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ORIGINAL RESEARCH

Adhesion of tooth fragment after trauma: effect of adhesion strategy and storage in the rescue box

Asli Kaya[1], Blend Hamza[2], Nadin Al-Haj Husain[1,3], Kiren J. Mätzener[1], Mutlu Özcan[1,*]

[1]Clinic of Masticatory Disorders and Dental Biomaterials, Center of Dental Medicine, University of Zurich, 8032 Zurich, ZH, Switzerland
[2]Clinic of Orthodontics and Pediatric Dentistry, Center of Dental Medicine, University of Zurich, 8032 Zurich, ZH, Switzerland
[3]Department of Reconstructive Dentistry and Gerodontology, School of Dental Medicine, University of Bern, 3010 Bern, BE, Switzerland

***Correspondence**
mutlu.ozcan@zzm.uzh.ch
(Mutlu Özcan)

Abstract

This study aims to investigate the impact of storage conditions for crown fragments (specifically, whether they were stored within a tooth rescue box or in tap water) on their adhesion to fractured teeth when subjected to two different adhesive systems (namely, total etch and self etch). Sixty maxillary premolars were sectioned to obtain tooth fragments. These fragments were stored briefly (2 hours) and reattached in the following groups: Group 1 (fragments stored in tooth rescue box and reattached with etch and rinse (E&R) technique), Group 2 (fragments stored in tap water and reattached with E&R technique), Group 3 (fragments stored in tooth rescue box and reattached with self-etch (SE) technique), and Group 4 (fragments stored in tap water and reattached SE technique). After reattachment, the bonded tooth fragments underwent thermal cycling (500 cycles, 5–55 °C) and bond strength testing using a universal testing machine. Two-way Analysis of Variance (ANOVA) and Tukey's tests were used for bond strength comparison ($p \leq 0.05$). A two-parameter Weibull distribution was conducted to evaluate the reliability of the storage medium and adhesion modality on bond strength. The results showed that measured shear bond values (MPa ± Standard deviation (SD); arranged in descending order) for each group were: Group 2 (Tap water/E&R = 6.5 ± 2.1), Group 1 (Rescue box/E&R = 6.0 ± 2.5), Group 4 (Tap water/E&R = 5.1 ± 2.8), and Group 3 (Rescue box/SE = 3.6 ± 3.2). Significant differences were found only between Groups 2 and 3 ($p = 0.002$). In conclusion, storing crown fragments in a tooth rescue box did not significantly affect the shear bond strength of the restored tooth. However, fragments reattached using the self-etch technique showed comparable shear bond strength but a higher rate of adhesive failures compared to the E&R technique.

Keywords

Dental trauma; Pediatric dentistry; Adhesion; Adhesive resin; Dental materials; Storage medium; Tooth rescue box

1. Introduction

Tooth fractures, often involving a maxillary incisor, represent the most prevalent type of dental trauma observed in permanent dentition [1]. When these fractures are confined to the crown, they may exclusively affect the enamel, manifesting as enamel infractions. In cases where both enamel and dentin are involved, it is termed an "uncomplicated crown fracture", while fractures of the enamel, dentin and dental pulp are referred to as "complicated crown fractures". Among these variations, uncomplicated crown fractures are the most frequently encountered [2]. Early attempts to address uncomplicated crown fractures through fragment reattachment date back to the 1980s, coinciding with the introduction of dentin adhesives. However, it was reported that the longevity of such reattachments was relatively short, with a survival half-life of approximately 2.5 years [3]. Subsequent advancements in dentin adhesive technology have since transformed fragment reattachment into the preferred treatment approach for both uncomplicated and complicated crown fractures, which now offers a reliable treatment option with long-term success [2, 4].

The dental adhesive bonding procedure typically involves an initial step known as "etch-and-rinse", during which 35–37% phosphoric acid is used to etch the enamel and dentin surfaces [5]. Following this acidic conditioning of both enamel and dentin, bonding systems are applied and meticulously rubbed onto the surfaces intended for bonding. To simplify the adhesive process, manufacturers have introduced "universal adhesives", which contain acidic monomers designed to eliminate the need for the etch-and-rinse step. Additionally, they offer the flexibility of choosing from various adhesive strategies, including etch-and-rinse, self-etch or selective enamel etching. Universal adhesives have been associated with several advantages, including reduced technique sensitivity for clinicians, decreased postoperative discomfort for patients, and shorter application times. Notably, their penetration into enamel and dentin occurs concurrently with the etching process [5, 6]. The chemical bonding between these adhesives and the hydroxyapatite within the tooth's hard tissue is facilitated by the presence of functional acidic monomers, such as Phenyl-P and 10-methacryloyloxydecyl dihydrogenphosphate (10-MDP), which typically possess a higher pH value compared to traditional phosphoric acid [7]. Self-etching adhesives are available in both one- and two-step application formats. In regard to the two-step self-etching adhesives, bond strength is achieved through the interaction of the adhesive monomers with the residual hydroxyapatite crystals in the thin submicron hybrid layer, in addition to micromechanical interlocking through hybridization [7]. However, there is limited available data regarding the dentin bond strength of one-step self-etching adhesives compared to etch-and-rinse adhesive systems.

The tooth rescue box, designed as a storage medium for avulsed teeth, was introduced in the 1990s [8]. It is composed of a tissue culture medium enriched with amino acids and vitamins, similar in composition to the medium used in islet cell transplantation procedures [9]. The tooth rescue box is widely used in kindergartens, schools and sports facilities in certain regions of Germany, Switzerland and Austria. Notably, while its original purpose was to preserve the vitality of the cells within the periodontal ligament of avulsed teeth, there have been instances where individuals, particularly those without dental expertise, have used it to store crown fragments due to their difficulty in distinguishing between different types of dental injuries [8, 9], which prompts the question of whether certain components in the tooth rescue box might interact with the hard tissues of teeth and influence the bonding of reattached fragments. To the best of our knowledge, there has been no prior report on this potential effect.

Thus, we designed this study to examine the impact of storing tooth fragments in the tooth rescue box on the adhesion of reattached fragments using both total etch and self-etch techniques. The null hypothesis posited that there would be no discernible difference in adhesion between bonded fragments stored in the tooth rescue boxes and those stored in tap water, nor between fragments reattached using the total etch technique and those reattached using the self-etch technique.

2. Materials and methods

A total of 60 maxillary human premolars were retrieved, which were extracted for orthodontic reasons and stored in a 0.5% Chloramine-T solution at a temperature of 4 °C for a maximum duration of three months until further use. All premolars were embedded in acrylic resin (Scandiquick; Scan-Dia, Hagen, Germany) to a depth of approximately 1 mm below the cement-enamel junction. To simulate a fractured tooth, each tooth was horizontally sectioned at the midpoint of the crown, utilizing a diamond saw with continuous water-cooling (Well; Walter Ebner, Locole, NE, Switzerland) to expose the enamel and dentin layers without affecting the pulp chamber, resulting in the creation of 60 fragments and 60 fractured teeth. Subsequently, all exposed surfaces were scanned, and the surface area of the exposed enamel and dentin was quantified and calculated using a digital microscope (Keyence vhx-2000d; Keyence, Tokyo, Japan) (Fig. 1).

In groups 1 and 3, the tooth fragments were stored in tooth rescue boxes (Miradent SOS Zahnbox; Hager Werken, Duisburg, Germany) for 2 hours, with two fragments placed in each box. In contrast, groups 2 and 4, which served as positive controls, stored their fragments in tap water for the same 2-hour period. Subsequently, the fragments were rinsed with water for 10 seconds and dried using a gentle air blow for 5 seconds. For groups 1 and 2, the reattachment process was carried out in the etch-and-rinse mode (E&R) using 37% phosphoric acid (applied for 30 seconds on enamel and 15 seconds on dentin) and the Syntac bonding system (comprising Syntac Primer, Adhesive, and Heliobond; Ivoclar Vivadent, Schaan, Liechtenstein), along with the appropriate flowable resin composite (Tetric Evo Flow; Ivoclar Vivadent, Schaan, Liechtenstein). Fragments in groups 3 and 4 were reattached in the SE mode using a universal adhesive (Adhese; Ivoclar Vivadent, Schaan, Liechtenstein) with the respective flowable resin composite (Tetric Evo Flow; Ivoclar Vivadent, Schaan, Liechtenstein). In contrast, the applied adhesive system was not photopolymerized to ensure a good adaptation of the fragment on the respective tooth. However, the flowable composite was subjected to photopolymerization for 10 seconds from all sides (buccal, mesial, distal and lingual). Following this procedure, any excess resin composite was carefully removed using a sharp scaler, and the reattachment interface was polished using Sof-Lex discs with progressively finer grit sizes, all while maintaining constant water-cooling (Sof-Lex Pop-on; 3M ESPE, Seefeld, Germany). A new Sof-Lex disc was employed after polishing every two teeth. The teeth that had not undergone fracture, along with their respective fragments, were subsequently randomized (Excel randomization table) into four groups (n = 15). The sample size was determined based on similar studies that assessed shear bond strength on dental hard tissues [10–12]. After randomization, the specimens were stored in artificial saliva, following the protocol established by Klimek [13].

After reattachment and polishing, the restored teeth underwent two key steps: thermocycling (500 cycles, 5–55 °C; dwell time 2 min) and shear bond testing using a Zwick/Roell Z010 universal testing machine (Zwick, Ulm, Germany). The tester tip was positioned buccally at the fragment's center, and a continuous force was applied at a rate of 1 mm/min until the fragment was fractured. The shear bond testing was performed by a single experienced operator who was blinded to the experimental settings. An overview of the experimental workflow is shown in Figs. 2,3 shows a specimen secured in the testing machine.

Shear bond strength (MPa) for each specimen was calculated by dividing the maximum load (N) leading to failure by the bonded surface area (in mm^2). Mean shear bond strength values and their corresponding standard deviations were then computed for each group. Significance among the groups was determined through a two-way ANOVA test (p = 0.02). Subsequent *post hoc* tests were conducted for multiple group comparisons, with p-values adjusted using Tukey's test. Importantly, the experimental factor itself was not subjected to further analysis and was not the primary focus of this study. Data were computed and analyzed using SPSS v.22.0

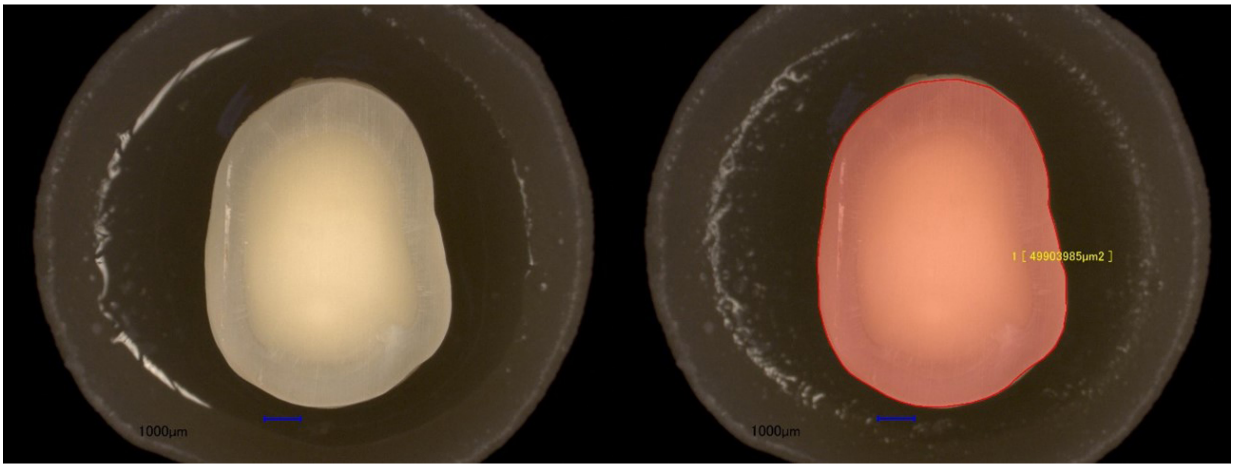

FIGURE 1. Illustration of a sectioned tooth and assessment of its surface area to be bonded (in μm^2).

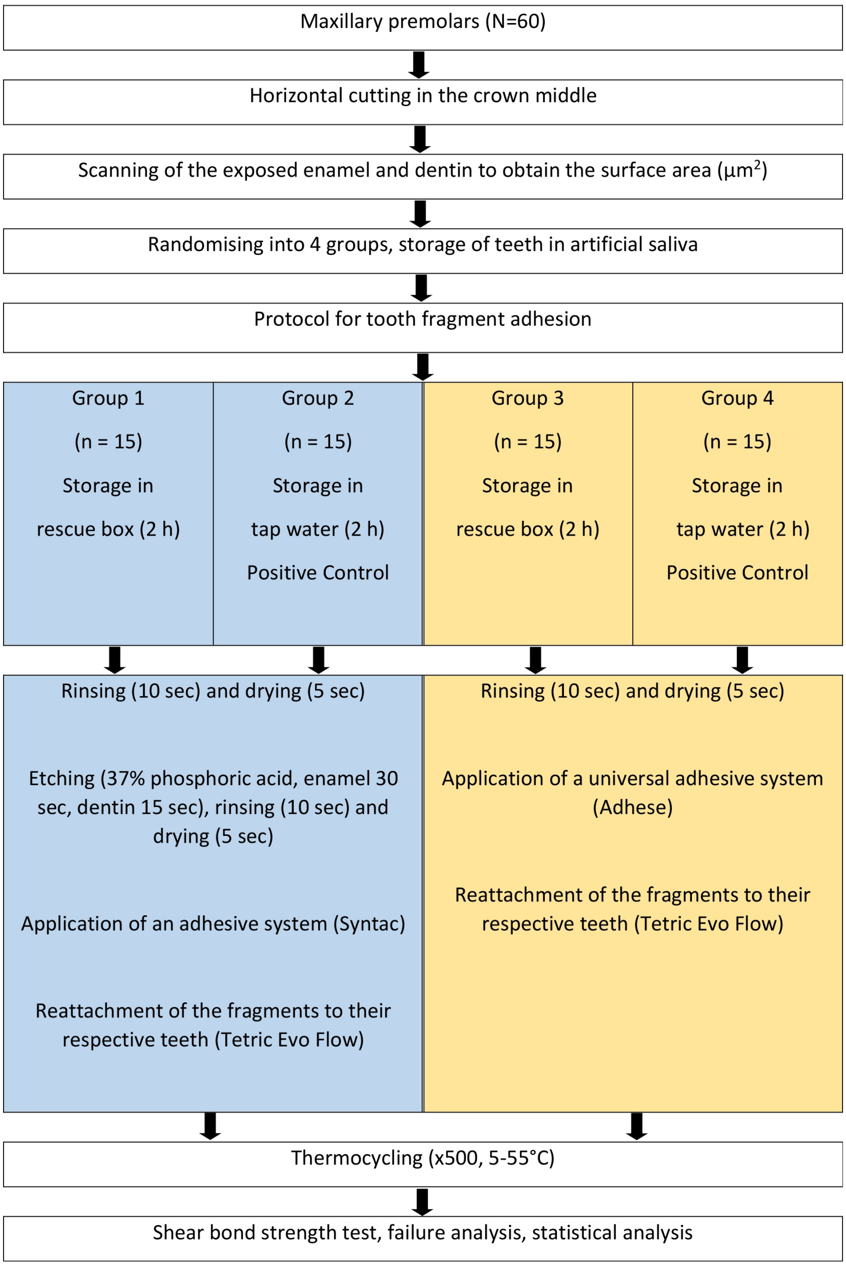

FIGURE 2. Experimental workflow of this study.

FIGURE 3. A specimen (arrow shows the acrylic embedding material) fixed during the shear bond testing using a universal testing machine.

(IBM, Armonk, NY, USA). The maximum likelihood estimation of the 2-parameter Weibull distribution, according to the Anderson-Darling tests, without a correction factor, and including the Weibull modulus, scale (m) and shape (0), was used to interpret the predictability and reliability of the effect of adhesive system and storage medium on VH (Fig. 4; Minitab Software V.16; Minitab LLC, Pennsylvania, USA).

3. Results

The mean exposed surface area of all fractured teeth was 51.0 $\pm$ 4.6 mm^2. Importantly, no significant differences were observed among the four groups of the exposed teeth surface areas ($p = 0.5$, one-way ANOVA test). Table 1 shows the mean, standard deviation, and the highest and lowest shear bond strength (MPa) of each experimental group, along with the observed failure types. Among the groups, the highest shear bond strength was recorded in group 2 (6.5 $\pm$ 2.1, Fragments stored in water, reattached in E&R mode), followed by group 1 (6.0 $\pm$ 2.5, fragments stored in tooth rescue box, reattached in E&R mode), group 4 (5.1 $\pm$ 2.8, fragments stored in water, reattached in SE mode), and group 3 (3.6 $\pm$ 3.2, fragments stored in tooth rescue box, reattached in SE mode). Notably, differences between groups where fragments were reattached with the same adhesive system (E&R or SE, group 1 *vs.* 2 and group 3 *vs.* 4) were not statistically significant. Similarly, differences between groups where fragments were stored in the same medium (Rescue box or Water, group 1 *vs.* 3 and group 2 *vs.* 4) did not reach statistical significance ($p > 0.05$). The only statistically significant difference among the groups was observed between group 2 (Water, E&R) and group 3 (Rescue box, SE) ($p = 0.002$). Additionally, it is worth noting that the highest incidence of adhesive failure was observed in the groups where the fragment was reattached using the SE technique (groups 3 and 4, as detailed in Table 1).

4. Discussion

Tooth rescue boxes were initially designed as an optimal storage medium for avulsed teeth. Nevertheless, it has been reported that crown fragments were occasionally stored in these boxes as well. However, the impact of such storage on the shear bonding strength of the reattached fragment has not been explored. Consequently, this study was conducted to investigate this specific effect while employing different bonding techniques (E&R and SE) for fragment reattachment. Considering the statistically significant difference observed between two of the groups, the null hypothesis of this study is rejected.

In clinical scenarios, the fractured tooth surface is typically devoid of a smear layer, which forms during tooth preparation or sectioning. Moreover, the fracture line follows the natural orientation of enamel prisms and is more distinct in dentin, which aids in precise fragment repositioning and reduces the gap between the fragment and the tooth, potentially influencing the bond strength of the reattached fragment [14]. However, it is important to acknowledge that sectioning the crowns, as performed in this study, was necessary to maintain uniformity among the fragments and has also been employed in similar *in vitro* investigations [15–19]. Nevertheless, it is worth noting that sectioning the crowns does not fully replicate a natural tooth fracture and thus represents a limitation of this study. While the shear bond test utilized in this study is a commonly employed method for assessing bond strength, it has faced criticism for its lack of standardized procedures and the variability of results it can yield [20]. To emulate clinical conditions more accurately, this study subjected the specimens to thermocycling, during which the specimens were exposed to extreme temperature variations, leading to contraction-expansion stresses, especially at the resin/dentin interface, due to differing coefficients of thermal expansion at various interfaces. This study utilized maxillary premolars, as they are readily available due to their extraction for orthodontic purposes. While permanent maxillary incisors would have more closely mirrored the clinical scenario, given that they are the most commonly affected teeth in crown fractures [1, 2], it can be argued that incisors may respond differently to shear testing due to their distinct shape and smaller bonded surface area when reattaching a fragment. However, given that the shear bond test accounts for the bonded surface area (in mm^2) and the study's primary aim was to investigate whether the tooth rescue box solution interacts with dental hard tissue in a manner that affects bond strength, it is reasonable to assume that premolars are a suitable choice

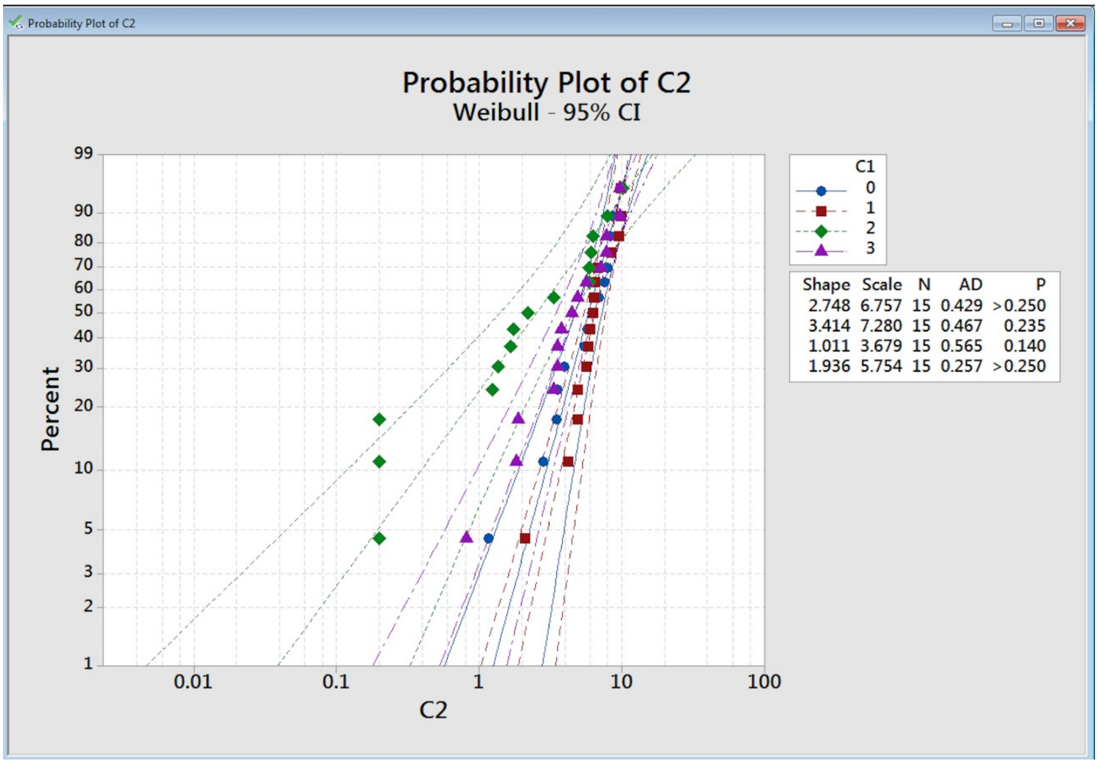

FIGURE 4. Distribution of two-parameter Weibull modulus for specimens from all tested groups. Rescue box, E&R (1); Water, E&R (2); Rescue box, SE (3); Water, SE (4). CI: Confidence Interval; AD: Acceleration Factor.

TABLE 1. Summary of mean shear bond strength, standard deviation, and frequency of observed failure types in each experimental group.

Groups	Shear bond strength (MPa)				Failure types n (%)			
	Mean	SD	Highest bond	Lowest bond	Score 1	Score 2	Score 3	Score 4
Group 1 (Rescue box, E&R)	6.0	2.5	7.4	4.6	4 (27%)	0 (0%)	7 (46%)	4 (27%)
Group 2 (Water, E&R)	6.5*	2.1	7.7	5.3	2 (13%)	1 (7%)	7 (46%)	5 (33%)
Group 3 (Rescue box, SE)	3.6*	3.2	5.4	1.8	3 (20%)	1 (7%)	1 (7%)	10 (66%)
Group 4 (Water, SE)	5.1	2.8	6.6	3.5	1 (7%)	0 (0%)	4 (27%)	10 (66%)

Mean values are denoted with an asterisk symbol (*) and exhibit statistical significance only when compared to each other. Failure Types: Score 1—Cohesive in enamel; Score 2—Cohesive in enamel and dentin; Score 3—Cohesive in the composite; and Score 4—Adhesive.

E&R: etch and rinse; SD: Standard deviation.

for this study setup.

In this study, fragments were stored in the tooth rescue box for 2 hours before their reattachment to the teeth. It is reasonable to assume that the impact of storing the fragment in the tooth rescue box could vary if the storage time were extended. Literature reports have indeed documented longer time delays, up to 24 hours, between the occurrence of dental trauma and treatment [21, 22]. However, the choice of a 2-hour time delay in this study was based on observations made at the Department of Pediatric Dentistry and the emergency department of the Children's Hospital at the University of Zurich, which aligns with situations where dental care is promptly sought after a dental trauma, thereby reflecting a clinically relevant scenario.

In this study, when fragments were stored in the tooth rescue box, no statistically significant difference in shear bond strength was observed between the two tested adhesive strategies (group 1 *vs.* group 3). It is important to note that, to the best of the authors' knowledge and after an extensive literature search, no prior studies have been conducted to investigate the impact of the tooth rescue box medium on adhesion. One of the components present in the tooth rescue box is sodium bicarbonate ($NaHCO_3$) [23], which has been found to have a protective effect against erosive tooth wear [24]. It is conceivable that the contact between tooth hard tissues and the sodium bicarbonate within the rescue box could influence how enamel and dentin respond to etching agents. A similar consideration applies to L-arginine, which has demonstrated an anti-erosive effect [25] and is also present in the rescue box. However, these hypotheses remain speculative and would require molecular tests for confirmation. Nevertheless, based on the current findings, it can be inferred that the ingredients within the tested rescue box did not interact with enamel and dentin in a manner that affects fragment adhesion when the fragment is stored in the box for up to 2 hours. On the other hand, it is also worth noting that the E&R bonding technique may result in superior shear bond strength when the fragment is stored in a tooth rescue box for extended durations. This conclusion is drawn from the observed higher bond strength when the E&R technique was employed, as well as the increased incidence of adhesive failures in the group where fragments were stored in the tooth rescue box and reattached using the SE technique.

Regarding the choice of bonding technique for fragment reattachment (E&R *vs.* SE), Tsujimoto *et al*. [26], Ernest *et al*. [27] and Yaseen *et al*. [28] have also reported that the shear bond strength of fragments reattached using either technique showed no statistically significant differences. As previously mentioned, in this study, a smear layer was present on both the fragments and the teeth due to the cutting procedure, which could have potentially acted as a physical barrier, limiting the deeper penetration of adhesive monomers and, in turn, affecting bond strength [29]. It is conceivable that in the absence of a smear layer, which corresponds to the clinical scenario of fractured teeth, the SE technique might exhibit even better performance than what was observed in this study. Additionally, it is also worth noting that the present study compared a SE bonding system to a three-step E&R bonding system, which could be considered a somewhat extreme and potentially "unfair" comparison. The inclusion of a group where the fragments were reattached using a universal adhesive in selective-enamel-etch mode could have provided some additional valuable insights. In addition, prior enamel etching before the application of universal adhesives has been shown to enhance the performance of self-etch adhesives by increasing enamel bond strength [7].

5. Conclusions

Based on the results of this study, within its inherent limitations, it can be concluded that storing a fragment inside a tooth rescue box for up to 2 hours does not significantly affect the shear bond strength of the restored tooth. Furthermore, fragments reattached using the SE technique demonstrate shear bond strength comparable to those reattached with the E&R technique, although it should also be considered that the SE technique might have a higher risk of adhesive failures compared to the E&R technique.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the results of this study is available from the corresponding author on request.

AUTHOR CONTRIBUTIONS

BH and MÖ—conceptualization; BH, NAH and MÖ—methodology, writing-review and editing; AK, NAH and KJM—investigation; NAH, MÖ and KJM—resources; NAH and MÖ—data curation; BH and AK—writing-original draft preparation; BH, NAH, MÖ and KJM—supervision; All authors have read and agreed to the published version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The patients gave their written consent for their teeth to be used for research purposes (for children aged under 16, informed consent was obtained from their parent or legal guardian). Ethical approval was obtained from Zurich cantonal ethics Committee (BASEC-Nr. Req-2022-00502).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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