ORIGINAL RESEARCH

Evaluation of Fracture Resistance of Reattached Fractured Tooth Fragment Stored in Different Storage Media: An *In Vitro* Study

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Abstract

Aim: To evaluate the fracture resistance of coronal fractured tooth fragments stored in five different storage media when reattached with nanohybrid flowable composite.

Materials and methods: The crown portion of 50 extracted human permanent maxillary central incisors were divided into three equal parts (incisal third, middle third and cervical third) and then marked incisal third were cut with the diamond disk. These were divided into five equal groups according to the type of storage media used i.e. dry storage, fresh tender coconut water, HBSS, milk, and propolis for 2 hours. Coronal fractured part with their respective apical parts were then reattached with flowable composite (G-aenial Universal Flo, GC India), then after thermocycling process samples were subjected to universal testing machine for testing fracture resistance. The collected data were subjected to statistical analysis using one way ANOVA and *Post-hoc* Tukey test.

Results: The obtained results revealed that large amount of force is required to fracture the reattached teeth which were stored in milk and fresh tender coconut water as compared to those which were stored in dry environment, HBSS and propolis.

Conclusion: In this study, maximum fracture resistance was seen in teeth stored in milk and fresh tender coconut water. Therefore, these two were considered as better storage media.

Clinical significance: Due to increased interest towards the use of tooth colored restoration, recently, fractured teeth reattachment treatment procedure gaining attention by preserving life like translucency of treated tooth.

Keywords: Coconut water, Fractured teeth, Hanks balanced salt solution, Milk, Propolis, Reattachment, Storage media.

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Introduction

Trauma to the oral cavity represents 5% of all injuries.[1] Among all dental hard tissue fracture, coronal fracture is the most common type of tooth fracture and it represents 18–22% of all traumatic injuries of teeth, approximately 96% of which involves the maxillary incisors.[2]

The potential for successful treatment of fractured teeth is highly favorable if treatment is facilitated immediately after injury.

There are many treatment options to restore the fractured teeth likelaminates,[3] resin/ceramic crowns, composite resin restoration with and without pins,[2] porcelain veneers, jacket crowns, post and core.[4] Treatment options for maintaining biologic width (crown lengthening, flap surgery, osteotomy/ostectomy, rapid orthodontic tooth extrusion)[5] are also some of the treatment options. All these options show variable degree of success, but are not conservative approaches.[6]

Reattachment of coronal fractured tooth fragment not only has esthetic value but also conservative and biologic values and also helpful in implementation of positive psychological response in patient.[3]

The anterior teeth face many unusual forces because of their unique position and function. Therefore, fragment reattachment technique still requires improvements to overcome unexpected situations.[7]

Various authors have suggested different methods for fragment reattachment of fractured teeth like adopting circumferential bevel prior to reattachment, placement of chamfer to fractured line after bonding, placing notch/internal groove/or superficial over contouring of fractured line, bonding of fractured fragment with no preparation at all are some of the methods.[8]

Success of the reattachment of a fractured fragment depends on the time interval of restoring the fractured part of teeth after

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trauma.[9] Factors which are responsible for bond strength of reattached fractured tooth fragment include hydration of fractured fragment and time period of rehydration[7] and this is influenced by method of storing these fractured fragments.[9]

Storage medium maintains the hydration of fractured fragment which in turn maintains the vitality and natural appearance of the teeth.[10] Various types of storage medium are available, some of them are: Milk, HBSS, coconut water, artificial saliva, egg white, saline, tap water, via span, propolis, various culture media, contact lens solution, etc.[11]

Unlike the well-established protocols of how to preserve the avulsed tooth before treatment, recent guidelines do not mention how a fractured fragment of tooth should be preserved, even-though most reports support the idea that the fragment should be kept hydrated.

Thus, this *in-vitro* study will focus on the importance of hydration of fractured coronal tooth fragment stored in different types of storage media and its effect on the fracture resistance after reattachment procedure.

Materials and Methods

In this *in-vitro* study 50 freshly extracted caries free human permanent maxillary central incisors were collected from the Department of Oral and Maxillofacial Surgery, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala which were extracted due to periodontal reasons.

All the collected teeth were disinfected and cleaned by ultrasonic tips and 0.2% thymol. After measuring the cervicoincisal distance of crown portion of all the samples using Vernier caliper the labial surface of crown divided into three equal parts (incisal third, middle third, and cervical third) and marked with the help of marker. Then the marked teeth were randomly divided into 5 groups of 10 each. To simulate Elli's class II fracture, samples were cut on the incisal one third marked line perpendicular to the long axis of teeth using low speed diamond disk with saline as coolant and preserved in five different types of storage media for 2 hours.

- Group I: Dry storage
- Group II: Hank's balanced salt solution
- Group III: Propolis
- Group IV: Milk
- Group V: Fresh tender coconut water

The remaining parts of the teeth were stored in artificial saliva. After 2 hours fractured fragments rinsed with distilled water and both the fractured surface of the fragment and remaining part of the teeth were etched using 37% phosphoric acid for 15 seconds, then rinsed with water for 10 seconds followed by air drying for 5 seconds. On the etched surface bonding agent was applied in two consecutive layers and each coat light cured for 20 seconds individually. Then nanohybrid flowable composite (G-aenial Universal Flo, GC India) was applied and light cured for 40 seconds on labial half, 40 seconds on lingual half after confirming the correct position for reattachment. All the reattached samples were subjected to thermocycling consisting of 100 cycles alternating between hot water (55°C) and cold water (5°C) for 15 seconds in each water bath. Root portions of all the samples were embedded into the acrylic resin block so that they can be easily placed into the universal testing machine.

In the next step, samples were subjected to universal testing machine (UTM) for testing fracture resistance. The rod of UTM was placed at the incisal third of the crown where the bonding line on the labial surface was seen and then the load was applied with the speed of 1 mm/minute until the debonding occurs. The value at which the debonding of reattached fragment occurred was recorded.

Statistical Analysis

The data was analyzed using SPSS (19.0 Version) and statistical analysis was done using Oneway ANOVA (Tables 1 and 2, Fig. 1) to compare the mean values between more than three groups and *Post-hoc* Tuckey test (Table 3) for further comparison between the groups (taking two groups at a time). The *p*-value was taken significant when it is less than 0.0001.

Results

All the groups were statistically significant except for group IV (milk) with mean value of 90.930 and group V (Fresh tender coconut water) with mean value of 86.775 having *p*-value 0.798 and mean difference of 4.155. No statistically significant difference seen between the groups IV and V, however, their obtained values were higher than the other groups i.e. group I (Dry storage) with mean value 21.994 and group II (HBSS) with mean value 67.686, have mean difference of 45.692, group I (Dry storage) with mean value 21.994 and group III (Propolis) with mean value 47.723 have mean difference of 25.729, group I (Dry storage) with mean value 21.994 and group IV (Milk) with mean value 90.930 have mean difference of 68.936, group I (Dry storage) with mean value 21.994 and group V (Fresh tender coconut water) with mean value 86.775 have mean difference of 64.781, group II (HBSS) with mean value 67.686 and group III (Propolis) with mean value 47.723 have mean difference of 19.963, group II (HBSS) with mean value 67.686 and group IV (Milk) with mean value 90.930 have mean difference of 23.244, group II (HBSS) mean value 67.686 and group V (Fresh tender coconut water) with mean value 86.775 have mean difference of 19.089, group III (Propolis) with mean value 47.723 and group IV (Milk) with mean value 90.930 have mean difference

Table 1: Descriptive analysis (mean fracture resistance and standard deviation among the groups)

					95% confidence interval for mean			
Group	*n*	*Mean*	*Std. deviation*	*Std. error*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*
Group I (Dry)	10	21.994	5.47544	1.73149	18.0771	25.9109	13.70	28.70
Group II (HBSS)	10	67.686	9.66870	3.05751	60.7694	74.6026	55.86	79.75
Group III (Propolis)	10	47.723	7.67827	2.42808	42.2303	53.2157	37.75	58.10
Group IV (Milk)	10	90.930	9.75419	3.08455	83.9523	97.9077	76.97	101.25
Group V (Coconut water)	10	86.775	8.37109	2.64717	80.7867	92.7633	76.42	98.73
Total	50	63.022	27.08658	3.83062	55.3237	70.7195	13.70	101.25

Table 2: One-way ANOVA

	Sum of squares	*df*	*Mean square*	*F*	*p value*
Between groups	32821.706	4	8205.426	118.016	<0.0001**
Within groups	3128.754	45	69.528		
Total	35950.459	49			

**$p < 0.001$; Highly significant

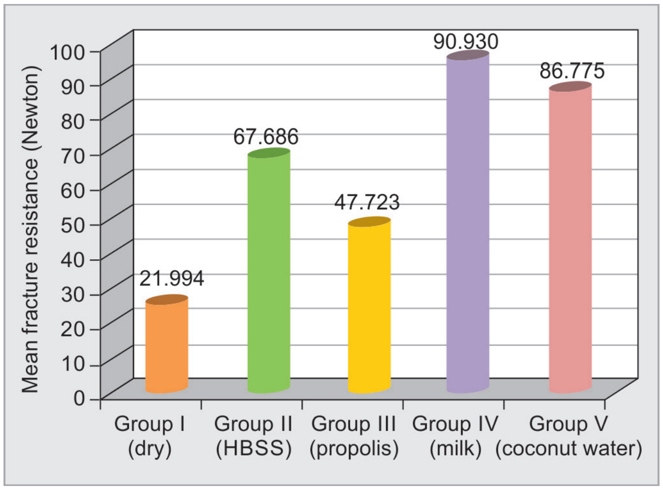

Fig. 1: Comparison of mean value of fracture resistance between five types of storage media

Table 3: *Post-hoc* Tuckey test for intergroup comparison level of significance

Intergroup	*Mean difference between groups*	*p value*
Group I (Dry storage) and II (HBSS)	45.692	<0.0001**
Group I (Dry storage) and III (Propolis)	25.729	<0.0001**
Group I (Dry storage) and IV (Milk)	68.936	<0.0001**
Group I (Dry storage) and V (Fresh tender coconut water)	64.781	<0.0001**
Group II (HBSS) and III (Propolis)	19.963	<0.0001**
Group II (HBSS) and IV (Milk)	23.244	<0.0001**
Group II (HBSS) and V (Fresh tender coconut water)	19.089	<0.0001**
Group III (Propolis) and IV (Milk)	43.207	<0.0001**
Group III (Propolis) and V (Fresh tender coconut water)	39.052	<0.0001**
Group IV and V (Fresh tender coconut water)	4.155	0.798

**$p < 0.001$; Highly significant

of 43.207, while group III (Propolis) with mean value 47.723 and group V (Fresh tender coconut water) mean value 86.775 have mean difference of 39.052. Least value obtained from group V followed by groups III and II.

Discussion

Now a days, reattachment procedure for fractured tooth fragment is gaining popularity by preserving life like translucency via exact restoration of coronal and surface morphology.[12] Therefore, if the fractured part of teeth is available then preserving it under moist condition is beneficial as it prevents collapse of collagen fibrils which in turn absorb energy, thus resisting fracture as stated by Jameson et al.). This enhance the bond strength.[2,13,14] Such moist condition is provided by storage media. Storage media is "the physiological solution that closely replicates the oral environment to help preserve the vitality of periodontal ligament cells following avulsion".[7]

Important properties of some storage media:

- *pH (Hydrogenionic potential):* pH of any solution depends on the concentration of hydrogen ion in that solution. If higher concentration of hydrogen ion present then that solution will have lower pH value. Mostly cells have pH approximately 7.0,i.e., hydrogen ion concentration and OH^- concentration is almost equal. This little variation in concentration of both the ions is hazardous to the cells.
- *Osmolality:* Osmolality and osmolarity both represents osmotically active solute molecules within a solution.

This study focused on achieving maximum bond strength and providing the better environment for storing the fractured part of the teeth. Different gold standard storage media were suggested like HBSS by "*International Association of Dental Traumatology*" and milk by *"American Association of Endodontics"* the reason behind this may be because:

- These two storage media has been popularly selected for studies of dental avulsion cases as reference media as these have approximately ideal osmolality as well as pH.
- Provide sufficient hydration.
- Maintain stability of collagen framework.[15–17]

Previous researches showed that some easily available storage media like human saliva, egg white, milk may work effectively in storing fractured fragments of teeth.[18–20] If Indian Scenario taken into account for the trouble of storing fractured part of tooth, there are high chances of availability of coconut water or milk at trauma site like schools or road side accidents.[21,22] Therefore, in this study coconut water, milk, HBSS were used as storage media. Although propolis is not new in dentistry, very little information is available about its efficacy as storage media for fractured tooth fragment. Hence, propolis is also selected as storage media.

It was found that dry storage had least fracture resistance values as compared to other groups. These results were in *agreement with the studies conducted by following authors*: Sharmin DD, Shrani et al.,[7] Prabhakar et al.,[2] Hedge et al.,[26] Shilpa et al.,[16] Daokar et al.,[14] Maia et al.,[27] Joseph et al.,[19] Kumar et al.[28] This is due to the absence of moisture which is responsible for brittleness of dentin[23,24] and collapsing of collagen fibrils, thus prevents formation of mechanical interlocking.[25,26] This shows the importance of moist dentin.

Although HBSS considered as gold standard storage media, in this study its fracture resistance values were lower from that of milk and fresh tender coconut water. Previous studies done by Shirani et al.,[9] Prabhakar et al.,[2] Hedge et al.,[26] Daokar et al.,[14] Chinchalkar et al.,[25] Thakre et al.[29] also found that milk was a better storage media than other storage media. This may be due to the greater amount of water content and higher proportion of calcium and phosphate ions in milk which contributes to the hardness of dentin.[27–29] The result of this study showed that propolis is less effective as compared to

HBSS, milk and fresh tender coconut water. Such observation may be due to the variability in composition of propolis according to climate, season, place, or presence of debris.[17,20,30,31]

However, there are some studies which does not support the result of present study like study done by Kumar et al.[28] and Mantri et al.[6] who observed that HBSS was the ideal storage media for fractured fragment when comparing it with coconut water, milk, open environment.

Probable causes of HBSS as good storage media are as follows:

- Ideal osmolality (320 mOSM kg^{-1}) and ideal pH (7.2)
- Like milk it also has physiologic pH and consists of essential nutrients
- Higher water content

According to this study milk and fresh tender coconut water were the best storage media for fractured teeth. There has been no comparative study for propolis and milk as a storage media done before. However, the probable reason for milk and coconut water as best storage media for fractured teeth rather than propolis is due to the greater osmolality and higher content of water due to which it showed better dentin wetting properties, thus forms better resin tags.[16] Also, the milk and the coconut water showed approximately similar pH. There is absence of toxic components in both the storage media.[24]

One of the major limitations of this study is its *in vitro* nature, therefore not mimic the oral. Apart from this, conditions like both the fragments to be attached were non vital and the storing time of the extracted teeth was not same, the oral temperature was maintained only for limited period of time (thermocycling) and artificial salivary components further added to the limitations of the study.

Conclusion

Based on the results and under the conditions of this study, it was concluded that hydration of fractured fragment improves the fracture resistance in contrast to dehydrated condition. Whereas, fracture resistance did vary within groups themselves; with the highest in milk and the lowest being with dry. Milk and fresh tender coconut water expressed no difference in their fracture resistance. Thus, fresh tender coconut water is a good alternative to milk as a storage media.

Further research is however needed with more studies and greater number of samples to compare these storage media especially for fractured teeth.

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