Effects of staining on white spot lesions treated with ICON® resin infiltration

Patra Vasiliki Alatsis, D.M.D

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Patra V. Alatsis, D.M.D

Masters of Science in Orthodontics Research Advisory Committee:

Signature: [redacted] Date: 12-22-14

Jack Ferracane, Ph.D.
Professor, Chair
Department of Restorative Dentistry
Oregon Health & Science University

Signature: [redacted] Date: 12-22-14

David A. Covell, Jr., Ph.D., D.D.S.
Associate Professor, Chair
Department of Orthodontics
Oregon Health & Science University

Signature: [redacted] Date: 2/13/15

Eric Burgin, D.D.S., M.S.
Clinical Assistant Professor
Department of Orthodontics
Oregon Health & Science University

Signature: [redacted] Date: 12/22/14

Ana Castilla, D.D.S., M.S.
Clinical Assistant Professor
Department of Orthodontics
Oregon Health & Science University

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Patra V. Alatsis[a], Jack Ferracane[b], David A. Covell, Jr.[a], Eric Burgin[a], Ana Castilla[a]

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

[b]Department of Restorative Dentistry, Division of Biomaterials and Biomechanics, School of Dentistry, Oregon Health & Science University, Portland, OR

Keywords: Resin Infiltration, White Spot Lesion, Demineralization, Staining

Corresponding Author:

David A. Covell, Jr.
Associate Professor, Chair
Department of Orthodontics
Oregon Health & Science University
2730 SW Moody Ave, SD-Ortho
Portland, Oregon 97201-5042
(v) 503-494-6155
(f) 503-494-5777
covelljr@ohsu.edu

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ABSTRACT

Background: The purpose of this in vitro study was to assess the esthetic appearance of a resin infiltrant used to restore enamel white spot lesions. Assessments were made immediately following resin infiltration and after extended exposure to several staining solutions.

Methods & Materials: White spot lesions were created on defined portions of the labial surfaces of 100 extracted bovine maxillary incisors using an enamel demineralization solution. Tooth surfaces blocked from the demineralization solution served as controls. Demineralization was conducted for either 2 weeks (n=50) or 6 weeks (n=50). The demineralized area was divided in half to form 2 windows. One window remained demineralized (WSL) and the other was restored using a resin infiltration technique (ICON; ICON® Smooth Surface Resin Infiltration System, DMG America, Englewood, NJ). For each tooth, photographs and color measurements (ΔE) of the 3 surface conditions, recorded with an intraoral spectrophotometer. Specimens from both demineralization groups were then divided into 5 subgroups of 10 teeth each and soaked for 2 weeks in one of 5 solutions: deionized water (control), coffee, red wine, energy drink, and 10% carbamide peroxide, after which photographs and color measurements were obtained. One specimen from each demineralization and staining group (n=10) was sectioned and the depth of stain penetration and the resin-tooth interface was assessed by light microscopy. Statistical analysis was performed using ANOVA with level of significance set at $\alpha \leq 0.05$.

Results: Initially ICON infiltration restorations showed significantly closer color matches to sound enamel relative to the untreated WSL, with 2-week demineralization showing ΔE=5.5 vs. 30.09, and 6-week demineralization showing ΔE=8.72 vs. 31.77, respectively ($p<.001$). ICON was less effective masking the lesions formed with 6 weeks relative to 2 weeks demineralization ($p=0.019$). Red wine produced the most staining in all 3 surface conditions for both demineralization groups ($p<0.001$).

Staining by red wine was significantly reduced for infiltrated lesions compared to untreated WSL or control ($p<0.001$). Staining by coffee was significantly reduced for infiltrated lesions compared to untreated WSL ($p<0.001$), but significantly increased for infiltrated lesions compared to the control for 6-week demineralized specimens ($p<0.001$). The effects of staining by the energy drink and carbamide peroxide where marginally evident. Microscopy revealed for 2-week demineralized specimens that WSL depths ranged from 142-377µm, with ICON penetration ranging from 118-225µm, and for 6-week specimens the ranges were 222-357µm and 110-303µm, respectfully.

Conclusions: Resin infiltration was able to significantly improve the appearance of WSLs in vitro. Staining remained superficial and different staining solutions produced variable changes in color. The greatest staining occurred with red wine and coffee, while energy drink, whitening solution and water had little effect. Resin infiltrated lesions are less susceptible to staining by coffee and red wine than WSL or sound enamel.

Keywords: Resin Infiltration, White Spot Lesion, Demineralization, Staining

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INTRODUCTION

Enamel white-spot lesions (WSLs) are a common sequelae of poor oral hygiene, often associated with fixed orthodontic appliances.[1,2] Enamel demineralization and subsequent formation of WSLs can occur whenever bacterial plaque is retained on the enamel surface for prolonged periods of time. The presence of fixed appliances during orthodontic treatment often leads to greater plaque retention on facial tooth surfaces due to decreased oral hygiene efficiency.[1] Reported prevalence of patients affected by WSLs after fixed orthodontic treatment ranges from 50%[1] to 97%,[4] with a recent study reporting 72.9% of patients developed a new WSL during orthodontic treatment.[5]

WSLs typically demonstrate an intact, remineralized surface layer over a subsurface porous lesion body where the lesion is caused by an imbalance between demineralization and remineralization as a consequence of the acidic environment created by cariogenic bacteria.[6] Their opaque chalky-white appearance is due to scattering of light within the subsurface demineralized enamel.[7] Formation of these lesions can occur quickly, with the first clinical signs detected as early as two weeks after initial biofilm formation.[8,9] In a small percentage of orthodontic patients, enamel demineralization can progress to the point of frank cavitation, which necessitates restoration.[1] Upon removal of fixed appliances, the cariogenic challenge ceases and WSLs may naturally regress via salivary remineralization and toothbrush abrasion.[10,11] Regression occurs predominately in the first three months and complete regression is unlikely to occur for most lesions,[12] as remineralization at the outer surface of the lesion inhibits the penetration of calcium and other ions into the deeper parts of the lesion, arresting the remineralization process.[2,13,14]

There are several treatment options available for treating WSLs. Topical remineralization therapy with fluoride,[15] casein phosphopeptide amorphous calcium phosphate (CPP-ACP),[16,17] and a

combination CPP-ACP with fluoride[18,19], have shown mixed success and are often clinically insignificant at producing cosmetic improvement.[16,20] Due to surface hypermineralization, deeper lesions do not remineralize completely, and long-term esthetics remain poor.[21,22] Bleaching results in limited esthetic improvement and has been associated with tooth sensitivity and reduction in enamel microhardness.[23-25] Microabrasion is effective for shallow WSLs,[26] however it can result in considerable enamel removal.[27,28] Likewise, traditional restorative options including composite restorations, veneers, or ceramic crowns lead to substantial loss of dental hard tissue, despite potentially excellent cosmetic results.[29,30] Due to the relatively small amount of demineralized enamel in WSLs, less invasive options would be preferred.

Recently, resin infiltration has been evaluated as a treatment of WSLs that formed during orthodontic treatment. The approach, originally developed to arrest proximal caries lesions,[31,32] has been termed a micro-invasive procedure as it involves penetration of a resin composite into the body of the WSL via capillary forces, with minimal removal of existing enamel.[33] The porous nature of active WSLs allows a low-viscosity resin to permeate into the previously demineralized enamel matrix filling the voids with polymer, creating a refractory index similar to healthy enamel.[34] In vitro studies have shown successful masking of WSLs using resin infiltration techniques.[31,35-37] Initial in vivo results have also shown immediate, reliable, and sometimes dramatic improvement of post-orthodontic WSLs.[38-40] A recent clinical trial reported complete masking of post-orthodontic WSLs in 61% of patients using the resin infiltration technique, while 33% had partial masking.[39] *Senestraro et al*[40] found resin infiltration was able to significantly improve the clinical appearance of post-orthodontic WSLs, reduce their size, and maintain favorable esthetics over an 8-week period.

More recently, in vivo studies have reported varying results regarding the effects of staining on resin infiltrated lesions. Results range from decreased staining of resin infiltrated enamel,[41] to mild staining by tea and red wine,[37] to increased staining compared to sound enamel.[42] Polishing of resin

infiltrated lesions has been found to increase their resistance to staining in vitro.[37,42] To date, no studies have looked at the effects of whitening solution on resin-infiltrated lesions.

The purpose of this in vitro study was to evaluate the color stability of a commercially-available resin infiltrant available to restore enamel white spot lesions. The restorations were assessed immediately after infiltration and after 2 weeks continuous exposure to one of five solutions: coffee, red wine, energy drink, overnight external bleaching, and water. The depth of stain penetration was also assessed from sections cut through the restorations. We hypothesized that ICON® resin infiltration would show reduced surface staining compared to control enamel or WSL, that staining would be greater with red wine and coffee than with energy drink, external bleaching, or water, and that the staining would be confined to superficial areas.

METHODS & MATERIALS

Specimen Preparation

100 extracted bovine incisors of the second dentition were selected having intact labial surfaces, void of stains or caries. Buccal enamel surfaces were cleaned of surface debris with a slow-speed handpiece, rubber prophy cup and plain pumice (Miltex, York, PA) then rinsed for 30 seconds and air dried for 10 seconds. To seal the root canal, the root apex was etched with 35% phosphoric acid (Ultra-Etch®, Ultradent, South Jordan, UT) for 30 seconds, rinsed for 30 seconds, air dried for 10 seconds, dental adhesive (Prime&Bond Elect™, Dentsply, Milford, DE) was applied using a flocked applicator tip (Dentsply, Milford, DE) and light cured (1600 mW/cm^2, Ortholux™ Luminous curing light, 3M Unitek, Monrovia, CA) for 10 seconds from the mesial and distal surfaces, and then low viscosity composite (TPH Spectra™, Dentsply, Milford, DE) was placed over the apex and light cured for 40 seconds. Two coats of acid-resistant nail varnish (SinfulColors, Revlon, New York, NY) were applied to the entire crown and root surface of each incisor with the exception of a 7x14mm window on the facial surface of the incisal half of the crown, which remained unprotected. The apical half of the facial surface was covered with varnish to allow each tooth to serve as its own control.

The tooth samples were randomly allocated to two treatment groups: 2-week and 6-week enamel demineralization groups (n=50 each). Artificial white spot lesions were created in the uncoated areas by storage of the teeth in 4L demineralizing solution containing 50mM acetic acid, 3mM $CaCl_2$ $+2H_20$, 3mM KH_2PO_4, and 6μM methylhydroxydiphosphonate (pH 4.95) maintained at 21˚C for either 14 days or 42 days. PH was monitored daily and adjusted, if necessary, with either hydrochloric acid (10%) or sodium hydroxide solution (1 M).

Resin Infiltration

At the end of the demineralization period the nail varnish was removed with acetone. Each specimen had 1 sound enamel surface at the apical half of the crown, which served as the control. The

7x14mm demineralized area on the incisal half of the crown was divided into two equal 7x7mm treatment windows: one window (WSL) remained demineralized, the other (ICON) was treated with the ICON® smooth surface resin infiltration system according to the manufacturer's instructions published July 7, 2014. 15% hydrochloric acid gel (ICON®-Etch, DMG America, Englewood, NJ) was placed on the demineralized lesion for 2 minutes. The etching gel was rinsed with water for 30 seconds, then air dried for 30 seconds. The etch, rinse and dry steps were repeated two more times, for a total etch time of 6 minutes per lesion, after which lesions were desiccated using 99% ethanol (ICON®-Dry, DMG America, Englewood, NJ) and air dried for 30 seconds. The resin infiltrant (ICON®-Infiltrant, DMG America, Englewood, NJ) was applied to the tooth surface and allowed to penetrate the lesion for three minutes. Excess material was removed from the surface using a cotton roll and the resin was light-cured perpendicular to the facial surface for 40 seconds. The resin infiltrant was re-applied for one minute, excess was removed, and the curing light applied for 40 seconds. The surface of the ICON window was polished using a one-step diamond composite polishing kit (ComposiPro, Brasseler, Savannah, GA; Fig. 1).

Specimens were numbered and an intraoral spectrophotometer (VITA Easyshade® Model #62011, Vident, Brea, CA; Fig 2) with 5mm inner diameter and 6mm outer diameter was used to measure and record baseline (T1) L*, a,* b* color values for the control, WSL, and ICON windows for each specimen against a white background. This measures the color of teeth or esthetic restorations using the CIElab (*Commission Internationale L'Eclairage)* system.

Staining and Colorimetric Analysis

Specimens from the 2-week and 6-week demineralization groups were randomly divided into 5 subgroups with 10 teeth each for staining in one of five different solutions- deionized water (pH=5.2; control), coffee (pH=5.4; purchased pre-brewed, Starbucks, Seattle, WA), red wine (pH=3.8; Dark Horse Wines, Modesto, CA), energy drink (pH=3.6; Monster®, Hansen Natural Corp., Corona, CA) and 12-hour

external whitening gel (pH=6.5; Opalescence® PF 10%, Ultradent, South Jordan, UT). Specimens were submerged in 350mL of their respective staining medium for 1 week at 21˚C and then removed from the staining solution. Whitening gel was painted on the buccal surface of the whitening group according to the manufacturer's instructions and applied at room temperature for 12 hours per day for 7 days to simulate the bleaching process. At the end of each whitening application, specimens were rinsed with tap water for 1 minute to remove the bleaching agent, blotted dry, and stored in 350mL deionized water.

At the end of one week (T2), buccal enamel surfaces were cleaned with a slow-speed handpiece, rubber prophy cup and plain pumice flour (Miltex, York, PA) then rinsed for 30 seconds and air dried for 10 seconds to remove any residue that may have precipitated out onto the enamel surface. The spectrophotometer (VITA Easyshade® Model #62011, Vident, Brea, CA) was used to measure and record L*, a,* b* color values for the control, WSL, and ICON windows for each specimen. The original staining mediums were discarded and refreshed with new solution and the specimens submerged again in 350mL of their respective staining solution for another week (21˚C). Whitening gel was applied at room temperature for 12 hours per day for another 7 days for the whitening group specimens. As before, at the end of each whitening application, specimens were rinsed with tap water for 1 minute to remove the bleaching agent, blotted dry, and stored in 350mL of deionized water.

At the end of week 2 (T3), specimens were again removed from solution, facial enamel surfaces were cleaned, and the spectrophotometer (VITA Easyshade® Model #62011) used to measure and record final L*, a*, b* color values.

Color changes were analyzed using the CIE-*L*a*b** color system. The CIE-*L*a*b** system records colorimetric parameters three-dimensionally: lightness (L*: 0-100), green-red chromaticity (a*: -150 to +100), and blue-yellow chromaticity (b*: -100 to +150)[43]. Any specimens with color values reported outside the L*a*b* spectrum were not included in statistical analysis. Color difference (ΔE) was then

calculated (Excel 2013, Microsoft, Redmond, WA) for the three surface treatments (control, WSL, ICON) over the three staining timepoints (T1, T2, and T3) using the equation $\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$. Color change between the control-WSL and control-ICON were also calculated at T1, prior to staining to determine how well the ICON infiltration masked the WSL. Intraoral differences of $\Delta E \leq 3.7$ are considered not clinically differentiable[44].

Photography

Sample images were obtained to illustrate clinical differences after two weeks of staining using a digital camera (Nikon D70, Nikon, Tokyo, Japan) and micro lens (AF Micro Nikkor 105mm, Nikon, Tokyo, Japan) in ambient lighting. Photographs were taken of groups of teeth according to staining group, as well as average-looking specimens from each staining group. If highly visible variation in staining was noted for a group, i.e., very light or dark stained specimens, pictures were made to illustrate this clinical variation (coffee and red wine).

Sectioning

One specimen from each of the demineralization and staining groups was selected at random for sectioning (n=10). The roots were sectioned from the crown of each tooth with a disc and handpiece, and the crowns were embedded in epoxy resin (EpoxyCure™, Buehler, Lake Bluff, IL) and allowed to set for 48 hours. The resin specimens were then sectioned vertically through the control and ICON windows and through the control and WSL using a precision table-top cut-off machine (Accutom-5, Struers, Cleveland, OH). The border between the C-ICON windows and C-WSL windows were examined under a stereozoom microscope (Nikon SMZ-10, Nikon, Tokyo, Japan) with 40x magnification, illuminated (L-150 microscope light source, Laser Precision Analytical, Utica, NY; with EEG2823 Fiber-Lite Fiber, Dolan Jenner, Boxborough, MA), and digitally photographed using a digital eyepiece (Dino-lite Eyepiece Camera, AnMo Electronics, Hsinchu, Taiwan) and DinoCapture 2.0 software (AnMo Electronics, Hsinchu, Taiwan) at a magnification of 175x.

Statistical Analysis

Statistical analysis was performed with Sigma Stat (Sigma Plot Software Version 12, Systat Software inc., San Jose, CA). The 5 specimens from the red wine group were not included in statistical analysis because their WSL a* values were >100.

Two-way ANOVA was used to analyze the effects of demineralization length (2-weeks vs. 6-weeks) and surface treatment (control, WSL, ICON) on initial color change prior to staining (T1). Three-way ANOVA was used to analyze the effect of staining duration, staining solution, and surface treatment within Group 1 and again within Group 2. Normal distribution was checked using the Shapiro-Wilk test. Because the data failed to display normal distribution, pairwise multiple comparison analysis was carried out with the Holm-Sidak method. Level of significance set at $\alpha<0.05$. Subsequently, the comparison of stains was accomplished using two-way ANOVA after one week (T2) and two weeks (T3) of staining within the 2 demineralization groups. Normal distribution was checked using the Shapiro-Wilk test and CIE-$L^*a^*b^*$ differences analyzed with the Tukey Test. Level of significance set at $\alpha<0.05$.

RESULTS

Baseline (T1)

At baseline, 6-week demineralized specimens showed significantly higher color differences ΔE between control and ICON than 2-week demineralized specimens (p=0.019; Table 1). ICON® infiltration showed significantly less color difference with the control than compared to untreated WSLs (p<0.001). Compared to the control, ICON had ΔE >3.7. No significant interactions were found between demineralization time and surface treatment (p=0.467).

Comparison of surface treatment and stain type

At T2, the 2-week demineralized specimens showed significant interaction between surface treatment group and type of stain (p=<0.001; Table 2). Significant differences in ΔE were observed within the surface treatment group for all surface treatments (p<0.005) and within all stain types for all interactions except energy drink vs. whitening (p=0.703) and whitening vs. water (p=0.321). Significant differences were found between surface treatment by staining group with the exception of whitening and water. In the coffee group, ICON and the control were both significantly lower than the WSL (p<0.001). In the red wine group, ICON was significantly lower than both the control and WSL (p<0.001). ICON was also significantly lower than the control for the energy drink group (p=0.002).

At T3, the 2-week demineralized specimens passed the Normality Test (p=0.107) and showed significant interaction between surface treatment group and type of stain (p=<0.001; Table 3). Significant differences in ΔE were observed within the surface treatment group for all surface treatments (p<0.001) except WSL vs. control (p=0.141) and within all stain types (p<0.005) for all interactions except energy drink vs. whitening (p=1.000). Significant differences were found when comparing surface treatments and staining group for most groups. ICON was significantly lower than the WSL in the coffee (p<0.001), whitening (p<0.001) and water groups (p=0.002), but similar to the

control. In the red wine group, ICON was significantly lower than both the control ($p<0.001$) and WSL ($p<0.001$). ICON was also significantly lower than the control ($p<0.001$) for the energy drink group.

At T1, the 6-week demineralized specimens showed significant interaction between surface treatment group and type of stain ($p=<0.001$; Table 4). Significant differences in ΔE were observed within the surface treatment group for all surface treatments ($p<0.001$) except control vs. ICON ($p=0.509$) and within all stain types for all interactions ($p<0.001$) except whitening vs. energy drink ($p=0.967$), whitening vs. water ($p=0.998$), and water vs. energy drink ($p=0.997$). Within the WSL and ICON groups, red wine and coffee stained significantly more ($p<0.001$) than whitening, energy drink or water. The only significant differences observed within the control group was an increase in staining with red wine ($p<0.001$). ICON was significantly less affected by staining with red wine ($p<0.001$) than the control or WSL. ICON was also significantly less affected by staining with coffee than the WSL ($p<0.001$), but was significantly higher than the control ($p<0.001$). Whitening and energy drink had no effect on surface treatment.

At T2, the 6-week demineralized specimens showed significant interaction between surface treatment group and type of stain ($p=<0.001$; Table 5). Significant differences in ΔE were observed within the surface treatment group for all surface treatments ($p<0.001$) except control vs. WSL ($P=0.068$) and within all stain types for all interactions except whitening vs. energy drink ($p=0.935$), water vs. energy drink ($p=0.121$), and whitening vs. water ($p=0.502$). Within the WSL and ICON groups, red wine and coffee stained significantly more than whitening, energy drink or water ($p<0.001$). The only significant differences observed within the control group were found with red wine and between energy drink vs. water ($p<0.001$). ICON was significantly less affected by staining with red wine ($p<0.001$) than the control or WSL, as well as less affected by staining with energy drink than the control ($p<0.001$). ICON was also significantly less affected by staining with coffee than the WSL ($p<0.001$), but was

significantly higher than the control ($p<0.001$). Whitening and energy drink had no effect on surface treatment.

Comparison of staining duration, surface treatment and stain type

Significant interactions were observed between duration of stain, surface treatment, and type of stain for the two-week demineralization group ($p<0.001$; Tables 2 & 3). ΔE for T2 was significantly higher than T1 ($p<0.001$). Significant differences in ΔE were observed within the surface treatment group for all surface treatments and within all stain types for all interactions ($p<0.001$) except energy drink vs. whitening ($p=0.330$). All three-way interactions were found significant ($p<0.001$) except for the interaction between stain time and surface treatment for water ($p=0.465$).

Within the two-week demineralization group, significant increases in staining were observed between T1 and T2 for the WSL with coffee (41.26±8.6 vs. 48.06±9.7; $p=0.035$), control with red wine (44.76±14.5 vs. 76.93±7.4), WSL with red wine (40.3±17.0 vs. 50.77±12.8; $p=0.005$), WSL with whitening (8.95±9.6 vs. 22.37±9.2; $p<0.001$), and the control with energy drink (16.23±4.8 vs. 25.14±6.0; $p=0.006$). Values for red wine, coffee, and energy drink decreased for ICON from 1 to 2 weeks of staining, however not significantly. The difference in mean ΔE of water after 1 and 2 weeks of staining, for the three surface treatments was significant ($p=0.001$). For water, WSL was significantly different than the control ($p=0.004$) and ICON ($p=0.003$; Fig 3).

Significant interactions were observed between duration of stain, surface treatment, and type of stain for the six-week demineralization group ($p=0.003$) (Tables 4 & 5). ΔE for T2 was significantly higher than T1 ($p<0.001$). Significant differences in ΔE were observed within the surface treatment group for all surface treatments and within all stain types for all interactions ($p<0.001$) except energy drink vs. whitening ($p=0.955$), energy drink vs. water ($p=0.439$), and whitening vs. water ($p=0.349$). All three-way interactions were found significant ($p<0.001$).

Fewer significant changes were observed with increased stain time in the 6-week demineralized group than the 2-week demineralized group. Within the 6-week demineralization group, significant increases in staining were observed between T1 and T2 for the WSL with coffee (35.74±8.5 vs. 45.75±7.2; $p=0.006$), control with red wine (41.24±19.9 vs. 69.22±12.0; $p<0.001$) and WSL with red wine (45.43±11.5 vs. 55.21±12.7; $p<0.009$). Values for red wine, coffee, whitening, and energy drink increased for ICON from 1 to 2 weeks, however not significantly. Whitening, energy drink, and water had no significant effects with increased stain time (Fig 4).

Visual Examination

At T2, photographs were taken for specimens in each staining group (Appendix I). Overall, wine and coffee had the most darkening effect. Whitening appeared to have a mild lightening effect.

Coffee caused increased staining of the WSL compared to ICON or control (Fig 5). A wide variation of staining was observed within the coffee group for both 2-week and 6-week demineralized specimens (Fig 6). Coffee showed the greatest marginal staining effects around ICON infiltrated areas (Fig 7).

While red wine caused the greatest staining of the control and WSL, ICON showed significantly less staining (Fig 8). As with coffee, a wide variation of staining was observed within the red wine group for both 2-week and 6-week demineralized specimens (Fig 9).

Energy drink appeared to demonstrate a mild staining effect, particularly on ICON (Fig 10). Whitening treatment (Fig 11) and water (Fig 12) however, showed little staining. For both whitening and water, ICON remained similar to the control, while the WSL remained visibly whiter than the control. In general, ICON did not appear to fully mask the WSL in 6-week demineralized specimens for the whitening or water groups.

Microscopic Examination

Microscopic examination showed variation in the depth of WSL and ICON penetration. While only a limited sample set was sectioned, the WSL depth of 6-week demineralization specimens was greater than 2-week demineralization specimens. However, the range varied considerably and overlap existed between the two groups. 2-week demineralized specimens showed WSL ranging in depth from 142μm to 377μm, with ICON penetration ranging from 118μm to 225μm. Penetration depth for the 6-week specimens showed similar values ranging in depth from 222μm to 357μm for WSL and 110μm to 303μm for ICON. For thicker WSLs, ICON did not fully penetrate the entire depth of the WSL, leaving a demineralized WSL beneath the layer of resin infiltration (Fig 13).

The greatest staining effects occurred with wine, followed by coffee. While wine demonstrated considerable surface staining, it remained superficial and did not appear to infiltrate into the WSL, control or ICON (Fig. 14-17). Coffee staining followed a similar pattern (Fig 13). Energy drink staining was the only stain that appeared to penetrate into the control, rather than remain superficial (Fig 18).

Marginal leakage was only observed for staining with red wine, and occurred between the control and ICON (Fig 16) as well as between the control and WSL (Fig 17).

DISCUSSION

Several methods can be used to evaluate color changes in WSLs including quantitative light fluorescence, colorimeters, spectrophotometers, image analysis, and visual comparison using color shade guides. A visible-range spectrophotometer was selected for use in this study due to ease of clinical application, as well as its ability to provide objective color assessment and precise quantitative data within the full visible spectrum.[45,46] The spectrophotometer uses CIE L*a*b* values to measure the perceived color in three-dimensional color space. These L*a*b* values can then be used to evaluate the color change ΔE between two time points using the equation $\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$.[43] Because the intraoral spectrophotometer functions best on smooth surfaces, bovine incisors were used as specimens due to difficulties obtaining a large number of extracted human incisors with intact facial surfaces. The chemical and physical properties of bovine substrate, including composition, density and microhardness have been shown to be very similar to human enamel[47]and demonstrate similar behavior with staining.[48]

Davila et al.[49] first proposed the use of resin infiltration as a restorative treatment for WSLs nearly 40 years ago. However, it was not until more recently that advances in its technique and materials have resulted in an effective approach for treating WSL caused by demineralization.[50] Previous clinical studies using resin infiltration to mask WSL report good, yet mixed results; some lesions were fully masked, while others responded only partly or not at all.[35,37,39,40,51] This study found ICON® infiltration was able to significantly improve the appearance of WSLs for all 100 specimens. While ΔE values at baseline for ICON vs control were greater than the clinically differentiable value of ΔE>3.7, they were significantly less than those reported for WSL vs control (Fig 1). The results are consistent with the findings of *Paris et al.*[37] who reported in vitro infiltrated lesions showed significantly better color match with sound enamel than untreated controls.

With respect to demineralization time, ΔE values increased significantly for the ICON® group as demineralization time increased from 2-6 weeks, indicating that ICON® was less successful at masking lesions with longer demineralization times. This may occur as a result of lesion depth. As demineralization time increases, lesion depth is also expected to increase. Surface layer thickness of natural enamel lesions has been shown to vary considerably from 10-197μm, with mean thickness of 45μm.[52] Using optical and microradiographical scanning, *Brinkman et al.*[53] determined that WSLs may be up to several hundred micrometers deep. This study found a range in WSL depth from 142-377μm for 2-week demineralization specimens and 222-357μm for 6-week demineralization specimens. This range is likely due to variability in enamel porosity or remaining tooth pellicle, which may have prevented artificial demineralization from occurring evenly.

In naturally occurring lesions, demineralization and remineralization cycles would also result in a remineralized enamel surface layer, reported to be 20-60μm,[52,54] which can act to seal off the subsurface demineralized area and prevent resin infiltrants. This process relies primarily on capillary forces for penetration of the full depth of the WSL.[13,52] Sufficient pretreatment removal of this layer by acid etching is necessary to increase porosity and enhance resin infiltration to subsurface areas of the lesion body.[31,52] *Paris et al.*[55] found that while resin penetration depths of incipient lesions varied considerably, resin infiltration remained superficial with an average penetration depth of 18±11μm and did not extend into the deeper portions of the lesion. This study found ICON penetration depths ranging from 118-225 μm for 2-week demineralized specimens and 110-303μm with 6-week demineralized specimens. The current study did not use a remineralization cycle. Without a hypermineralized surface, specimens demonstrated much higher ICON penetration depths compared to those reported by *Paris et al.*[55] However, with thicker WSLs, ICON did not fully penetrate the entire depth of the WSL, leaving a demineralized WSL beneath the layer of resin infiltration, which impeded the ability of ICON to fully mask the lesion (Fig 13).

This study used the most up-to-date manufacturer's protocol for ICON® infiltration. Recently the manufacturer changed the recommended protocol to include a third cycle of etching with 15% hydrochloric acid gel for a total etch time of 6 minutes per lesion. This step was most likely added as an attempt to fully remove any remineralized surface area and allow for better penetration of the infiltrant into the body of the lesion. Previous studies by *Senestraro et al.*[40] and *Paris et al.*[37] were based on two etch cycles, while other studies only used a single 2-minute etch cycle.[42,56] While the current study did not include a remineralization cycle, manufacturer's protocol was followed with three etch cycles. Without a hypermineralized surface layer, we most likely had increased penetration of the ICON resin into the lesion.

To simulate prolonged clinical life of the specimens in this study, continuous immersion in staining solutions occurred for a period of two weeks. *Ertas et al.*[57] determined 24h of continuous immersion of a resin specimen in coffee solution was approximately equivalent to 1 month of daily coffee consumption, while *Ardu et al.*[58] concluded that 99 days of immersion was equivalent to resin staining over 8 clinical years. Using this conversion, the two weeks of continuous staining used in this study is roughly the equivalent of 14 months of stain exposure.

Coffee, red wine, and energy drink were chosen as staining solutions because of their frequent consumption. Coffee, red wine, tea and other beverages have been shown to cause discoloration of various aesthetic composite materials.[59-65] Red wine is reported to cause the most severe discoloration of composite materials,[60-62] while coffee exhibits a strong potential for staining both tooth structure and resin materials.[66,67] This study showed red wine had the greatest staining effect followed by coffee, for the control and WSL surfaces. With regard to ICON® infiltrated lesions, coffee demonstrated the highest staining effect, however ICON® was significantly more resistant to staining by all stains relative to control or WSL surfaces. The results are consistent with *Paris et al*[37] who reported resin infiltrated lesions demonstrated slight staining by tea and red wine, whereas polished infiltrated lesions were

resistant to staining in vitro. *Borges et al*[42] reported significant differences for surface treatment and dye after staining and repolishing, with immersion in wine and coffee resulting in increased ΔE compared with water.[42] Unlike other studies however, they reported higher staining with resin-infiltrated enamel, although polishing minimized the staining effect.[42] Another study examining the color stability of WSLs treated by resin infiltration immersed in saliva, coffee or wine for 4-8 weeks, showed significant changes in color and lightness values with ΔE wine>coffee>saliva.[41] The variability in staining noted within the red wine and coffee groups, may likely be due to the presence of an enamel pellicle remaining on some specimens after pumicing, which may have caused increased staining. Staining by energy drink demonstrated the ability to penetrate sound enamel, which is likely due to its acidic pH (Fig 18).

In this study, ICON® infiltrated lesions showed greater staining resistance and maintained ΔE values closer to the baseline control values. This is likely due to reduction in surface porosity, decreased roughness, removal of the oxygen inhibition layer, and hydrophobic nature of the resin. However, because the control and WSL showed heavy staining, resin infiltrated lesions were highly visible as they did not stain to match the control tooth structure (Fig 8). This is supported by the findings of *Cohen-Carneiero et al*[41] who warned that the staining resistance of ICON® may pose a significant esthetic disadvantage.

While many significant changes in ΔE occurred, L*a*b* values changed differently depending on the staining solution. In general, L* (brightness) was most affected by staining. Coffee staining caused L* to decrease over time, while a* and b* both increased (red and yellow respectively). L* and b* decreased with red wine, but it caused an increase in a*. Energy drink L* values decreased for the control and WSL, and increased slightly for the ICON group. These findings are expected based on the intrinsic color of the staining solutions.

Overall, staining significantly increased from one to two weeks for the control with regard to coffee and for the WSL with regard to coffee and red wine, with the greatest discoloration occurring

during the first week of staining. Staining by red wine and coffee remained primarily on the surface and did not penetrate the control, WSL or ICON. This is consistent with previous findings by *Chan et al*[65] who also determined that staining remained superficial, with penetration depths estimated between 3-5µm.[65] *Nasim et al*[63] found that a progressive increase in color variation could be expected with increase in immersion time. In this study, 2-week demineralization specimens showed similar staining values to 6-week demineralized specimens for the control and WSL groups, however staining by coffee and red wine showed a greater effect on 6-week demineralized ICON treated specimens. This is consistent with baseline data, which indicated a significantly increased color change between ICON infiltrated 2-week and 6-week demineralized specimens. Because ICON cannot fully penetrate the WSL in 6-week demineralization specimens, changes in the light refraction index caused by the demineralized area remaining beneath the ICON layer may cause staining by wine and coffee to have an increased effect.

External whitening solution was selected as a staining group because after orthodontic treatment many patients express an interest in whitening their teeth. The current literature contains no studies on the effects of external bleaching on resin infiltrated WSL, however several studies report perceptible color changes for composite resins bleached with various whitening agents as a result of the successful removal of surface stains.[61,68,69] While this study found mean increases in L* values for whitening of all three surface treatments, the only significant increase in ΔE over two-weeks occurred in the 2-week demineralized WSL group, which may be due to surface porosity.

The present in vitro study investigated the effects of various staining solutions on artificial white spot lesions treated with ICON® resin infiltration. Although resin infiltrated areas showed a general resistance to staining, specimens demonstrated the ability for significant color alteration between ICON treated areas and sound control enamel with regard to coffee and red wine. Red wine specimens also demonstrated the potential for marginal leakage (Figs 16, 17). Although this is unlikely to be unique to red wine alone, as the most potent stain used in the study, red wine was able to emphasize subtle

marginal leakage around the ICON restoration. While staining susceptibility may have been exacerbated by the use of continuous immersion and absence of tooth brushing, [64] patients should be aware of the potential for staining to occur over time. Further studies are recommended to assess the effects of microleakage at margins between infiltrated areas and sound enamel.

CONCLUSIONS

1. ICON® resin infiltration was able to significantly improve the appearance of WSLs in vitro.
2. Staining remained superficial and different staining solutions produced variable changes. The greatest staining occurred with red wine and coffee, while energy drink, whitening solution and water had little effect.
3. Resin infiltrated lesions are less susceptible to staining by coffee and red wine than WSL or sound enamel, thus the restorations will likely become noticeable when the surrounding tooth structure stains.

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Figure Legends

Figure 1: Specimen after resin-infiltration

Figure 2: Intraoral spectrophotometer (VITA Easyshade® Model #62011, Vident, Brea, CA)

Figure 3: Mean and standard deviation for 2-week demineralization specimens

Figure 4: Mean and standard deviation for 6-week demineralization specimens

Figure 5: Average staining for coffee. #1 and 10: 2-week demineralized group. #52 and 57: 6-week demineralized group. Resin-infiltrated areas shown on left incisal, WSL on right incisal, except for #57 (resin-infiltration performed on right incisal, WSL shown on left incisal).

Figure 6: Range of clinical staining for coffee. From left to right: darkest to lightest. #6 and 8: 2-week demineralized group. #54 and 58: 6-week demineralized group. Resin-infiltrated areas shown on left incisal, WSL on right incisal, except for #58 (resin-infiltration performed on right incisal, WSL shown on left incisal).

Figure 7: Effects of coffee staining on the margins (left:#10, right:#56). #10: 2-week demineralized group. #56: 6-week demineralized group. Resin-infiltrated areas shown on left incisal.

Figure 8: Average staining for red wine. #12 and 27: 2-week demineralized group. #62 and 63: 6-week demineralized group. Resin-infiltrated areas shown on left incisal, WSL right incisal.

Figure 9: Range of clinical staining for red wine. #11 and 15: 2-week demineralized group. #65 and 68: 6-week demineralized group. Resin-infiltrated areas shown on left incisal, WSL right incisal.

Figure 10: Average staining with energy drink. #33 and 38: 2-week demineralization group. #83 and 87: 6-week demineralization group. Resin-infiltrated areas shown on left incisal, WSL right incisal.

Figure 11: Average results of whitening treatment. #22 and 25: 2-week demineralization group. #77 and 80: 6-week demineralization group. Resin-infiltrated areas shown on left incisal, WSL right incisal.

Figure 12: Average staining with water. #42 and 48: 2-week demineralization group. #92 and 98: 6-week demineralization group. Resin-infiltrated areas shown on left incisal, WSL right incisal.

Figure 13: Coffee stained, 6-week demineralized specimen. ICON treatment surface only. Superficial surface staining present on ICON. Total WSL depth= 0.246mm with depth of ICON penetration= 0.110mm. Depth of WSL remaining underneath ICON= 0.136mm.

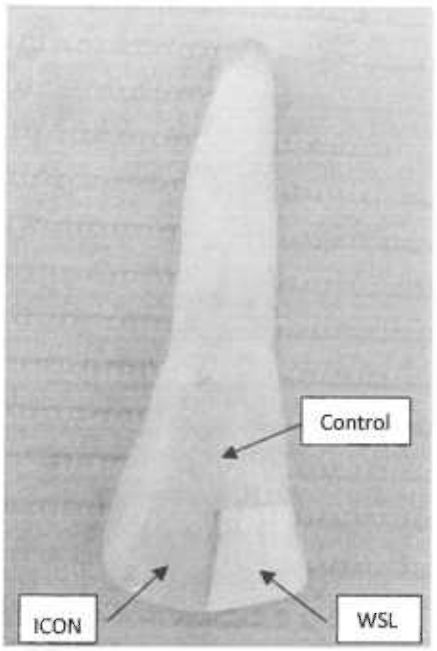
Figure 14: Wine stained, 2-week demineralized specimen. L: control, R: ICON. Arrow indicates margin between control and ICON. Surface staining present on control. Depth of ICON= 0.159mm.

Figure 15: Wine stained, 2-week demineralized specimen. L: control, R: WSL. Arrow indicates margin between control and WSL. Surface staining present on control and WSL. Depth of WSL= 0.377mm.

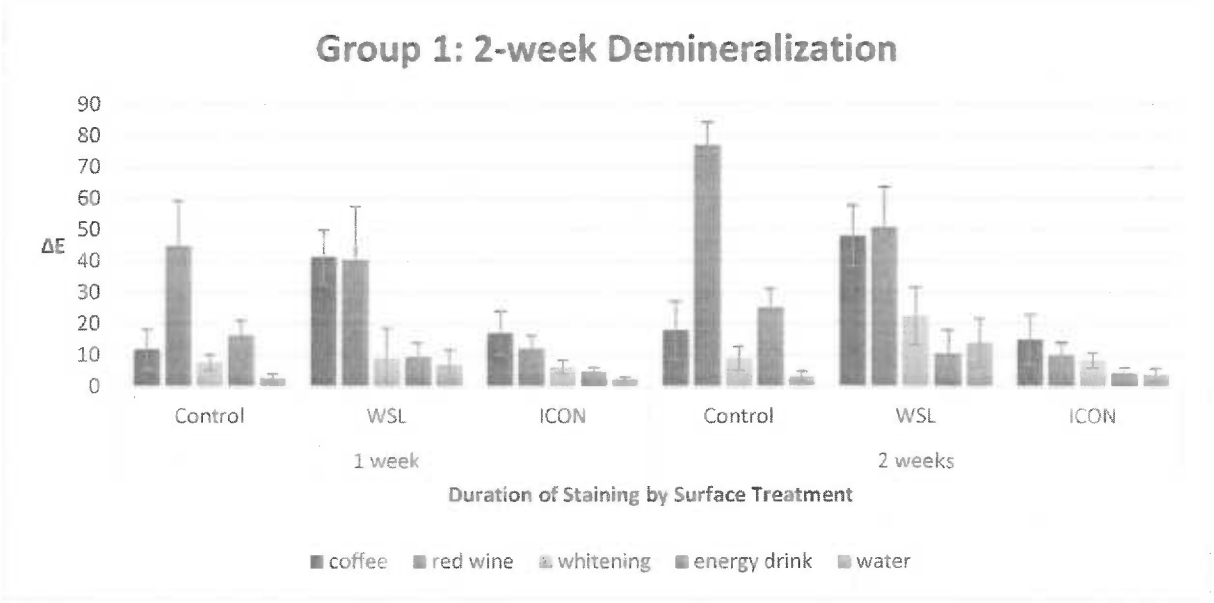
Figure 16: Wine stained, 6-week demineralized specimen. L: control, R: ICON. Surface staining present on control, lighter staining present on ICON. Depth of ICON= 0.222mm. Demineralized WSL band remains underneath ICON penetrated area. Arrow indicates slight marginal staining between control and WSL.

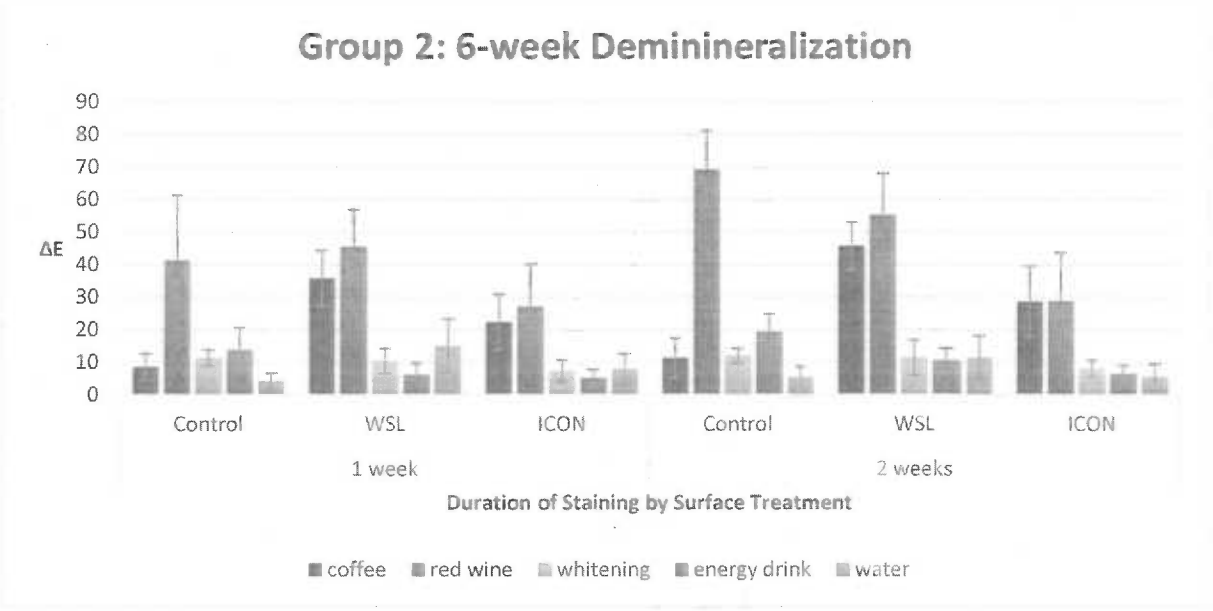
Figure 17: Wine stained, 6-week demineralized specimen. L: control, R: WSL. Surface staining present on control and WSL. Depth of WSL= 0.342mm. Arrow indicates marginal staining between control and WSL.

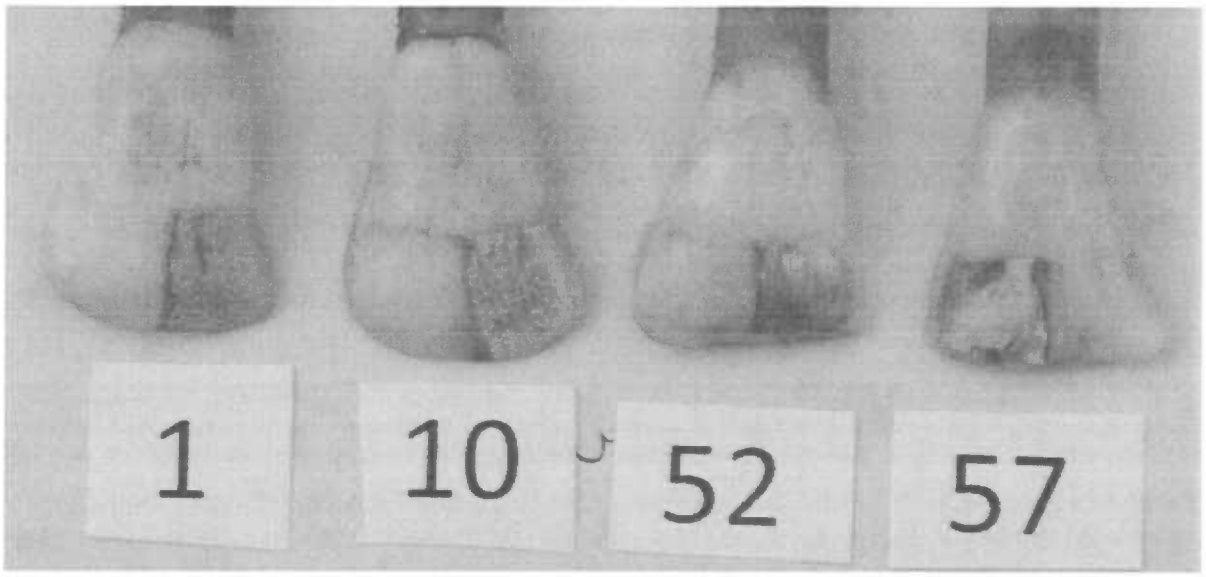
Figure 18: Energy drink stained, 2-week demineralized specimen. L: control, R: ICON. Staining present and penetrating into control. Depth of ICON= 0.118mm.

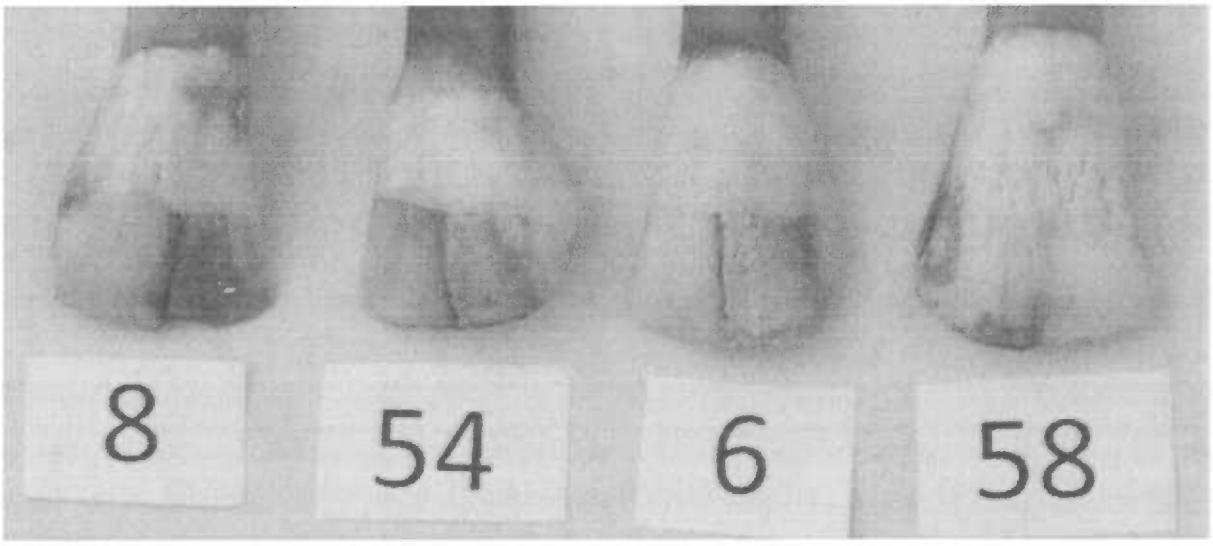

Control
ICON
WSL

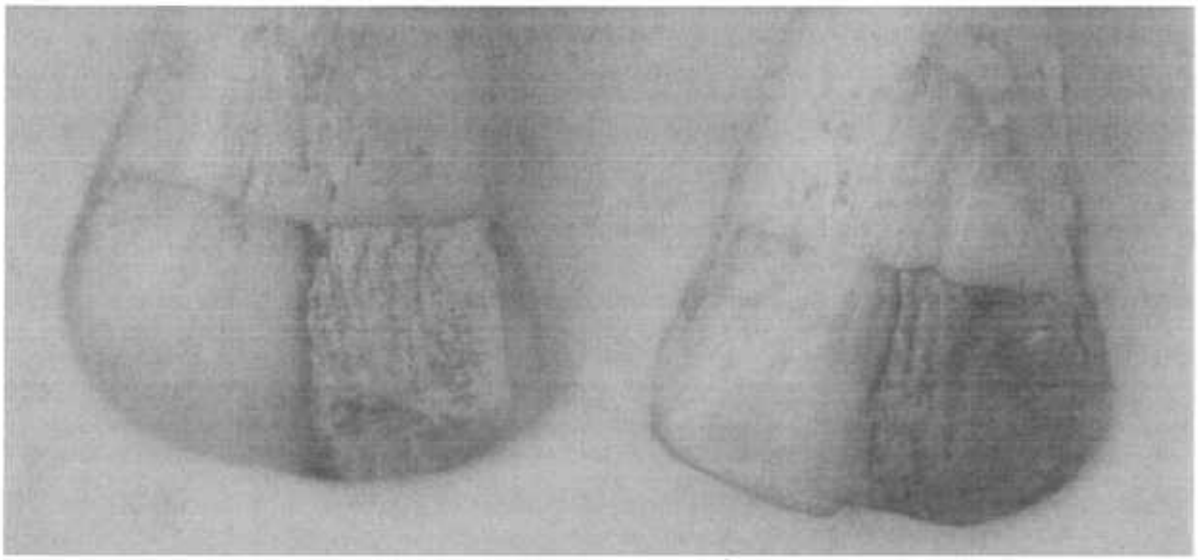

VITA Easyshade

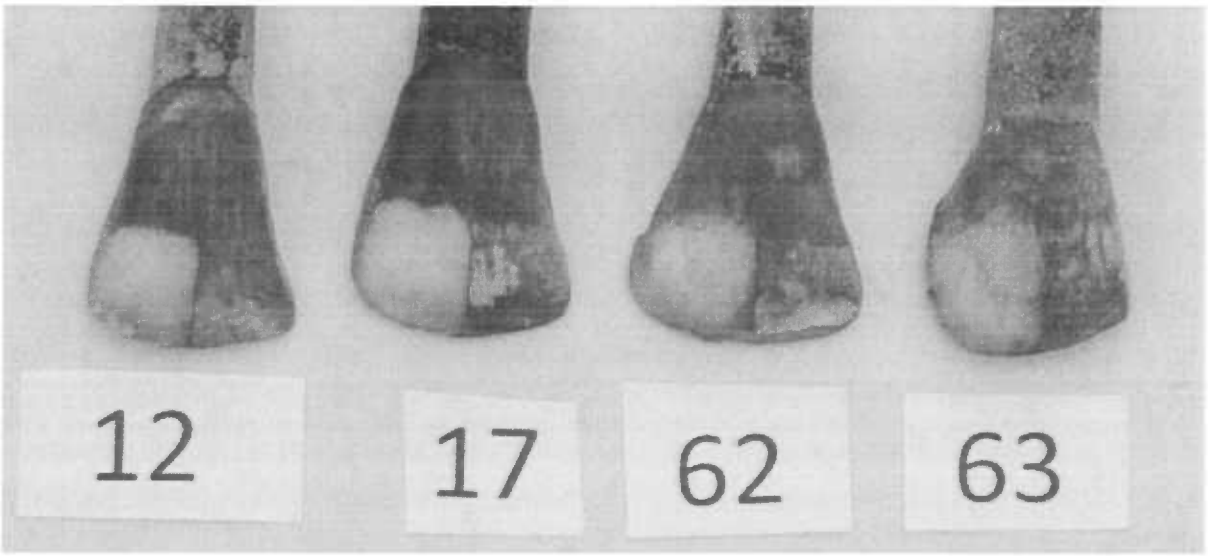

Group 1: 2-week Demineralization
ΔE
90
80
70
60
50
40
30
20
10
0
Control
WSL
ICON
Control
WSL
ICON
1 week
2 weeks
Duration of Staining by Surface Treatment
coffee
red wine
whitening
energy drink
water


Group 2: 6-week Demineralization
ΔE
90
80
70
60
50
40
30
20
10
0
Control
WSL
ICON
Control
WSL
ICON
1 week
2 weeks
Duration of Staining by Surface Treatment
coffee
red wine
whitening
energy drink
water


1
10
52
57


8
54
6
58




12
17
62
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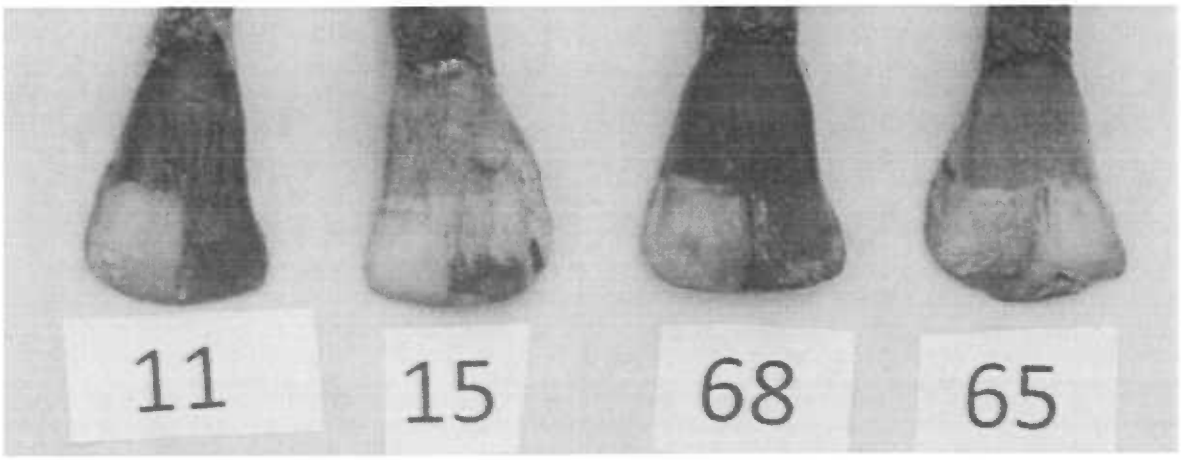
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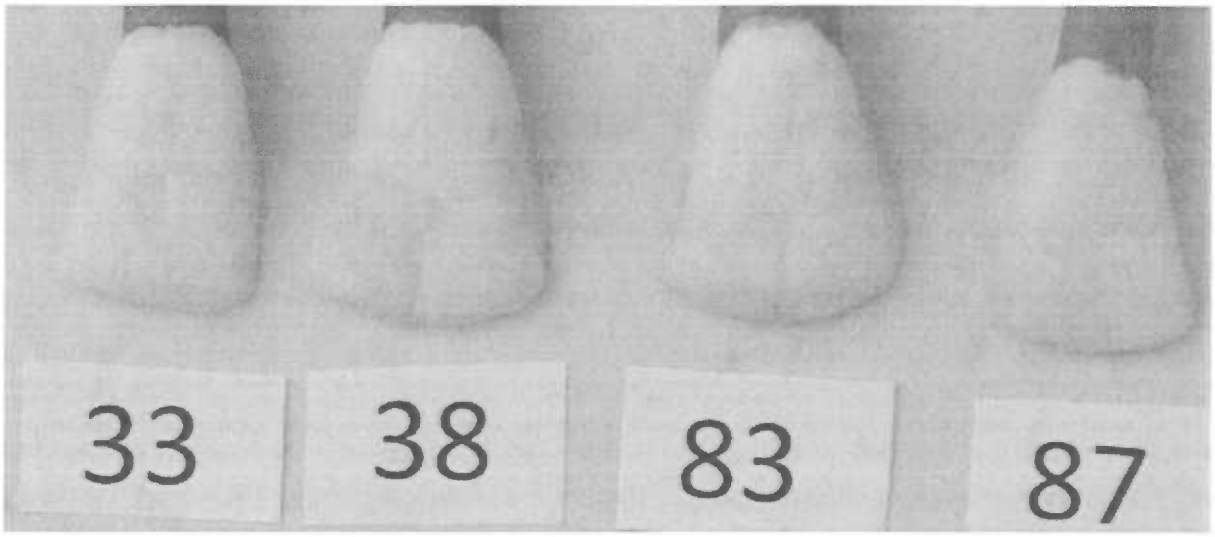
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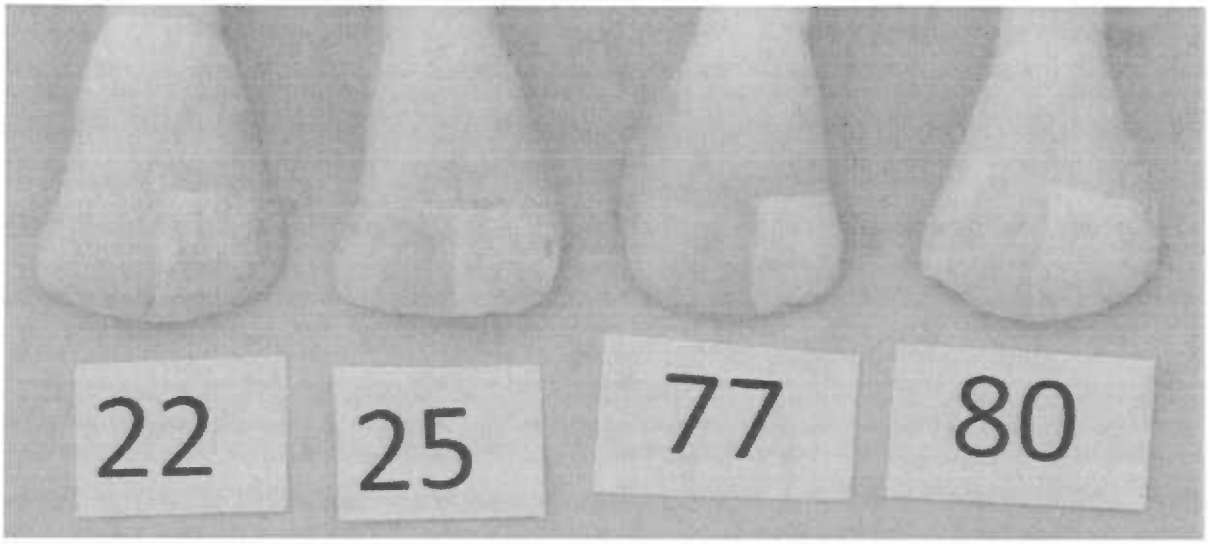
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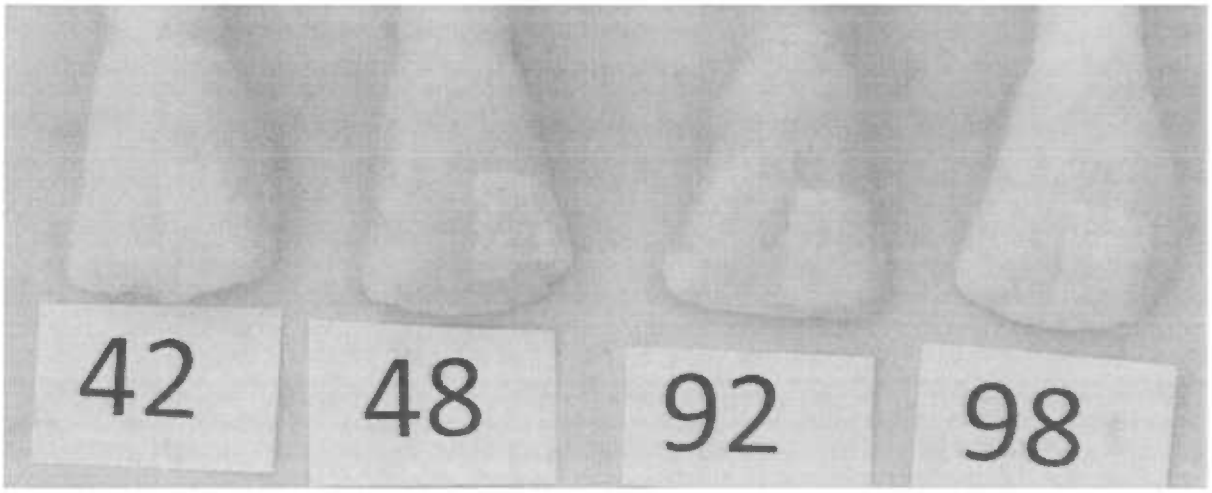
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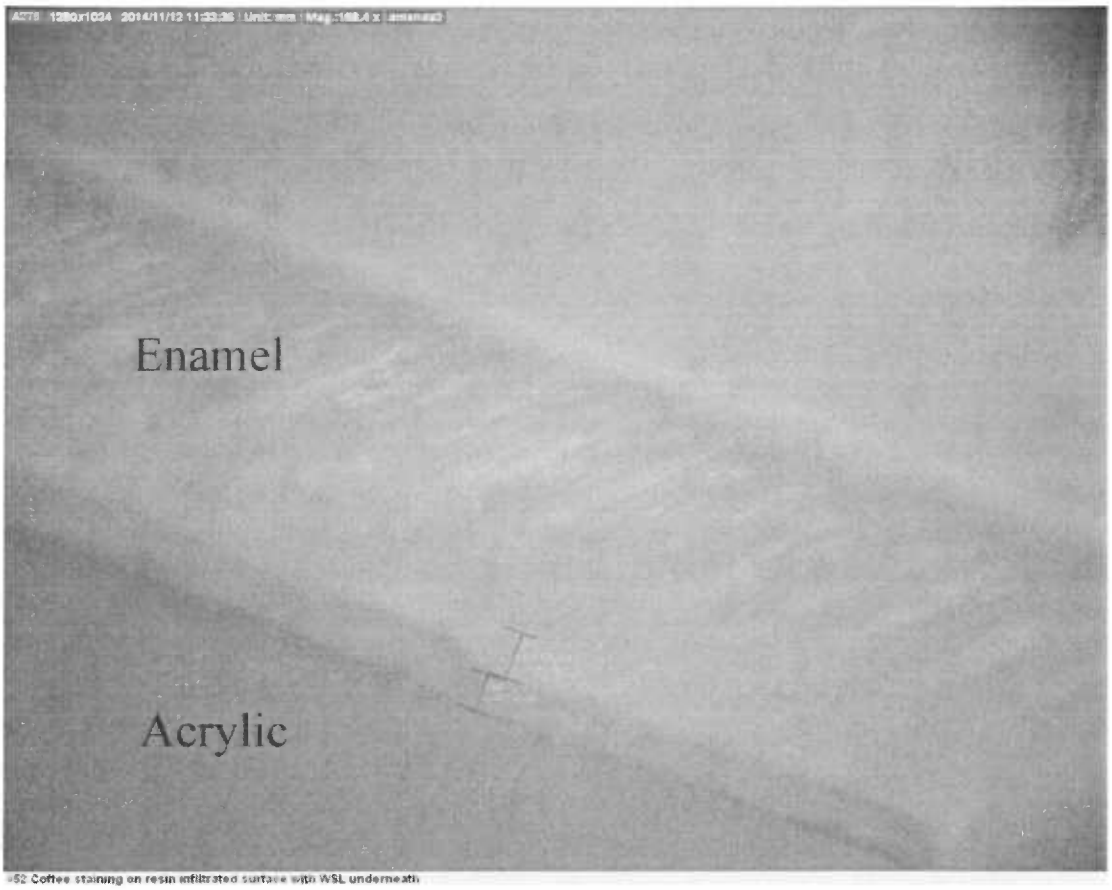
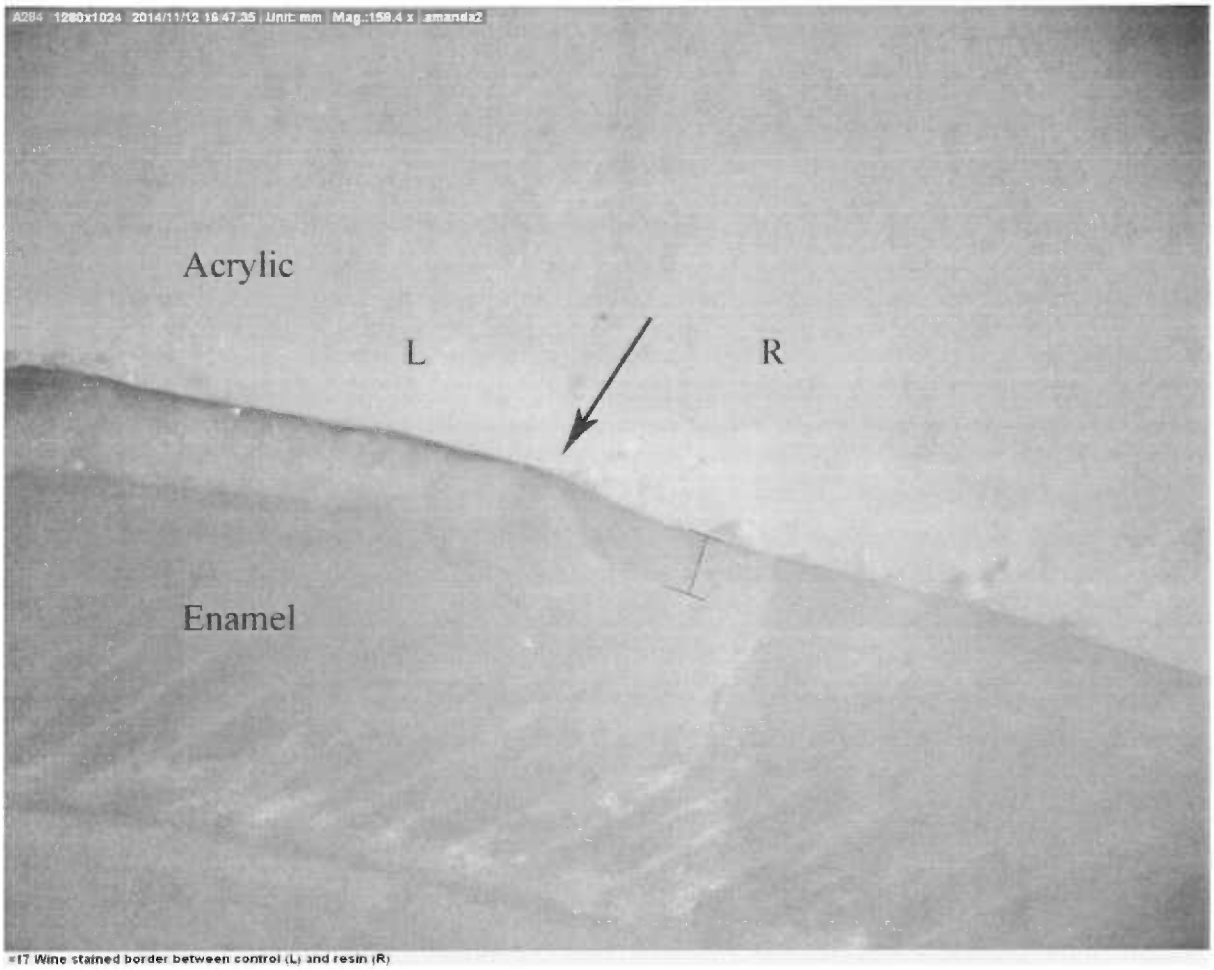
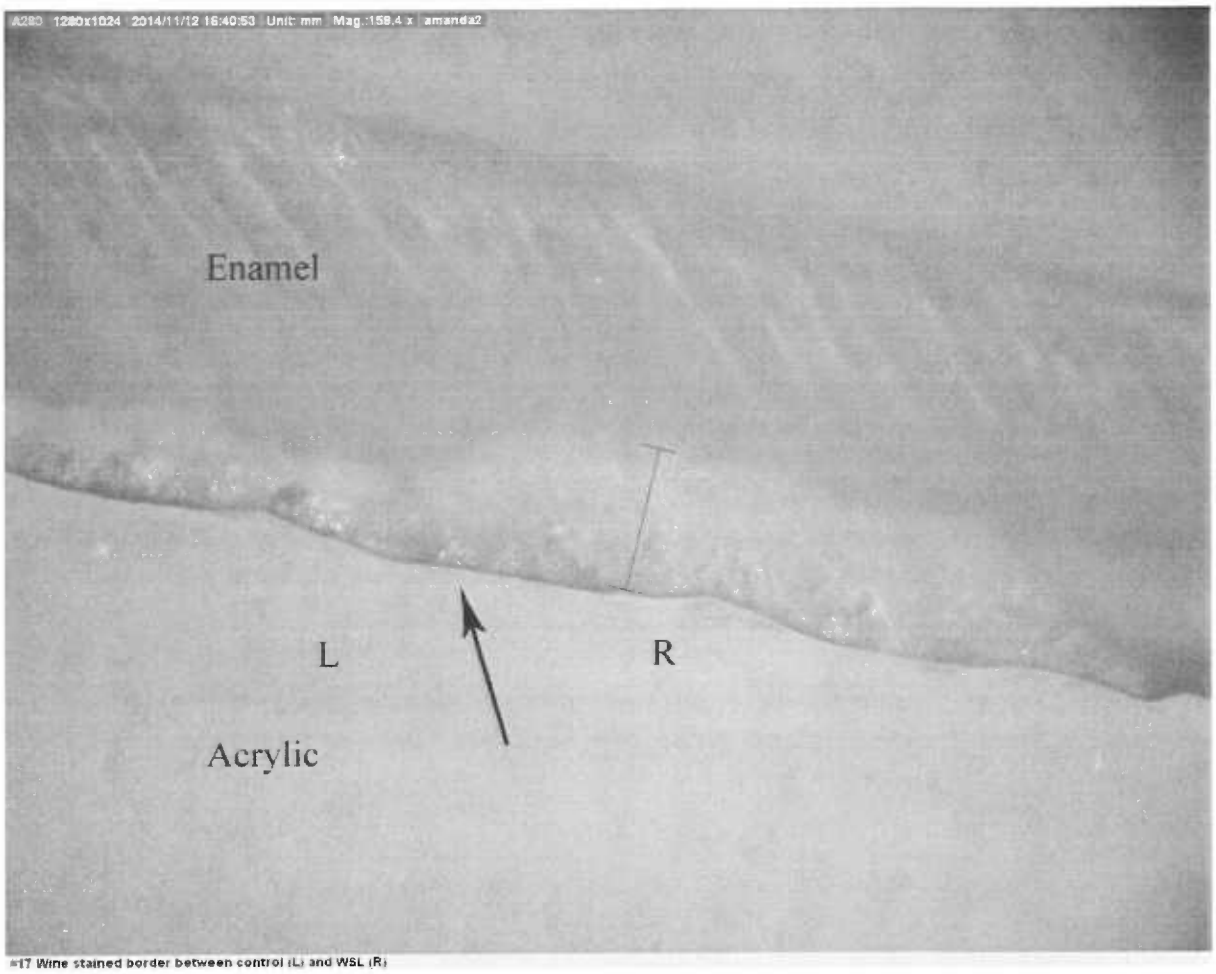


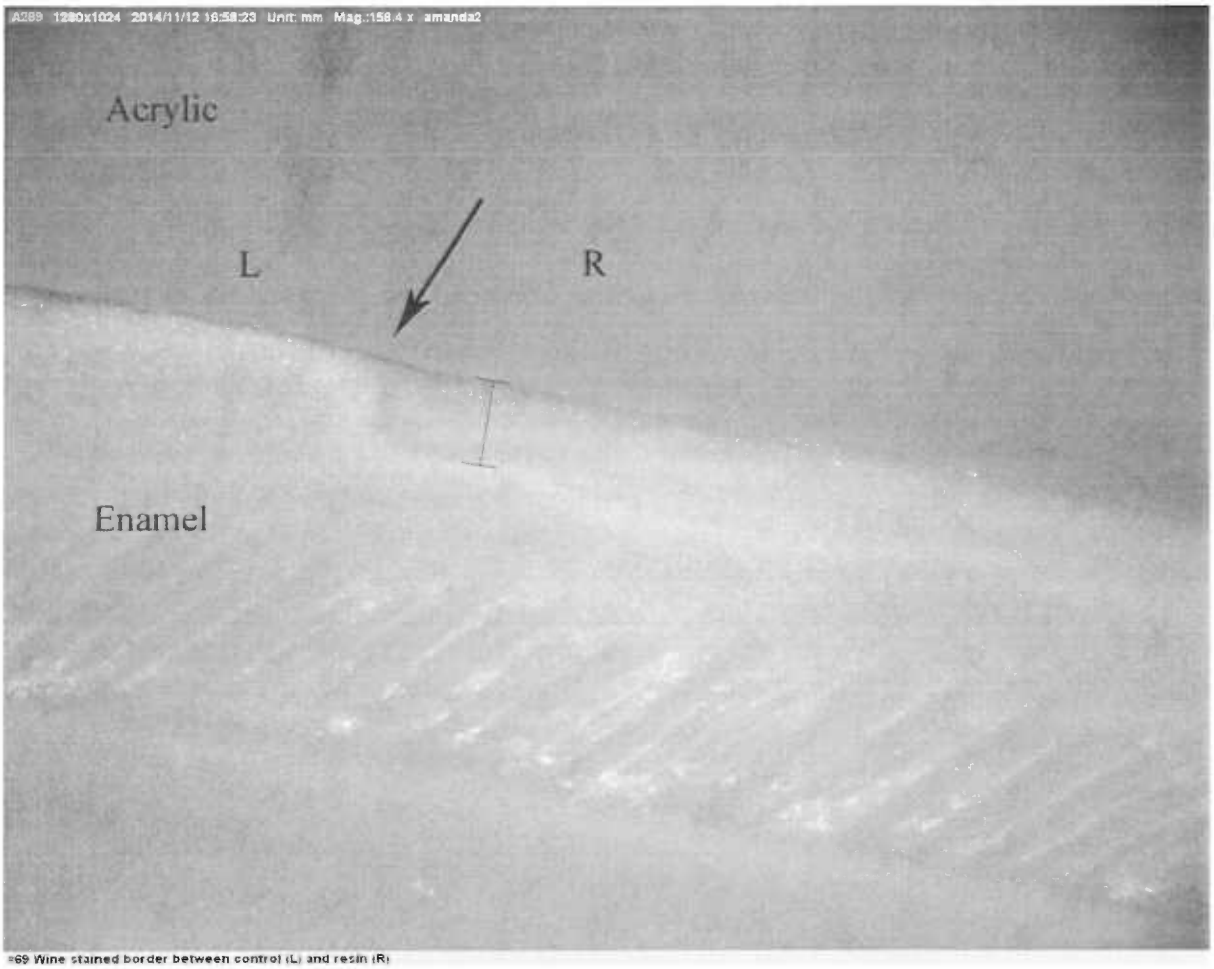
52 Coffee staining on resin infiltrated surface with WSL underneath



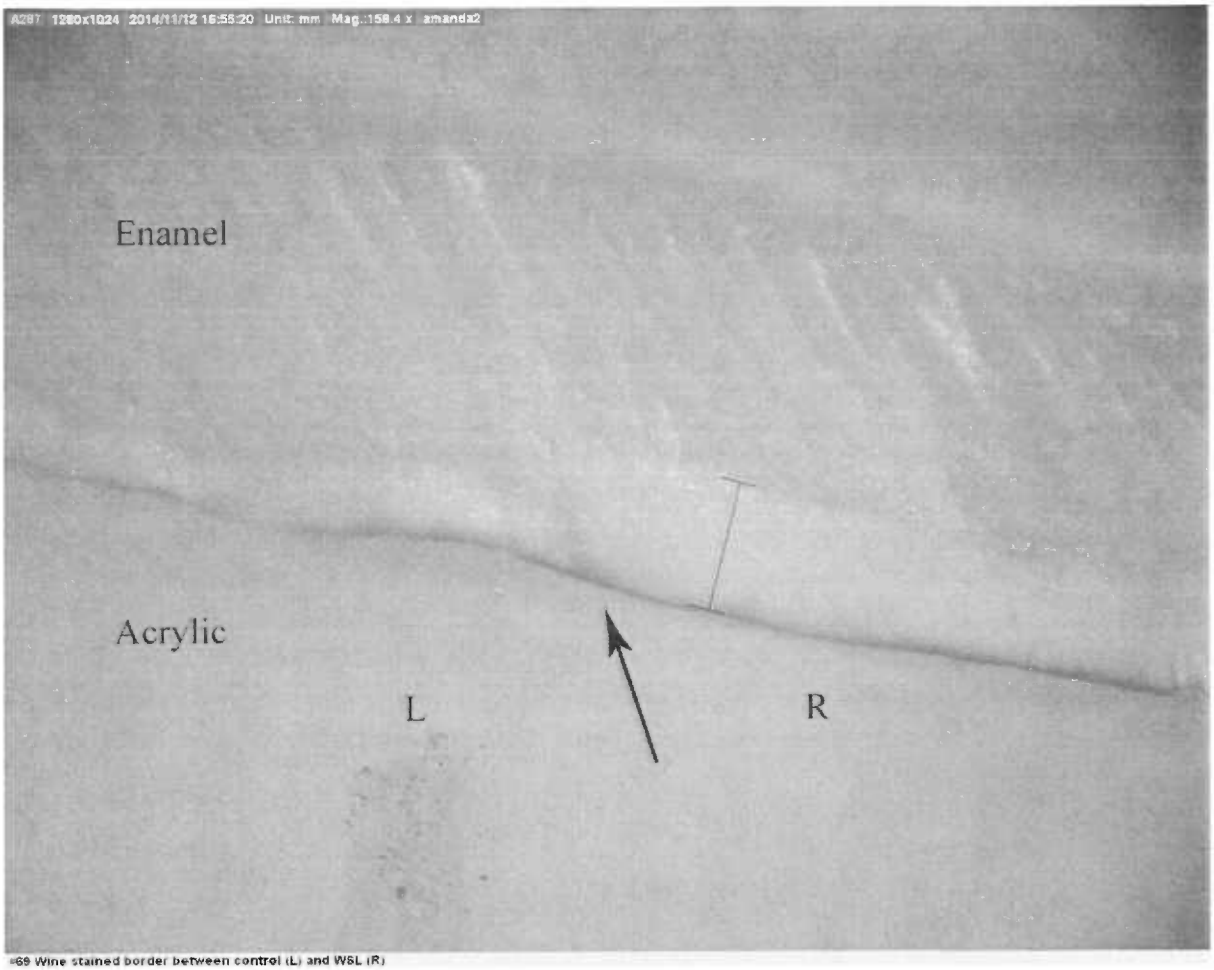
#17 Wine stained border between control (L) and resin (R)



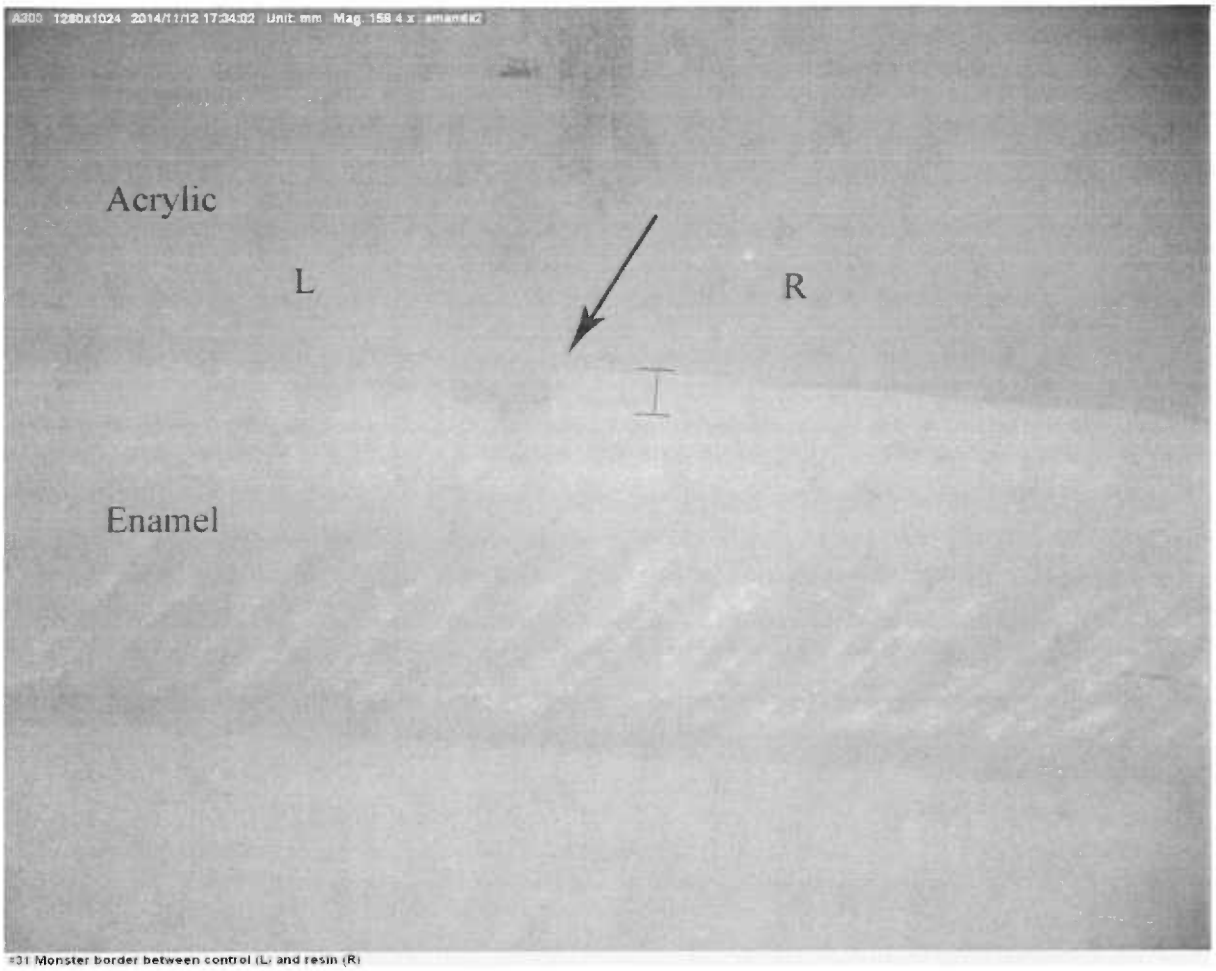
#17 Wine stained border between control (L) and WSL (R)



#69 Wine stained border between control (L) and resin (R)



#69 Wine stained border between control (L) and WSL (R)



#31 Monster border between control (L) and resin (R)

Table 1: Results of Tukey Test at baseline using mean and standard deviation. Superscript denotes significant differences found between ΔE, $p<0.05$. Lowercase denotes significance across rows, uppercase denotes significance within columns.

	ΔE (Control and WSL)	ΔE (Control and ICON)
2 week demin	$30.09 \pm 9.6^{a,A}$	$5.55 \pm 3.6^{b,A}$
6 week demin	$31.77 \pm 8.8^{a,A}$	$8.72 \pm 5.2^{b,B}$

Table 2: Results of Tukey Test with 2-week demineralization group after 1 week of staining using mean and standard deviation. Superscript denotes significant differences found between ΔE, $p<0.05$. Lowercase denotes significance within staining group (by rows), uppercase denotes significance within surface treatment (by columns).

		ΔE after 1 week of staining		
		Control	WSL	ICON
2-week demineralization group	coffee	$11.86 \pm 6.3^{b,B}$	$41.26 \pm 8.6^{a,A}$	$17.10 \pm 6.9^{b,A}$
	red wine	$44.76 \pm 14.5^{a,A}$	$40.30 \pm 17.0^{a,A}$	$11.95 \pm 4.2^{b,A,B}$
	whitening	$7.64 \pm 2.5^{a,B,C}$	$8.95 \pm 9.6^{a,B}$	$6.21 \pm 2.2^{a,B,C}$
	energy drink	$16.23 \pm 4.8^{a,B}$	$9.44 \pm 4.5^{a,b,B}$	$4.61 \pm 1.4^{b,B,C}$
	water	$2.61 \pm 1.4^{a,C}$	$6.78 \pm 4.8^{a,B}$	$2.36 \pm 0.6^{a,C}$

Table 3: Results of Tukey Test with 2-week demineralization group after 2 weeks of staining using mean and standard deviation. Superscript denotes significant differences found between ΔE, $p<0.05$. Lowercase denotes significance within staining group (by rows), uppercase denotes significance within surface treatment (by columns).

		ΔE after 2 weeks of staining		
		Control	WSL	ICON
2-week demineralization group	coffee	$17.95 \pm 9.2^{b,B}$	$48.06 \pm 9.7^{a,A}$	$15.00 \pm 7.8^{b,A}$
	red wine	$76.93 \pm 7.4^{a,A}$	$50.77 \pm 12.8^{b,A}$	$9.95 \pm 4.0^{c,A,B}$
	whitening	$8.92 \pm 3.8^{b,C}$	$22.37 \pm 9.2^{a,B}$	$8.30 \pm 2.4^{b,A,B}$
	energy drink	$25.14 \pm 6^{a,B}$	$10.43 \pm 7.7^{b,C}$	$4.22 \pm 1.8^{b,B}$
	water	$3.36 \pm 1.6^{b,C}$	$13.96 \pm 7.8^{a,C}$	$3.76 \pm 1.9^{b,B}$

Table 4: Results of Tukey Test with 6-week demineralization group after 1 week of staining using mean and standard deviation. Superscript denotes significant differences found between ΔE, $p<0.05$. Lowercase denotes significance within staining group (by rows), uppercase denotes significance within surface treatment (by columns).

		ΔE after 1 week of staining		
		Control	WSL	ICON
6-week demineralization group	coffee	$8.50 \pm 4.1^{c,B}$	$35.74 \pm 8.5^{a,A}$	$22.23 \pm 8.5^{b,A}$
	red wine	$41.24 \pm 19.9^{a,A}$	$45.43 \pm 11.5^{a,A}$	$27.06 \pm 12.8^{b,A}$
	whitening	$11.21 \pm 2.5^{a,B}$	$10.30 \pm 3.9^{a,B}$	$7.32 \pm 3.4^{a,B}$
	energy drink	$13.65 \pm 6.9^{a,B}$	$6.01 \pm 3.7^{a,B}$	$4.97 \pm 2.8^{a,B}$
	water	$4.09 \pm 2.5^{b,B}$	$14.92 \pm 8.2^{a,B}$	$7.82 \pm 4.7^{a,b,B}$

Table 5: Results of Tukey Test with 6-week demineralization group after 2 weeks of staining using mean and standard deviation. Superscript denotes significant differences found between ΔE, $p<0.05$. Lowercase denotes significance within staining group (by rows), uppercase denotes significance within surface treatment (by columns).

		ΔE after 2 weeks of staining		
		Control	WSL	ICON
6-week demineralization group	coffee	$11.22 \pm 6.0^{c,B,C}$	$45.75 \pm 7.2^{a,A}$	$28.53 \pm 10.8^{b,A}$
	red wine	$69.22 \pm 12.0^{a,A}$	$55.21 \pm 12.7^{b,A}$	$28.64 \pm 15.0^{c,A}$
	whitening	$12.02 \pm 2.2^{a,B,C}$	$11.31 \pm 5.5^{a,B}$	$7.95 \pm 2.5^{a,B}$
	energy drink	$19.25 \pm 5.6^{a,B}$	$10.43 \pm 3.8^{b,B}$	$6.30 \pm 2.5^{b,B}$
	water	$5.13 \pm 3.4^{a,C}$	$11.36 \pm 6.7^{a,B}$	$5.28 \pm 4.1^{a,B}$

LITERATURE REVIEW

OVERVIEW

Enamel white-spot lesions (WSLs) are a common sequelae of poor oral hygiene, often associated with fixed orthodontic appliances.[1,2] WSL development has been regarded as "the most important iatrogenic effect of fixed orthodontic appliance therapy".[3]

The formation of these visible, chalky-white, opaque lesions can compromise the esthetic goals of orthodontic treatment, as they predominately affect the labial surfaces of maxillary incisors.[1] Lesions generally demonstrate an apparently intact surface layer, with a subsurface porous lesion body. The lesion's opaque white appearance is due to scattering of light within the subsurface demineralized enamel.[4] In a small percentage of orthodontic patients, enamel demineralization can progress to the point of frank cavitation, which necessitates restoration.[1] To date, it can be difficult if not impossible to completely remineralize WSL.[5]

The prevalence of WSLs in orthodontic patients ranges considerably in the literature from 49.6%[1] to 97%[6] after bonded or banded orthodontic treatment. A recent study by *Richter et al*[7] reported 72.9% of post-orthodontic patients developed a new WSL during treatment, with an incidence of 2.3% for cavitated lesions. *Richter*[7] asserted that "this widespread problem is alarming and warrants significant attention from both patients and providers."

Enamel demineralization and subsequent formation of WSLs can occur whenever bacterial plaque is retained on the enamel surface for a prolonged period of time.[1] The presence of fixed appliances during orthodontic treatment can lead to greater plaque retention on smooth tooth surfaces generally not susceptible to caries, decreasing oral hygiene efficiency, and thereby increasing the patient's susceptibility to WSL formation.

WSLs associated with subsurface enamel porosities are caused by a cyclical imbalance between demineralization and remineralization, resulting from the acidic environment created by cariogenic

bacteria.[8] Formation of these lesions can occur quickly, with the first clinical signs detected as early as two weeks after initial biofilm formation.[9,10] Once orthodontic treatment has concluded and the fixed appliances removed, the cariogenic challenge ceases. Over time, remineralization at the outer surface of the lesion inhibits the penetration of calcium and other ions into the deeper parts of the lesion, arresting the remineralization process.[2,5,11] Regression of WSLs after removal of orthodontic appliances occurs predominately in the first three months owing to salivary remineralization and toothbrush abrasion; however lesions present after this time are likely to remain and complete regression does not occur for most lesions.[12-14]

With regard to preventative measures, the literature demonstrates considerable variation in its effectiveness. While there is general agreement that fluoride can reduce the occurrence and severity of WSL during orthodontic treatment and can improve remineralization of early WSL, there is little consensus on its effectiveness, method of delivery, or dose.[15] In their systematic review, *Benson et al*[15] concluded that there is a definite need for more high quality trials concerning WSL prevention protocols. WSL prevention remains an extremely complex and multi-factorial problem, involving prevention protocols, clinical factors, clinician factors, patient factors and their respective interactions.

There are a range of treatment options available to patients for post-orthodontic WSL after fixed appliance removal. These can range from reliance on natural remineralization alone to placement of a restoration. Salivary remineralization alone is highly variable ranging from continued demineralization resulting in cavitation[16] to complete regression of the WSL.[2] However, for the majority of patients, complete remineralization of WSL will not naturally occur.[2] Remineralization products including fluoride, casein phosphopeptide amorphous calcium phosphate (CPP-ACP), and a combination CPP-ACP with fluoride, have indicated mixed success in the remineralization treatment of PO WSL.[17-23] While fluoride or CPP-ACP have been shown to positively influence caries arrest, clinical studies have not shown a significant cosmetic improvement or considerable reduction of the carious lesions according to

the International Caries Detection System.[24,25] Recent studies provide in-vitro evidence that CPP-ACP with fluoride shows increased remineralization compared with CPP-ACP or fluoride treatments alone.[19,26,27] Restorative options for WSL treatment include acid micro-abrasion, composite restoration, veneers, and full coverage crowns. While microabrasion is effective for shallow WSLs,[28] it is technically demanding and can result in considerable enamel removal.[29,30] Likewise, restorative options involving composite or ceramic lead to substantial enamel loss, despite their excellent cosmetic results.[31,32]

Recently, resin infiltration has been evaluated as a treatment for PO WSL. Originally developed to arrest proximal caries lesions,[33,34] resin infiltration is a micro-invasive procedure involving penetration of a resin composite into the body of the WSL via capillary forces, with minimal removal of existing enamel.[35] The porous nature of active WSL allows a low-viscosity resin to permeate into the previously demineralized enamel matrix filling the voids with polymer. This creates a refractory index similar to healthy enamel, which results in improvement to the appearance of the lesion.[36] In vitro studies have shown successful masking of WSL using resin infiltration techniques.[33,37,38] Initial in vivo results have also shown immediate, reliable, and sometimes dramatic improvement of PO WSL.[39-41] In a clinical trial, *Kim et al*[40] found complete masking of PO WSL in 61% of patients with the resin infiltration technique, while 33% had partial masking. *Paris et al*[41] found that resin infiltration was able to reliably mask artificial WSL and demonstrated that polished infiltrated lesions proved resistant to staining influences. In a recent in vivo clinical trial by *Senestraro et al,*[42] resin infiltration was found to significantly improve the clinical appearance of PO WSLs, reduce their size, and maintain their stability over an 8-week period. Although resin infiltration has been used to restore interproximal caries for some time, research for its use in treating PO WSL is just beginning.

White Spot Lesions

White spot lesions are defined as subsurface enamel porosities caused by an imbalance between demineralization and remineralization.[43] WSL is a broad term that can include developmental

enamel lesions, localized areas of demineralization or caries in non-orthodontic patients, or localized areas of demineralization or caries related to fixed orthodontic appliances.[36] WSL associated with orthodontic appliances are not distinct types of carious lesions, rather they are a result of enamel demineralization at an early stage of the carious process occurring around fixed appliances.[36] *Fejerskov and Kidd*[44] defined WSL as the "first sign of a caries lesion on enamel that can be detected by the naked eye." The characteristic opaque white appearance of the WSL is due to subsurface demineralization and its subsequent optical changes within the body of the lesion, primarily caused by differences between the refractory index of healthy enamel and that of demineralized enamel.[8,36,45] When a light photon enters sound enamel, it travels an average distance of 0.1mm before being scattered.[46] Because demineralized enamel is more porous than sound enamel, voids in its crystalline structure are partially filled with water resulting in a decreased refractive index, higher light scattering, and a whiter appearance.[45] When the lesion is dried, the refractive index declines even more as the water is replaced by air, resulting in an even whiter lesion.[45] The surface of the WSL may appear rough or chalky with surface erosion that can be detected tactilely compared with noncarious white spots that are generally smooth and shiny.[8,44]

Based on their appearance, WSL can be clinically assessed and categorized further as active or inactive lesions.[47] Active WSL have a whitish/yellowish opaque surface with loss of luster, rough appearance, and increased porosity. Inactive WSL are more yellowish/brownish than white with a shiny surface that appears smooth.[47,48] Its distinct color and texture, along with distribution pattern, shape, stability over time, and association with fixed orthodontic appliances facilitate discernment between developmental opacities and orthodontic WSL.[8] As stated by *Lovrov et al,*[49] despite improvements in materials and preventative efforts, orthodontic treatment continues to carry the considerable risk of enamel demineralization.

White Spot Lesion Etiology

Demineralization during orthodontic treatment is primarily a dietary carbohydrate and saliva-modified bacterial infectious disease, much like that of the smooth surface proximal carious lesion.[8] When bonded to the facial surfaces of teeth, fixed orthodontic appliances create greater surface area for plaque adherence and an increased oral hygiene challenge.[36] Similar to those found interproximally, this niche is more difficult to mechanically clean and provides an area for carbohydrate retention. Meanwhile, the irregular shape of the appliance itself limits the self-cleansing ability of the saliva, lips, tongue and cheeks. Together, these obstacles can lead to increased risk of incipient caries on the smooth facial surfaces of teeth not usually prone to carious attack.[36]

Acidogenic bacteria, specifically *Streptococcus mutans* and *Lactobacilli*, have long been identified as the primary causative agents in the initiation and progression of the caries process.[8,50,51] Mutans streptococci is significantly associated with caries prevalence and caries increment.[50,51] By creating increased retention sites for plaque and carbohydrate accumulation, fixed orthodontic appliances promote the colonization of bacteria and change the diffusion properties of the plaque matrix itself.[8] Studies have shown a significant increase in proliferation of the mutans streptococci and lactobacilli species in patients undergoing fixed orthodontic therapy, and also corresponds with the number of orthodontic attachments applied and overall duration of treatment.[52-54] This bacterial increase results in a caries progression that is more rapid than in patients without fixed appliances.[52] Interesting to note, a recent study reported reduced incidence of WSL in orthodontic patients treated with lingual appliances where the tongue and saliva flow are able to provide better self-cleansing of the tooth surfaces adjacent to the fixed appliances.[55]

Streptococcus mutans and *Lactobacilli* are both acidogenic and aciduric; they produce acid and thrive in an acidic environment, as well as synthesize extracellular glucans from sucrose that shield the bacteria from the buffering effects of saliva and allow the pH to drop.[8] At pH 5.5 remineralization and demineralization are in equilibrium. Below pH 5.5, saliva is undersaturated relative to enamel, resulting

in a net diffusion of calcium and phosphate from the enamel to the oral environment resulting in demineralization.[11,56,57] Without regular mechanical removal of the biofilm, the tooth surface is exposed to an acidic environment for a prolonged period of time leading to a shift in the demineralization/remineralization cycle towards a net mineral loss as the biofilm adapts to favor more acidogenic and aciduric bacteria.[8,44,58] Under such a cariogenic challenge, enamel lesions progress quickly from initial demineralization, to noncavitated carious lesions, to frank cavitation.[52,58] Using optical and microradiographical scanning, *Brinkman et al*[59] determined that WSL may be several hundred micrometers deep. Even within 4 weeks, WSL with a depth of 100μm may develop under orthodontic bands.[60] Upon removal of the cariogenic challenge via hygiene or appliance removal, the surface of these lesions are preferentially remineralized in the presence of calcium and phosphate from the pellicle and saliva.[11] Unfortunately, surface remineralization can decrease or eliminate sufficient access for ions to remineralize the subsurface lesion, resulting in an arrested WSL.[11]

Development of WSL is multifactorial and complex involving patient behavior and physiology, preventative measures, clinician behavior, and clinical factors, amongst others.[8] Not all individuals share the same caries risk, and individual host factors include salivary flow and composition, enamel solubility, immune response, genetic susceptibility, diet, and medication history.[36] WSL have been shown to be associated with treatment time, salivary flow, bacterial load, specific bacterial species, preventative measures, hygiene and diet.[8]

White Spot Lesion Prevalence/Incidence

There exists considerable variation in the literature regarding the prevalence and incidence of WSL. *Benham et al*[61] reported prevalence ranges from 2%-96% due to variation in sample size, assessment method, presence of pre-existing decalcification, and use of prevention methods including fluoride supplements during treatment. In a more recent study by Julien et al the prevalence of WSLs based on post-treatment evaluation ranged from 0-97%, with the reported prevalence after controlling

for pretreatment decalcification ranging from 26%-89%.[62] In a 1982 study, *Gorelick et al*[1] compared WSLs in a control group of 50 children with 121 orthodontic patients reporting the percentage of WSL to be 24% and 49.6% respectively. *Ogaard et al*[21] found an 88% prevalence at the time of appliance removal in patients treated without fluoride application, while *Richter*[7] reported 72.9% of 350 orthodontically treated patients developed at least one new WSL with a 2.3% incidence of cavitation. While the presence of WSLs are usually assessed using visual examination or photographs, *Boersma et al*[6] used quantitative light-induced fluorescence (QLF) to better detect WSLs immediately after removal of fixed appliances, as QLF detects decalcification before it is visible to the naked eye. They reported a 97% prevalence post orthodontics, with the number of WSL detected by QLF far outnumbering that found by visual examination, however the distribution pattern was similar for both groups.[6] In contrast, with rigorous OHI and fluoride applications, *Lovrov* et al[49] reported a 24.9% prevalence of WSL post-orthodontics.

Post-orthodontic WSLs most commonly occur between the gingiva and fixed appliance on the facial surface of the tooth.[1] In most cases, lesions are small and restricted to thin bands surrounding the bracket bases or to areas extending between the bracket and gingival margin.[5] As with prevalence, there is considerable variation reported in the distribution of WSLs. In non-orthodontically treated patients, *Gorelick*[1] reported the highest incidence of WSL naturally occurred among the maxillary incisors at 7%. Post-orthodontics, *Gorelick* found the highest incidence occurred in the maxillary lateral incisor at 23%, followed by the mandibular canine and mandibular first premolar at 18%, and the lowest incidence of 1%-3% in the maxillary posterior segment. No incidence of WSL were found on the lingual surfaces of mandibular canines and incisors after prolonged use of a bonded canine-to-canine retainer.[1] *Boersma*[6] reported 30% of the buccal surfaces in a person were affected. On average, in males 40% of surfaces and in females 22% showed white spots ($p < 0.01$). *Ogaard*[5,60] found that generally the first molars (51% maxillary, 48% mandibular), upper lateral incisors (25.5%) and lower canines and first premolars (28.4-

29.5%) are the teeth most affected by WSL. While the incidence for maxillary lateral incisors is high, *Ogaard* reported only 9.8% of maxillary central incisors developed WSL. *Heymann et al*[36] reported that maxillary anterior teeth are most commonly affected, with the order of incidence being lateral incisors, canines, premolars and central incisors. Overall, it is generally agreed that maxillary lateral incisors and canines along with mandibular canines and premolars experience a relatively high WSL incidence compared to other teeth, excluding first molars.[1,12,60,63]

White Spot Lesion Prevention

The best predictor for WSL development during orthodontic treatment is the presence of visible plaque and mutans streptococci around fixed appliances.[21] Fixed appliances predispose teeth to increased biofilm accumulation by introducing new retention sites in the mouth and altering the site ecology. Both the number of orthodontic attachments and overall duration of treatment can influence this process. Since plaque is the primary cause of WSL, regular mechanical removal of this biofilm is critical.[8] Good oral hygiene and fluoride have a synergistic effect on caries development in orthodontic patients.[21] A recent study by *Julien et al*[62] found that patients who exhibited fluorosis before treatment were significantly ($p<.001$) less likely to develop new WSLs during treatment compared to patients without pretreatment fluorosis. This is consistent with the finding that caries in general occur less frequently in populations exhibiting obvious fluorosis.[64]

It is generally agreed upon that fluoride treatment is the most effective agent in preventing WSL in orthodontic patients.[5,8,15,49] Regular use of fluoride toothpastes alone have been shown to be insufficient at inhibiting WSL development around orthodontic brackets.[65,66] Daily mouthrinses with sodium fluoride (.05% or 0.2%) and/or weekly acidulated phosphate fluoride rinses (1.2%) have been found to reduce the incidence of demineralization in patients during active treatment.[2,65] Fluoride varnish has also been shown to be effective at preventing WSL.[67] *Todd at el*[68] found that teeth treated with fluoride varnish exhibited 50% less demineralization than control teeth. A systematic review by

Benson et al[15] indicated that the use of a fluoride-releasing glass ionomer cement for bracket bonding may reduce the occurrence and severity of WSL during orthodontic treatment, however the evidence is weak and more clinical studies are needed.

Oral hygiene and patient compliance are major factors in WSL prevention. *Geiger et al*[69] reported that only 13% of 206 patients rinsed daily with fluoride as requested, and 42% followed the fluoride rinse protocol every other day; all of which had significantly fewer lesions than those who rinsed less frequently. While *Chang*[8] recommends risk assessment, regular OHI, dietary education and daily fluoride treatments, the systematic review by *Benson et al*[15] recommends daily 0.05% sodium fluoride rinses as the most effective way to prevent WSL. Overall, more high quality clinical trials regarding WSL prevention are needed.

White Spot Lesion Treatment

Several studies have followed the changes in WSL after the removal of fixed appliances. Evidence indicates that post appliance removal, demineralization ceases and the WSL can naturally regress to some extent.[8,12,70] This arrest can be attributed to several factors related to the elimination of the cariogenic challenge, including physical removal of overlying acid-producing plaque and improved accessibility of saliva.[8] Both remineralization and toothbrush abrasion have been shown to contribute to the partial amelioration of PO WSL. [12,60,70,71] However, clinical evidence indicates that the majority of WSL regression is primarily due to natural and toothbrush surface abrasion of the partially dissolved enamel surface of the WSL rather than from natural remineralization. [8,12,70] Polishing or abrasion of the dull and irregular external surface enamel leads to exposure of more tightly packed enamel crystals which give a harder and glossier clinical appearance.[70] However, with surface abrasion and natural remineralization, the relatively well-mineralized surface layer may form a diffusion barrier against the subsurface uptake of salivary minerals. This prevents passage of ions into the deeper layers of the lesion, resulting in an arrested WSL that may remain an aesthetic problem for years after treatment,

especially in advanced WSL. [8,60,70] *Al-Khateeb et al*[14] used quantitative laser fluorescence to evaluate longitudinal in vivo changes of PO WSL and found that remineralization partly contributes to WSL regression, primarily in the first three months following appliance removal, and remineralization ability decreases over time. *Willmot*[25] reported a wide variation in the response of naturally occurring PO WSL regression; lesions consistently diminished in size during the six months immediately following the cessation of orthodontic treatment, and lesion area reduced by approximately one-third after 12 weeks and half after 26 weeks.

Fluoride

The use of fluoride to treat PO WSL remains controversial. While it is generally agreed that fluoride can promote enamel remineralization, there is little consensus as to its effectiveness, method of delivery, timing or dose. *Willmot*[25] found no difference in PO WSL regression between lesions treated with a low fluoride (50ppm) toothpaste versus a non-fluoride mouthrinse/toothpaste regime, suggesting this level of fluoride was insufficient to improve WSL. Toothpastes with higher fluoride concentrations (1,500-5,000ppm) have been shown to have greater ability to inhibit demineralization and promote remineralization.[72] Other studies report high concentrations of fluoride can remineralize the subsurface zone of incipient carious lesions.[73,74] Likewise, fluoride varnish has also been shown to improve remineralization of incipient lesions.[75,76] It is important to note, however, that while remineralization of the outer enamel layer increased, demineralization decreased in the inner enamel layer, indicating preferential mineralization of the outer enamel layer.[77] Some evidence suggests the use of higher concentration fluoride rinses results in greater remineralization of the outer enamel layer, which prevents ions from permeating into the subsurface areas of the WSL.[77,78] *Clarkson* found that high fluoride concentrations in the surface layer of sound enamel are depleted during WSL formation and appear to be redistributed into the subsurface body of the WSL.[79] During remineralization, high exogenous concentrations of fluoride appear to establish a larger concentration gradient, depositing

more fluoride into the surface zone of the lesion relative to sound enamel. [79] *Ogaard et al* warned against treating visible WSL with concentrated fluoride agents, since this arrests (hypermineralizes) the lesion and prevents complete repair.[2] *Willmot*[80] found that such arrested PO WSL lesions regress very little and frequently become stained and unsightly. To avoid arresting the PO WSL, several studies recommend low dose fluoride applications to enhance sub-surface remineralization.[25] In contrast to these findings, a recent study showed that 5000ppm fluoride showed greater remineralization of incipient caries than a 1500ppm fluoride concentration.[81] However, this study did not examine the distribution of mineralization within the lesion.

CPP-ACP

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is a recently developed remineralizing agent. This amorphous form of calcium phosphate (ACP) stabilized by a phosphopeptide from the milk protein casein (CPP) is deliverable as a caries preventive and mineralizing agent through mouthwash solutions, dental creams and commercially available chewing gum.[82] CPP is a ligand which can ionically bind up to 25 calcium ions, 15 phosphate ions and 5 fluoride ions per molecule. [82] CPP is important in stabilizing ACP and producing a highly water-soluble calcium phosphate phase.[18] *Cross*[57] summarized the proposed mechanism of anticariogenicity for CPP-ACP as one where "they localize ACP at the tooth surface, where ACP buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization. In effect the CPP-ACP complex behaves as a delivery vehicle for calcium and phosphate ions". [57] *Reynolds et al*[18] found that low concentrations of CPP-ACP in solution, could increase mineral deposition in enamel caries by 44% (0.1% CPP-ACP) to 64% (1.0% CPP-ACP) and with the concentration provided in commercially available chewing gum, mineral deposition increased 46%. Several other studies have shown significantly greater remineralization of incipient caries treated with CPP-ACP containing treatments including mouthrinse solutions, dental creams, and chewing

gum.[17,18,23,24,83-85] *Shen et al*[84] conducted one of the first studies examining the ability of CPP-ACP to remineralize enamel subsurface lesions using a human *in situ* model. They found the addition of CPP-ACP to sorbitol- or xylitol-based gum resulted in dose-related increases in enamel remineralization.[84] *Iijima*[85] later reported that gum containing 18.8mg of CPP-ACP per piece produced approximately twice the level of remineralization as sugar-free gum without CPP-ACP, and lesions remineralized with the mineral were more resistant to subsequent acid challenges. The literature also reports significantly more regression of PO WSL after CPP-ACP remineralizing treatment.[24] After directly comparing 0.05% sodium fluoride mouthwash, fluoridated toothpaste, and CPP-ACP dental cream regimens as treatments for PO WSL, *Andersson et al*[23] concluded that although all regimens could promote regression of PO WSL, CPP-ACP had more aesthetically favorable outcomes. A small number of recent studies however, report no significant differences in remineralization using CPP-ACP treatments.[20,43] In addition, there is some question about the ideal concentrations of calcium and phosphate that should be used to restore PO WSL. While low calcium, low phosphate and low fluoride concentrations provide an ideal environment for remineralization, *Garcia-Godoy*[11] warns that excessively high concentrations of CPP-ACP may lead to surface mineralization and WSL arrest by mechanisms similar to those proposed for high fluoride concentrations.

CPP-ACP + Fluoride

Recently it has been demonstrated that the CPP-ACP complex is able to incorporate fluoride ions, forming a CPP-stabilized amorphous calcium fluoride phosphate (CPP-ACFP) nanocomplex. Binding of fluoride results in minimal changes to the CPP structure compared to when it is bound to calcium and phosphate ions alone, making it an effective delivery vehicle for fluoride, calcium, and phosphate ions to the tooth surface. [57] Early data has supported the synergistic effects of fluoride and CPP-ACP. *Reynolds et al*[86] showed a dentifrice containing 2% CPP-ACP with 1100ppm fluoride was superior to CPP-ACP or fluoride treatments alone at remineralizing enamel subsurface lesion, while 2% CPP-ACP alone showed

similar remineralization to 2800ppm fluoride alone. Microradiography of the remineralized lesions demonstrated that fluoride only groups had predominately surface remineralization while CPP-ACP groups alone and in combination with fluoride "produced a more homogenous remineralization throughout the body of the lesion." [86] Another study found that CPP-ACP demonstrated a higher remineralizing potential when applied as a topical coating after the use of a fluoridate toothpaste.[26] Contradictory evidence however, is presented by *Pulido et al*[20] and *Beerens et al,*[43] who found no evidence of increased remineralization with CPP-ACP alone or in combination with fluoride. While early studies on CPP-ACP are promising, there remains limited independent research on the topic to date. In a systematic literature review, *Azarpazhooh et al*[87] concluded that "the quantity and quality of clinical trial evidence are insufficient to make conclusions regarding the long-term effectiveness of casein derivatives, specifically CPP-ACP."

Microabrasion

Microabrasion has many applications and has been widely used for the removal of superficial noncarious enamel defects.[88-90] This technique involves using a slurry of pumice or silicon carbide particles and hydrochloric acid to create surface dissolution of enamel defects,[91] and has been advocated for the removal of PO WSL.[92,93] However, few quantitative studies have assessed the success of microabrasion treatment in improving the cosmetic appearance of PO WSL, and its effectiveness has been mainly empirical and anecdotal.[94] *Murphy et al* conducted one of the first studies to quantify changes in PO WSL after application of the well-accepted 18% hydrochloric acid and pumice microabrasion technique previously described by *Croll et al* and *Welbury et al.*[80,94] The teeth were first cleaned with pumice and water using a rubber cup in a slow contra-angle handpiece and a rubber dam was applied for isolation. 18% hydrochloric acid was mixed with a fine pumice powder to form a slurry which was then applied to the buccal surface of each affected tooth and agitated into the tooth for 10 seconds before being washed off with an air-water spray. The cycle of acid pumice application,

agitation, and washing was repeated 10 times per tooth. Finally the tooth was washed for 30 seconds, treated with a 3-minute application of nonacidulated fluoride, and polished.[94] The results showed a significant reduction in the size of the demineralized lesion and marked improvement in its appearance, confirming that the microabrasion technique is effective for the cosmetic treatment of PO WSL.[94] Criticism of the hydrochloric acid and pumice technique relates to potential tooth-tissue loss, however *Murphy et al*[94] reported the precise amount of enamel removed by microabrasion did not appear to exceed 250 µm. Another recent study advocated use of a 37.5% phosphoric acid etching and pumice technique instead of the 18% hydrochloric acid mixture to minimize tooth-tissue loss during microabrasion.[95] While microabrasion is effective for shallow WSLs,[28] it is technically demanding and early reports suggest that it can result in considerable enamel removal.[29,30]

Restoration

Traditional restorative options for WSL include composite fillings, composite veneers, ceramic veneers, and full coverage crowns. While these options can provide an esthetic improvement, tooth preparations require considerable removal of sound enamel extending beyond the demineralized zone into dentin. Because PO WSL primarily affect a young patient population, the long-term prognosis of the restored teeth becomes a significant concern, and a less invasive restorative technique is preferable.[42]

Resin Infiltration

Resin infiltration has recently been marketed as a novel and minimally invasive restorative treatment option, bridging the gap between non-operative and operative procedures.[96] The clinical protocol involves etching of the PO WSL with a 15% hydrochloric acid solution to remove the outer layer of sound remineralized enamel, exposing the subsurface demineralized lesion body, and using a resin mixture with a high penetration coefficient to fill the lesion via capillary action.[35] The porous nature of the PO WSL allows the low-viscosity resin to permeate into the previously demineralized enamel matrix, displacing the air or water and filling the voids with resin of a similar refractory index as apatite

crystals.[36,41] This creates an overall refractory index similar to that of healthy enamel, reducing light scattering, and improving lesion appearance.[36,41] While the technique was originally developed and shown to be effective for the treatment of incipient carious lesions, it has more recently been proposed as a treatment for PO WSL. Icon™ (DMG America, Englewood, NJ, USA) is currently the only product on the market that uses this approach.[36]

Early descriptive studies dating back to the 1970s demonstrate the potential for infiltration of incipient lesions using low viscosity resins. *Davila et al*[97] concluded that the "plastification" technique using adhesive penetration "offers potential use in preventing, arresting, and restoratively infiltrating incipient proximal enamel lesions." Several in vitro studies further demonstrated the ability for artificial carious lesions to be infiltrated by polymerisable resins; including several commercially available adhesives and fissure sealants.[33,98-100] Resin infiltration was also shown to reduce further acid demineralization and lesion progression when teeth were exposed to a cariogenic environment in vitro.[97,98,101-103] *Martignon et al*[104] was one of the first to demonstrate reduction in the progression of proximal lesions infiltrated with a commercially available resin *in situ*.

Paris et al[105] found that while resin penetration depths of incipient lesions varied considerably, infiltration remained superficial and did not extend into deeper portions of the lesions. This incomplete infiltration can be explained by preferential remineralization at the enamel surface of incipient lesions, which seals off the subsurface demineralized area.[11] Because infiltration of enamel lesions is mainly driven by capillary forces, a remineralized surface layer can prevent resin penetration due to its relatively low pore volume.[106] Therefore, a major factor contributing to incomplete resin infiltration is inadequate removal of the remineralized surface layer. Sufficient pretreatment removal of this layer by acid etching is necessary to increase porosity and enhance resin infiltration to subsurface areas of the lesion body.[33,106]

Surface layer thickness of natural enamel lesions varies considerably from 10-197μm, with mean thickness of 45μm. The majority of lesions have a surface layer thickness between 30-40μm, with 29% exhibiting a surface layer thickness greater than 50μm.[106] In addition, the remineralized surface layer of PO WSL is more resistant to acid etching than adjacent sound enamel.[107] Two recent studies showed that etching with 15% hydrochloric acid gel for 2 minutes led to more effective surface layer erosion compared with 37% phosphoric acid gel.[105,106] While *Paris et al* reported no successful resin infiltration without pretreatment acid-etching, etching with 37% phosphoric acid allowed for superficial resin infiltration only, with an average penetration depth of 18±11μm. After etching with 15% hydrochloric acid gel, however, the mean penetration depth was significantly greater, with an average adhesive penetration depth of 58±37μm.[33] In both studies, WSL acid-etched with hydrochloric acid showed significantly greater surface layer reduction and subsequent increase in penetration depth.[34,106] While surface layers were not completely removed in all cases, likely due to variation in surface layer depth,[106] lesions with complete surface layer removal showed the greatest penetration depths.[34] Use of hydrochloric acid etchant coupled with improvements in the resin infiltrant material itself have led to overall greater resin penetration depths.[100]

Once sufficient access to the body of the lesion is obtained by etching away the surface layer, the lesion is desiccated and the applied infiltrant allowed to permeate into the lesion via capillary action. The penetration of an uncured resin into a porous enamel lesion can be described by the Washburn equation, where the penetration coefficient (PC) is dependent on viscosity, surface tension, and contact angle.[100,105] A comparison of commercially available adhesives with experimental resin infiltrants of varying PCs demonstrated that resins with higher PCs are strongly correlated to greater penetration speed and depth.[100,105,108] The addition of ethanol as an adjunct to desiccation with forced air, has been shown to more completely remove water in the porous structure and increase the capillary potential of the infiltrant. *Paris et al*[105] found the addition of ethanol decreased viscosities, surface tensions, and

contact angles of all monomer combinations, resulting in increased PCs. However, the addition of solvents should be approached with caution, as while ethanol can increase the PC of the resin mixture and improve penetration, high amounts may prevent complete polymerization of the material in deeper parts of the lesion where the intensity of the polymerization light is low.[105,109] A recent in vitro study concluded that resin infiltrants with high PCs, mainly consisting of triethylene glycol dimethacrylate, are capable of almost completely penetrating the enamel lesion body of natural carious lesions.[109] These recent advances in methods and materials for resin infiltration have made it possible to predictably and successfully infiltrate incipient lesions. While initial data reported penetration depths ranging from 20-60µm,[105] *Meyer-Lueckel*[109] reported penetration depths of 400-600µm and greater depending on the infiltrant and initial lesion depth.

A positive side effect of resin infiltration treatment is the cosmetic camouflage of incipient enamel carious lesions.[37,108] Because the low-viscosity resin displaces air and water as it fills the voids in the subsurface demineralized enamel, it creates a refractory index similar to healthy enamel, reducing light scattering and masking the WSL.[36,37,41] As a result, PO WSL lose their whitish appearance and more closely resemble sound enamel.[37] Several recent studies have demonstrated the ability for resin infiltration to be an effective treatment for improving or completely masking WSL.[37,38,40-42,110]

Paris et al[37] were one of the first to suggest the use of the resin infiltration technique as a novel approach to treat smooth-surface white spot lesions. In an extensive review, *Kielbassa et al*[108] also recommends resin infiltration for a minimally invasive approach for "improved esthetic outcome when used as a "masking" resin on demineralized labial surfaces with orthodontic patients." A case study demonstrated the ability for resin infiltration of PO WSL to completely mask less severe WSL; the visual appearance of moderate-severe WSLs was improved though they still remained visible after treatment.[110] *Torres et al*[38] found superior aesthetic results in an in vitro study, using resin infiltration to mask artificial white spots when compared with remineralization after fluoride application alone. More

recently, *Paris et al*[41] assessed the influence of various refractive indices of experimental and commercial infiltrants to mask the appearance of artificial WSLs. They found a moderate correlation between the refractive index and ΔE of infiltrated lesions, polished infiltrated lesions are more resistant to staining, and that the resin infiltration technique is suitable for masking artificial WSL. *Kim et al*[40] conducted one of the first in vivo clinical studies to demonstrate successful masking of PO WSL using infiltration treatment. Using a commercially available resin infiltration system (Icon™) to treat 18 teeth with PO WSL, they categorized 61% (11 teeth) as "completely masked", 33% (6 teeth) as "partially masked" and 6% (1 tooth) as unchanged.[40] Despite their small sample size, the results are encouraging with 94% of teeth demonstrating marked esthetic improvement after resin infiltration. In light of recent evidence and advances in resin infiltration technology, researchers are beginning to advocate the use of resin infiltration as a preferred alternative to traditional treatment modalities for PO WSL.[37,40-42,108]

Long-Term Wear/Durability

More recently, a clinical study conducted by *Senestraro et al*[42] confirmed that resin infiltration significantly improved the clinical appearance of PO WSL and remained stable over the eight-week study period, with a mean reduction in WSL area of 61.8% immediately after treatment, and 60.9% eight weeks later. A similar randomized long-term controlled in vivo study published by *Knösel et al*,[111] confirmed the esthetic improvement of WSLs using resin infiltration, and showed sufficient esthetic durability over a six-month period. While large and deep WSL lesions showed some improvement with resin infiltration, smaller and more superficially located lesions were treated more effectively from an esthetics standpoint. *Knösel et al*[111] recommended that the time between debonding and infiltration be as short as possible to minimize surface changes to the WSL from toothbrush abrasion, as resin infiltration becomes less effective once the porous enamel surface is smoothed and remineralized.

Because WSL occur in esthetic areas, they are exposed to significant wear challenges. *Belli et al*[112] conducted a study to evaluate the toothbrush wear resistance of artificial WSL following infiltration

with two different techniques. After 10,000 abrasion cycles, resin infiltration showed a vertical wear loss of 25.7μm±7.1μm against unabraded enamel, and 18.3μm±7.3μm against abraded enamel. Statistically higher abrasion depths were recorded after 20,000 abrasion cycles than at 10,000 cycles, with resin infiltration demonstrating a loss of 42.6μm±20.7μm against unabraded enamel and 27.3μm±10.0μm against abraded enamel. While both materials showed similar vertical wear loss of the restorative material after 20,000 abrasion cycles, the resin infiltration technique showed improved color matching, infiltration depths, and surface stability over the adhesive technique. *Belli et al*[112] also suggest that resin infiltration acts like pit and fissure sealants in that neither techniques prevent caries progression by fully infiltrating the lesion, but rather serve to isolate the lesion from acidic sources. Success in the sealing protocol is highly dependent on the retention of the pit and fissure sealant, which has demonstrated high retention rates in prospective clinical trials.[113-115] Because resin infiltration of WSL exhibits penetration depths that can exceed 400μm, retention of the infiltrant material is unlikely to pose a problem.[116].

Staining

While resin infiltration has been shown to effectively transmit the natural shade of the tooth because of its optical properties and unfilled nature, little is known how these color esthetics may change over time.[37] Discoloration of tooth-colored resin-based composites may be caused by intrinsic or extrinsic factors.[117] Intrinsic factors involve the discoloration of the resin material itself, by altering the resin matrix or matrix-filler interface with age under various physical-chemical conditions.[118] Extrinsic factors include adsorption or absorption of colorants from exogenous sources which can cause discoloration.[119] Using the CIE-L*a*b* color system, which records colorimetric parameters three-dimensionally, values for ΔE_{ab}*> 3.7 are considered to be clinically significant.[120]

Intrinsic factors

Color stability of resin composite is associated with resin matrix, filler size, degree of polymerization, and exposure to aqueous environment.[117,121] When composites are exposed to the intraoral environment, the hydrophilic portion of the matrix and matrix-filler interface absorbs water. This leads to separation at the matrix-filler interface and discoloration.[122] *Yamamoto and Takahashi*[123] reported higher degrees of water absorption are observed with resin infiltrants because they lack fillers. In addition, the main ingredient of resin infiltrants is hydrophilic triethylene glycol dimethacrylate (TEGDMA), which is subject to degeneration by temperature change in the oral cavity long term.[124] A recent study found the color of resin infiltrants changed significantly after thermal cycling, because the resin infiltrants absorbed water, however the color change was not clinically significant.[125] *Bak et al*[125] concluded that "resin infiltrated lesions showed recovery of CIE value close to that of healthy enamel over time."

Extrinsic factors

Several studies have been conducted to determine the effects of extrinsic staining on composite resin restorative materials. Red wine, coffee, tea and other beverages have all been shown to cause discoloration of various composite materials, with different types exhibiting different degrees of stain based on resin composition and filler type.[117,121,126-128] Overall, red wine is reported to cause the most severe discoloration of composite materials.[117,121,127] *Chan et al*[129] reported that while staining increased with time, the greatest discoloration occurred during the first week and extended to the second week. Staining remained superficial, with penetration depths estimated between 3-5µm. A recent study by *Paris et al*[41] examining the color stability of resin infiltrated WSL in vitro, indicated infiltrated lesions were only slightly stained by tea and red wine. They suggest that "reducing subjective visibility of white spots and preventing their staining using resin infiltration therapy is a suitable way of treating such lesions."

Surface finish is also reported to have a significant effect on composite stainability, however this remains controversial. While some studies indicate surface roughness of resin composites has a direct influence on its susceptibility to staining,[126,130] others report no such correlation.[131,132] In looking at the effects of extrinsic staining on resin infiltration of WSL, *Paris et al*[41] determined that polished infiltrated lesions are more resistant to staining in vitro. This is most likely due to reduction in surface porosity and possible removal of the oxygen inhibition layer. One must be cautious however, as abrasion of the resin infiltrant over time can lead to increased surface roughness and increased stain susceptibility.

Whitening

External bleaching therapies utilizing 3-35% hydrogen peroxide solutions or hydrogen peroxide releasing agents, including carbamide peroxide or sodium perborate, have been shown to safely and successfully whiten discolored teeth.[133-135] As a treatment approach for post-orthodontic WSL, external bleaching alone results in limited esthetic improvement and has been associated with tooth sensitivity and reduction in enamel microhardness.[136-138] At the conclusion of orthodontic treatment however, bleaching therapy to brighten discolored teeth has become an increasingly popular modality. The current literature contains no studies on the effects of external bleaching on resin infiltrated WSL. Several studies however, have examined the effects of external bleaching on various other resin composite materials.

A systemic review conducted by *Attin et al.*[135] examined the effects of peroxide releasing bleaching agents on dental restorative materials and restorations. They found mixed evidence regarding the effects of external bleaching on microfilled and hybrid composite resins regarding their surface roughness, porosities, microhardness, color change, and marginal integrity. A similar review by *El-Murr et al*[139] also concluded that "while many studies found potential changes in the physical properties of composite-resin restorations after bleaching, they could not demonstrate the clinical relevance of these changes and recommended further clinical research." Some studies warned that

bleaching therapy may increase the surface roughness of composites, leading to increased stain susceptibility after bleaching.[117,140] *Villalta et al*[117] reported that Esthet-X microhybrid composite and Filtek Supreme nanocomposite both returned to baseline with bleaching even after substantial discoloration by staining. While they found that various bleaching agents were effective at removing exterior staining on dental resin composites, they concluded that the color change after bleaching was most likely due to superficial cleansing of the composite, rather than an intrinsic color change. Perceptible color change has also been reported for composite resins bleached by 10% or 35% hydrogen peroxide solutions.[141,142] *Klukowska et al*[143] found that bleaching with 20% carbamide peroxide or 38% hydrogen peroxide had no effect on microleakage at the margins of Class V composite restorations. Care must also be taken when bleaching, as the composite restoration may no longer match the surrounding bleached tooth structure.

Studies to Date

The majority of esthetic studies to date have focused on abrasion, extrinsic staining, and external bleaching of various resin composites, however few studies have investigated their effects on resin infiltration. Previous resin infiltration studies predominately focus on its use to inhibit the progression of incipient carious lesions, rather than its potential cosmetic effects. PO WSL are essentially incipient carious lesions. While they may differ in etiology, location and progression, their major differences are that PO WSL are located in the esthetic zone and tend to regress naturally. As such, the current evidence on resin infiltration of incipient proximal carious lesions is applicable to PO WSL.[42]

Because WSL occur in esthetic areas, they are exposed to significant wear and esthetic challenges unlike the majority of incipient carious lesions. To date very few studies have evaluated the long-term esthetic outcomes for resin infiltration of PO WSL. There have been limited studies examining

abrasion and external staining, and no studies examining the effects of external bleaching on resin infiltration of PO WSL.

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Appendix I: Additional Figures

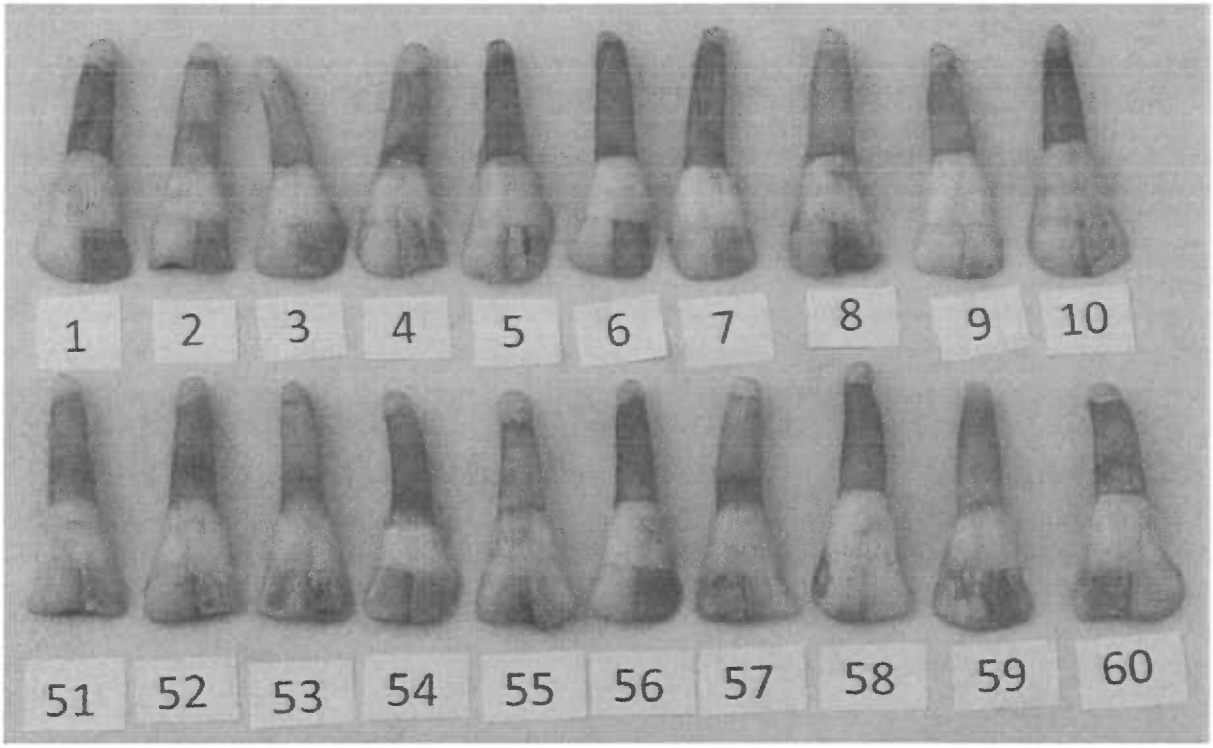

Fig A. Effects of staining with coffee. Top: 2-week demineralized specimens. Bottom: 6-week demineralized specimens. Resin-infiltrated areas shown on left incisal except for #57, 58, and 60 (resin-infiltration performed on right incisal, WSL shown on left incisal).

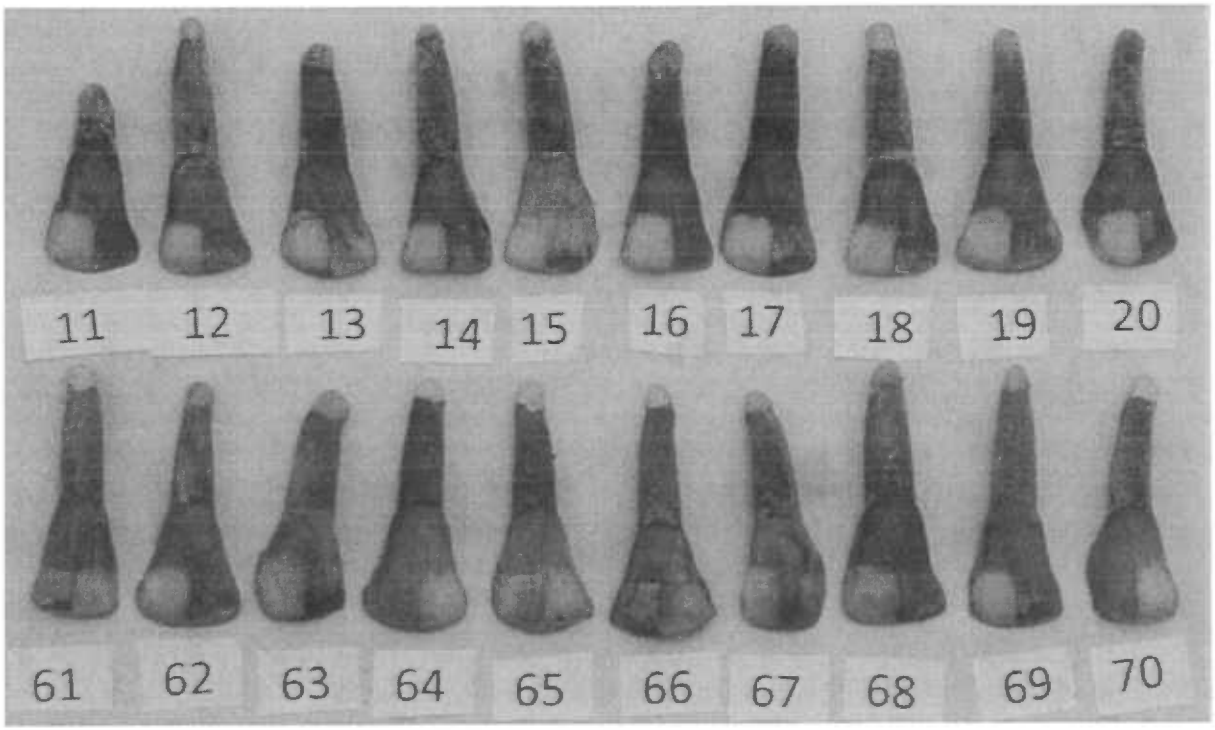

Fig B. Effects of staining with red wine. Top: 2-week demineralized specimens. Bottom: 6-week demineralized specimens. Resin-infiltrated areas shown on left incisal except for #61, 64, 70(resin-infiltration performed on right incisal, WSL shown on left incisal).

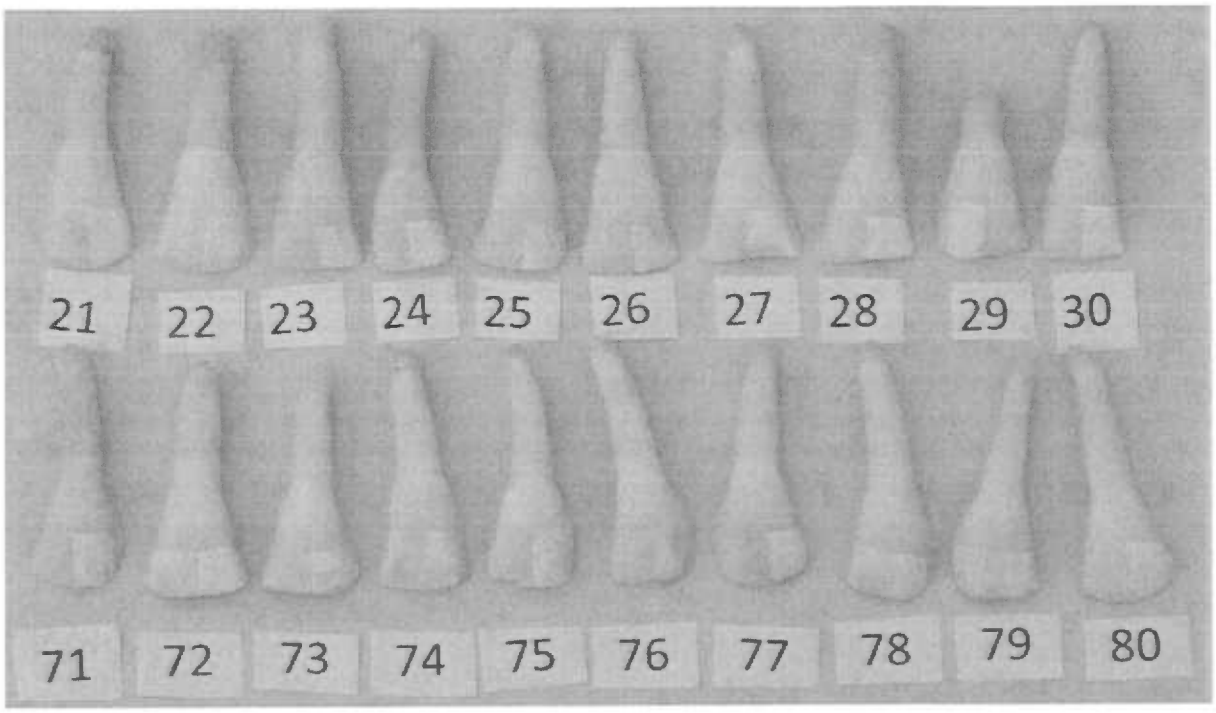

Fig C. Effects of whitening treatment. Top: 2-week demineralized specimens. Bottom: 6-week demineralized specimens. Resin-infiltrated areas shown on left incisal except for #29 (resin-infiltration performed on right incisal, WSL shown on left incisal).

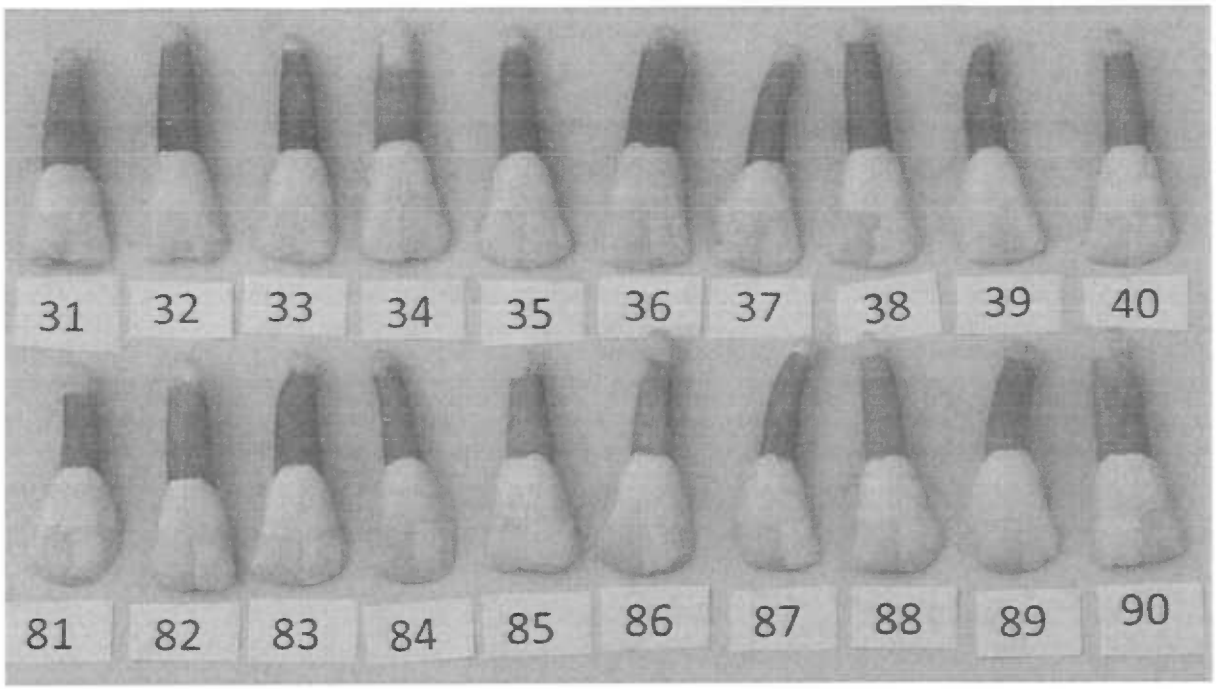

Fig D. Effects of staining in energy drink. Top: 2-week demineralized specimens. Bottom: 6-week demineralized specimens. Resin-infiltrated areas shown on left incisal except for #34, 90 (resin-infiltration performed on right incisal, WSL shown on left incisal).

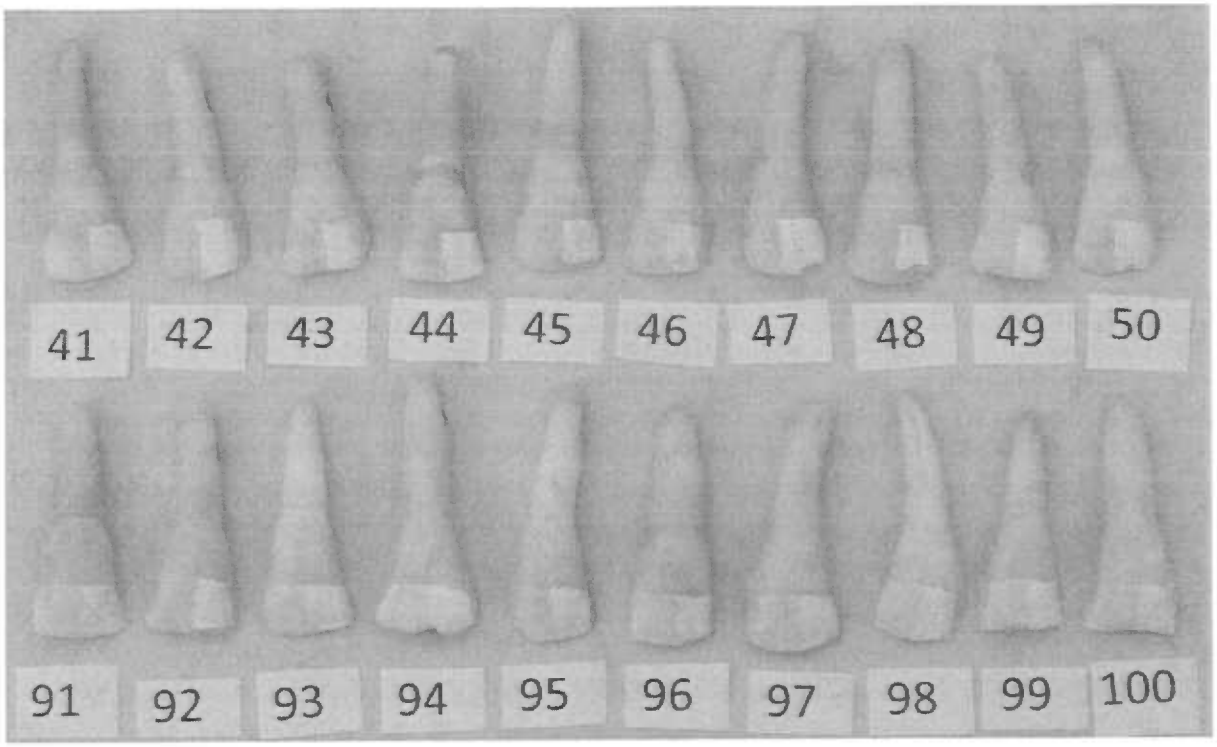

Fig E. Effects of staining in water. Top: 2-week demineralized specimens. Bottom: 6-week demineralized specimens. Resin-infiltrated areas shown on left incisal.