Trade Name & Synonyms Ace Plastic	**MSDS Code Number** Refer to part number
Chemical Name NA	**Manufacture / Distributor** GAC International Inc.
C.A.S. Number NA	**Address** 355 Knickerbocker Ave. Bohemia, NY USA 11716
Grades or Minor Variant Identities NA	**Information Telephone Number** 1-631-419-1700
Product Use (for Canada) Plastic	**Emergency Telephone Number** 1-631-419-1700

2. Composition of Ingredients

Hazardous Components	C.A.S. Number	Exposure Limits	%
Trade Secret	Proprietary	NA	5
Copolyester	Proprietary	NA	95

3. Hazard Identification

Emergency Overview

Molten material will produce thermal burns.

Routes of Exposure	Signs and Symptoms	Single, Repeated, or Lifetime Exposure	Severity (Mild, Moderate, Severe)	Acute and Chronic Health Effect(s)	Target Organ(s)
Eye	NA	NA	NA	NA	NA
Skin	NA	NA	NA	NA	NA
Inhalation	NA	NA	NA	NA	NA
Ingestion	NA	NA	NA	NA	NA
Other	NA	NA	NA	NA	NA

Medical Conditions Aggravated by Exposure
NA

Carcinogenicity (IARC, NTP)
NA

Potential Environmental Effects
NA

4. First Aid Measures

Routes of Exposure	First Aid Instructions	Immediate Medical Attention	Delayed Effects
Eye	If molten material contacts the eye, immediately flush with plenty of water for at least 15 minutes. If easy to do remove contact lenses.	Get medical attention immediately.	NA
Skin	If burned by contact with molten material, cool as quickly as possible. Do not peel material from skin.	Get medical attention.	NA
Inhalation	If symptomatic, move to fresh air.	Get medical attention if symptoms persist.	NA
Ingestion	Material is not expected to be absorbed from the gastrointestinal tract so that induction of vomiting should not be necessary.	NA	NA
Other	NA	NA	NA

APPENDIX A (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. I

Product Name: Ace Plastic

Part Number(s):
25-025-35,25-420-41,25-450-45,25-325-30,25-020-35,25-050-35,25-478-40,25-425-40,25-320-31,25-350-35,25-347-30,25-347-35

Release Date: December14, 2005

Note to Physicians (Treatment, Testing, and Monitoring)
Burns should be treated as thermal burns. The material will come off as healing occurs; therefore, immediate removal from the skin is not necessary.

5. Fire and Explosion Data

Flashpoint & Method:	Flammable (Explosive) Limits in Air	Autoignition Temperature	Other
°C / °F N/A; combustible solid	LEL: NA UEL: NA	NA	NA

Flame Propagation or Burning	Properties Contributing to Fire Intensity	Flammability Classification
NA	NA	Health- ,1Flammability-1, Reactivity-0

Extinguishing Media	Extinguishing Media to Avoid
Water spray, dry chemical	NA

Protection and Procedures for Firefighters
Wear self- contained breathing apparatus and protective clothing.

Unusual Fire and Explosion Hazards
Powdered material may form explosive dust- air mixtures. Hazardous combustion products: Carbon dioxide, carbon monoxide

6. Accidental Release Measures

Containment Techniques
NA

Spill/Leak Clean-Up Procedures and Equipment
Sweep or scoop up and remove.

Evacuation Procedures
NA

Special Instructions
NA

Reporting Requirements
NA

7. Handling and Storage

Handling Practices and Warnings
Personal Precautionary Measures: Avoid contact with molten material.

Prevention of Fire and Explosion: Keep from contact with oxidizing materials. Minimize dust generation and accumulation. In the United States of America refer to NFPA R Pamphlet No. 654, " Prevention of Fire and Dust Explosions in the Chemical, Dye, Pharmaceutical and Plastics Industries".

Storage Practices and Warnings
(°C / °F). Keep container closed.

8. Exposure Control/Personal Protection

Ventilation	Other Engineering Controls
Good general ventilation (typically 10 air changes per hour) should be used.	Ventilation rates should be matched to conditions. Supplementary local exhaust ventilation, closed system, spaces mechanical generation of dusts, heating, drying etc.

Routes of Entry:	Personal Protective Equipment (PPE) for Normal Use:	PPE for Emergencies:
Eye/Face	Wear a face shield when working with molten material.	NA
Skin	When material is heated, wear gloves to protect against thermal burns.	NA

APPENDIX A (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Product Name: Ace Plastic

Part Number(s):
25-025-35,25-420-41,25-450-45,25-325-30,25-020-35,25-050-35,25-478-40,25-425-40,25-320-31,25-350-35,25-347-30,25-347-35

Release Date: December14, 2005

Inhalation	If engineering controls do not maintain airborne concentrations to an acceptable level, an approved respirator must be worn. In the United States of America, if respirators are used, a program should be instituted to assure compliance with OSHA Standard 63 FR 1152, January 8, 1998. Respirator type: Dust, organic vapor.	NA

General Hygiene Considerations and Work Practices
Recommended Decontamination Facilities: Eye bath, washing facilities.

Protective Measures During Repair and Maintenance of Contaminated Equipment
NA

Other Protective Measures and Equipment
NA

9. Physical and Chemical Characteristics

Appearance
Solid (pellet). Color: Varies with formulation

Odor
Odorless

Normal Physical State:
☐ Liquid ☐ Gas
☒ Solid ☐ ____ (Other)

Boiling Point °C / °F NA
Melting Point °C / °F NA
Freezing Point °C / °F NA

Specific Gravity or Density (H_20=1)	Solubility in Water	pH
>1	Negligible	NA

Vapor Pressure (mm Hg @ 20°C)	Vapor Density (AIR= 1)	Evaporation Rate (Butyl Acetate = 1)
NA	NA	NA

Other
Thermal Decomposition Temperature: Thermal stability not tested. Low stability hazard expected at normal operating temperatures.Softening point: Varies with formulation.

10. Stability and Reactivity Data

Incompatibility (Materials to Avoid)
Material reacts with strong oxidizing agents.

Hazardous Products Produced During Decomposition
NA

Hazardous Polymerization?		Conditions to Avoid
☐ May Occur	☒ May Not Occur	°C / °F NA
Stability?		**Conditions to Avoid**
☒ Stable	☐ Unstable	°C / °F NA

11. Toxicological Information

Toxicity Data, Epidemiology Studies, Carcinogenicity, Neurological Effects, Genetic or Reproductive Effects, or Structure Activity Data
Acute toxicity data if available are listed below. Additional toxicity data may be available on request.

APPENDIX A (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Product Name: Ace Plastic

Part Number(s):
25-025-35,25-420-41,25-450-45,25-325-30,25-020-35,25-050-35,25-478-40,25-425-40,25-320-31,25-350-35,25-347-30,25-347-35

Release Date: December14, 2005

12. Ecological Information

Toxicity, Environmental Fate, Physical/Chemical Data, or Other Data Supporting Environmental Hazard Statements

Acute toxicity data if available are listed below. Additional toxicity data may be available on request. This material has not been tested for environmental effects.

13. Disposal Considerations

Regulations

Discharge, treatment, or disposal may be subject to national, state, or local laws. Incinerate.

Properties (Physical/Chemical) Affecting Disposal

NA

14. Transport Information

Regulated for shipping?	Proper Shipping Name	Packing Group	
☐ Yes ☒ No	Ace Plastic	None	
Do changes in quantity, packaging, or shipment method change product classification?		**Hazard Class**	**Identification Number**
☐ Yes ☒ No		None	None

Other

DOT (USA): Class not regulated. ICAO Status: Class not regulated. IMDG Status: Class not regulated

15. Regulatory Information

Federal Regulations

WHMIS (Canada) Status: noncontrolled.

SARA 313: None, unless listed below.

International Regulations

NA

Other

Carcinogenicity Classification (components present at 0.1% or more): none, unless listed below.

TSCA (US Toxic Substances Control Act): This product is listed on the TSCA inventory. Any impurities present in this product are exempt from listing.

DSL (Canadian Domestic Substances List) and CEPA (Canadian Environmental Protection Act): This product is listed on the DSL. Any impurities present in this product are exempt from listing.

EINECS (European Inventory of Existing Commercial Chemical Substances): This product is listed on the EINECS. Any polymer present in this has regulatory clearance under Directives of European Union.

AICIS/NICNAS (Australian Inventory of Chemical Substances and National Industrial Chemicals Notification and ASSESSMENT Scheme): This product is listed on AICS or otherwise complies with NICNAS

MITI (Japanese Handbook of Existing and New Chemical Substances): This product is listed in the Handbook or has been approved in Japan by new substance notification.

ECL (Korean Toxic Substances Control Act): This product is listed on the Korean inventory or otherwise complies with the Korean Toxic Substances Control Act.

APPENDIX A (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Product Name: Ace Plastic

Part Number(s):
25-025-35,25-420-41,25-450-45,25-325-30,25-020-35,25-050-35,25-478-40,25-425-40,25-320-31,25-350-35,25-347-30,25-347-35

Release Date: December14, 2005

16. Other Information

Supplier Number: 40/25 **Supplier Release:** December 8, 2005

N/A = not applicable. **NA** = not available, **N/E** = not established. **N/D** = not determined.

Appendix B

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Part Number(s): 25-131-25, 25-134-78, 25-135-00

Product Name: Essix C Plastics

Release Date: April 13, 2007

1. Product and Company Identification

Trade Name & Synonyms Type " C" + Plastic	**MSDS Code Number** Refer to part number
Chemical Name NA	**Manufacture / Distributor** GAC International Inc.
C.A.S. Number 9010-79-1	**Address** 355 Knickerbocker Ave. Bohemia, NY USA 11716
Grades or Minor Variant Identities NA	**Information Telephone Number** 1-631-419-1700
Product Use (for Canada) NA	**Emergency Telephone Number** 1-631-419-1700

2. Composition of Ingredients

Hazardous Components	C.A.S. Number	Exposure Limits	%
Polypropylene/ethylene copolymer	9010-79-1	NA	>95
Stabilizers (trade secret)	NA	NA	<5
This product is not considered a hazardous material by Raintree Essix according to the U.S occupational safety and health act definitions and regulation including the hazard communication standard 29CFR 1910.1200. Raintree Essix does not contain consider this product a controlled substance according to Canada's WHMIS regulations. Threshold limit value (TLV) or permissible exposure limit (PEL) values are not established. This material is not expected to cause physiologic impairment at low concentration. Until a specific TLV is adopted by ACGIH (American conference of government hygienists) , or an OSHA PEL standard is issued, raintree essix suggest that this material be treated as a nuisance dust or particle in accordance with the recommendations of ACGIH.			

3. Hazard Identification

Emergency Overview

Physical appearance: Translucent to white solid pellets.

Immediate concerns:Spilled material may present a slipping hazard. This product as shipped is not classified as a combustible dust however, a combustible concentration of dust may occur if fines are suspended in air. Avoid contact with strong oxidizing agents. When working with the material at elevated temperatures, the material will begin to decompose producing fames that can contain carbon dioxide, carbon monoxide, ketones, acrolein, aldehydes and other unidentifiable organic compounds that come from the breakdown of the material. Adequate room and extrude ventilation should be provided to minimize exposure.

WARNING AUTION LABELS: Burn risk-Avoid contact with molten resin. Explosion risk- Prevent accumulation of dust particles. Slipping risk-Keep walking surfaces free of spilled material. Vapor risk- Provide ventilation to avoid exposure to process vapors. Physical hazards: Spilled material may present a slipping hazard. Health hazards: None known.

Routes of Exposure	Signs and Symptoms	Single, Repeated, or Lifetime Exposure	Severity (Mild, Moderate, Severe)	Acute and Chronic Health Effect(s)	Target Organ(s)
Eye **Yes**	Irritation or redness	NA	NA	Acute: May cause eye irritation.	None known.
Skin	N/A	NA	NA	NA	NA
Inhalation **Yes**	Irritation of the nose, throat and respiratory tract irritation.	NA	NA	Acute: May cause respiratory tract irritation.	NA
Ingestion	N/A	NA	NA	NA	NA

APPENDIX B (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Part Number(s): 25-131-25, 25-134-78, 25-135-00

Product Name: Essix C Plastics

Release Date: April 13, 2007

Other	NA	NA	NA	NA	NA

Medical Conditions Aggravated by Exposure
None known.

Carcinogenicity (IARC, NTP)
This product is not considered to be a carcinogen by OSHA, IARC or NTP. Irritancy: None known. Sensitization: None known. Subtonic/chronic toxicity: Chronic: None known. Teratogenicity: None known. Reproductive toxin: None known. Mutagenicity: None known.

Potential Environmental Effects
Potential health effects: Inhalation: Remove to fresh air. If breathing is difficult or has stopped, administer artificial respiration (mouth-to-mouth) or oxygen is indicated. Call a physician. **Skin**: Exposure to molten resin may cause thermal burns. **Ingestion:** None known. **Inhalation**: None known.

4. First Aid Measures

Routes of Exposure	First Aid Instructions	Immediate Medical Attention	Delayed Effects
Eye	Flush eye with water for 15 minutes.	Get medical attention.	NA
Skin	If molten material comes in contact with the skin, cool under ice water or a running stream of water. Do not attempt to remove the material from the skin. Removal could result in severed tissue damage.	Get medical attention.	NA
Inhalation	Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficulty, give oxygen.	Give medical attention.	NA
Ingestion	Not applicable.	NA	NA
Other	NA	NA	NA

Note to Physicians (Treatment, Testing, and Monitoring)
None. Antidotes: N/A. Additional information: None

5. Fire and Explosion Data

Flashpoint & Method:	Flammable (Explosive) Limits in Air	Autoignition Temperature	Other
°C / °F N/D	LEL: N/D UEL: N/D	N/D	Sensitive to static discharge: Static discharge could be in ignition source for a combustible concentration of dust. Sensitivity to impact: N/A

Flame Propagation or Burning	Properties Contributing to Fire Intensity	Flammability Classification
Hazardous combustion products: Carbon monoxide, carbon dioxide, ketones, acrolein, aldehydes, and unidentified organic compounds.	NA	Health-1, Flammability-0, Reactivity-0.

Extinguishing Media	Extinguishing Media to Avoid
Use alcohol, foam, carbon dioxide, or water spray when fighting fire involving this material.	NA

Protection and Procedures for Firefighters
Standard procedures for a class A fires. Fire fighting equipment: As in any fire, wear self-contained pressure demand breathing apparatus, (MSHA/NIOSH approved or equivalent) and full protective gear.

Unusual Fire and Explosion Hazards
Product as shipped is not a combustible dust. However, a combustible concentration of dust may occur when fires are suspended in air.

APPENDIX B (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Product Name: Essix C Plastics

Part Number(s): 25-131-25, 25-134-78, 25-135-00

Release Date: April 13, 2007

6. Accidental Release Measures

Containment Techniques

Environmental precautions: Water spill: Keep pellets out of waterways. Land spill: Not yet determined. Air spill: Not yet determined.

Spill/Leak Clean-Up Procedures and Equipment

Small spill: Vacuum or sweep up material and place in a disposal container.

Large spill: Vacuum or sweep up material and place in a disposal container.

Evacuation Procedures

NA

Special Instructions

Vacuum or sweep up material and place in a disposal container. Release notes: None. Special protective equipment: None

Reporting Requirements

NA

7. Handling and Storage

Handling Practices and Warnings

NA

Storage Practices and Warnings

(°C / °F). NA

8. Exposure Control/Personal Protection

Ventilation	**Other Engineering Controls**
Provide adequate room ventilation. Provide adequate ventilation at the extrude to minimize exposure to process vapors. Eliminate ignition sources during repair and maintenance operations.	NA

Routes of Entry:	**Personal Protective Equipment (PPE) for Normal Use:**	**PPE for Emergencies:**
Eye/Face	Wear safety glasses with side shields or goggles.	NA
Skin	When handling and/or processing resins at elevated temperatures or in a molten state, wear protective clothing over the skin to prevent contact. Protective clothing: When handling and /or processing resins at elevated temperatures or in a molten state, wear protective clothing over the skin to prevent contact.	NA
Inhalation	A respiratory protection program that meets OSHA 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant a respirator's use.	NA

General Hygiene Considerations and Work Practices

N/E

Protective Measures During Repair and Maintenance of Contaminated Equipment

NA

Other Protective Measures and Equipment

Eyewash fountains and safety showers should be easily accessible.

APPENDIX B (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Product Name: Essix C Plastics

Part Number(s): 25-131-25, 25-134-78, 25-135-00

Release Date: April 13, 2007

9. Physical and Chemical Characteristics

Appearance
Physical state: Solid (film or sheet) Color: Translucent to white. Appearance: Pellet.

Odor
Slight waxy odor

Normal Physical State:
☐ Liquid ☐ Gas
☒ Solid ☐ ____ (Other)

Boiling Point °C / °F N/A

Melting Point 120°C / >248

Freezing Point °C / °F N/A

Specific Gravity or Density (H_20=1)	Solubility in Water	pH
0.88 to 0.92	N/A	N/A
Vapor Pressure (mm Hg @ 20°C)	**Vapor Density (AIR= 1)**	**Evaporation Rate (Butyl Acetate = 1)**
N/A	N/A	N/A

Other
Density: N/D Viscosity: N/A VOC notes: Not yet determined. Percent volatile: <0.4%. Physical state: None. Molecular weight: N/A

10. Stability and Reactivity Data

Incompatibility (Materials to Avoid)
Oxidizing materials.

Hazardous Products Produced During Decomposition
At elevated temperatures the material will begin to decompose producing fumes that can contain carbon dioxide, carbon monoxide, ketones, acrolein, aldehydes, and unidentified organic compounds.

Hazardous Polymerization?		Conditions to Avoid
☐ May Occur	☒ May Not Occur	°C / °F Will not occur
Stability?		**Conditions to Avoid**
☒ Stable	☐ Unstable	°C / °F NA

11. Toxicological Information

Toxicity Data, Epidemiology Studies, Carcinogenicity, Neurological Effects, Genetic or Reproductive Effects, or Structure Activity Data

Environmental data: N.A

Ecotoxicological information: N.A

Distribution: N.A

Chemical fate information: Not readily biodegradable.

12. Ecological Information

Toxicity, Environmental Fate, Physical/Chemical Data, or Other Data Supporting Environmental Hazard Statements
NA

APPENDIX B (CONT)

DENTSPLY GAC

MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Part Number(s): 25-131-25, 25-134-78, 25-135-00

Product Name: Essix C Plastics

Release Date: April 13, 2007

13. Disposal Considerations

Regulations

Disposal method: 1. Recycle (reprocess)

2. Incineration including energy recovery of waste material in a permitted facility in accordance with local, state or provincial and/or federal regulations.

RCRA hazard class: This product is not judged to be a hazardous waste by any local, state or federal regulations. However, it may be listed as industrial waste in some state or provinces. This product is not listed in the U.S. federal hazardous waste regulations, 40 CFR 261.33 paragraphs (e) or (f), i.e., chemical products that are considered hazardous if they become wastes. It does not exhibit any of the hazardous waste characteristics listed in 40 CFR subpart C. State or local hazardous waste regulations may apply if different from the federal.

Properties (Physical/Chemical) Affecting Disposal

NA

14. Transport Information

Regulated for shipping?	Proper Shipping Name	Packing Group	
☐ Yes ☐ No NA	Essix C Plastics	NA	
Do changes in quantity, packaging, or shipment method change product classification? ☐ Yes ☐ No NA		**Hazard Class** NA	**Identification Number** NA

Other

Special shipping notes: This product is not regulated by DOT, IMO, IATA, and Canadian TDG and associated regulation, ADR or RID.

15. Regulatory Information

Federal Regulations

Regulatory information united states: SARA title III (Superfund amendments and reauthorization act)
Title III notes: This product is not subject to SARA title III requirements.
TSCA Status: This product appears on the TSCA.
OSHA hazard comm. rule: Raintree essix does not judge this product hazardous according to OSHA definitions.
Clean water act: This product is regulated under EPA's clean water act/NPDES rules as floating material. In addition, this product is considered significant material under EPA's storm water permit rules.

International Regulations

Regulatory information Canada: WHMS: This product is not considered a controlled substance under WHMS. This MSDS meets WHMS format requirements.
Canadian environmental protection act: All ingredients in this product are listed under CEPA on the DSL.
Regulations: International regulations: All ingredients are in compliance with EINECS/ELINCS.

Other

NA

16. Other Information

Supplier Number: 40/25 **Supplier Release:** NA

N/A = not applicable. **NA** = not available, **N/E** = not established. **N/D** = not determined.

VITA

NAME: Laurie Susarchick

EDUCATION:
B.A., Public Relations, Rowan University, Glassboro, New Jersey, 2009

D.D.S., Columbia University, New York, New York, 2018

M.S., Oral Sciences, University of Illinois at Chicago, Chicago, Illinois, 2021

Certificate, Orthodontics, University of Illinois at Chicago, Chicago, Illinois, 2021

AWARDS:
AAOF Research Aid Award, 2020

Columbia University College of Dental Medicine Summer Research Fellowship, 2015

PROFESSIONAL MEMBERSHIPS:
American Association of Orthodontists
American Dental Association
Chicago Dental Society