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Evaluation of Stain Penetration by Beverages in Demineralized Enamel Treated With Resin Infiltration

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Clinical Relevance

Clinicians are provided useful information about the staining potential of common beverages on resin infiltration–treated enamel surfaces.

SUMMARY

Purpose: To evaluate stain penetration by different beverages in artificially demineralized human teeth treated with resin infiltration.

Methods and Materials: Sixty extracted human permanent molars were demineralized, treated with resin infiltration (Icon), and immersed in four different beverages (coffee, grape juice, iced tea, and distilled water; N=15) for four weeks. After aging, teeth in the distilled water group were stained with 2% methylene blue for 24 hours. All teeth were sectioned, and stain penetration was evaluated under light microscopy. Chi-square test, independent and paired sample *t*-test, analysis of variance with the Fisher least significant difference *post hoc* test, and the Kruskal-Wallis test were used to analyze the results ($p<0.05$).

Results: Resin infiltration–treated surfaces (Icon surfaces) had statistically significant fewer samples with presence of stain penetration compared to untreated surfaces (control surfaces) ($p<0.001$). There was also a significant decrease in depth of stain penetration in Icon surfaces compared to the control surfaces ($p<0.001$). Among tested beverage groups, iced tea showed significantly greater depth of stain penetration (0.134 ± 0.029 mm), followed by grape juice (0.118 ± 0.047 mm), methylene blue (0.022 ± 0.019 mm), and coffee (0.008 ± 0.017 mm; $p<0.001$).

Conclusion: Both Icon and control surfaces exhibit stain penetration by different beverag-

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es (iced tea, grape juice, and coffee). However, resin-infiltrated enamel surfaces allow significantly less depth of stain penetration compared to untreated surfaces. The iced tea group presents greatest depth of stain penetration, followed by grape juice, methylene blue, and coffee.

INTRODUCTION

While the overall prevalence of dental caries in the United States has declined dramatically in the past few decades, dental caries remains the number one chronic childhood disease.[1,2] Historically, addressing the caries challenge has relied on prevention and restoration, with no intermediary means to stop lesion progression.[2] Recently, a technique called resin infiltration (Icon, DMG America, Englewood, NJ, USA) was introduced as a conservative way for arresting dental caries.

The concept of resin infiltration was first developed in Germany and later was brought into the US market in 2009.[2] The resin infiltration technique was developed with the aim of filling the noncavitated pores of an incipient lesion with a low-viscosity resin by capillary action.[2-4] Since porosities of enamel caries lesions act as diffusion pathways for acids and dissolved minerals, infiltration of these pores with light-curing resins might occlude the pathways and thus arrest caries progression.[5,6] In contrast to the application of sealants,[8] where the diffusion barrier remains on the enamel surface as a covering resin coat, the resin infiltration creates a diffusion barrier within the enamel lesion and thus prevents further caries progression.[4,5] In addition, resin infiltration has been proven to have high penetration depths up to 800 μm into lesion bodies, strengthen demineralized enamel, and withstand new acid challenges.[7,9] Resin infiltration is indicated for arresting incipient caries, postorthodontic white spot lesions, and noncavitated smooth surface lesions.[2]

In clinical dentistry, a commonly encountered problem in dental restorative materials is microleakage. Microleakage is defined as the passage of bacteria, fluids, chemical substances, molecules, or ions in the interface between the tooth and its restorative material.[10,11] Factors that can contribute to microleakage include polymerization shrinkage of restorations, different linear coefficients of thermal expansion from the tooth, water absorption, mechanical loading, and manipulation of materials by operators.[12,13] *In vitro* studies have shown that microleakage may cause marginal discoloration, hypersensitivity, recurrent caries, adverse pulpal response, and accelerated deterioration of some restorative materials.[11] This property has been used by many clinicians and researchers to predict the performance of a restorative material.

A common problem with resin materials is that, after months and years of use and exposure to a variety of different foods and beverages, the materials become stained.[14-16] This discoloration of restorations, which can be due to either extrinsic or intrinsic factors, is a frequent reason for replacement of resin materials.[17,18] Color stability of the infiltrated area can be a particular factor for the long-term clinical success and acceptability of teeth in the esthetic zone.[3,4] Intrinsic factors can involve the discoloration of the resin material itself as well as microcracks and microvoids located at the interface between the filler and matrix acting as penetration pathways for staining agents.[19,20] On the other hand, extrinsic factors for discoloration include staining by adsorption or absorption of colorants as a result of contamination from exogenous sources.[19,20] The degree of discoloration from exogenous sources varies according to individual oral hygiene; eating, drinking, and smoking habits; and the type of chromogenic bacteria.[19] The staining of polymeric materials by colored solution in the form of beverages (ie, coffee, tea, red wine, and cola), mouth rinses (ie, chlorhexidine), and medications has been reported in many studies.[14,19,20,21]

To ensure optimal restoration longevity and esthetics, it is necessary for a restorative material to minimize microleakage, maintain color stability, and be resistant to surface staining. The current body of literature dealing with microleakage in resin infiltration remains scarce. To date, there has been no published study investigating its stain penetration and microleakage potential when under chronic challenges by colored and acidic beverages. Therefore, the purpose of this *in vitro* study was to evaluate the extent of stain penetration by common beverages in artificially demineralized human teeth treated with resin infiltration.

METHODS AND MATERIALS

Preparation of Teeth

Sixty extracted noncavitated human permanent molars were collected, cleaned, and stored in 0.1% thymol solution prior to study to avoid dehydration. Apices of the teeth were covered with utility wax,

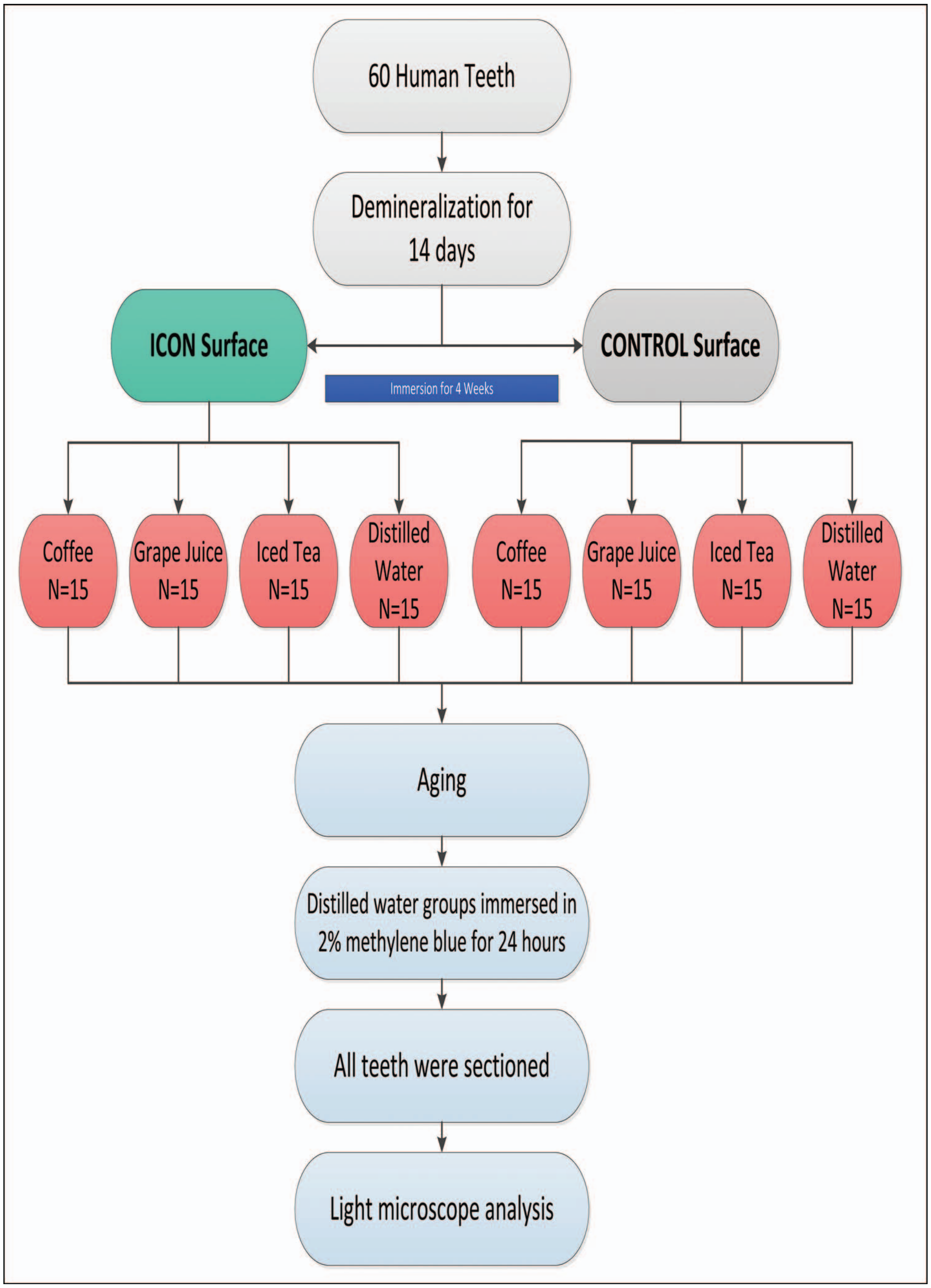

Figure 1. *Study design.*

and root surfaces were coated with acid-resistant varnish (Revlon 730 Valentine, Revlon Consumer Products Corporation, New York, NY, USA). Demineralization was created on the crowns of teeth by storing specimens in 1L of a demineralizing solution (pH=5) containing 100 mL of 1 mol aged lactic acid and 1% carbopol (C907) for 14 days (Loma Linda University demineralization formula). The total volume of solution used was estimated to immerse the entire sample group. Demineralization was confirmed, as clinically detectable white discoloration appearing on the crowns of teeth.[22] Teeth were stored overnight in a 37°C incubator to simulate the oral environment. For the study design, refer to Figure 1.

Resin Infiltration

After all specimens were demineralized, resin infiltration (Icon) was accomplished according to manufacturer's recommendations on randomly selected buccal or lingual surfaces on each specimen. First, Icon-Etch (15% HCl) was applied on the tooth

Table 1: *Summary Table of Beverages*

Beverage	Brands	Flavor	Color	pH	Sugar Content (Teaspoons in 12 Ounces)
Coffee	Folgers	Instant coffee	Brown	4.5	0.0
Grape juice	Welch	100% natural grape	Purple	3.4	11.9
Iced tea	Snapple	Raspberry	Red	3.2	7.6
Water	Sparkletts	Distilled water	Clear	7.0	0.0

surface for two minutes followed by water rinsing for 30 seconds. The etching step was performed twice for two minutes each. Next, Icon-Dry (99% ethanol) was applied for 30 seconds followed by air drying. Then, Icon-Infiltrant was applied on the tooth for three minutes followed by light curing for 40 seconds. Excess material was removed with an explorer prior to light curing. A second application of Icon-Infiltrant was applied for one minute followed by light curing for 40 seconds. The contralateral surface of the tooth did not receive any treatment and served as its own control. Polishing was done with aluminum oxide polishing strips (Sof-Lex Finishing Strips Fine/Superfine, 3M ESPE, St Paul, MN, USA). The batch number was not recorded since all Icon material used was from the same box.

Immersion of Specimens in Beverages

On completion of specimen preparation, teeth were randomly divided into four groups (n=15) according to storage solutions: 1) coffee, 2) grape juice, 3) iced tea, and 4) distilled water. Beverages and their respective colors and pH values are summarized in Table 1. Samples were immersed in beverages continuously for four weeks, during which all beverages were replaced with fresh solutions daily. Samples were stored in a 37°C incubator to simulate oral conditions while immersed in these beverages.

Aging, Dye Penetration, and Sectioning

After four weeks of staining, all teeth were put in separate containers for aging. After six months, teeth in the distilled water group were immersed in 2% methylene blue for 24 hours.[10,21] The sectioning was done using a diamond wheel of 0.3-mm thickness (part no. DWH4123, Southbaytech, San Clemente, CA, USA) mounted in a low-speed diamond wheel saw (Model 650, Southbaytech). Five buccal-lingual cuts were made in the center of the occlusal surfaces, resulting in four sections of 1-mm width per tooth. Each section was examined on both sides to evaluate both Icon and control surfaces so that each tooth underwent 16 examinations. The cut surfaces were polished using a roll grinder (Handimet II, Buehler, Lake Bluff, IL, USA) with running water to ensure similar texture and smoothness.

Light Microscope Analysis

A light microscope, at a magnification of 30× (Optometric Tools, Inc, Rockleigh, NJ, USA) connected to a magnescale LH10 (Sony, Tokyo, Japan), was used. All sections were evaluated by a single observer and examined for the following:

a) Presence or absence of stain penetration on both Icon and control surfaces:
   1 = no stain penetration
   2 = stain penetration only on treated enamel surface
b) Rank scale of stain penetration on both Icon and control surfaces:
   Rank 1 = 0 to 0.049 mm
   Rank 2 = 0.050 to 0.099 mm
   Rank 3 = 0.1 to 0.149 mm
   Rank 4 = 0.150 to 0.2 mm
   Rank 5 = 0.2 mm and beyond
c) Actual depth of stain penetration in millimeters for both Icon and control surfaces. The average depth from the three deepest penetration points was recorded in each reading.

Statistical Analysis

The Statistical Package for the Social Sciences (IBM SPSS Statistics, ver 20, SPSS Inc, Chicago, IL, USA) computer software was used for statistical analysis. Descriptive statistics for the presence or absence of stain, rank scale, and actual depth of stain penetration included mean, median, mode, and standard deviation. Inferential statistical tests used in the study included chi-square test, independent and paired sample *t*-test, analysis of variance (ANOVA) with the Fisher least significant difference (LSD) *post hoc* test, and the Kruskal-Wallis test. The significance level was set at $p < 0.05$.

RESULTS

A total of 960 data points from both the Icon surfaces (n=480), and the control surfaces (n=480) were included in the statistical analysis. Representative

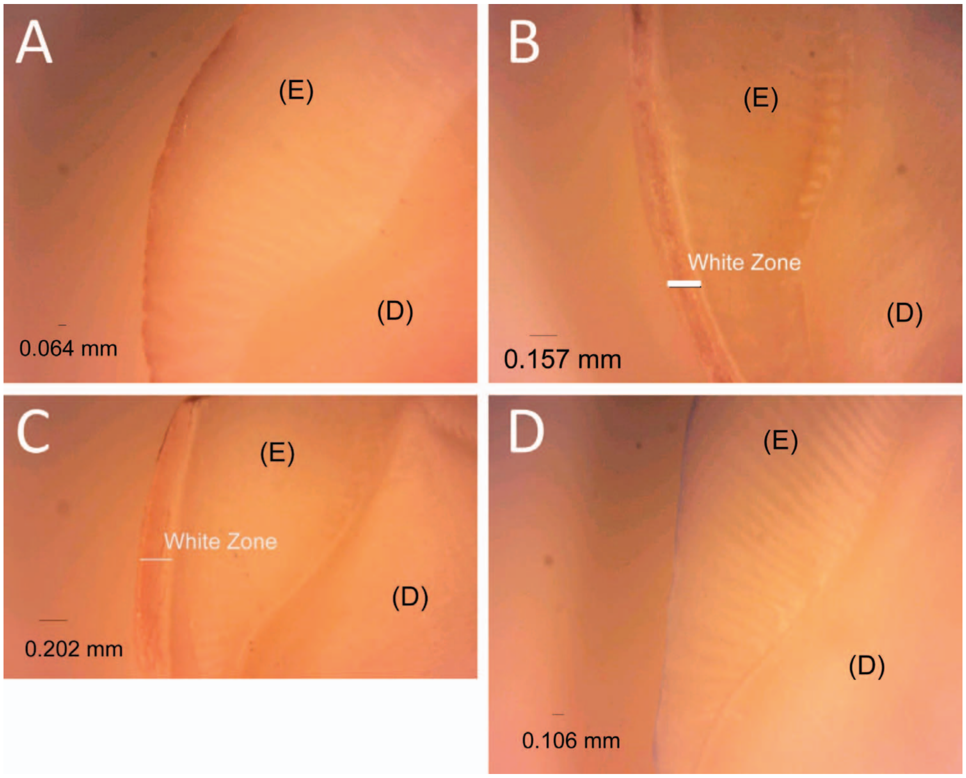

Figure 2. *Slide samples from different beverage group: A) Coffee, B) Grape Juice, C) Iced tea, D) Methylene blue. (E= enamel; D=dentin)*

slide samples from different beverage groups are shown in Figure 2a,b,c,d.

As summarized in Table 2, the majority of data points in the control surfaces (n=421, 87.7%) showed stain penetration on enamel surfaces, and only a small number (n=59, 12.3%) had no stain penetration. A similar pattern was also observed in the Icon surfaces, where the majority of data points (n=340, 70.8%) had stain penetration on enamel surfaces, and fewer (n=140, 29.2%) showed no stain penetration. Nevertheless, according to the chi-square test, the Icon surfaces had significantly fewer data points with stain penetration compared to the control surfaces ($p<0.001$).

When comparing different beverage groups (Table 2), most of the data points in iced tea (n=240, 100%), grape juice (n=239, 99.6%), and methylene blue (n=197, 82.1%) had stain penetration on treated enamel surfaces, whereas fewer data points in the coffee group (n=85, 35.4%) showed stain penetration. Chi-square test indicated that these different stain penetration patterns between coffee group and the rest of beverage groups were statistically significant ($p<0.001$).

Table 2: *Presence or Absence of Stain Penetration and Rank Scale*

	Icon Treatment, N (%)		Beverage Groups			
	Icon Surfaces	**Control Surfaces**	**Coffee**	**Grape Juice**	**Iced Tea**	**Methylene Blue**
No stain penetration	140 (29.2)	59 (12.3)	155 (64.6)	1 (0.4)	0 (0)	43 (17.9)
Stain penetration	340 (70.8)	421 (87.7)	85 (35.4)	239 (99.6)	240 (100)	197 (82.1)
p-value, chi-square test	<0.001		.001			
Rank and depth (mm)						
1: 0-0.049	234 (48.8)	86 (17.9)	180 (75)	8 (3.3)	1 (0.4)	131 (54.6)
2: 0.05- 0.099	64 (13.3)	130 (27.1)	49 (20.4)	45 (18.8)	14 (5.8)	86 (35.8)
3: 0.1-0.149	118 (24.6)	53 (11.0)	5 (2.1)	64 (26.7)	79 (32.9)	23 (9.6)
4: 0.15-0.199	56 (11.7)	93 (19.4)	4 (1.7)	62 (25.8)	83 (34.6)	0 (0)
5: >0.2	8 (1.6)	110 (24.6)	2 (0.8)	61 (25.4)	63 (26.3)	0 (0)
p-value	<0.001, chi-square test		<0.001, Kruskal-Wallis test			

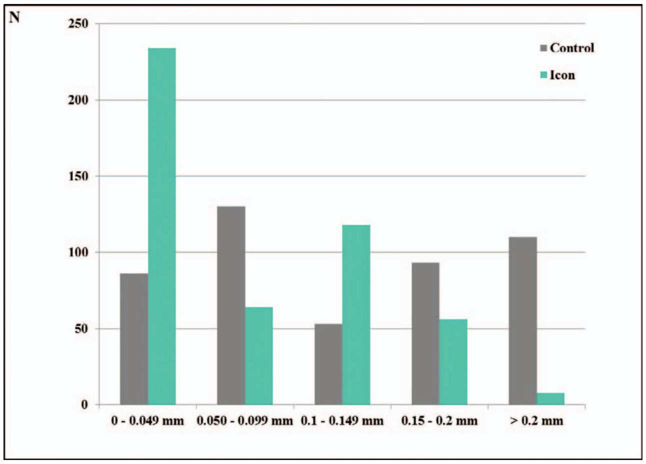

Figure 3. *Bar chart of stain penetration between Icon and control surfaces.*

The results of the stain penetration rank scale are summarized in Table 2. Data points in the Icon surfaces (n=234, 48.8%) had most of their stain penetration in rank 1 (0-0.049 mm), whereas the control surfaces had stain penetration evenly distributed among the five ranks (Figure 3). This indicated that stain penetration in the Icon surfaces tended to be shallower than the control surfaces ($p<0.001$, chi-square test). Among different beverage groups, coffee (n=180, 75%) and methylene blue (n=131, 54.6%) had most of their stain penetration in rank 1. In comparison, both grape juice and iced tea had most of the stain penetration evenly distributed in higher ranks 3, 4, and 5 (Figure 4). This demonstrated that stain penetration in both the grape juice and the iced tea groups was significantly deeper than in the coffee and the methylene blue groups ($p<0.001$, Kruskal-Wallis test).

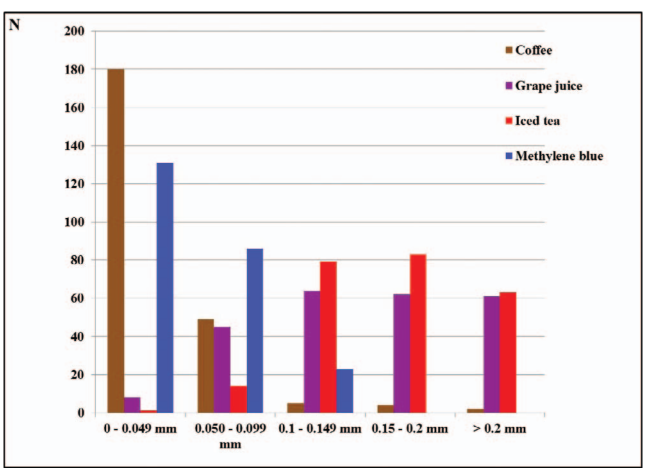

Figure 4. *Bar chart of stain penetration among beverage groups.*

Table 3: *Summary Table of Stain Penetration*

Beverage	Actual Depth (mm) Mean	Standard Deviation	95% Confidence Interval Lower Bound	Upper Bound
Icon surfaces				
Coffee	0.0078	0.01677	0.0048	0.0108
Grape juice	0.1176	0.04737	0.1091	0.1262
Iced tea	0.1339	0.02930	0.1286	0.1391
Methylene blue	0.0216	0.01931	0.0181	0.0251
Total	0.0702	0.06387	0.0649	0.0765
p-value	<0.001, LSD post hoc test			
Control surfaces				
Coffee	0.0469	0.07874	0.0326	0.0611
Grape juice	0.1925	0.06428	0.1809	0.2041
Iced tea	0.2033	0.03556	0.1968	0.2097
Methylene blue	0.0731	0.02845	0.0680	0.0783
Total	0.1289	0.08914	0.1210	0.1369
p-value	<0.001, LSD post hoc test			

In regard to the actual depth of stain penetration (Table 3), the Icon surfaces showed statistically significant less stain penetration depth (0.070±0.064 mm) compared to the control surfaces (0.129±0.089 mm; $p<0.001$, paired sample *t*-test). One-way ANOVA showed that there was a statistically significant difference of actual stain penetration depth between different beverage groups when considering all surfaces together (Icon and control surfaces; $p<0.001$). The LSD *post hoc* test indicated that iced tea presented the greatest depth of stain penetration (0.134±0.029 mm), followed by grape juice (0.118±0.047 mm), methylene blue (0.022±0.019 mm), and coffee (0.008±0.017 mm; $p<0.001$; Table 3). According to the two-way ANOVA, there was also a statistically significant difference of stain penetration depth among different beverage groups (Icon vs. control surfaces; $p<0.001$) (Figure 5). The stain penetration patterns of four beverages in between the Icon and the control surfaces were similar. Iced tea also presented the greatest depth of stain penetration, followed by grape juice, methylene blue, and coffee (Figure 5).

"White zones" were observed microscopically as areas of continuous, white-color bands within the outer one-third of enamel (Figure 2b,c). Interestingly, only the grape juice and the iced tea groups had white zones observed, and stain penetration was confined to the white zones. The *t*-test indicated that there was a statistically significant difference of white zone width between the iced tea and the grape juice groups ($p<0.001$). Iced tea showed greater width of the white

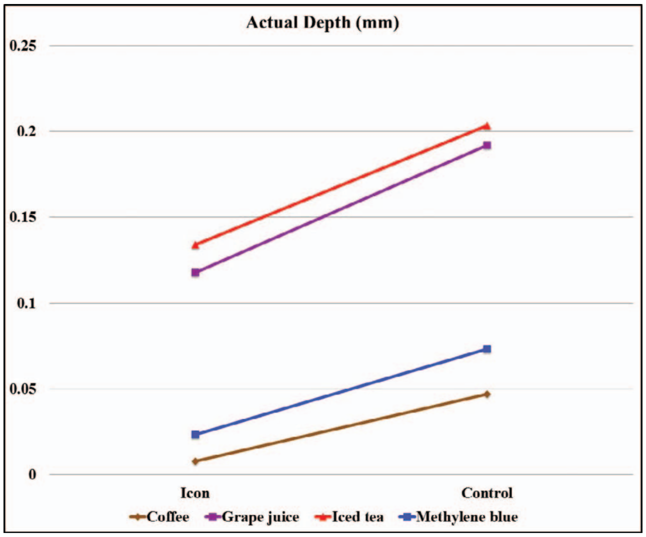

Figure 5. *Actual depth of stain penetration.*

zone (0.255±0.053mm) compared to grape juice (0.217±0.079 mm). In addition, the Icon surfaces overall showed less width of the white zone (0.205±0.053 mm) compared to the control surfaces (0.267±0.065 mm), though the difference was not statistically significant ($p>0.05$, paired sample t-test).

DISCUSSION

According to the results of this study, although most of resin-infiltrated enamel surfaces (70.8%) were susceptible to stain penetration by different beverages, they had statistically significant fewer samples with stain penetration compared to the control surfaces ($p<0.001$). Also, when there was stain penetration, the Icon surfaces showed statistically significant less depth of stain penetration compared to the control surfaces ($p<.001$). This is an important finding, as it is consistent with the manufacturer's claim that Icon can occlude the diffusion pathway when light cured.[1,2,5] Therefore, resin infiltration might help to prevent or lessen stain penetration by beverages.

Previous studies have suggested the potential of erosion from acidic beverages in the development of microleakage.[10,11] In vitro studies have shown that microleakage may cause marginal discoloration along with many other issues in dental restorative materials.[10] Since resin infiltration has been introduced recently in dentistry, there have not been any published studies on microleakage of Icon. According to the results (Table 3), beverages with lower pH, such as iced tea and grape juice (pH=3.2 and 3.4, respectively), showed significantly greater stain penetration than beverages with higher pH (coffee pH=4.5; $p<0.001$). In addition, iced tea, having the lowest pH value among the tested beverages (pH=3.2), had greatest depth of stain penetration on both the Icon and the control surfaces. The acidity of the beverages ranged from pH 3.2 to 4.5 in the present study (Table 1). The acidic environment could have negatively affected the surface integrity of the resin-infiltrated enamel. This detrimental effect can possibly lead to microleakage at the interface between the resin infiltration and tooth structure and thus increase staining potential of the infiltrated surfaces.

Other factors that can contribute to the increase of microleakage include filler loading of the resin matrix and the extent of polymerization shrinkage. Studies have shown that lower filler content of the resin matrix has demonstrated higher polymerization shrinkage and therefore contributes to greater microleakage of the material.[23] Other studies have also shown that resin with the lowest filler content had poor color stability due to more water absorption in the filler-matrix interface, consequently leading to filler-matrix debonding or hydrolytic degradation of the filler.[18,24] Since the infiltrant is unfilled resin, it is likely that the material would have greater polymerization shrinkage and also be susceptible to stain penetration.

In addition, some studies have shown that the degree of water sorption and hydrophilicity of the resin matrix may influence the stain susceptibility of the resin materials.[25-27] The resin matrix used as an infiltrant has hydrophilic characteristics that may allow beverages to absorb into resin matrix and lead to discoloration. Thus, it is not surprising to find that both the Icon and the control surfaces had the majority of samples with stain penetration by different beverages (coffee, grape juice, and iced tea) in this study. Also, some studies[19] have noted that there is more water sorption as the proportion of triethylene glycol dimethacrylate (TEGDMA) increases in the resin matrix. Kalachandra and others[25] found that incorporation of greater amounts of TEGDMA results in an increase in water uptake in Bis-GMA-based resins. Mazato and others[26] explained that the ethoxy group in TEGDMA shows an affinity with the water molecule by hydrogen bonding to oxygen, thereby increasing its surface hydrophilicity. While DMG America has yet to disclose the composition of the Icon infiltrant, based on Meyer-Luckel and Paris's[6] work it is evident that the Icon infiltrant is a TEGDMA-based resin. Therefore, the extent of discoloration and stain penetration from different beverage groups in this study might be attributed to the infiltrant's high-TEGDMA content and its hydrophilic property.

There are only a few reported studies on stain potential and color stability of teeth treated with resin infiltration. Luebbers and others[28] found that artificial lesions treated with resin infiltration were not sensitive to discoloration by sunlight. Our study demonstrated that stain from beverages could not only occur on external surfaces of teeth but also penetrate into the infiltrated surfaces, indicating that both intrinsic and extrinsic factors can influence stain penetration. The beverages (coffee, iced tea, and grape juice) used in this study are consumed regularly in our daily lives and have been recognized as strong staining agents.[14,16] Stain penetration in the iced tea group showed the greatest depth of penetration, followed by grape juice and coffee. The findings from this study were similar to the results of a project conducted by Kuriya and others[29] demonstrating the discoloration potential of teeth treated with resin infiltration. According to the study, all tested beverages (grape juice, iced tea, coffee, and distilled water) affected the color stability of enamel treated with resin infiltration. As immersion time increased, color changes became more intense.

While most of the samples in the iced tea, grape juice, and methylene blue groups had stain penetration into treated enamel surfaces, only a small number of samples in the coffee group showed stain penetration. Even when samples in the coffee group had stain penetration, the depth of penetration was shallower than the rest of the groups. It is interesting to point out that the pH of coffee (pH=4.5) was lower than that of distilled water (pH=7). If acidity was the main factor influencing stain penetration, the depth of stain penetration in the coffee group should be deeper than the distilled water group; however, this was not the case. One of the possible explanations was that coffee's pigments may be larger than the rest of the beverages as well as methylene blue and therefore they remained on the surface and did not penetrate into the resin-infiltrated enamel as much as the other beverages.

White zones were incidental findings observed microscopically only in the grape juice and iced tea groups—the two groups having lower pHs and higher sugar content compared to the other groups. Since the samples used in this study were not sterile, the combination of low pH, high sugar content, and the presence of microorganisms could have caused further demineralization of the enamel surfaces, leading to increased staining potential of the infiltrated enamel. It is worth pointing out that the iced tea group showed a significantly greater white zone width compared to the grape juice group. Since the iced tea group had lower pH but less sugar content than the grape juice group, the pH factor seemed to play a more important role in creating the white zone.

This *in vitro* study was done under controlled laboratory settings utilizing demineralized enamel treated with resin infiltration. The results indicated that some common beverages have stain penetration potential into resin infiltrated enamel and therefore can contribute to discoloration. The study, however, did not simulate any saliva-buffering effect or consider any relevant clinical situations, such as dietary habits, oral hygiene, xerostomia, or other effects caused by systemic diseases and so on. This study did not consider the bacteria type and loading amount in the samples. Thus, future studies are needed to investigate other factors that could potentially contribute to the extent of stain penetration, such as the surface integrity and roughness of the infiltrated surface in relation to stain penetration after an acid challenge, the composition of the white zone, the effect of chromogenic bacteria, and the effect of beverage temperature.

CONCLUSION

Stain penetration by different beverages in resin-infiltrated enamel was evaluated using light microscopy. Based on the findings of this study, the following conclusions were drawn:

- Both the Icon and the control surfaces show stain penetration by different beverages (iced tea, grape juice, and coffee).
- Resin-infiltrated enamel surfaces allow significantly less depth of stain penetration than untreated enamel surfaces.
- The iced tea group exhibits the greatest depth of stain penetration, followed by grape juice, methylene blue, and coffee.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Loma Linda University. The approval code for this study is IRB#5130272.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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