Original Article

Effect of Different Hydrating Solutions and Immersion Time Periods on Bond Strength of Reattached Fragment to the Tooth - An *In Vitro* Study

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Abstract

Aim: The aim of the study was to evaluate the effect of different hydrating solutions and immersion time periods on bond strength of reattached fragment to the tooth. **Materials and Methods:** Sixty human extracted maxillary central incisors with similar dimensions and free of any structural deformities were selected and fractured 5 mm from the incisal edge and divided into six groups: (n = 10): I — Whole milk: For 1 h (IA) or 24 h (IB); II — skimmed milk: For 1 h (IIA) or 24 h (IIB); III — powdered milk: For 1 h (IIIA) or 24 h (IIIB); IV — honey milk: For 1 h (IVA) or 24 h (IVB); V — normal saline: For 1 h (VA) or 24 h (VB); and VI — control group (dry tooth): For 1 h (VIA) or 24 h (VIB). Tooth fragments were then re-attached using self-etching technique with nanohybrid composite resin remained the same in all groups. Fracture resistance was evaluated in a universal testing machine under a compressive load (1 mm/min). **Statistical analysis:** The data were submitted to one-way ANOVA, the *post hoc* Tukey test and sub group comparison by independent sample t-test. **Results:** The highest fracture resistance value was demonstrated by Group I (whole milk) showed statistically significant difference (2671.60 ± 530.24) compared with honey milk Group III (1990.80 ± 518.95), powdered milk Group IV (1872.30 ± 301.03), skimmed milk Group II (1631.00 ± 554.95), normal saline Group V (1551.60 ± 455.65), and least fracture resistance values were observed in dry tooth Group VI (1267.90 ± 309.13). For sub group comparison, Independent t-test was applied and no significant difference between 1 and 24 h duration, except there was a statistically significant difference in fracture resistance values between Group IIA and Group IIB (P = 0.041 P <0.05), and Group IIIA and Group IIIB (P = 0.016 P <0.05).

Keywords: Activated acrylic resin, Self etching, Nanohybrid composite

Introduction

Traumatic dental injuries (TDIs) most commonly occur in preschool, school-age children, and young adults comprising 5% of all injuries for which people seek treatment.[1] Coronal fractures of permanent incisors represent 18–22% of all the traumas affecting dental hard tissues; of these, 80% involve maxillary central incisors and 16% lateral incisors.[2,3] Different techniques have been used to restore the fractured crown such as usage of resin, stainless steel and ceramic crowns, orthodontic bands, and composite restorations with and without pins. However, all these interventions tend to remove a lot of healthy tissue structure.[4] With the development of adhesive dentistry, came the concept of "fragment reattachment." If the fractured crown fragment is retrieved at the time of injury, its reattachment provides several benefits over other forms of restoration.[5] Success rate of fragment reattachment is dictated by the firmness of its' attachment to the tooth and also mode of storage medium immediately following trauma. Storage medium acts as hydrating media. It maintains the vitality, esthetic, and the bond strength of the tooth.[4] No studies have yet been reported on the different types of milk such as whole milk, skimmed milk, honey milk, powdered milk, and normal saline in which parents should store the fractured fragments before referring to the dentist like as in case of avulsion injury.[5] The aim of this study was to evaluate the effect of different hydrating solutions in different immersion time periods on bond strength of reattached fragments and teeth.

The specific objective was to test the following two null hypotheses: Fragment storage time does not influence fracture

Access this article online

Website: www.jrad.co.in

DOI: https://doi.org/10.53064/jrad.2022.13.1.92

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Received: 12 Oct, 2021; **Accepted:** 15 Nov, 2021; **Published:** 01 Dec, 2021

How to cite this article: Yadav SS, Trinath T, Veeraiyan M, Jaiswal M, kemasaram D, Priyanka Y. Effect of different hydrating solutions and immersion time periods on bond strength of reattached fragment to the tooth - An *in vitro* study. J Res Adv Dent 2022;13(1):58-62.

resistance and fragment storage solution does not influence fracture resistance.

Materials and Methods

A total of 60 permanent human maxillary central incisors, free of structural deformities, were selected [Figure 1]. Cleaning and removal of tissue remnants on the root surfaces were carried out using curettes and ultrasonic tips. The teeth were disinfected using 0.2% thymol and stored in artificial saliva until the time they were intentionally fractured. The teeth were placed inside a mold made with a stainless-steel cylinder using chemically activated acrylic resin. The intentional fracture was done by sectioning the tooth 5 mm away from the incisal edge of the tooth using a lowspeed diamond disk perpendicular to the long axis using saline as a coolant [Figure 2], followed by storage of the fractured fragments separately marked with appropriate storage medium (whole milk, skimmed milk, powdered milk, honey milk, normal saline, and dry tooth) for the time intervals of 1 h and 24 h.

Following groups were made [Table 1]:

- Group I: The fractured fragments stored in whole milk subgroups IA and IB fragments stored for 1 h and 24 h, respectively
- Group II: The fractured fragments stored in skimmed milk subgroups IIA and IIB fragments stored for 1 h and 24 h, respectively
- Group III: The fractured fragments stored in powdered milk subgroups IIIA and IIIB fragments stored for 1 h and 24 h, respectively
- Group IV: The fractured fragments stored in honey milk subgroups IVA and IVB fragments stored for 1 h and 24 h, respectively
- Group V: The fractured fragments stored in normal saline subgroups VA and VB fragments stored for 1 h and 24 h, respectively
- Group VI: The fractured fragments stored in dry environment

Fragments were reattached by means of simple reattachment technique without any additional preparation. Before reattachment, the fragment was removed from respective storage media, rinsed with distilled water for 10 s, and dried with paper towel. Both the surfaces of the tooth fragment were etched using 37% phosphoric acid (3M ESPE scotch bond) for 15 s and then rinsed with distilled water for 10 s, followed by air drying for 5 s [Figure 3]. Two coats of bonding agent (3M ESPE Adper Single) were applied, and then, the surfaces were dried for 5 s using an air syringe to allow solvent evaporation. The bonding agent was light cured for 20 s in the fractured fragment and 20 s in the tooth remnant. The flowable composite (3M Filtek) of matching shade was applied on the fractured surface of fragment, tooth remnant was reattached, and a brush was used to remove the excess composite. The fragments were approximated together and light cured for 10 s on mesial, distal, buccal, and lingual sides each [Figures 4 and 5]. All samples were then subjected to fracture resistance test using universal testing machine at the speed of 1 mm/min [Figure 6].

Results

The values for each group were tabulated, and the mean and standard deviation were calculated for each of the groups. Statistical tests were then applied. The highest fracture resistance value was demonstrated by Group I (2671.60 ± 530.24), followed by Group III (1990.80 ± 518.95), Group IV (1872.30 ± 301.03), Group II (1631.00 ± 554.95), Group V (1551.60 ± 455.65), and least fracture resistance values were observed in Group VI (1267.90 ± 309.13). For group-wise comparison, *post hoc* Tukey's test was applied and it was seen that there was a statistically significant difference in fracture resistance values between Group and other groups Group I and Group II ($P = 0.000$ and $P < 0.05$), Group I and Group III ($P = 0.018$ and $P < 0.05$), Group I and Group IV ($P = 0.003$ and $P < 0.05$), Group I and Group V ($P = 000$ and $P < 0.05$), Group I and Group VI ($P = 0.000$ and $P < 0.05$), Group III and Group VI ($P = 0.010$ and $P < 0.05$), and Group IV and Group VI ($P = 0.050$ and $P < 0.05$). For sub group comparison, Independent t-test was applied and it was seen that there was a statistically significant difference in fracture resistance values between Group IIA and Group IIB ($P = 0.041$ and $P < 0.05$), and Group IIIA and Group IIIB ($P = 0.016$ and $P < 0.05$) [Table 2].

Discussion

In the present study, the influence of both the storage medium used to hydrate the fragment and the hydration time was evaluated to determine the fracture resistance of teeth after reattachment. The null hypotheses partially accepted, since no difference was found between the values for the storage times (1 h or 24 h) except Group II. Moreover, there was a statistically significant difference between different types of storage medium. Hydrating the fragment increased the fracture resistance of the tooth compared with bonding a dehydrated fragment. Maxillary central incisors were used in this study. Because the majority of dental injuries involves the anterior teeth, especially the maxillary central incisors.[2] Bond strength evaluation after fragment reattachment is relevant because most often reattachment failures occur due to repeated trauma.[6] Tooth fragments were obtained by sectioning with a low speed diamond disk with water coolant instead of fracturing. Badami *et al*.[7] and Reis *et al*.[8] stated that the surface of a sectioned tooth is different from that of a fractured tooth. In a fractured region, the fracture line tends to run parallel to the direction of enamel prisms, whereas the direction in a sectioned tooth is dictated by the position of the diamond disk. However, this direction does not represent the actual clinical situation of the trauma, since it does not always occur linearly or with perfect adaptation. Nevertheless, fracture simulation with a cutting diamond disk allowed the standardization of the fragments required to reduce confounding bias.[9] According to Garcia *et al*., 2018, and de Sousa *et al*., 2018, fragment reattachment using a technique with no preparation and an adhesive system associated with an intermediate resin composite with good mechanical properties can restore part of the resistance of the fractured tooth.[10,11] The proposed

Table 1: Means comparison of Compressive force (N) among Group I, Group II, Group III, Group IV, Group V, and Group VI

Groups	Sample size	Min	Max	Mean	SD	F value	*P* value
GROUP I	10	1960	3660	2671.60	530.24	11.202	<0.001
GROUP II	10	820	2680	1631.00	554.95		S
GROUP III	10	1200	2900	1990.80	518.95		
GROUP IV	10	1450	2500	1872.30	301.03		
GROUP V	10	876	2040	1551.60	455.65		
GROUP VI	10	873	1874	1267.90	309.13		

Table 2: Mean comparisons of Compressive force (N) between the subgroups of Group I, Group II, Group III, Group IV, Group V, and Group VI

Groups	Sample size	Mean	SD	Mean difference	*P* value
GROUP IA	5	2463.60	221.87	−416.00	0.235
GROUP IB	5	2879.60	689.35		NS
GROUP IIA	5	1288.00	464.24	−686.00	0.041
GROUP IIB	5	1974.00	428.11		S
GROUP IIIA	5	2352.00	432.80	722.40	0.016
GROUP IIIB	5	1629.60	304.06		S
GROUP IVA	5	1742.00	201.79	−260.60	0.185
GROUP IVB	5	2002.60	347.45		NS
GROUP VA	5	1412.60	499.59	−278.00	0.365
GROUP VB	5	1690.60	411.40		NS
GROUP VIA	5	1268.80	409.81	1.80	0.993
GROUP VIB	5	1267.00	216.96		NS

technique for fragment reattachment was selective etching in enamel, followed by active application of single bond universal as a self-etching adhesive. Because acidity (pH 2.5) of most self-etching adhesives is insufficient to promote de-mineralization of the enamel to the extent achieved by phosphoric acid. Therefore, the selective application of phosphoric acid to the enamel surface has been recommended based on *in vitro* study.[12] The intermediate material of choice was nanohybrid composite resin because, its lower viscosity provided better remnant-fragment adaptation. In the present study, different types of milk such as whole milk, skimmed milk, honey milk, and powdered milk were chosen as it is easily available and has better physiologic properties, including pH and osmolality. Normal saline was chosen as one of the storage media for hydration of the fractured tooth fragments as it is easily available in dental clinic. Dry environment was also tested for 1 and 24 h in the present study, to test the effect on the fracture resistance of the reattached tooth fragment to simulate the clinical scenario in which patient might report immediately or the next day. In this study, Group I (whole milk) had a statistically significant difference when compared to other groups. Shirani *et al*., 2013,[18] proved that milk elements, such as calcium and phosphate, can harden and stiffen both demineralized and healthy dentin by permeating

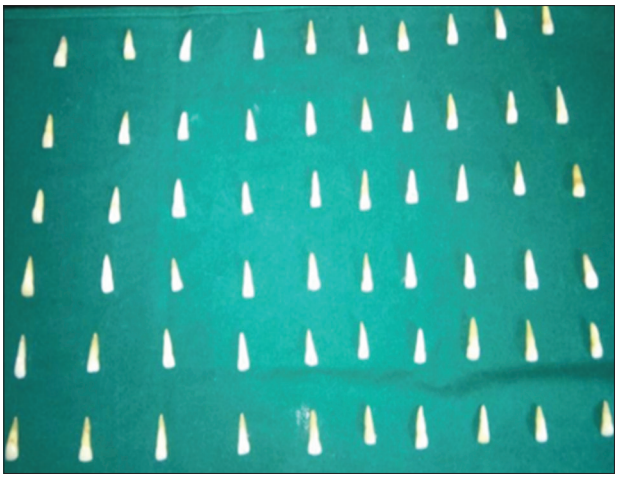

Figure 1: Sixty maxillary central incisors

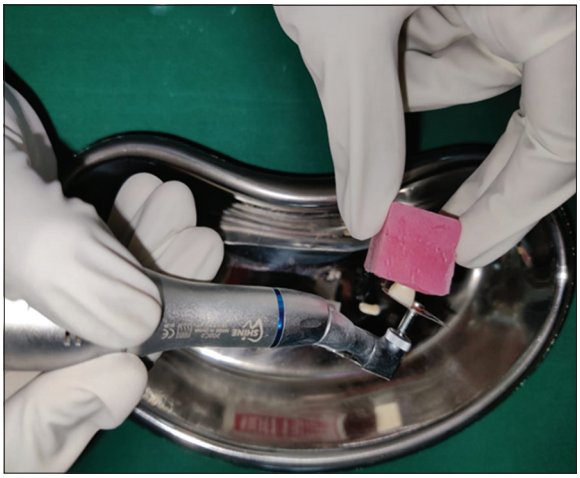

Figure 2: Crown fractured with diamond disc 5 mm from the incisal edge

Figure 3: Etchant was applied to the enamel for 30 s, rinsed for 30 s, and then dried for 10 s

the surface.[2] Shirani *et al*., 2013, and Jalannavar and Tavargeri 2019 reported a statistically significant difference between the solutions, with the highest mean values being obtained for

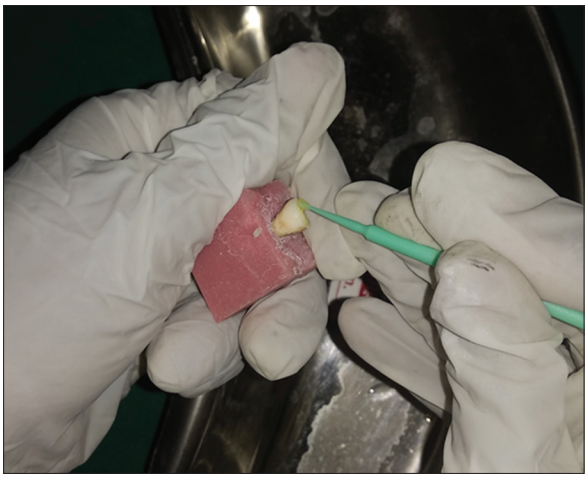

Figure 4: Single bond universal adhesive was then applied for 20 s and light polymerized for 20 s

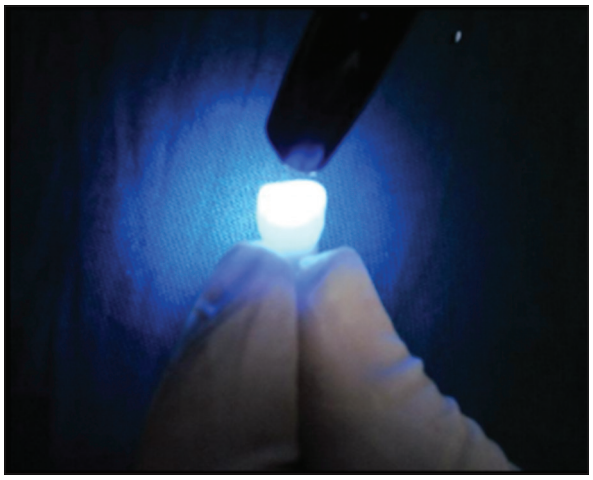

Figure 5: Flowable composite applied then the reattached surfaces were light cured for 40 s

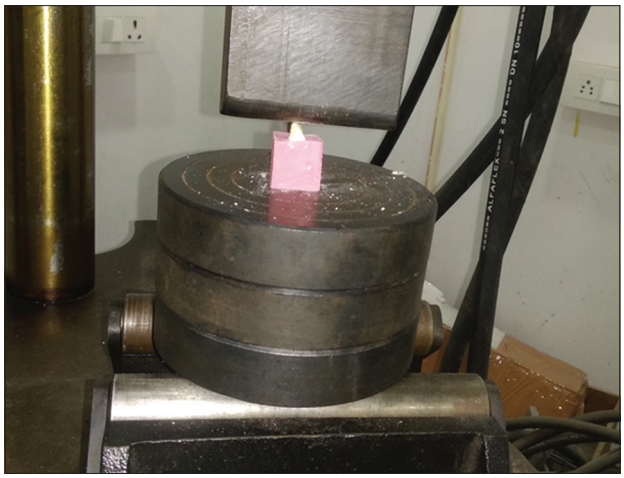

Figure 6: The reattached teeth were mounted in a universal testing machine (1 mm/min) to evaluate resistance to impact[15]

milk and saliva [Figure 7].[2,13] Results showed that fracture resistance value for Group I was observed to be statistically

Figure 7: Materials

significant over Group II, Group III, and Group IV. It can be attributed to the isotonicity of whole milk with high water content which allowed adequate rewetting of the dentinal tubules.[2] All four types of milk showed better fractured resistance than normal saline. The results of this study were in accordance with a study conducted by Lee *et al*., 2007, where they concluded that residual chlorine from saline and sodium hypochlorite can negatively influence the bond strength when used as a storage medium.[14] Another parameter evaluated in this study was the duration (1 h and 24 h) of fragment in the media on the bond strength of the reattached fragment. It was observed that 24 h Group IIB showed statistically significant bond strength mean values compared with the 1 h Group IIA. However, there were no statistically significant differences in other groups. According Poubel *et al*., 2017, 15 min of rehydration was sufficient to increase the fracture resistance, even after a 24-h dehydration period.[18] This can be explained by the action of water and its ability to establish polar bonds through hydrogen bridges with the peptides from collagen fibrils, causing a rapid re-expansion of fibrils that had collapsed due to dehydration and allowing the formation of an optimized hybrid layer.[16,17] Result of the present study showed that 1 h rehydration was sufficient to hydrate fractured fragments. Limitation of this study was the amount of load which was applied using a universal testing machine at the crosshead speed of 1 mm/min, which did not simulate a clinical traumatic scenario.[4]

Conclusion

However, it is evident that the use of storage media for the hydration of the fractured tooth fragment does improve its fracture resistance significantly. In the present study, all types of milk offer the highest fracture resistance. Normal saline offers fracture resistance higher than dry storage media but lesser than milk.

Clinical significance

Milk can be considered as a better storage media till patient reports to the dental office.[18]

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