Original Article

Effect of different rehydration protocols and their immersion times on the fracture resistance of fragment reattachment in uncomplicated crown fractures: An in vitro study

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Abstract

This study aims to evaluate the effects of different rehydration media and their immersion times on fracture resistance of fragment reattachment in uncomplicated crown fractures. 110 extracted sound human maxillary and mandibular incisors were randomly divided into 11 groups (n=10). The teeth were fractured 3mm away from and parallel to the incisal edge using diamond disc. Afterwards, the apical segments were stored in artificial saliva (Saleva), while the coronal segments underwent the dehydration-rehydration stages and grouped randomly into 5 (n=10, 10, 30, 30, 30). Group 1: control group (intact teeth); Group 2: 24 hours dehydration, no rehydration; Group 3: skimmed milk rehydration; Group 4: saline(0.45%) rehydration; Group 5: dextrose solution(50%) rehydration. Group 3,4,5 further sub-divided into A: 15 min, B: 30 min, C: 24 hours. Re-attachment was done with flowable composite resin. Fracture resistance tested with universal testing machine. Statistical analysis (p=0.05) done by One-way ANOVA, Two-way ANOVA and post-hoc Tukey's test HSD. Fracture resistance: Group 1 (highest), Group 5 (higher), Group 2(least). Within study limitations, it is conclusive that preservation of tooth fragment in dextrose solution(50%) results in higher fracture resistance, followed by saline(0.45%) and skimmed milk, respectively.

Keywords: Fracture resistance, reattachment, rehydrating media, uncomplicated crown fracture.

INTRODUCTION

Chosack and Eidelman [1] proposed the technique of reattaching a tooth fragment for the first time in 1964. Reattachment has the advantage of being a highly conservative approach that does not require any form of preparation, allowing for the preservation of natural tooth structure, acceptable aesthetics, and patient acceptability. The success of fragment reattachment is determined by the fragment's firm attachment to the tooth, as well as strong bonding between the two segments and the tooth preparation. For the union of fractured segments, various research have been conducted employing various materials and tooth preparation techniques. The type of storage of the fragment post trauma is one of the critical determinants in the effectiveness of fragment reattachment [2]-[4].

The aim of the study was to evaluate the effect of different rehydration media and their immersion times on the fracture resistance of fragment reattachment in uncomplicated crown fractures. The null hypothesis was that different rehydration medium and their immersion times does not affect the fracture resistance of fragment reattachment in uncomplicated crown fractures.

MATERIAL AND METHOD

This study was approved by the Ethical Committee and was carried out in the Department of Conservative Dentistry and Endodontics, K.D. Dental College and Hospital, Mathura, Uttar Pradesh and at the Apex Assessment Labs Private Limited, Anand Industrial Estate, Ghaziabad.

In this experimental study, 110 extracted sound human maxillary and mandibular incisors, free of any cracks, caries or structural defects were selected and randomly divided into eleven groups (n=10). The teeth were cleaned of any debris and calculus, and then stored in artificial saliva (Saleva) for two weeks until use.

The tooth crowns were prepared for fracturing. The teeth were measured on the labial side from the cervical to the incisal edge with a metal scale and divider. A line was traced around each tooth, 3mm away from and parallel to the incisal edge. The teeth were fractured along this line using diamond disc (Ajex & Turner Wire Dies Co., New Delhi, India). (Fig 1) The teeth that were showing any fracture pattern other than along the predetermined line were discarded at this stage.

Fig 1: Tooth sectioned 3 mm from the incisal edge using diamond disc. a) 3mm measured with metal scale and divider, b) 3mm measured on tooth, c) tooth fractured using diamond disc, d) tooth fragment and apical portion

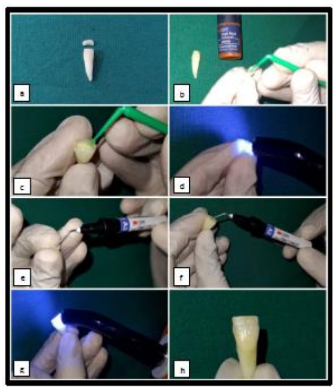
Afterwards, the apical segments were stored in artificial saliva (Saleva), while the coronal segments underwent the dehydration and rehydration stages and grouped randomly into 5 groups. (Fig 2)

- **Group 1**:- control group (intact teeth) [n=10]
- **Group 2**:- dehydrated for 24 hours and no rehydration done [n=10]
- **Group 3** :- rehydration in skimmed milk [n=30]
- **A** :- 15 minutes
- **B** :- 30 minutes
- **C** :- 24 hours
- **Group 4** :- rehydration in saline (0.45%) [n=30]
- **A** :- 15 minutes
- **B** :- 30 minutes
- **C** :- 24 hours
- **Group 5** :- rehydration in dextrose solution(50%) [n=30]
- **A** :- 15 minutes
- **B** :- 30 minutes
- **C** :- 24 hours

Fig 2: Tooth fragments of the specimens in respective storage media at 15 minutes, 30 minutes and 24 hours

After this stage was completed, the fragments were reattached to the teeth utilizing a single technique; each fragment (including the control group) was rinsed under water for 5 seconds to remove any of the remaining storage media. The fractured surface and the corresponding apical segment were coated with bonding agent (Single bond universal, 3M ESPE, St. Paul, MN, USA) which was common for all the groups. Each surface was light cured for 20seconds. A layer of flowable composite resin (Filtek Flow, 3M ESPE, St. Paul, MN, USA) was placed on both the reattachment bonding surfaces, and fragments were pressed together into their proper position. The realignment of the two bonded fragment and the traced lines were checked to ensure an exact fit and then the labial and lingual surfaces were each light cured for 40 seconds. (Fig 3)

Fig 3: Fragment reattachment with respective tooth specimens using flowable composite. a) tooth fragment and apical portion, b & c) bonding agent application on fragment and apical portion, respectively, d) tooth fragment and apical portion light cured, e & f) flowable composite application on fragment and apical portion, respectively, g) fragments light cured, h) reattachment completed



The teeth were dipped in molten wax to 1 mm apical to the cement-enamel junction (CEJ), which resulted in the formation of a wax layer. The self-curing polymethyl-methacrylate resin (DPI-RR Cold Cure) was mixed in a jar and poured into the custom-made mould. All the teeth were embedded vertically into the mould to a level 1 mm apical to the CEJ, positioned so that the long axis of the tooth would be parallel to the central axis of the block, and at the same time the fracture line would be parallel to the block surface. After the resin had been set, the teeth were removed from the block, and wax was eliminated from the teeth and the base block. Elastomeric impression material was loaded in the resin block cavity, and the teeth were re-seated in position. The flash paste was trimmed with a blade. (Fig 4)

Fig 4: Teeth embedded in acrylic blocks

Each tooth was exposed from 1mm below its fracture line. The samples were stored in artificial saliva for 24 hours to ensure complete polymerization of the acrylic resin before the debonding test.

All the samples were fixed with the help of a clamp and positioned in a universal testing machine. A 1mm diameter round tip stainless steel metal rod was positioned perpendicular to the lingual surface of the tooth adjacent to the reattachment line. (Fig.

5) The force was applied to the center of the sample at a crosshead speed of 1 mm/min. The peak-load fracture was recorded in Newton (N) for each sample.

Fig 5: Tooth placed in Universal testing machine

STATISTICAL ANALYSIS

Data obtained were statistically analyzed by One-way ANOVA, Two-way ANOVA and *post hoc* Tukey's test HSD using statistical package for social sciences (Version 20.0, SPSS Inc, New York, USA) for Windows. The level of statistical significance was set at 95% (*p*=0.05).

RESULTS

Table 1 represents the means and standard deviations of fracture resistance (N) of various groups according to their rehydrating media. One-way ANOVA revealed that the fracture resistance of the coronal fragment was affected by the rehydration prior to reattachment (p=0.00). Group 1 (control group) showed the highest fracture resistance of 167.10 ± 36.48 N, followed by Group 5 (50% dextrose solution) having fracture resistance of 162.60 ± 39.26 N ; and the least fracture resistance of 77.40 ± 16.10 N was seen in Group 2 (dehydration). Group 4 and Group 5 showed increased mean fracture resistance with increase in time duration except for Group 3.

Table 2 represents the multiple comparison among various groups by using *post hoc* Tukey's test HSD and it was seen that there was a statistically significant difference in fracture resistance values between Group 1 and Group 2 (p = 0.000, p< 0.05); Group 1 and Group 3 (p = 0.000, p< 0.05); Group 1 and Group 4 (p = 0.010, p< 0.05); Group 2 and Group 4 (p = 0.029, p< 0.05); Group 2 and Group 5 (p = 0.000, p< 0.05); Group 3 and Group 5 (p = 0.000, p< 0.05); Group 4 and Group 5 (p = 0.024, p< 0.05).

In **Table 3**, two-way ANOVA revealed that rehydrating media (p = 0.000, p< 0.05) had significant effect on the fracture resistance of various groups while rehydrating time duration (p = 0.282, p> 0.05) shows non-significant effects.

Table 1: Means and standard deviations of fracture resistance (N) of various groups according to their rehydrating media using One-way ANOVA

Groups	N	Mean	S.D.	Min.	Max.	F-value	P-value	Inferences
GROUP 1 (Control group)	10	167.10	36.48	123.00	229.00	17.923	0.000	S
GROUP 2 (dehydration)	10	77.40	16.10	46.00	97.00			
GROUP 3 (skimmed milk)	10	88.70	19.34	56.00	108.00			
GROUP 4 (0.45% saline)	10	119.50	34.99	62.00	154.00			
GROUP 5 (50% dextrose solution)	10	162.60	39.26	75.00	221.00			

Group	Time	N	Mean	S.D.
GROUP 3 (skimmed milk)	15 Min	10	90.00	9.73
	30 Min	10	84.20	12.90
	24 Hours	10	77.40	16.10
GROUP 4 (0.45% saline)	15 Min	10	104.90	42.05
	30 Min	10	106.30	41.72
	24 Hours	10	119.50	34.99
GROUP 5 (50% dextrose solution)	15 Min	10	123.60	39.23
	30 Min	10	146.60	38.72
	24 Hours	10	162.60	39.26

Table 2: Level of significance on intergroup comparison using *post hoc* Tukey's HSD

Group	Vs	Group	P-Value	Inferences
GROUP 1 (Control group)	Vs	GROUP 2 (dehydration)	0.000	S
GROUP 1 (Control group)	Vs	GROUP 3 (skimmed milk)	0.000	S
GROUP 1 (Control group)	Vs	GROUP 4 (0.45% saline)	0.010	S
GROUP 1 (Control group)	Vs	GROUP 5 (50% dextrose solution)	0.997	NS
GROUP 2 (dehydration)	Vs	GROUP 3 (skimmed milk)	0.923	NS
GROUP 2 (dehydration)	Vs	GROUP 4 (0.45% saline)	0.029	S
GROUP 2 (dehydration)	Vs	GROUP 5 (50% dextrose solution)	0.000	S
GROUP 3 (skimmed milk)	Vs	GROUP 4 (0.45% saline)	0.184	NS
GROUP 3 (skimmed milk)	Vs	GROUP 5 (50% dextrose solution)	0.000	S
GROUP 4 (0.45% saline)	Vs	GROUP 5 (50% dextrose solution)	0.024	S

Table 3: Two-way ANOVA results for fracture resistance according to different experimental protocols for hydration

Source	df	Mean Square	F	P-value	Inferences
Group	2	27508.14	25.180	0.000	S
Time	2	1404.84	1.286	0.282	NS
Group x Time	4	1742.58	1.595	0.184	NS

DISCUSSION

The type of rehydrating solution utilized for the rehydration of fragments following trauma is one of the most important aspects in the effectiveness of fragment reattachment. If the coronal fragment is allowed to dry out before reattachment, it will desiccate, and in vitro experiments have shown that such reattached fragments have lower fracture resistance. As a result, the fragment should be kept moist during the time between retrieval and reattachment. Storing the fragment in a moist environment ensured that the collagen fibres in the dentin would not collapse, resulting in improved fracture resistance and bond strength. Furthermore, it protects the fragment from drying out, which can be detrimental to the aesthetics [5]. Farik et al.[6] found that drying the fragment for more than 1 hour before bonding reduced fracture strength.

In this study, permanent incisors were selected because of their greater involvement in traumatic episodes [7].

When utilized as a storage medium, residual chlorine from saline and sodium hypochlorite can have a deleterious impact on fracture resistance and bond strengths, according to Lee et al.[8] As a result, in the current intervention, artificial saliva was chosen as the storage medium for freshly extracted teeth in order to imitate the intra-oral environment and prevent affecting the fracture resistance of the samples to be examined.

Instead of fracturing, the tooth fragments were sectioned using a low-speed diamond disc in this study. The surface of a sectioned tooth differs from that of a fractured tooth, according to Badami et al.[9] and Reis et al.[10] In a fractured region, the fracture line tends to run parallel to the direction of enamel prisms, whereas in a sectioned tooth, the direction is dictated by the position of the disk [11]. However, because trauma does not usually proceed linearly or with perfect adaption, this orientation does not accurately depict the actual circumstances of the trauma [12],[13]. Nonetheless, fracture simulation using a cutting disk allowed for the necessary fragment standardisation to reduce confounding bias.

We used three rehydrating medium in this study: 50 percent dextrose solution, 0.45 percent saline, and skimmed milk, all of which are widely available. The fragments' rehydration periods of 15 minutes, 30 minutes, and 24 hours were designed to mimic diverse scenarios in real life. The shortest clinical condition is a 15-minute rehydration interval [14], the 30-minute rehydration period is similar to the time that treatment is carried out at the same appointment (with a 30-minute rehydration carried out by the dentist prior to treatment), and the 24-hour rehydration period is similar to the time between an initial visit and a second appointment for treatment scheduled by the dental practitioner [15].

The results showed that the fracture resistance of the coronal fragment was affected by the rehydration prior to reattachment ($p=0.00$). Group 1 (control group) showed the highest fracture resistance of 167.10 ± 36.48 N, followed by Group 5 (50% dextrose solution) having fracture resistance of 162.60 ± 39.26 N; and the least fracture resistance of 77.40 ± 16.10 N was seen in Group 2 (dehydration). Group 4 and Group 5 showed increased mean fracture resistance with increase in time duration except for Group 3. The null hypothesis is partially rejected because two-way ANOVA revealed that rehydrating media ($p = 0.000$, $p< 0.05$) had significant effect on the fracture resistance of various groups while rehydrating time duration ($p = 0.282$, $p> 0.05$) shows non-significant effects.

The higher fracture resistance in Group 4 (50% dextrose solution) may be related to the high osmolality of this solution. This is in accordance with a study done by Shirani et al.[16] where they concluded that preservation of the fractured tooth fragment in hypertonic solutions (50% dextrose solution) results in a higher strength of the bond between the restoration and the tooth as compared to storage in water or dried conditions.

In our study, the fracture resistance of the samples stored in 0.45% saline was higher compared with those stored in skimmed milk. This can be due to the amount of water content in milk, which is less than saline. The hydration of the collagen fibers could have been better achieved in saline leading to better fracture resistance.[5] This is in accordance to the previous studies [16],[17].

The statistically significant difference between the dehydrated group (Group 2) and the other experimental groups corroborates the findings of previous studies [5],[18],[19] where dehydration of the fragment led to lower fracture resistance values. Once the fragments were kept dehydrated for 24 hours, the collagen fibers collapsed [6],[20], thus preventing penetration of the resin monomers between the collagen fibrils [19],[20]. This accounted for the least fracture resistance values amongst the tested groups.

The results also revealed that the duration of time spent rehydrating the fractured fragments had no effect on their fracture resistance. This is in line with the findings of a study conducted by Brasil Maia et al.[21], who found that hydration of the tooth fragment increased fracture resistance regardless of storage solution or immersion period (1 hour or 24 hours). Poubel et al.[14], on the other hand, found that rehydrating a tooth fragment for 15 minutes before bonding with a multimode adhesive appeared to keep enough moisture in the tooth fragment to boost reattachment strength. Shirani et al.[15] also found that a 24-hour rehydration of the tooth fragment before treatment appeared to salvage enough moisture to result in an increase in reattachment strength when compared to a 30-minute period.

The proposed technique for fragment reattachment was active application of Single Bond Universal (3M/ESPE, SP, Brazil) as a self-etching adhesive. The intermediate material of choice was Filtek Z350 XT flowable composite resin (3M ESPE, SP, Brazil) because its lower viscosity provided better remnant-fragment adaptation compared with conventional composite resins and because its inorganic filler content was higher compared with resin and ionomeric cements [22]. According to Garcia et al.[23] and Souza et al.[24] 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.

Another limitation of the current study was the 1 mm/min compression stress applied to the specimens in the universal test machine, which did not replicate clinical conditions.[16] Furthermore, spontaneous fracture happens frequently and with an instantaneous overload on the tooth. In contrast, the study's intentional fracture was accomplished at a slow and consistent speed, with a force that gradually rose as the machine's contact with the tooth increased. Dental trauma, on the other hand, is not usually caused by a high-energy impact. Malocclusion and parafunctions like bruxism can cause persistent overload on the teeth, which can lead to coronal fracture [21].

Both laypeople and caregivers appear to have inadequate awareness of dental emergencies and how to address them [25]. Despite the higher occurrence of crown fractures, understanding of tooth fracture management appears to be lower than that of tooth avulsion [26]. The most common example of dental injury management education is tooth avulsions, which show the proper rehydrating media and condition for keeping the avulsed tooth alive. Other dental tissues obtained in an accident (such as tooth fragments) may be advised to be stored in a storage medium until reaching a dentist. Having a unified protocol for the management of the traumatized tooth (in both cases of tooth avulsion and crown fractures) would help keep the protocols as basic as possible, allowing for easier and faster dissemination of information.[16] Further research in this aspect can be done to find out better rehydrating media.

CONCLUSION

Within the limitations of this study, it can be concluded that:

- Rehydration of the fragment does improve its fracture resistance significantly.
- Preservation of the tooth fragment in 50% dextrose solution results in higher fracture resistance, followed by 0.45% saline and skimmed milk, respectively.
- Rehydrating immersion times does not improve fracture resistance of reattached fragments significantly.

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