

Comparisons of retention, microleakage, penetration and caries inhibition of fissure sealant and resin infiltration in pit and fissure caries: an in vitro study

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Abstract

Objectives

The *in vitro* study aimed to investigate the retention, penetration, microleakage and the ability in inhibiting caries progression of fissure sealant, resin infiltration and their combined application.

Materials and methods

One-hundred-and-eight extracted human teeth with non-cavitated occlusal caries were randomly allocated to six groups: resin-based fissure sealant (FS), resin infiltration with 60s etching (RI 60), resin infiltration with 120s etching (RI 120), resin infiltration with 180s etching (RI 180), combined resin infiltration and resin-based fissure sealant (RI + FS), and the control group without sealant application. Following thermocycling, retention was evaluated via tactile and visual inspection, and microleakage was assessed visually under stereomicroscopy. Furthermore, sealant penetration was examined using scanning electron microscopy (SEM) while mineral density changes (Δ MD) after pH cycling and bacterial challenge, which reflected caries inhibition ability, were analyzed through Micro-CT.

Results

Retention of sealants did not differ significantly among groups. Compared with the control group, significantly milder microleakage was observed in FS, RI 60, and RI 120, whereas RI 180 and RI + FS revealed the comparable microleakage level to the control group. Penetration rates were determined as 50% (0.00%), 50% (4.17%), 50% (12.86%), 50% (26.41%), and 50% (72.45%) in RI 120, RI 60, RI 180, FS, and RI + FS, respectively, with no significant differences detected. Following pH cycling, significantly lower Δ MD were found in FS, RI 60, RI 120, RI 180, and RI + FS compared to the control group. However, no significant differences in Δ MD were detected among groups after bacterial challenge.

Conclusion

Fissure sealant and resin infiltration with etching times of 60s and 120s provided comparable protection against microleakage. Additionally, resin infiltration, fissure sealant and their combination exhibited equivalent effectiveness in caries inhibition.

Clinical relevance

Fissure sealant and resin infiltration demonstrate similar advantages in minimally invasive management of initial occlusal caries.

Introduction

Dental caries is a disease mediated by the biofilm, in which acid-producing bacteria lead to demineralization of dental hard tissues and cavitation. Despite a notable decrease in its prevalence over the past few decades, dental caries still affects approximately 3 billion people worldwide [1–3].

The fossae and grooves on the occlusal surface of molars and premolars are particularly prone to dental caries development compared to other tooth surfaces. The susceptibility is attributed to the accumulation of food debris and dental plaque in these intricate structures [4, 5]. Molars with deep fissures are three times more likely to develop caries compared to those with shallow fissures [6]. Additionally, more than 12% of maxillary molars and 19% of mandibular molars suffered from occlusal caries among children under 6 years old [7].

Fissure sealants are commonly used for teeth with deep occlusal fissures, especially in patients with high caries risk [8]. These sealants act by sealing the fossae and grooves, creating a diffusion barrier on enamel that reduces the accumulation of plaque [9]. This results in a reduction of caries risk by approximately 90% within 1 year and 50% within 4 years [10, 11]. The barrier also blocks nutrient delivery from the oral cavity to the cariogenic bacteria in the incipient carious lesions, resulting in caries arrest [9, 12, 13]. However, the anti-caries efficacy of fissure sealants is limited by their shallow penetration depth, as they only cover the outermost layer of the teeth [14, 15]. Furthermore, fissure sealants may dislodge or deteriorate over time, resulting in potential microleakage [16]. Studies have shown that 70% of sealants remain after 4.5 years, and only 39% remain after 9 years [17]. The unsatisfactory longevity of fissure sealants continues to be a notable drawback.

Comparing to fissure sealants, resin infiltrations demonstrate a deeper penetration depth that can reach the inner layer of non-cavitated fissure caries [18, 19]. A barrier is formed not on the superficial surface of the enamel [20], but within the caries lesion itself, sealing the porous, demineralized structure and enhancing the microhardness of the enamel [15]. Solid evidence supports the efficacy of infiltrant in arresting non-cavitated proximal caries lesions in both primary and permanent teeth [21]. However, when addressing non-cavitated lesions on occlusal surfaces, the specific morphology and the presence of air bubble caused by surface tension may hinder proper wetting following etching with hydrochloric acid and complete sealing of fissures [14, 22]. Various approaches, such as different pretreatments with brushing and abrasion, modified etching methods, and use of flowable composite materials, have been introduced to address these limitations [14, 15, 23–25]. Nevertheless, the literature indicates a lack of thorough investigation into long-term anti-caries outcomes of these interventions. Furthermore, while etching time is reported to affect the performance of fissure sealant [26], its influence on resin infiltration remains insufficiently investigated, as the optimal etching time has not yet been established. Minimizing the duration of etching is critical for patients with poor cooperation, where patients' compliance significantly impacts the treatment quality and clinical outcomes.

The present study aimed to evaluate and compare the commercial resin-based fissure sealant, resin infiltration, combined resin infiltration and fissure sealant regarding their retention, microleakage and

penetration depth in non-cavitated fissure caries. In addition, the influence of these sealants on enamel demineralization and remineralization was investigated. The null hypotheses tested were 1) There is no difference in the retention, microleakage, penetration and occlusal caries inhibition among resin infiltration, fissure sealant and resin infiltration + fissure sealant. 2) The etching time has no effect on the retention, microleakage, penetration and occlusal caries inhibition on resin infiltration.

Materials and Methods

Study design

Figure 1 outlines the key steps of this *in vitro* study. The study adhered to the Declaration of Helsinki and was reviewed and received approval from the Institutional Review Board (IRB) of The University of Hong Kong – Hospital Authority Hong Kong West Cluster (IRB number: UW 20–293).

Sample size calculation

Sample size was calculated using G*Power 3.1 (Franz Faul, Germany) based on a prior study [27], with significance level set at 0.05 and power at 0.95, respectively. Based on the primary variable, mineral density changes, derived from previous study [27], the minimum sample size was measured to be 14 tooth sections per group, derived from 6 molars. In total, 108 teeth (6 teeth x 6 groups x 3 studies) were collected for the study.

Specimen preparation

Freshly extracted human third molars with non-cavitated occlusal carious lesions (ICDAS 1–2) were collected from dental practitioners in Hong Kong and China Mainland. Informed consents were obtained verbally and in writing from donors. The teeth were stored in 0.5% thymol solution at 4°C until use [28]. All samples were examined under a stereomicroscope (Carl Zeiss Stereo 475002, ZEISS, Germany), with the assessment strictly based on the ICDAS criteria. Teeth with cavitated caries or developmental defects, such as hypoplasia and hypomineralization, were excluded. The occlusal surface of each crown was thoroughly cleaned using ultrasonication. The crowns were then sectioned and separated from the roots at the cemento-enamel junction with a 150-µm thick saw microtome (SYD Mikki Pulley, Japan) for subsequent tests. Afterwards, all specimens were coated with nail varnish (Revlon, USA), leaving occlusal surfaces exposed.

Treatments

Teeth were randomly distributed into 6 groups (Table 1). The sealants were applied according to the manufacturers' instructions. Briefly, specimens in group FS were etched with 37% phosphoric acid (H₃PO₄), thoroughly rinsed and air-dried, and subsequently applied with fissure sealant. The sealant was

cured for 20s using a Blue Style curing light (Ivoclar Vivadent, Liechtenstein) with an output of 1100 mW/cm². In group RI 120, specimens were etched with 15% hydrochloric acid (HCl) for 120s, rinsed, air-dried, and further dried with Icon-Dry. Infiltrant was applied and left undisturbed for 180s, then light-cured for 40s. Subsequently, a second layer of infiltrant was applied, left undisturbed for 60s and light-cured. Specimens in groups RI 60 and RI 180 were etched with 15% HCl for 60s and 180s, respectively, and subjected to the same procedure as group RI 120. Group RI + FS followed the identical protocol as group RI 120, but with the second application of infiltrant replaced by the application of fissure sealant, which was placed over the first infiltrant layer and light-cured for 20s. The control group did not receive any treatment.

Table 1
Protocol of treatments

Group	Etching	Sealant
FS	37% H ₃ PO ₄ (60s)	Helioseal pit and fissure sealant (Ivoclar Vivadent, Liechtenstein)
RI 60	15% HCl (60s)	ICON resin infiltration (DMG, Germany)
RI 120	15% HCl (120s)	ICON resin infiltration (DMG, Germany)
RI 180	15% HCl (180s)	ICON resin infiltration (DMG, Germany)
RI + FS	15% HCl (120s)	ICON resin infiltration (DMG, Germany) + Helioseal pit and fissure sealant (Ivoclar Vivadent, Switzerland)
Control	—	—

Retention and microleakage assessment

After treatments, specimens were stored in distilled water in the incubator at 37°C with a humidity of 100% for 1 day. 10000 cycles of thermocycling was then performed at the temperatures of 5°C and 55°C, the specified dwell time was 30 seconds and transfer time was 5 seconds [29]. The retention of sealants was evaluated via visual and tactile examinations under a stereomicroscope on the integrity and marginal sealing. Results were classified according to the reported criteria [30]: completely intact,

partially intact, missing. For microleakage assessment, specimens were immersed in a 2% solution of methylene blue for 24 hours [31]. Specimens were then rinsed with deionized water for two minutes, and sectioned buccolingually with a low-speed diamond disc under water cooling. A stereomicroscope was utilized for examining the microleakage by observing the dye penetration on the sections. Microleakage was measure based on the following standard [32]:

0: no dye penetration

1: dye penetration < 1/3 of the fissure depth

2: dye penetration between 1/3 and 2/3 of the fissure depth

3: dye penetration > 2/3 of the fissure depth

Penetration assessment

Of all sections obtained from tooth specimens, two to three sections were selected for scanning. The sections were then sputter-coated with platinum-palladium and observed under a scanning electron microscopy (SEM, SU1510, Hitachi, Japan). Representative images were captured at 70x and 550x magnifications. The penetration rate was determined using the established method [33]. Briefly, three reference points were identified: a) the deepest point on the outer margin of the sealant, b) the uppermost point of the lower margin of the sealant, c) the deepest point of the fissure. Horizontal lines were drawn through these points and labeled line a, line b, line c accordingly. The sealant depth was measured as the distance between line a and line b. The fissure depth was measures as the distance between line a and line c (Fig. 2). The penetration rate was calculated as follow:

$$\text{Penetration rate} = \text{sealant depth} / \text{fissure depth}$$

pH cycling and mineral density assessment

After the application of sealants, the specimens were assessed using a Micro-CT scanner (SkyScan 1276, Bruker, Belgium) with the scanning parameters set to 90 kV tube voltage, 200 μ A tube current, 3630 ms exposure time, and 14 μ m spatial resolution.

A modified version of a reported pH cycling procedure was applied for 28 days [34–36]. Briefly, after treatments, the specimens were exposed to 6 hours of demineralization solution (2.2 mmol/L CaCl_2 , 2.2 mmol/L KH_2PO_4 and 50 mmol/L acetic acid, pH adjusted to 4.4 with 5 mol/L KOH) for cariogenic challenge, followed by 18 hours of artificial saliva (1.5 mmol/L CaCl_2 , 0.9 mmol/L NaH_2PO_4 and 150 mmol/L KCl, pH adjusted to 7.0 with 5 mol/L KOH) for remineralization. The specimens were rinsed with deionized water between each phase. The pH cycling was conducted at a temperature of 37°C with agitation of 150 rpm, and the artificial saliva and demineralization solution were renewed daily. The superficial demineralization was inspected under a stereomicroscopy.

Following pH cycling, the specimens were rescanned using Micro-CT. The resulting data were then compared with those obtained before pH cycling. The teeth were reconstructed with CTvox software (Bruker, Belgium), and the occlusal surfaces were visually inspected for any structure changes around the fissures. DataViewer software (Bruker, Belgium) was employed to examine the datasets in the transaxial, sagittal, and coronal planes. The datasets obtained after pH cycling were spatially adjusted to ensure the consistent positions and orientations with the pre-cycling datasets. Once the alignment was completed, the sagittal sections were captured and imported into CTAn software (Bruker, Belgium) for the evaluation of mineral density. The selected regions of interest were defined, and the mineral density value (MD) was subsequently determined. The change in the mineral density (ΔMD) was measured through the formula:

$$\Delta MD = MD_{before\ pH\ cycling} - MD_{after\ pH\ cycling}$$

Bacterial challenge and mineral density assessment

As with the pH cycling, Micro-CT scanning was performed prior to the bacterial challenge, following the same parameters as described above. The bacterial challenge was performed using *Streptococcus mutans* (ATCC 35668) [27, 37]. To ensure pure bacteria cultures, a single colony was harvested from the agar plate and transferred into brain heart infusion broth for further cultivation until sufficient bacteria were available for subsequent testing. Following sterilization with 75% ethanol and ultraviolet irradiation, each specimen was anaerobically cultured with 1.5 ml bacterial suspension (6×10^8 cells/mL) at 37°C for 7 days. After incubation, tooth surfaces were cleaned with flowing water. The superficial demineralization was inspected under a stereomicroscopy. Afterwards, the specimens were rescanned using Micro-CT, MD was computed and ΔMD was calculated using the following formula:

$$\Delta MD = MD_{before\ bacterial\ challenge} - MD_{after\ bacterial\ challenge}$$

Statistical analysis

Statistical comparisons were performed using SPSS v. 29 (IBM SPSS® Statistics Inc, USA). Data were expressed as mean $\pm$ standard deviation for parametric variables, and median with interquartile range (IQR) for non-parametric variables. The normality of data was evaluated using Shapiro-Wilk test, while the equality of variances was examined with Levene's test. One-way ANOVA with Tukey's post-hoc tests were conducted for the data that met the assumptions of normality and equality of variances. For non-parametric data, Kruskal-Wallis test was utilized, followed by Dunn's multiple comparisons test. To investigate the correlations between the results of different tests, Spearman's rank correlation was performed, followed by robust regression if indicated. The value of $p < 0.05$ was considered statistically significant ($*p < 0.05$, $**p < 0.01$, and $***p < 0.001$).

Results

Retention

After thermocycling, the retention of sealants was visually assessed under the stereomicroscope and tactilely assessed with the strip probe. For RI 60 and RI 180, among the six specimens tested, two were partially intact, while the other four were completely intact. Half of the specimens displayed complete integrity in RI 120, and the remaining half exhibited partial integrity. Lower retention rates were observed in the remaining two groups. Specifically, two specimens maintained complete integrity and four remained partially intact in FS, whereas only one specimen showed complete retention and five were partially intact in RI + FS. However, intergroup comparisons indicated no statistically significant differences ($p = 0.354$) (Fig. 3c). It is noteworthy that the partially debonded parts of the resin infiltrant were confined to the junctions between the occlusal surfaces and the smooth surfaces. Conversely, fissure sealant revealed more extensive debonding across the entire occlusal surfaces (Fig. 3a).

Microleakage

The buccolingual sections were prepared after thermocycling and dye penetration. Typical images illustrating the scoring criteria of microleakage are shown in Fig. 3b. Figure 3d reveals the comparable portions of sections with no microleakage across all groups. Nevertheless, a large portion (78%) of specimens in the control group displayed microleakage extending into the lower 1/3 of the fissures, while the incidences of such severe microleakage were below 35% in the remaining groups, with the lowest percentage at 13% observed in RI 60. Statistically, significant differences were shown between the control group and FS ($p = 0.025$), RI 60 ($p < 0.001$), and RI 120 ($p = 0.034$), whereas no significant differences were indicated between the control group and RI 180 ($p = 0.053$) or RI + FS ($p = 0.062$).

As shown in Fig. 3b, the penetration pattern resulted in a blurred interface between the resin infiltrant and the fissures, in contrast to a distinct boundary found between the fissure sealant and the fissures. Additionally, more prominent white demineralization regions were present adjacent to the fissure in RI 180 compared with other groups, with most sections showing dye penetrating into these regions.

Penetration

The specimens further underwent SEM scanning for the analysis of sealant penetration. Figure 4b shows the fissure depth within each section. Despite substantial within-group variation, no significant differences were observed among groups ($p = 0.167$), providing a reliable baseline for the penetration assessment. As presented in Fig. 4c, penetration rates were comparable across different groups, with half of the sections in each group reaching 100% penetration. Specifically, RI 120 exhibited the greatest penetration (IQR: 0.00%), followed by RI 60 (IQR: 4.17%), RI 180 (IQR: 12.86%), FS (IQR: 26.41%), and RI + FS (IQR: 72.45%), respectively. While the penetration rates surpassed 75% in the majority of the sections, distinctly low penetration rates ($< 50\%$) were detected in some sections from FS and RI + FS, presumably as a result of the intricate morphology of individual fissures. However, no significant differences in penetration rates were identified among all groups ($p = 0.712$). To investigate whether the penetration rates were affected by fissure depths, a correlation test was performed. The result demonstrated a

significant negative correlation between fissure depths and penetration rates ($\rho = -0.397$, $p = 0.003$). However, further regression analysis produced a slope close to zero (6.629×10^{-19}), indicating no obvious linear relationship between fissure depths and penetration rates.

Notably, detachment of the sealants from the fissures was observed in FS, which was less frequently detected in RI groups. Such detachment was less pronounced in RI + FS with evidence of uniform integration between fissure sealant and fissures. However, some partial detachment still remained with linear gaps at the interfaces. Besides, RI groups revealed satisfactory fusion of the two-layer resin infiltrant, and RI + FS also exhibited favorable integration between fissure sealant and resin infiltrant, with no evidence of detachment after thermocycling. Furthermore, polymerization shrinkage was observed in both fissure sealant and resin infiltrant, which typically induced the formation of small gaps at the sealant-tooth interfaces, most notably at the base of the sealants (Fig. 4a).

Caries inhibition (pH cycling)

As shown in Fig. 5a, enamel demineralization extended to perifissural enamel and fissure walls in the control group, whereas such demineralization in sealed specimens was confined to the vicinity of cusps and occlusal margins. The findings were further validated through the 3D reconstruction images, which revealed structural defects, indicative of reduction in mineral density, within the fissures in the control group. In contrast, the defects did not extend into fissures in other groups (Fig. 5b, 5c). Following pH cycling, the control group revealed significant differences in ΔMD compared to other 5 groups treated with sealants ($p < 0.001$). However, no significant differences were detected among FS, RI 60, RI 120, RI 180, and RI + FS ($p > 0.823$) (Fig. 5d).

Caries inhibition (Bacterial challenge)

In contrast to the outcomes following pH cycling, less severe demineralization was noted in the specimens subjected bacterial challenge, and no distinct white lesions were found within fissures in any group (Fig. 6a). The 3D reconstruction images demonstrated consistent results, with no noticeable volume loss in the specimens after bacterial challenge, as compared to before the challenge (Fig. 6b, 6c). Statistically, no significant differences were identified among all groups ($p = 0.390$), with both sealed and unsealed teeth exhibited comparable decrease in mineral density after exposure to bacterial challenge (Fig. 6d).

Discussion

The objective of the present study was to assess the penetration ability, microleakage prevention, and caries inhibition of fissure sealant and resin infiltration applied through different methods. In this context, although the outcomes for retention and caries inhibition did not differ among the experimental groups, the null hypotheses were partially rejected, as variations in etching time of resin infiltration and the combined application of resin infiltration and fissure sealant increased microleakage.

The effectiveness of sealants in inhibiting caries largely depends on sealant retention [38], since only the well-retentive sealants can serve as a physical barrier against the nutrients and bacteria, thus preventing the progression of caries [39]. After 10,000 cycles of thermocycling, which simulated one year of clinical use [40], adequate retention was shown in the fissure sealants, with majority of the sealants remained adherent to the fissures and debonding only limited to the marginal regions. The findings align with a previous clinical study, which reported a 74.3% one-year success rate of fissure sealant, with most failures resulting from marginal discoloration [41]. Notably, no complete loss or significant debonding of fissure sealants was observed when applied directly over the resin infiltrant, suggesting the feasibility of the combined treatment.

Attributed to the increased shear bond strength induced by HCl [42], resin infiltration exhibited better retention compared with fissure sealant, as debonding was restricted to occlusal-smooth surface junctions and no marginal debonding was detected elsewhere on the occlusal surfaces. Nevertheless, no significant differences were shown between fissure sealant, resin infiltration and the combined treatment. This may be owing to the limited accuracy of the evaluation method, which failed to consider the extent of the debonded area. Besides, the duration of the thermocycling may not be long enough to reveal significant differences among the different groups. Apart from the bond strength that affects sealant retention, mechanical and chemical wear can also contribute to dislodgement of sealants [43]. Chewing conditions, saliva and acid exposure, and other relevant factors should also be considered prior to the determination of sealant retention. Regarding etching time, the plateau effect may explain the present findings, as prolonged etching does not further increase bond strength once a relatively short etching time has already achieved maximum bond strength [44].

Although the integrity of sealants was well maintained, the presence of marginal debonding may further facilitate microleakage at the tooth-sealant interface. Where microleakage is present, bacteria can be trapped beneath the sealants without being eliminated, thereby initiating caries [45, 46]. During the etching process, porosities are created on exposed enamel as the smear layer is removed and the enamel rods are dissolved by acid action. Capillary attraction then facilitates the penetration of fissure sealant into these porosities, resulting in the formation of an interlocking structure between the sealant and enamel after light polymerization [47]. The sealed configuration significantly reduces the infiltration and accumulation of bacteria deep within the fissures.

Following stronger conditioning with HCl at 120s as per the manufacturer's instructions, and a shorter conditioning time of 60s, resin infiltration revealed a less defined interface with the enamel under microscopic observation, indicating that the resin infiltrant penetrated the etched enamel structure. However, no significant difference in microleakage was observed among FS, RI 60 and RI 120, possibly because both the fissure sealant and resin infiltration, being resin-based materials, may have reached the maximum achievable level within the constraints of polymerization shrinkage [42, 48]. These findings conflict with a previous study which reported reduced microleakage of resin infiltration compared to fissure sealant on smooth surfaces [49]. The reason lies in the more intricate morphology of the occlusal surface, which is more difficult to be thoroughly cleaned, which hinders the penetration of infiltrant into

lesions [23], thereby restricting the effectiveness of resin infiltration. Conversely, microleakage was also not reduced when the etching time of HCl was extended to 180s, possibly attributed to lesions caused by over etching, which were not infiltrated by resin [50]. Furthermore, the strong erosive effect of HCl alters not only the micromorphology but also the macroscopic shape of occlusal fissures. The removal of the outer layer of enamel weakens the structural integrity of fissure margins, which may induce collapse of the fissure edges, resulting in a modified fissure contour [51, 52]. Although HCl etching blunts sharp fissure margins and expands the entrance, thus reducing capillary resistance and enhancing infiltrant penetration, the associated structural collapse can yield irregular gaps [53]. Low-viscosity infiltrant may fail to adapt and compensate for polymerization shrinkage within these irregular gaps, ultimately resulting in unsealed regions. Similarly, in the case of combined application of resin infiltration and fissure sealant, the sealant was applied over enamel pretreated with HCl, which may also be considered over-etching, resulting in no significant difference from the control group.

The unfilled resin endows fissure sealant with low viscosity, which further ensures its deep penetration into narrow fissures [54]. Besides viscosity, the penetrativity of sealants also relies on contact angle of the liquid with the solid as well as surface tension, which, along with viscosity, are referred to as the penetration coefficient [55]. While fissure sealant showed a relatively high penetration coefficient at 4 cm/s (Helioseal) [56], resin infiltration revealed a much higher figure of 147 cm/s (ICON) [14]. Accordingly, although no significant differences were found among different treatments, resin infiltration displayed more stable penetration rates. A longer application time of resin infiltration, which provided adequate time for infiltrant to flow into the porosities, also contributed to the current findings [14]. Another explanation for the better penetration of resin infiltration was that the remaining space not filled by the first layer of infiltrant, as well as the gap formed after polymerization shrinkage, could be compensated by the second application of resin infiltration. In contrast, such compensation by fissure sealant was relatively poor, which accounted for the remarkably low penetration rate in RI + FS. While low viscosity offers certain benefits, it might also compromise the complete filling of infiltrant over fissures [14, 57]. Nevertheless, the drawback was circumvented through the two-layer application of resin infiltration, achieving the occlusal filling level comparable to that of fissure sealant.

Consistent with the findings under the stereomicroscope, marginal debonding was also evident under SEM, occurring more frequently in fissure sealant. The debonding was less pronounced in RI + FS, likely due to the enhanced interlocking structure formed after HCl etching [58, 59]. Unlike previous study, resin infiltration exhibited superior adaption with minimal marginal debonding [15]. Sharing compatible chemical foundations, the fissure sealant integrated with resin infiltrant effectively, with marked polymerization shrinkage [42, 60] shown in both sealants along the walls and bases of fissures. Although a significant correlation was observed between fissure depths and penetration rates, most sealants exhibited penetration rates near 100%, irrespective of the fissure depth. The results may be influenced by certain sections showing lower penetration rates within narrow fissures, which were more commonly associated with greater depth. In addition, discrepancies in the fissure type may be another factor contributing to the non-linear relationship between fissure depths and penetration rates [61].

A 28-day pH cycling regimen was employed to simulate long-term caries progression in a high caries risk oral environment [62]. Demineralization was progressively induced via ion exchange in the acidic solution with a pH below 5.5 [63], commencing at the outermost layer of enamel on the occlusal surface. After 28 days, demineralization spread to the fissure walls in the control group, while it was limited to regions uncovered by sealants in other groups. Even at the sealant margins, distinct demineralization was not detected beneath the translucent sealants, suggesting effective marginal sealing achieved by both fissure sealant and resin infiltration. Although the prolonged etching time may increase the risk of microleakage in resin infiltration, such impact appeared minimal for caries inhibition, as indicated by the lack of significant differences in ΔMD among various resin infiltration groups.

Apart from good marginal sealing, structural stability under high caries risk conditions is equally essential in inhibiting caries development. Under continuous acid attacks, degradation of resin-based sealants is accelerated, resulting in increased structural porosity and deterioration of mechanical properties [64, 65]. Moreover, acidic environment further increases the surface roughness of sealants and facilitates bacterial adhesion, which later produces acid on tooth surfaces [66]. Nevertheless, compared to the outcomes observed after pH cycling, less pronounced demineralization was noted following bacterial challenge, with consistently low ΔMD across all groups. The findings may be attributed to the biofilm acting as the physical barrier, which limited matrix exchange on both sides and thereby restricted acid production. Furthermore, bacterial activity might be influenced by the sugar content in the culture medium and frequency of medium changes, making it challenging to optimize the experimental parameters to maintain a persistently low pH level.

Despite the outstanding performance of resin infiltration in preventive dentistry, certain considerations are essential in clinical practice. Different from applications on smooth surfaces where indications for resin infiltration can be verified via visual, tactile, and radiographic examinations [67, 68], the precise diagnosis of occlusal caries can at times be challenging. While the superficial layer of occlusal surfaces displays sound structure or initial carious lesions, hidden caries may be concealed within deep fissures or dentine [69], which can lead to the misjudgment in making treatment decisions. Prior to infiltrant applications, the complex anatomy of fissures, comprising cusps and isthmuses, hinders thorough removal of plaque and calculus, as well as proper etching of the fissures [70]. The complete etching process is further impaired by the rather high viscosity and surface tension of HCl gel, impeding its penetration into fissures and distribution over the enamel surface [14, 23]. While the absence of filler improves the penetrability of resin infiltrant, accompanying low abrasiveness may compromise its durability when subject to occlusal force [24].

In clinical practice with paediatric patients, optimal outcomes are constrained by their limited cooperation and tolerance for the etching process and extended chair time. Specifically, excessive tongue movement and high salivary flow may result in inadequate moisture control, which in turn compromises the retention and marginal adaptation of fissure sealants [71]. While for resin infiltration, the risk of the gingival ulceration induced by HCl necessitates rigorous rubber dam isolation, thereby restricting its general use by general practitioners and in young patients [23]. Moreover, regular recall and

occasional repair or reapplication of sealants can be challenging for patients with poor compliance and insufficient access to dental care [72]. Optimizing the sealant properties with improved moisture resistance and simplified application procedure may promote the feasibility and effectiveness of these treatments in paediatric patients.

Although the present study was designed to simulate sealant performance under the oral conditions, the interpretation of the findings might be limited by several confounding variables. In particular, surface morphology and fissure type of the included third molars could not be standardized. Additionally salivary flow and masticatory force could not be fully simulated. The results may vary due to differences in sealant flowability across various fissure structures, as well as the mechanical load and abrasion from chewing and saliva. As caries development is a multifactorial process resulting from an extended period of interaction between bacteria and sugar [73], using thermocycling, pH cycling, or bacteria challenge individually cannot fully replicate this process. The results would be more convincing if the study design incorporates all these etiology factors together. Furthermore, due to the distinct color differences between resin-based fissure sealant and resin infiltration, blinding of outcome assessor could not be achieved, which may potentially introduce evaluation bias.

In contrast to clinical trial, patient-related factors, including immune response, dietary habits, and measures taken in response to sealant failure, which are crucial to clinical outcomes, are difficult to mimic and replicate in *in vitro* studies. In addition, *in vitro* studies do not account for patient compliance and variability in operator technique, which may also influence the outcomes. Although thermocycling and pH cycling can simulate long-term fatigue and caries progression, they cannot fully replicate the intricate and dynamic intraoral environment over time. Clinical trials with longer observation periods may provide a more accurate assessment of long-term effectiveness of sealants.

Conclusion

In summary, resin infiltration showed similar efficacy in preventing microleakage with both 60s and 120s etching durations compared to fissure sealant. Prolonged etching to 180s or combined application of resin infiltration and fissure sealant did not result in additional therapeutic benefit. Similar penetrativity was observed between resin infiltration and fissure sealant, regardless of fissure depth. Fissure sealant, resin infiltration, and their combined application were similarly effective in inhibiting occlusal caries.

Declarations

Ethical approval

The present study was reviewed and approved by the Institutional Review Board (IRB) of The University of Hong Kong – Hospital Authority Hong Kong West Cluster (IRB number: UW 20–293).

Informed consent

Verbal and written informed consents were obtained from all donors involved in this study.

Competing interest

The authors declare no conflict of interest.

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Author Contribution

The conception and design of the study were accomplished by Y.T., P.P.Y.L. and C.K.Y.Y.. Material preparation was performed by Y.T., M.Y. and Z.C.. Data collection and analysis were conducted by Y.T.. The first draft of manuscript was prepared by Y.T., and further revised by P.P.Y.L. and C.K.Y.Y.. All authors reviewed and approved the final version of the manuscript.

Data availability

The data supporting the findings of the present study are available from the corresponding author on reasonable request.

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Figures

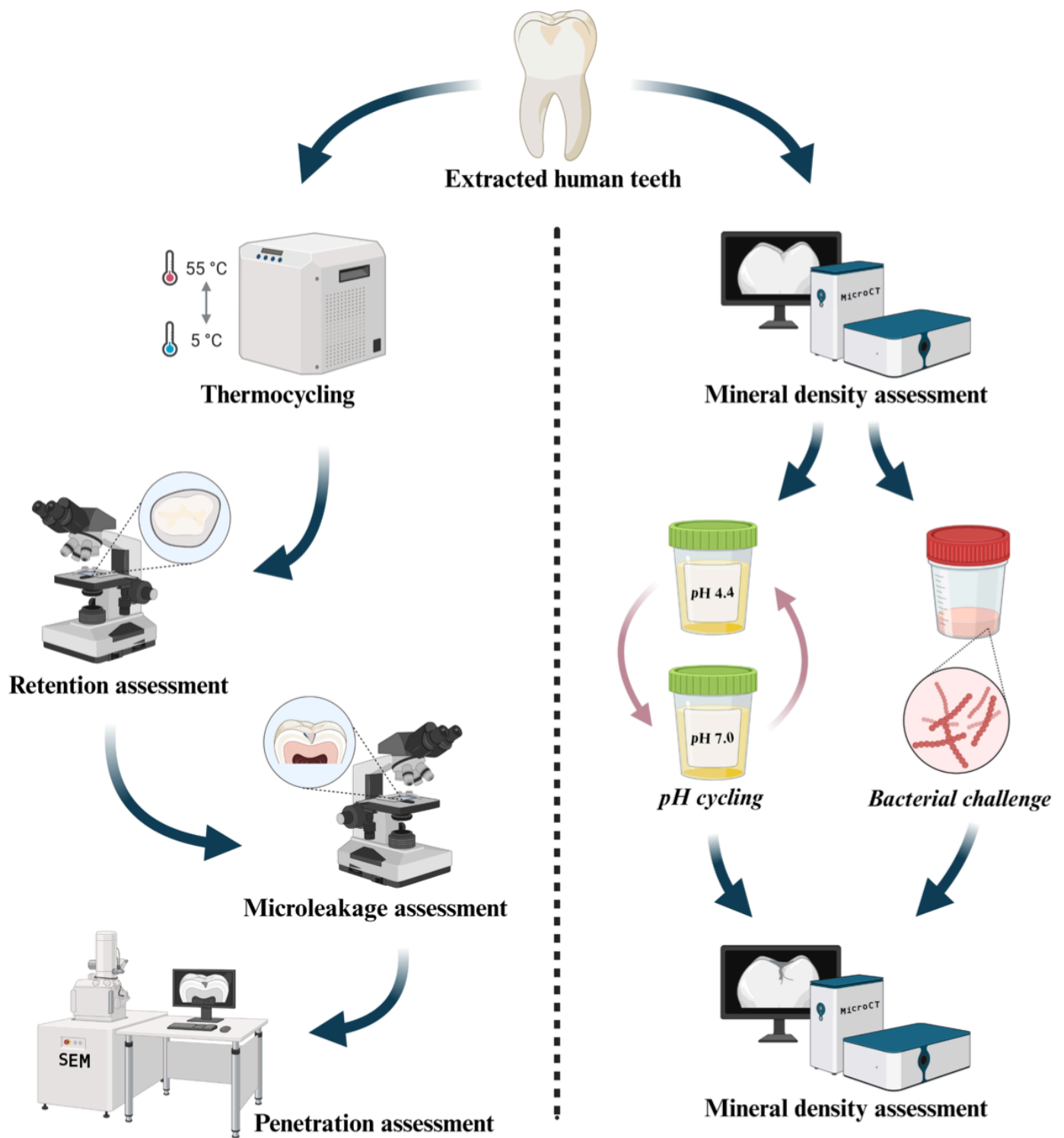


Figure 1

Key steps of the study

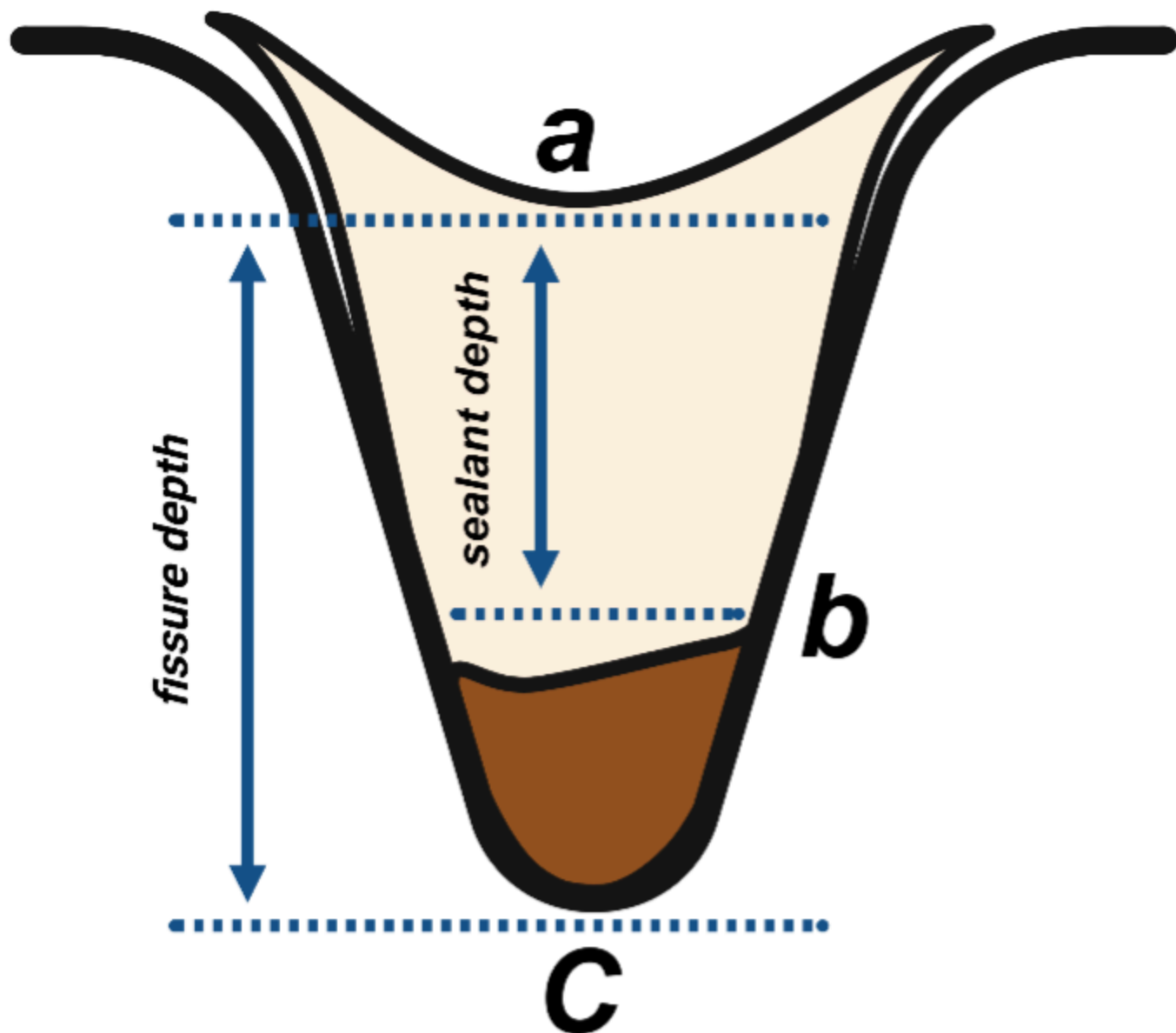


Figure 2

Schematic illustration for the measurement of fissure depth and sealant depth

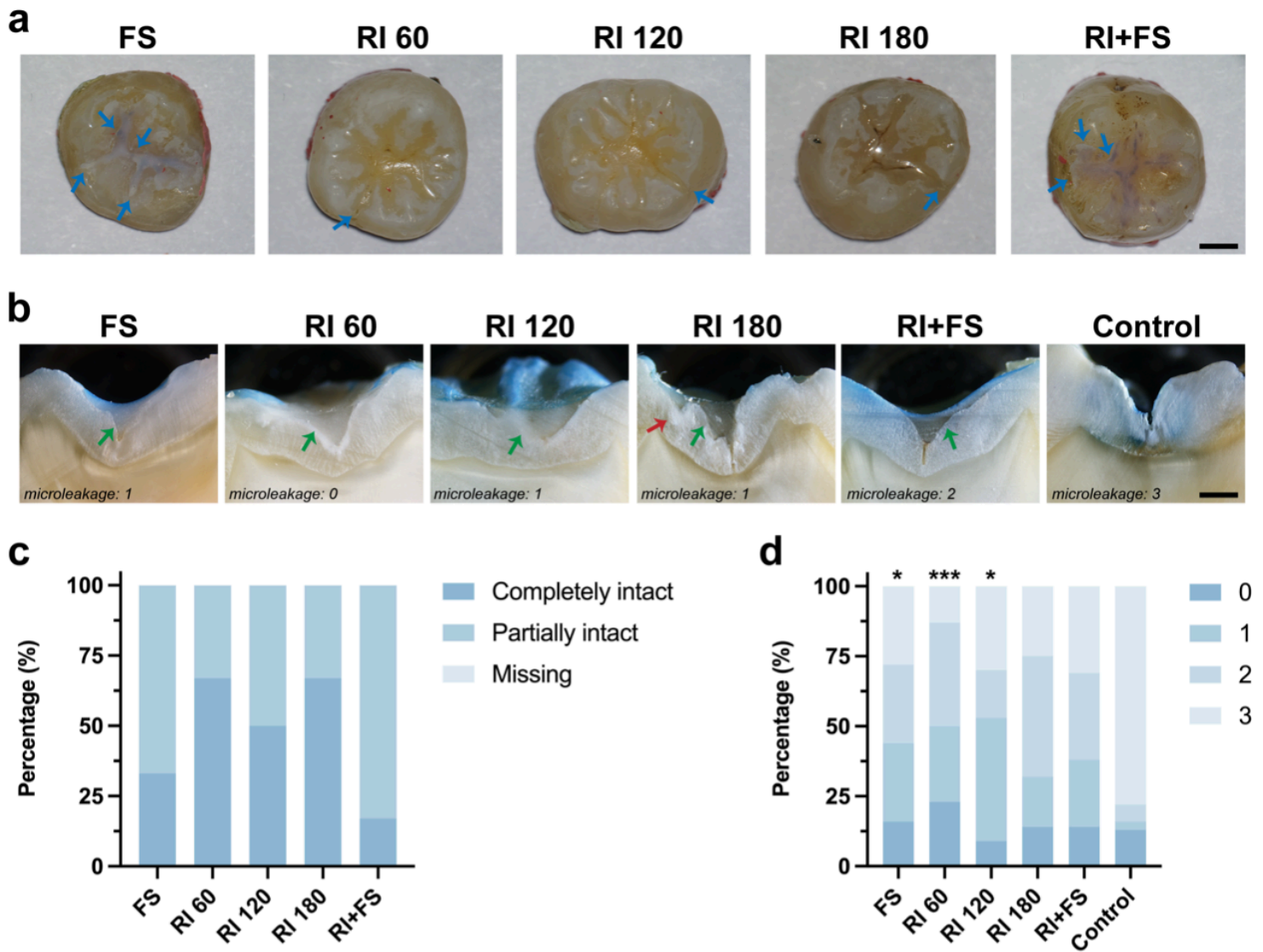


Figure 3

(a) Microscopic images of tooth specimens after thermocycling (scale bar: 3 mm). Blue arrows refer to debonded sealants. (b) Microscopic images of tooth sections after dye penetration (scale bar: 8 mm). Green arrows refer to interface between sealants and enamel, and the red arrow refers to over-etched enamel. (c) Percentage distribution of sealant retention grades. (d) Percentage distribution of microleakage grades. * $p < 0.05$, and *** $p < 0.001$ compared with the control group

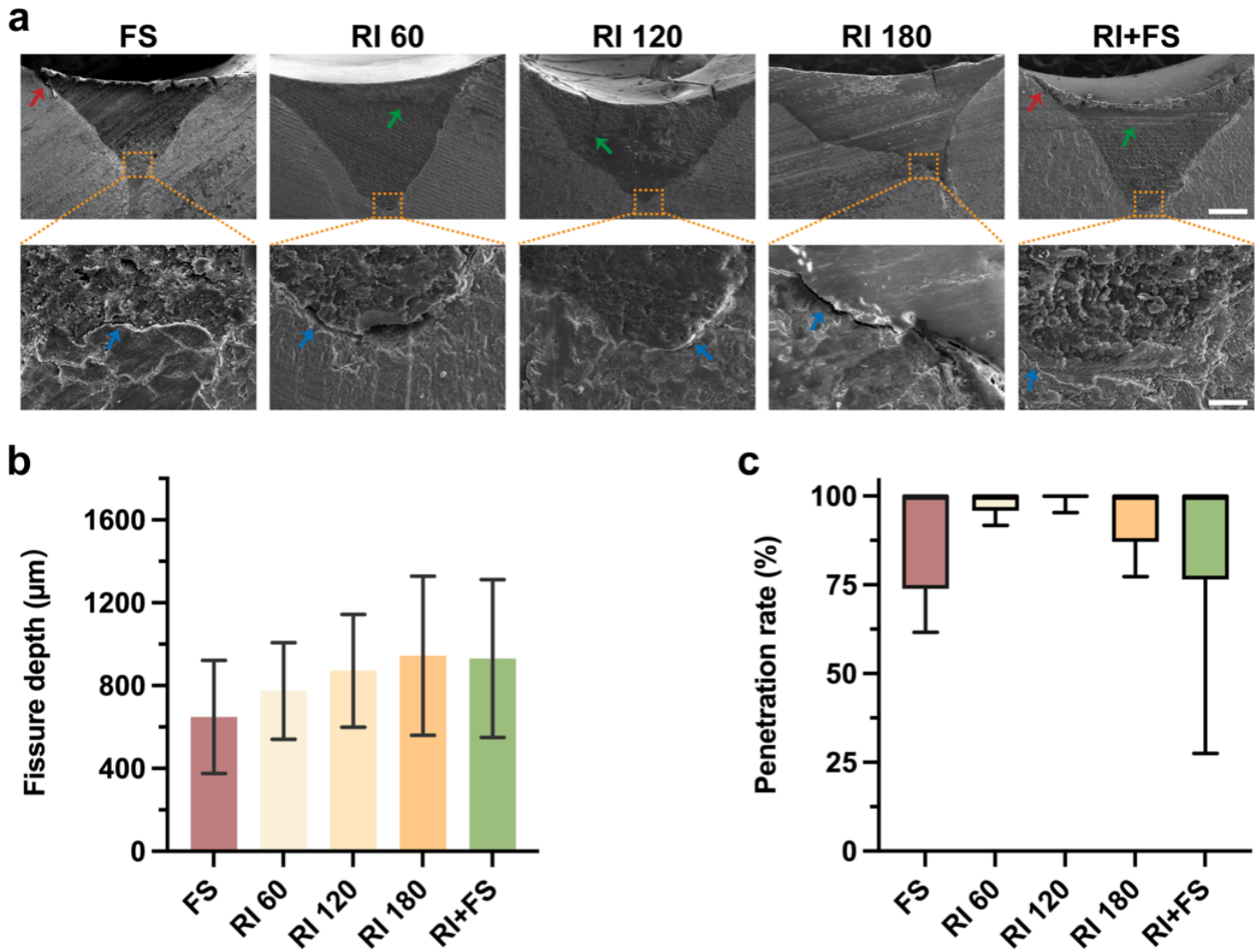


Figure 4

(a) SEM images of tooth sections (scale bar: 300 μm) and magnified SEM images of tooth sections (scale bar: 40 μm). Red arrows refer to marginal debonding of sealants, green arrows refer to interface between sealants, and blue arrows refer to polymerization shrinkage (b) Fissure depth in different treatment groups. (d) Penetration rates of sealants under different treatments

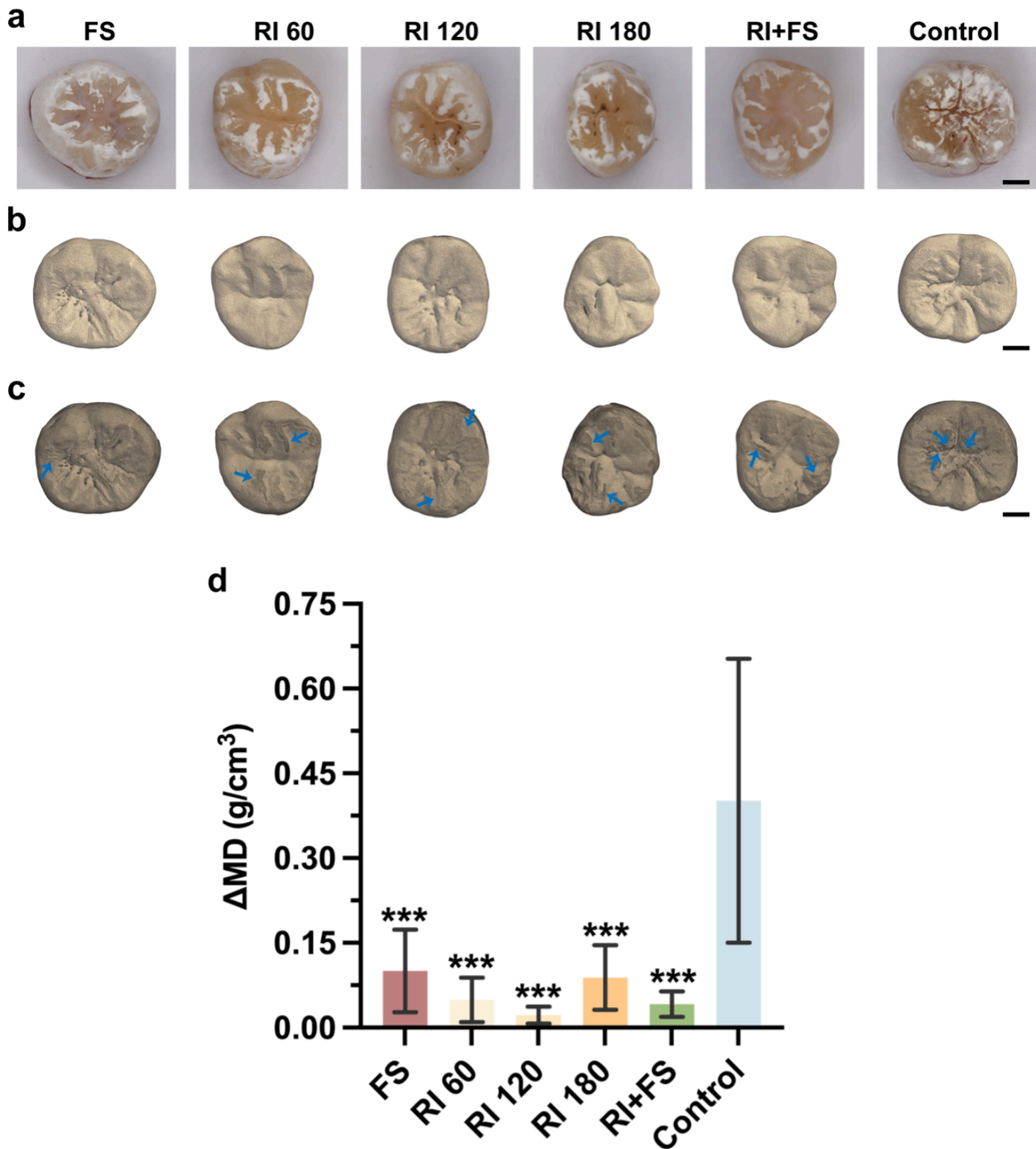


Figure 5

(a) Digital photographs of tooth specimens after pH cycling (scale bar: 3 mm). (b) 3D reconstruction images of tooth specimens before pH cycling (scale bar: 3 mm). (c) 3D reconstruction images of tooth specimens after pH cycling (scale bar: 3 mm). Blue arrows refer to demineralization regions. (d) Mineral density changes within fissures in different treatment groups following pH cycling. *** $p < 0.001$ compared with the control group

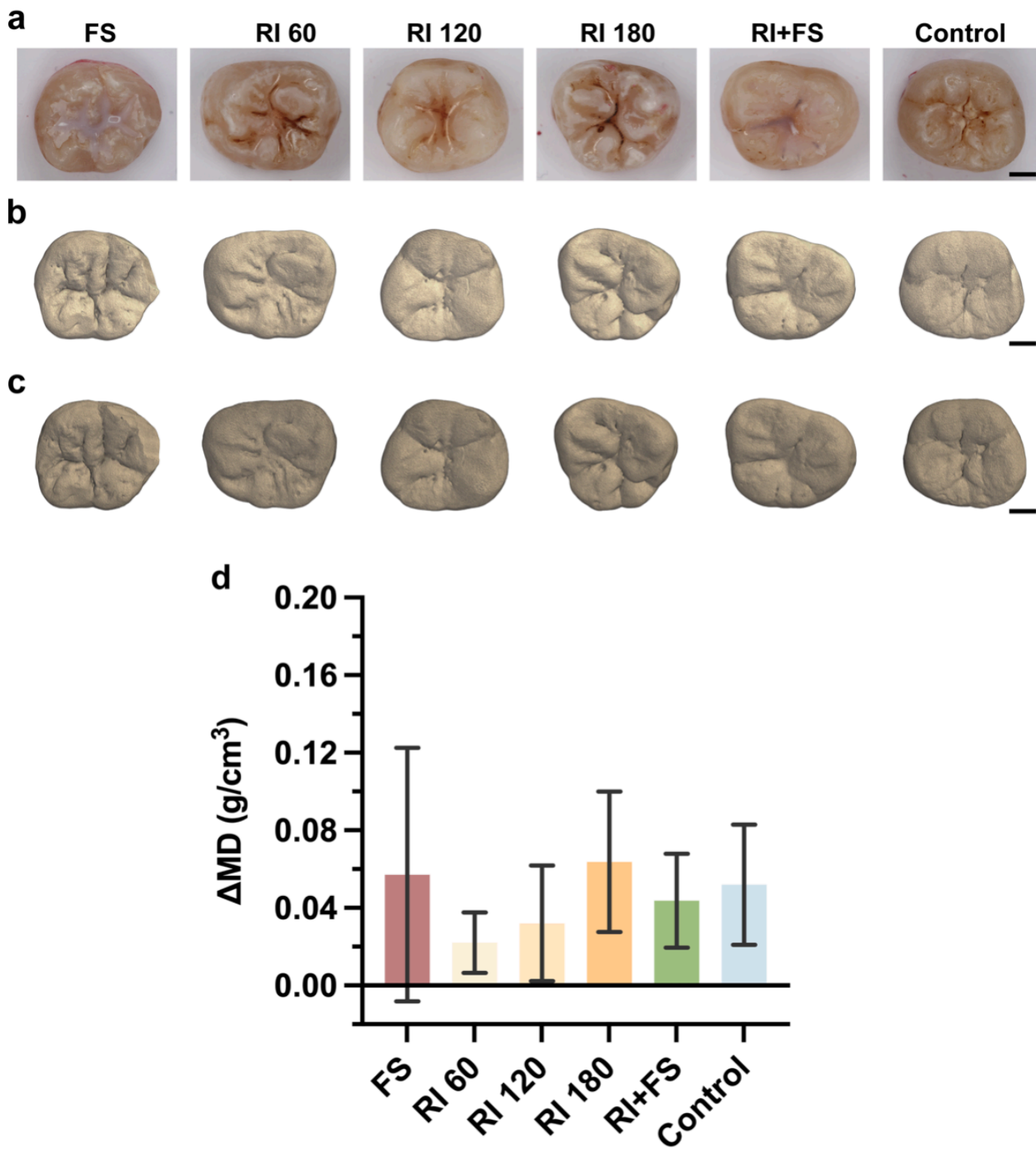


Figure 6

(a) Digital photographs of tooth specimens after bacterial challenge (scale bar: 3 mm). (b) 3D reconstruction images of tooth specimens before bacterial challenge (scale bar: 3 mm). (c) 3D reconstruction images of tooth specimens after bacterial challenge (scale bar: 3 mm). (d) Mineral density changes within fissures in different treatment groups following bacterial challenge