Effect of Resin Infiltration on Bleached Enamel: An In Vitro Evaluation of Micromorphology, Resin Penetration and Microhardness

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Research article

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Abstract

Background: Tooth bleaching is a promising aesthetic treatment for shaded teeth; however, demineralized lesions also occur after bleaching, and Icon resin can infiltrate into the demineralized lesions and then improve the color of teeth. This study aims to evaluate the effectiveness of resin infiltration on bleached teeth that are managed with different protocols by assessing the micromorphology of enamel surfaces, the depth of resin penetration and the microhardness of enamel after bleaching and Icon resin infiltration.

Methods: A total of 150 noncarious premolars with sound coronal structure, which were extracted for orthodontic purposes, were obtained in this study in three parts: part Ⅰ“micromorphology of enamel surfaces”（n=60）；part II“observation the depth of the resin penetration”（n=30）；part III “Vickers hardness number values”（n=60). Both part Ⅰ and part III were divided into six subgroups. Part II was divided into three subgroups. 10 samples per subgroup. Data were analyzed using SPSS 22.0, Mann-Whitney test.

Results: The surface roughness of the teeth increased after bleaching. When etched with 15 % HCl before the resin infiltration, it would result in further increase in roughness, but the surface of the bleached teeth could be as smooth as that of normal teeth after resin infiltration. There was a statistically significant increase in the mean resin penetration depth of the bleached teeth with 5-day delay of resin infiltration over the bleached teeth with resin infiltration immediately (Mann-Whitney test, $P<0.05$). Otherwise，the VHN values of delayed ones were similar to the normal one.

Conclusions: It suggested that Icon resin infiltration treatment should be delayed after tooth bleaching but not performed right after tooth bleaching.

Background

Tooth bleaching is an aesthetic way to change the color of teeth without cutting hard tissue, and its success is directly related to the diffusion capacity of hydrogen peroxide (HP)[1)]. After the bleaching gel is applied on the tooth surface, the pH then becomes acidic, achieving the effectiveness of the gel whitening function but in the meantime possibly damaging the tooth structure[2–4)]. Extensive literatures[5–9)]have reported that the surface of teeth often show decreased enamel hardness and mineral loss after bleaching. Many studies have also found that after a certain degree of demineralization appeares in the enamel, dissolution and surface roughness increased after the application of bleaching material, causing micromorphology alterations[10–12)]. When the concentration of the bleaching reagent increases and the bleaching time increases, the dissolution of enamel intensifies, becoming similar to fish scales. Additionally, the interspace ratio of teeth also increases[13)].

In addition to tooth bleaching, resin infiltration (RI) can also improve the color of teeth. In recent years, as a much more tissue-preserving approach, RI is developed to improve the color of teeth when applied to rest and control white spot lesion, shallow caries lesion, dental fluorosis and demineralization. For this treatment, a new resin, Icon®-DMG Germany has a lower viscosity and is mainly composed of bisphenol

A glycidyl methacrylate, triglyceride dimethacrylate and ethanol. After etching the enamel surface of shallow caries with 15% hydrochloric acid(15% HCl), some white spots can be removed and fully expose surface micropores. Then the Icon resin could penetrate into the pores and fill in the hard tooth tissue lost, and preventing the entry of acid and ending caries[14)]. Normal tooth enamel is translucent, with a refractive index of 1.62; the refractive index of Icon resin is 1.475[15)]. After the Icon resin infiltrates into the demineralized enamel and solidifies, the overall refractive index of the enamel is close to that of normal enamel, with the chalky appearance and color of the enamel having been greatly improved. Since the refractive index of Icon resin is close to that of natural tooth enamel, it has been gradually used combined with tooth bleaching in the therapy of shaded teeth. Wang et al. suggested that it seemed to be a minimally invasive therapy for severe dental fluorosis by combining the tooth bleaching and resin infiltration to achieve the esthetic appearance[16)].

With the knowledge that both HP applying in bleaching technique and 15%HCl using in RI causing certain amounts of acid erosion and subsequently resulting in demineralization, will the performance of Icon resin infiltration right after tooth bleaching cause excessive etching and do harm to the restorative function of Icon resin? Otherwise, Lai et al.[17)] reported that the decomposition of oxygen in HP diffusing in enamel, inhibits the polymerization of resin. It led to the decrease in the resin bonding tensile strength after bleaching and it was indicated that it may need to be delayed for at least 7 days to make sure the enamel adhesion ability recover[18)], while data from Bargih et al.[19–20)] have shown that the use of ethanol-based adhesives might reverse the drop in bond strength found immediately after bleaching, in which the ethanol accounted for. For Icon resin is a new kind of resin material with excellent fluidity that could achieve the penetration into the demineralized lesions and stabilize the tooth color. Will the decomposition of oxygen in HP diffusing in enamel inhibit the penetration depth of Icon resin, too? At present, there is still a lack of studies concerning the effect of immediate infiltration on the restorative function of Icon, and the questions warrant extensive research to formulate evidence-based guidelines for their combined application.

In conclusion, the aim of this in vitro study was to evaluate the effectiveness of resin infiltration on bleached teeth with or without 15% HCl and researching whether the delay of RI is necessary or not. The study assessed the depth of resin penetration into bleached enamel, the microhardness and the micromorphology of post-infiltration to determine whether a better effect could be achieved with a delay of RI and etching with 15% HCl after bleaching.

Materials And Groups

1 Preparation and groups of specimens

A total of 150 noncarious premolars with sound coronal structure, which were extracted for orthodontic purposes, were obtained. These teeth were stored below room temperature in distilled water until they were subjected to any intervention. This present experiment consisted of 3 parts.

Part I: **Evaluating the micromorphology of enamel surfaces.** In this part, 60 samples were divided into 6 subgroups and 10 samples for each subgroup: Ia: control group;Ib:10 samples were subjected to a bleaching agent with 40% hydrogen peroxide (HP);Ic:10 samples were subjected to a bleaching agent with 40% HP and immediately etched with 15% hydrochloric acid (15% HCl); Id:10 samples were bleached with 40% HP and infiltrated with Icon®-DMG Germany; Ie:10 samples were subjected to a bleaching agent with 40% HP and immediately etched with 15% HCl, and then infiltrated with Icon®-DMG Germany; If (10 samples)were placed in artificial saliva for 5 days after being bleached with 40% HP and etched with 15% HCl, after which were infiltrated with Icon®-DMG Germany.

Part II: **Evaluating the depth of resin penetration tags.** In this part, 30 samples were divided into 3 subgroups and 10 samples for each subgroup: IIa – bleached and infiltrated with Icon®-DMG Germany; IIb – post-Icon resin infiltration after being bleached and etched with 15% HCl; IIc– post-Icon resin infiltration after being bleached and etched with 15% HCl as well as a placing in artificial saliva for 5 days. The depth of resin penetration and the micromorphology of the surface were studied using scanning electron microscopy (SEM).

Part III: **Evaluating Vickers hardness number (VHN) values of enamel.** In this part, other 60 teeth were equally divided into six subgroups (10 samples/subgroup) as group I and VHNvalues were obtained to measure the surface microhardness.

Table 1
Protocols in Part I& Part III

\	Protocols	Samples (n)
a	Control group	10
b	Bleaching	10
c	Bleaching + 15% HCl	10
d	Bleaching + immediate RI	10
e	Bleaching + 15%HCl + immediate RI	10
f	Bleaching + 15%HCl + delayed RI	10

Table 2
Protocols in Part II

\	Protocols	Samples (n)
a	Bleaching + immediate RI	10
b	Bleaching + 15%HCl + immediate RI	10
c	Bleaching + 15%HCl + delayed RI	10

2 Methods And Procedure

After allocation, the specimens were treated respectively. Before the bleaching procedure, specimens were gently dried with air for moisture control. For I (b, c, d, e, f), II (a, b, c) & III (b, c, d, e, f), the 40% HP were applied evenly on the enamel surface for office bleaching, obtaining a uniform gel layer of 2-mm thickness for 20 minutes and then repeated once more. After bleaching. I(c,e), II b,and □ (c, e) were etched with 15% HCl for 2 minutes. After each application time, all specimens were abundantly rinsed with distilled water for 30 seconds until the products were completely removed, and were air-dried. In the last session of each protocol, the specimens (Id, Ie, II a,II b & □d, □ e) were submitted to application of Icon®-DMG Germany for 3 minutes immediately after etching and then the samples above were maintained in distilled water at 37℃ for the duration of the entire experiment. If, IIc and IIIf were placed in artificial saliva at 37℃ for 5 days before etching and RI. The resin was applied as follows: a solution of 99% ethanol was applied for 30 seconds, and the specimen was air-dried followed by Icon resin infiltration application, which was left on the enamel for 3 minutes before curing. The resin was light-cured for 40 seconds. The application of resin was repeated for 1 minute and again light-cured for 40 seconds. 90 samples from the total were randomly selected for SEM analysis (I & II), and a sample size of 60 was selected for the microhardness test (III).

3 Scanning Electron Microscopy

Samples from I were sectioned in the mesial-distal plane. Samples from II were sectioned in the buccolingual plane using a hard tissue microtome (Leica 1600). All the blocks were then subjected to a drying process by placing them in a hot air oven (Heathron Industrial Heaters) for 10 minutes at 110 °C. Samples were then ready to be subjected to SEM (JEOL, JSM-6380LA) to investigate the micromorphology of the enamel surface and the depth of penetration of the infiltrant.

4 Surface Microhardness Evaluation

A Vickers microhardness tester (CLEMEX CMT HD) was used to investigate the change in hardness after bleaching and Icon resin infiltration. Teeth in III were tested for enamel microhardness. For the microhardness testing, a load of 100 gm was applied for 10 seconds as recommended. Three indentations were performed on the enamel surface, and the microhardness even values were recorded.

5 Statistical Analysis

The data were statistically processed using SPSS 22. 0 software. A Mann-Whitney test was performed for the comparison of values between two subgroups. $P < 0.05$ indicated that the difference was highly significant.

Results

SEM revealed the roughness of the bleached enamel increased in Ib, compared with which, the roughness of the bleached enamel etched with 15% HCl in Ic was obviously increased at 50 × magnification(Fig. 1).

Upon comparing the microsurfaces among Ia-f, it was determined that the microsurface after Icon resin infiltration (Id, Ie and If) could be as smooth as that of normal teeth (Ia) at 50 × magnification (Fig. 2).

The SEM observation of resin infiltration samples showed resin-infiltrated tags. InIIa, the deepest depths of infiltration were determined to range from 245 to 317 μm, and the mean depth was 284 μm(Table 3). Figure 3 shows the depth of resin penetration in one sample fromIIa at two different magnifications of 250 × and 500×.

Table 3
Depth of penetration of resin tags in IIa

Sample no.	Penetration depth
1	245
2	300
3	268
4	290
5	317
6	254
7	266
8	305
9	289
10	315
mean	284

Table 4
Depth of penetration of resin tags in IIb

Sample no.	Penetration depth(μm)
1	397
2	391
3	430
4	422
5	540
6	507
7	534
8	395
9	402
10	417
mean	443
Figure 4 showed the depths of resin infiltration in one sample fromIIb at two different magnifications of 250 × and 500×.	

Otherwise, the deepest depths of resin infiltration in IIc ranged from 256 to 402 μm, and the mean depth of resin infiltration was 371 μm(Table 5).

Table 5
Depth of penetration of resin tags in IIc

Sample no.	Penetration depth(μm)
1	400
2	370
3	256
4	385
5	374
6	423
7	368
8	402
9	379
10	356
mean	371

Figure 5 showed the depths of resin infiltration in one sample from IIc at two different magnifications of 250 × and 500×.

In total, the mean depths of penetration were 284 μmin IIa, 443 μm in IIb and 371 μm in IIc. There was a statistically significant increase in IIc over IIa and IIb in the depth of resin penetration (Mann-whitney test, $P < 0.05$) (Table 6).

Table 6
Mean depths of resin infiltration of IIa-c

Groups	Mean ± SD	
IIa	284 ± 6.3	p 0.05(comparing IIa and IIb, IIb and IIc,IIa and IIc)HS
IIb	443 ± 14.2	
IIc	371 ± 14.7	
HS: Highly significant; SD: Standard deviation		

A Vickers microhardness tester was used to measure the change in hardness of the bleached enamel and enamel post- Icon resin infiltration. Table 7showed the VHN values for III, and Table 6 presents the mean values of microhardness for each subgroup and the intergroup comparisons. Intergroup comparisons were performed between IIId and IIIe, IIIa and IIIe, IIIa and IIId, IIIb and IIId, IIId and IIIf, IIIe and IIIf, using Mann-Whitney test. Compared with the IIIa, the VHN values of IIIb-f were low (Mann-Whitney test, $P <$

0.05). Otherwise the VHN values of IIIb were higher than IIIc and there was statistically significant between them (Mann-Whitney test, $P < 0.05$). There was a statistically significant VHN values increase of the teeth with a 5-day delay of RI than that of other groups except the control group. (Mann-Whitney test, $P < 0.05$). In particular, the VHN values of the bleached and etched teeth with a 5-day delay of RI in III were similar to the normal one, which was not statistically significant (Mann-Whitney test, P 0.05).

Table 7
VHN values of each sample of each subgroup(III)

Sample no.	IIIa	IIIb	IIIc	IIId	IIIe	IIIf
1	364	354	313	354	344	355
2	358	340	314	350	349	360
3	361	345	317	348	355	358
4	367	345	317	352	350	364
5	365	338	316	356	351	353
6	366	337	312	357	352	365
7	362	347	309	358	350	364
8	354	330	313	356	356	355
9	353	339	314	355	349	361
10	360	343	313	358	347	362

Table 8
Mean of VHN values of IIIa-f

Groups	Mean ± SD	
IIIa	361 ± 1.2	p 0.01(comparing IIIb and IIIc, IIIa and IIIe, IIIe and IIIf) HS
IIIb	340 ± 6.4	p 0.05(comparing IIIa and IIId ,IIId and IIIe IIId and IIIf)HS
IIIc	315 ± 2.4	p 0.05(comparing IIIa and IIIf)
IIId	355 ± 3.4	
IIIe	350 ± 3.5	
IIIf	360 ± 4.3	
HS: Highly significant; SD: Standard deviation		

Thus, these results indicated that a 5-day delay of RI after tooth bleaching by placing in artificial saliva did significantly affect the surface microhardness of the bleached teeth. If perform the RI immediately, the VHN of the non-etched ones was higher. Though the resin penetration depth of the teeth whose resin

infiltration was applied immediately after bleaching and etched with 15% HCl were highest, they were at the cost of the worse enamel damage before RI (Fig. 1B).In conclusion, based on this experiment, it was indicated that Icon resin infiltration treatment should be delayed after tooth bleaching but not performed right after tooth bleaching generally. If the patients required to do the resin infiltration treatment after tooth bleaching immediately, it was suggested to infiltrate without 15% HCl etching.

Discussion

The RI technique is a new technique used to prevent the development of caries, which can treat early caries without drilling or sacrificing healthy tooth structure around caries[21].And now is demonstrated that restoring demineralization, dissolution and abrasion on enamel by resin infiltration is a promising approach for the aesthetic treatment of post-bleaching teeth. It confirms the results obtained in this study. The infiltrant resin used for restoration possesses very low viscosity, high surface tension, and low contact angle with the enamel, all of which are vital properties for penetration of the resin into the body of the enamel.

As is known to all, micropores could occur in the surfaces of enamel after tooth bleaching, which are visible by SEM. The relatively intact enamel surface is slightly damaged. There are many tiny pores that provide the channels for organic acid and minerals diffusing and moving, which can promote bacterial adhesion. After applied with RI, the color of the demineralization area can be similar to the normal teeth because the refractive index of Icon resin is 1.475, close to the refractive index of enamel (1.62). RI uses low-viscosity resin to penetrate, plug, and fill the micropores of the demineralization to form a barrier and avoid disintegration of the enamel[22]. The present experiment found that the Icon resin penetrated into the demineralization area through SEM observation. The resin penetration depth of etched teeth was deeper than that of the bleached ones without etching (Mann-Whitney test, $P < 0.05$). The result of deeper penetration may due to the acid. The acid etching technique can punch holes in the enamel surface and can completely remove the surface layer and fully expose the demineralization area[23], which can make the resin penetrate into the micropores as deep as possible. While for the VHN value, though the penetration depth increased, the VHN value of the etched ones was less than the bleached ones without etching at the same time(Mann-Whitney test, $P < 0.05$). Moreover, we also found that Icon resin sometimes only penetrated to a certain depth, while some micropores still existed deep in the enamel. Based on our SEM result, etching with 15% HCl might cause excessive erosion. Though the resin penetration depth of the bleached teeth with etching were highest, it came at the cost of worse damage of micromorphology after etching. Tamer et al. created the artificial initial caries lesion with an average depth of 200 μm and it showed that the Icon penetration depth in the lesion was about 70%, 140 μm which was deep enough to prevent the further demineralization[24]. However, there was still some demineralization deep in the enamel due to the 15% HCl erosion. Whether deep demineralization can further affect the long-term function of bleached teeth is still unclear and needs further studies. Another reason we took into account was the saliva. The constant presence of saliva and fluoride may minimize changes in the enamel through the deposition of calcium fluoride crystals, mitigating the effects of

demineralization, prolonging tooth health, and preventing changes that may damage the structure of bleached teeth[25]. Furthermore, extensive studies indicated that the bond strength of bleached teeth with a delay of resin bonding were higher than that of the bleached teeth bonded immediately, which had been attributed to the decomposition of oxygen free radicals accountable for the whitening effect in HP diffusing in enamel and adversely influencing the penetration of the bonding agent into the tooth surface and then inhibiting the polymerization of resin[26,27].

Current study used artificial saliva for the experiment. The results showed that with a 5-day delay of RI by placing in artificial saliva for 5 days, the VHN values of the bleached teeth could regain closed to the normal one and better than the other groups(Mann-Whitney test, P 0.05). Additionally, the penetration depths of the delayed ones were deeper than the bleached teeth which were resin infiltrated immediately Mann-Whitney test, P 0.05 . Based on the results, we inferred that several elements might account for the result. First, excessive erosion with 15% HCl leading the demineralization caused the VHN values decrease. Secondly, decomposition of oxygen free radicals inhibited the polymerization of resin which decreased the bonding strength. As for the delayed ones, it could be attributed to the partial loss of oxygen diffusion layer over time at the tooth and composite interface[28] and the constant presence of saliva mitigating the effects of demineralization would account for it. Thus, it is suggested to delay the Icon resin infiltration treatment after tooth bleaching. However, many clinical doctors choose to perform the resin bonding after tooth bleaching immediately. Because both tooth bleaching and RI containing erosion, the immediate combination of these two techniques may cause excessive erosion, decreasing VHN value and polymerization. Barghi et al.[19,20] indicated that ethanol could eliminate the oxygen free radicals. Thus it is suggested that if the patients required to perform the Icon resin infiltration after tooth bleaching immediately, 15% HCl etching was not needed.

In addition, all resins, including the Icon®-DMG Germany, have a disadvantage: aging. Furthermore, clinical long-term follow-up is lacking. Therefore, further studies of the combined application of tooth bleaching and Icon resin infiltration should be performed including long-term follow-ups to study the effects.

Declarations

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Hospital of Stomatology, Guangzhou Medical University (KY-2017-012). Consent was obtained from all participants and informed consent was signed.

Consent for publish

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from

the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’contributions

WZ contributed to the conception and design of the work. GJW contributed

to the acquisition and analysis of data. LYH drafted the Manuscript. WWD and YXC revised the manuscript substantively. All authors read and approved the final manuscript.

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Figures

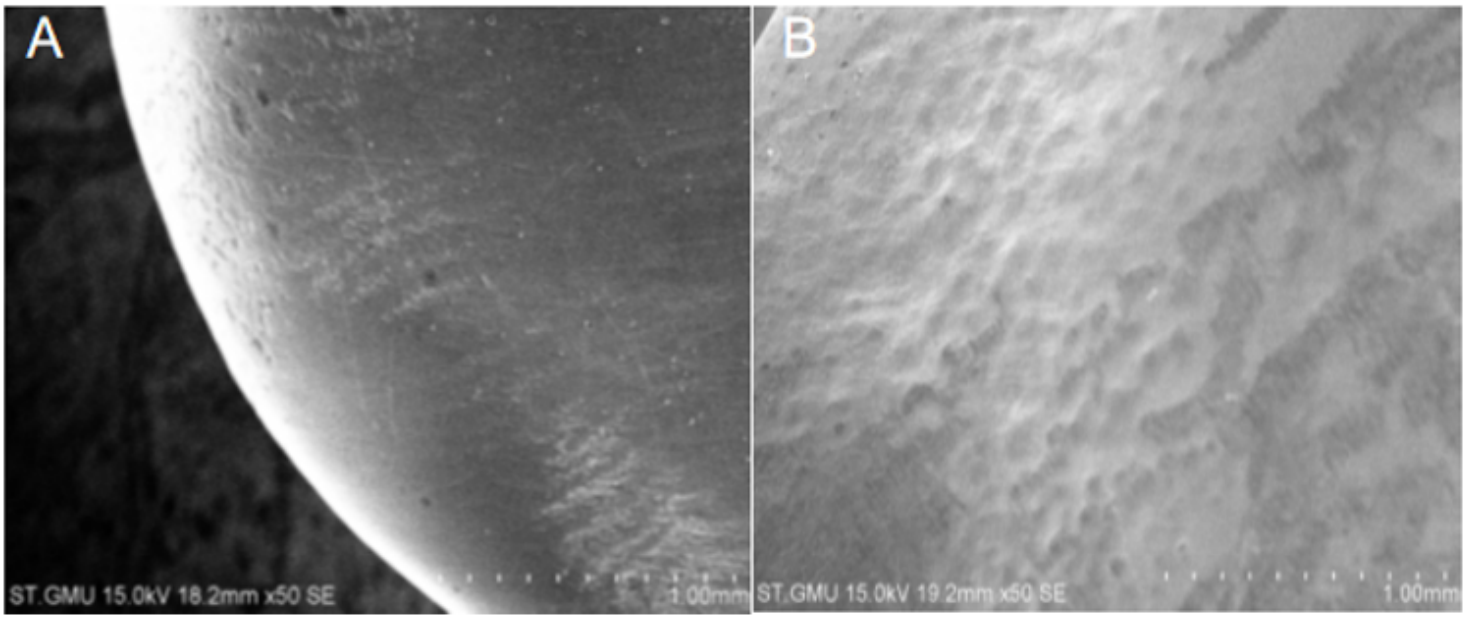


Figure 1

A SEM view of the surface of bleached enamel (Ib 50×) B SEM view of the surface of bleached and etched enamel (Ic 50×)

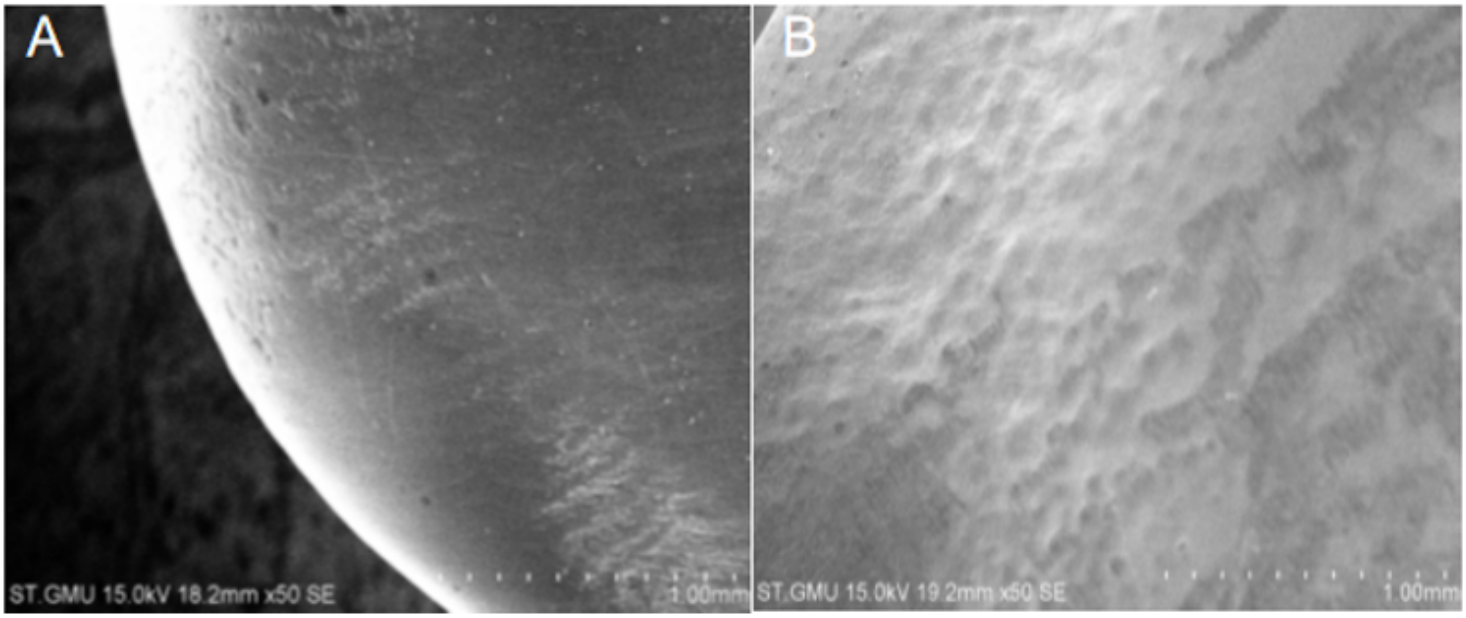


Figure 1

A SEM view of the surface of bleached enamel (Ib 50×) B SEM view of the surface of bleached and etched enamel (Ic 50×)

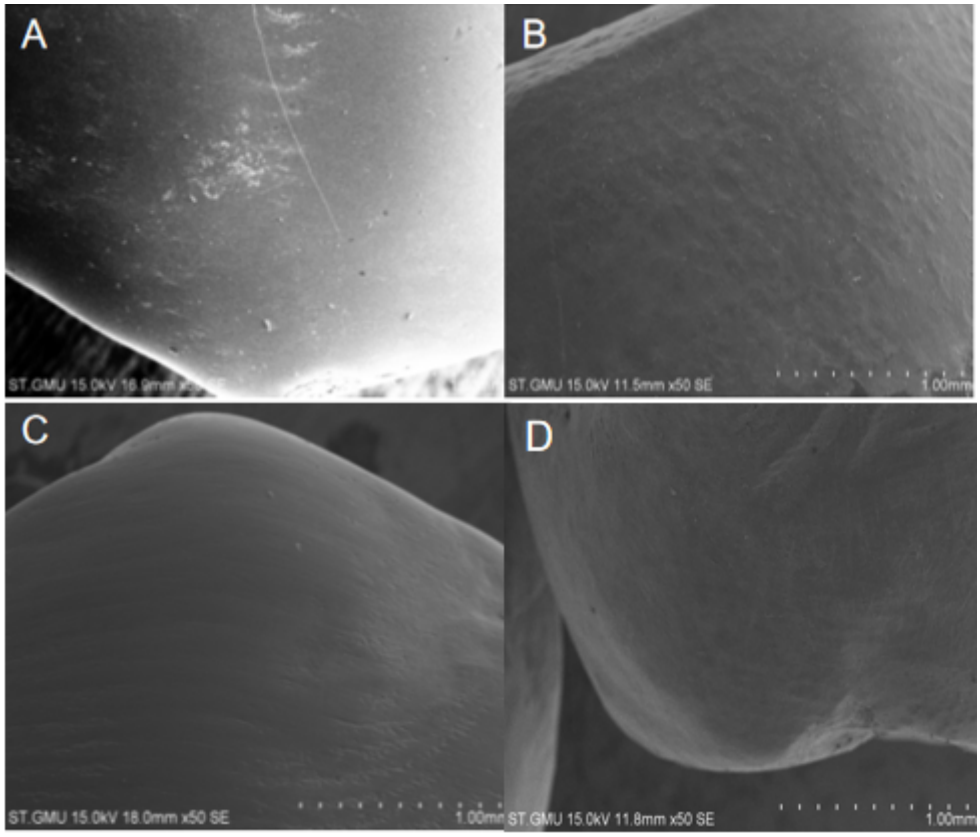


Figure 2

A SEM view of the surface of normal enamel (Ia 50×) B SEM view of the surface of bleached enamel post resin infiltration (Id 50×) C SEM view of the surface of bleached and etched enamel post resin infiltration (Ie 50×) D SEM view of the surface of bleached and etched enamel with a 5-day delay of RI (If 50×)

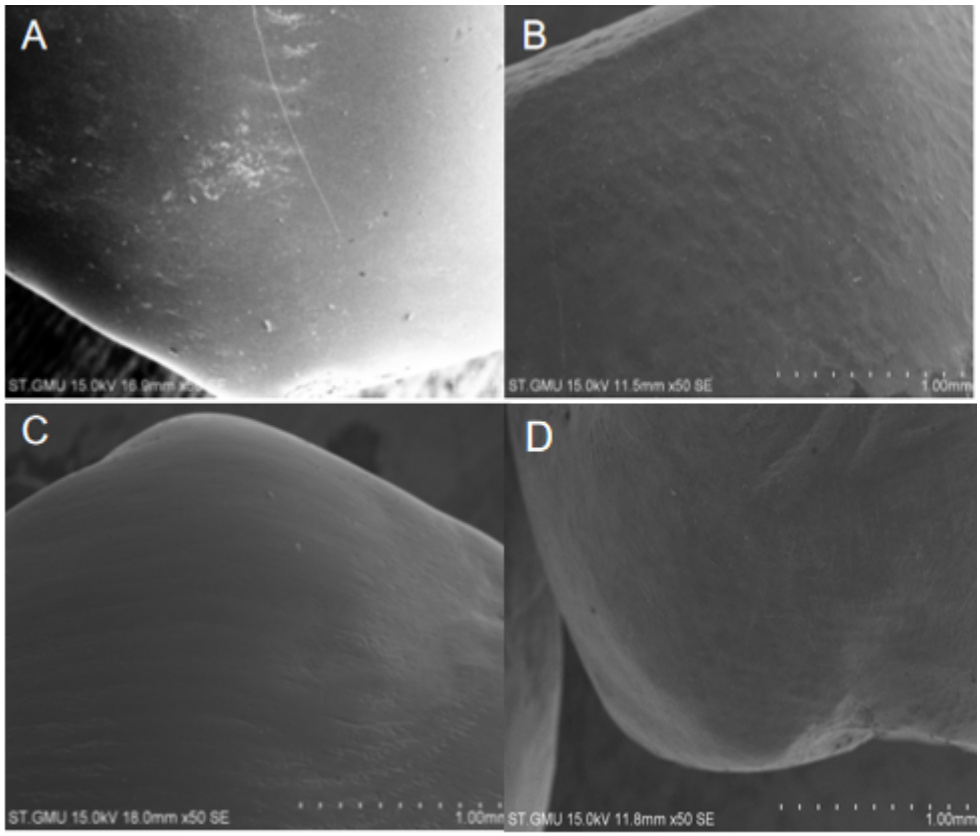


Figure 2

A SEM view of the surface of normal enamel (Ia 50×) B SEM view of the surface of bleached enamel post resin infiltration (Id 50×) C SEM view of the surface of bleached and etched enamel post resin infiltration (Ie 50×) D SEM view of the surface of bleached and etched enamel with a 5-day delay of RI (If 50×)

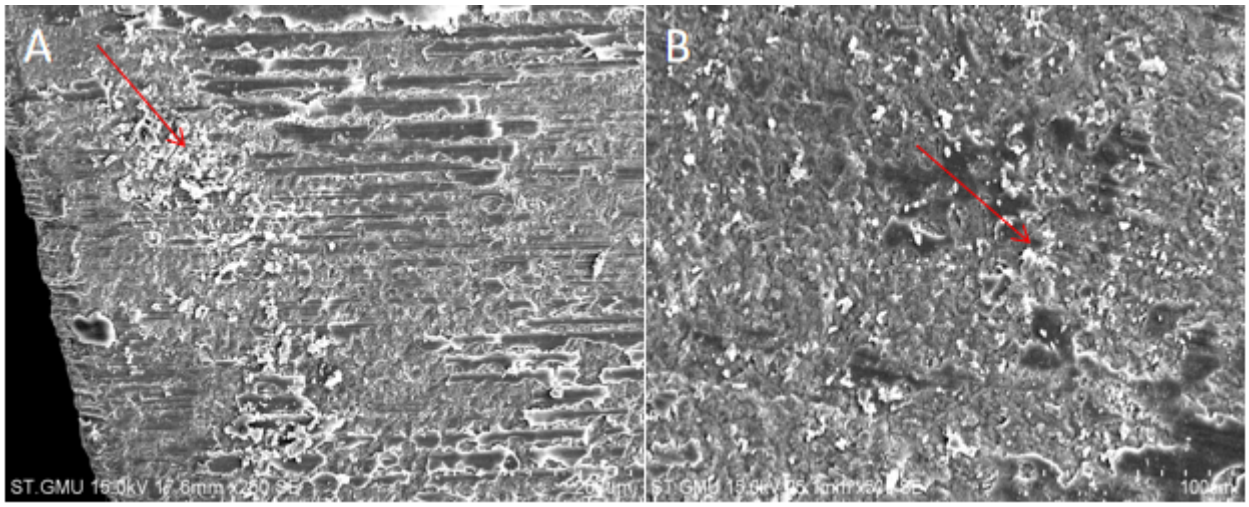
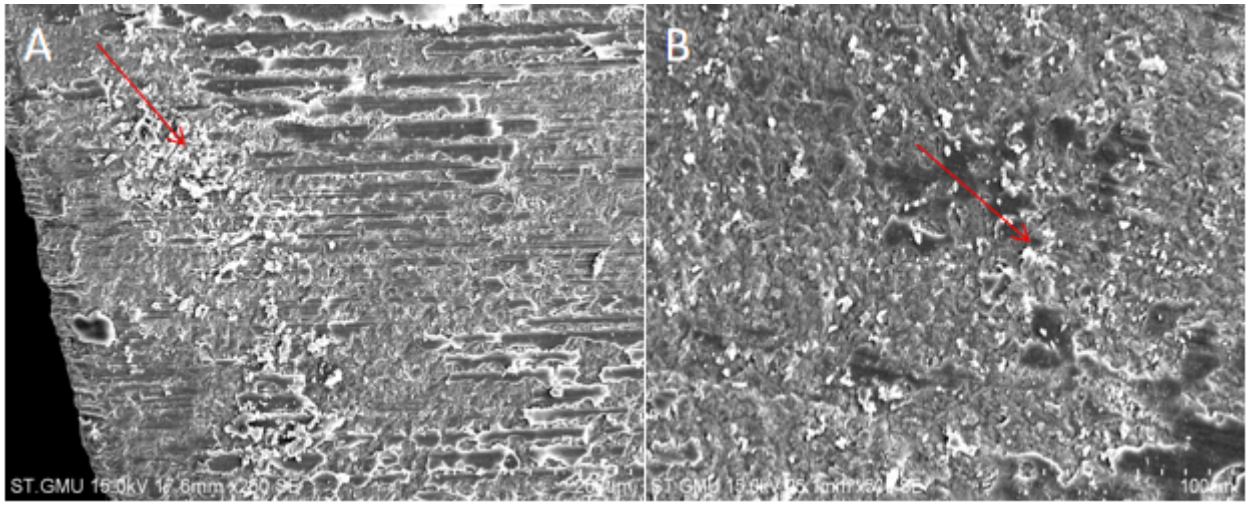


Figure 3

A：SEM view of the penetration of resin tags in bleached enamel（IIa 250×) B：SEM view of the penetration of resin tags in bleached enamel（IIa 500×) In contrast, the deepest depths of resin penetration in IIb ranged from 391 to 540 μm, and the mean depth of resin infiltration was 443 μm(Table 4).



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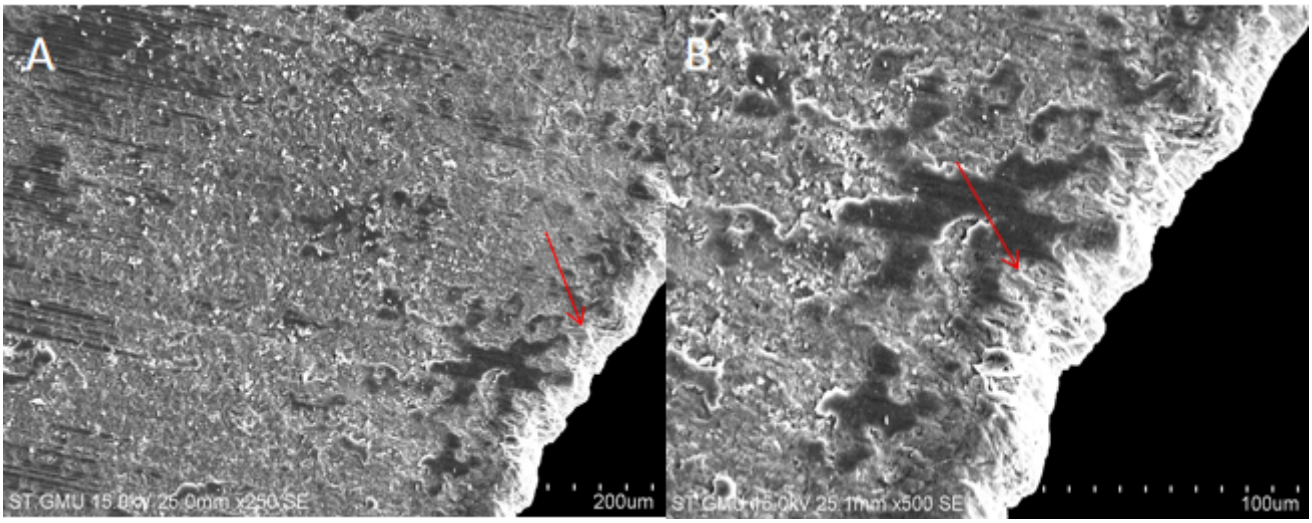

Figure 4

A： SEM view of the penetration of resin tags in bleached and etched enamel (IIb 250×) B： SEM view of the penetration of resin tags in bleached and etched enamel (IIb 500×)

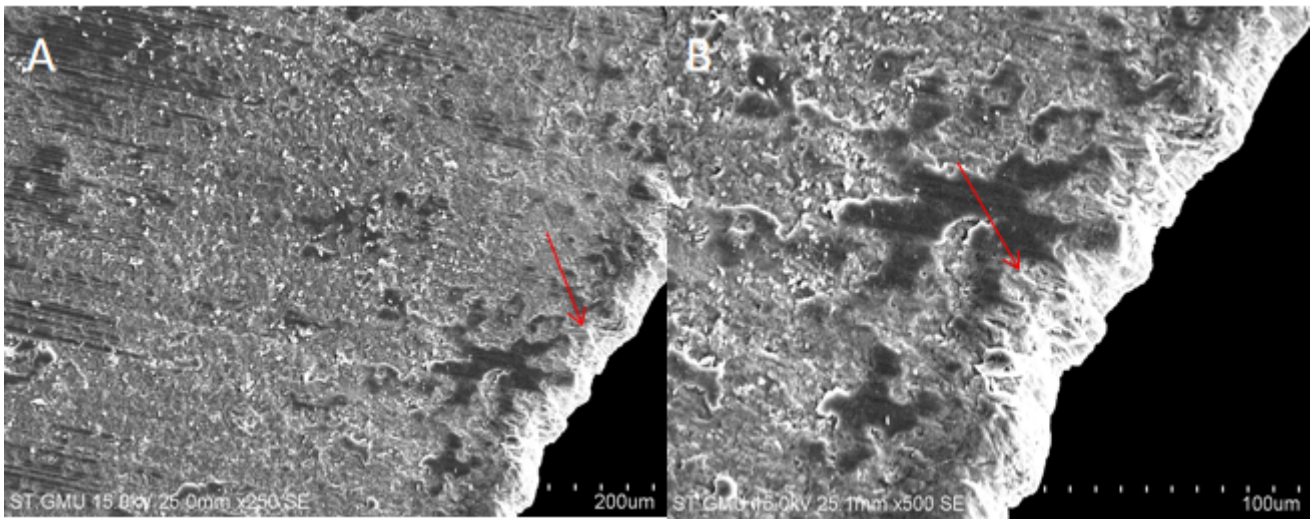

Figure 4

A SEM view of the penetration of resin tags in bleached and etched enamel (IIb 250×) B SEM view of the penetration of resin tags in bleached and etched enamel (IIb 500×)

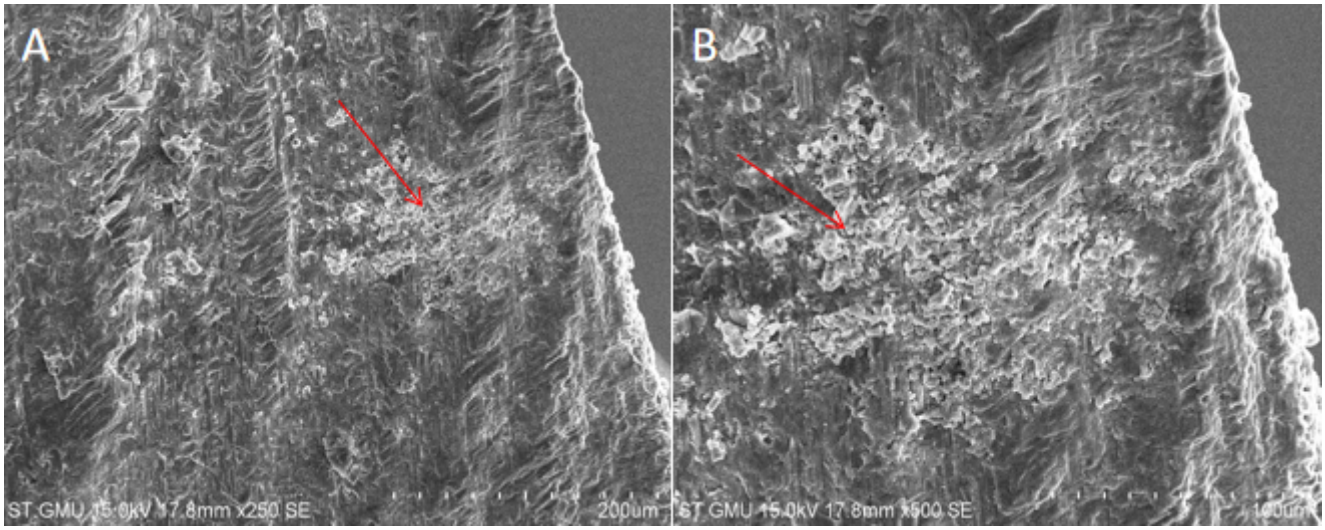

Figure 5

A SEM view of the penetration of resin tags in bleached and etched enamel placed in artificial saliva for 5 days (IIc 250×) B SEM view of the penetration of resin tags in bleached and etched enamel placed in artificial saliva for 5 days (IIc500×)

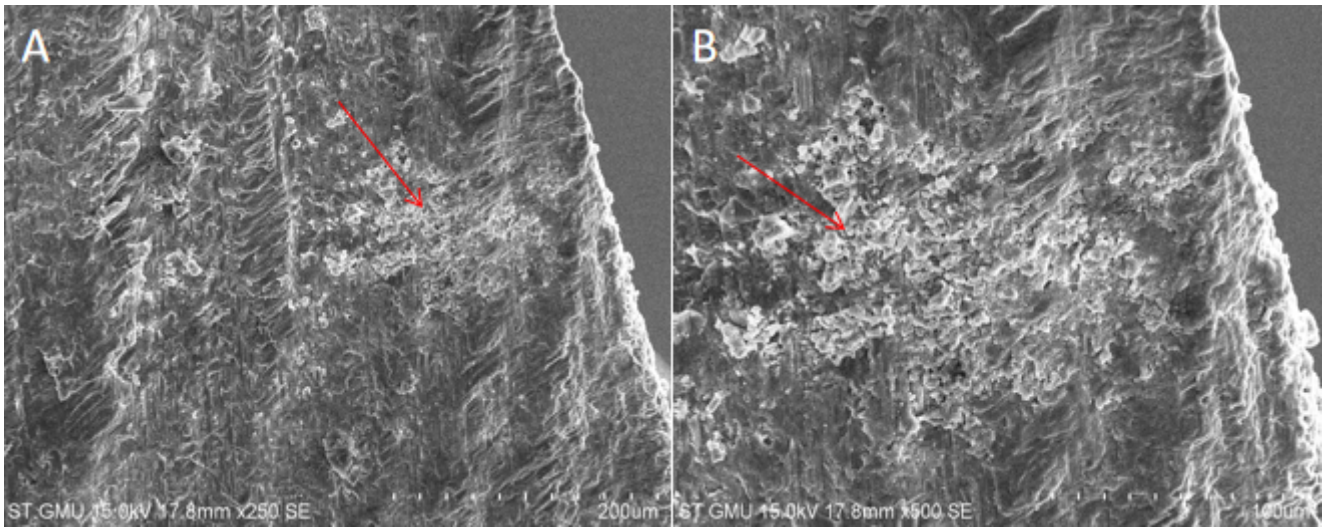

Figure 5

A SEM view of the penetration of resin tags in bleached and etched enamel placed in artificial saliva for 5 days (IIc 250×) B SEM view of the penetration of resin tags in bleached and etched enamel placed in artificial saliva for 5 days (IIc500×)