



EFFECT OF VARIOUS STORAGE MEDIA ON FRACTURE RESISTANCE OF THE REATTACHED TOOTH FRAGMENTS: AN IN-VITRO STUDY.

Dental Science

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ABSTRACT

AIM: The aim of the present study is to evaluate the effect of storage media on fracture resistance of the reattached tooth fragments stored in five different storage medium: dry environment, milk, green tea extract, coconut water and oral rehydration salt.

MATERIAL AND METHODS: Fifty freshly extracted maxillary incisor teeth were divided into 5 groups of 10 each. Teeth were fractured at incisal third of crown; the fracture fragments were stored for 2 hours in dry environment (Group 1), milk (Group 2), green tea extract (Group 3), coconut water (Group 4) and oral rehydration salt (Group 5). By using simple reattachment technique, fragments were reattached with flowable composite resin. The fracture resistance was measured by a universal testing machine. Statistical analysis was performed using one way analysis of variance (ANOVA) test and Tukey HSD post hoc test.

RESULTS: The maximum mean fracture resistance was seen in milk (Group 2) (314.84 ± 4.398), followed with coconut water (Group 4) (282.45 ± 40.250), oral rehydration salt (Group 5) (271.62 ± 6.704), green tea extract (Group 3) (268.48 ± 4.889) and the least fracture resistance was seen in dry environment (Group 1) (209.16 ± 3.277).

CONCLUSIONS: From the present study it can be concluded that, fractured fragments stored in milk showed maximum fracture resistance and dry environment showed least fracture resistance. Oral rehydration salt, coconut water and green tea extract can be considered a viable alternative for storage of fractured tooth fragment.

KEYWORDS

Reattachment, Fractured Fragments, Dry Environment, Milk, Green Tea Extract, Coconut Water, Oral Rehydration Salt.

INTRODUCTION:

Traumatic fracture of anterior tooth is the most common problem amongst different age groups and gender which impede the esthetics and psychology of the individual^{1,2}. Of all the trauma affecting the dental hard tissues, percentage of coronal fractures of permanent incisors is 18 to 22%; of these, maxillary incisors involve 96%³. Injuries may be caused by falls, contact sports, automobile accidents and striking of foreign bodies^{4,5}.

There are several techniques for restoring the fractured tooth, including pin retained restorations, crowns, porcelain veneers, orthodontic bands, composite resins, each technique showing some amount of diverse degrees of success.⁶ With the development of adhesive dentistry came the concept of 'fragment reattachment'⁷. When the tooth fragment is available with no or minimal violation of the biological width, reattachment of the fractured fragment is the most conservative option.⁸ It has several advantages over other techniques as it provides good and long-lasting esthetics because the tooth's original anatomic form, color, translucency and surface texture are maintained. It also restores function, provides a positive psychological response, results in harmonious wear of the incisor edge, preserves the pulp vitality, economical, less chair side time and is a relatively simple procedure.^{9,10,11}

Prognosis of the fragment reattachment depends on the intact retrieval of the fragment at the time of injury, time of restoring the fractured part, type of storage medium for adequate hydration of the fragment outside the mouth, firm attachment and strong bond of the fragment to the tooth and the patient's awareness¹². As the hydration maintains the vitality and original esthetic appearance of the tooth⁷.

Very few studies have been reported on the type of storage medium for the fractured parts of teeth like saliva, water, or normal saline. Storage

medium were selected based on natural, readily available and publicly accessible materials. Milk is rich in calcium and phosphate which harden and stiffen both demineralized and healthy dentin by permeating the surface¹³. Green tea extract has been reported to have remarkable antibacterial, anti-inflammatory, and anticariogenic effects and it prolong allograft survivals.¹⁴ Coconut water and oral rehydration salt are suitable medium for maintaining the PDL cell viability in simulated avulsed teeth¹⁵.

Many patients were unaware of storage medium for fracture reattachment and they bring the fracture fragment in dry environment, Hence the focus of the present in-vitro study was to evaluate the effect of storage media on fracture resistance of the reattached tooth fragments stored in five different storage medium: dry environment, milk, green tea extract, coconut water and oral rehydration salt.

MATERIALS AND METHODS:

In the present study, 50 freshly extracted permanent maxillary central incisors were selected. All the teeth were free from caries or any other structural deformities which were extracted for periodontal reasons. Surface of all the extracted teeth were cleaned with curettes and ultrasonic tips and were randomly divided into five groups of 10 teeth each. The teeth were marked 3 mm apical to the incisal edge and were sectioned using a diamond disk at slow speed (Fig. 1) which were stored in already labeled trays having five storage medium (Fig. 2) as follows:

- Group 1 - dry environment
- Group 2 - milk
- Group 3 - green tea extract (Healths Harmony Ltd.)
- Group 4 - coconut water
- Group 5 - oral rehydration salt (Cipla Ltd.)

All the fragments were preserved in the respective storage media and

the apical portions of teeth were stored in distilled water. After 2 hours, all the fragments were rinsed and dried. Both the fractured surfaces were etched with 37% phosphoric acid (3M ESPE, USA) for 15 seconds then rinsed with water and dried. After application of bonding agent (Single Bond, 3M ESPE, USA), both the etched surfaces were dried for 5 seconds by an air syringe to allow solvent evaporation and cured with light curing unit (Ivoclar Vivadent). The fragments were approximated with their respective apical portion using a flowable composite (Filtek Flow, 3M ESPE, USA) and held by the gutta percha stick and light curing was done for 40 seconds buccally and palatally (Fig. 3). Reattached samples were then stored in distilled water.

All the samples were mounted on an acrylic block up to 1 mm apical to the cingulum (Fig. 4), and specimens were loaded on the universal testing machine (Servo Computerized TUF-C-1000-kN, India). In order to evaluate the impact, a crosshead speed of 1mm/minute was selected and the compressive load was applied perpendicular on the incisal third of teeth specimen using universal testing machine to simulate the traumatic impact.

The fracture resistance of each specimen was tested and measured Newton. This load represented the fracture resistance of the reattached tooth. Collected data were tabulated and statistically analysed.

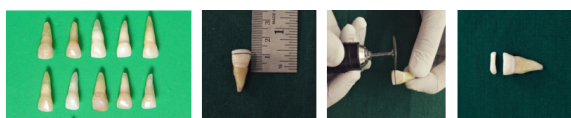


Fig.1: Tooth sectioned 3 mm from the incisal edge using diamond disk.



Fig.2: Tooth fragments of the specimens in respective storage media for 2 hours.

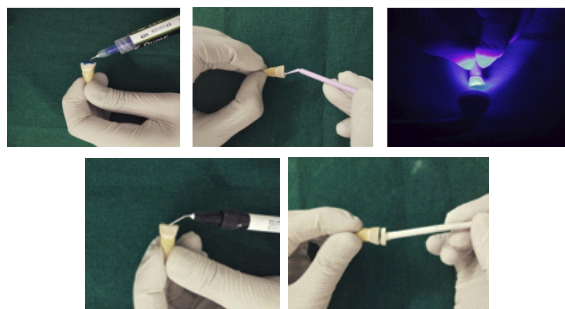


Fig.3: Fragment reattachment with respective tooth specimens using flowable composite.



Fig.4: Reattached tooth samples mounted on acrylic blocks

RESULTS:

The results were tabulated and statistically analysed using one way analysis of variance (ANOVA) test and Tukey HSD post hoc test using statistical package for social sciences (SPSS) for windows, version 21.0 software (IBM Corp., USA) and confidence intervals were set at 95 % and values of $p < 0.05$ were interpreted as statistically significant. The values for each group were tabulated, and the mean and standard deviation were calculated in Newton for each of the groups (Table 1). Statistical tests were then applied.

The maximum mean fracture resistance was seen in milk (Group 2) (314.84 ± 4.398), followed with coconut water (Group 4) (282.45 ± 40.250), oral rehydration salt (Group 5) (271.62 ± 6.704), green tea extract (Group 3) (268.48 ± 4.889) and the least fracture resistance was seen in dry environment (Group 1) (209.16 ± 3.277).

When ANOVA test was applied, there was a significant difference in fracture resistance among the groups ($p = 0.00$).

On Tukey HSD post hoc test, it was found, that milk (Group 2) demonstrated the maximum fracture resistance values having a statistically significant difference with respect to dry environment (Group 1), green tea extract (Group 3), coconut water (Group 4) and oral rehydration salt (Group 5). Dry environment (Group 1) shows the least fracture resistance values having a statistically significant difference with respect to green tea extract (Group 3), coconut water (Group 4) and oral rehydration salt (Group 5). There was no statistically significant difference in fracture resistance values between green tea extract (Group 3) with respect to coconut water (Group 4) ($p = 0.453$) and oral rehydration salt (Group 5) ($p = 0.995$); also coconut water (Group 4) and oral rehydration salt (Group 5) ($p = 0.689$).

Table 1: Mean fracture resistance and the standard deviation among the groups

	n	Mean(in Newton)	Std. Deviation
Group 1 - Dry environment	10	209.1600	3.27794
Group 2 - Milk	10	314.8400	4.39854
Group 3 - Green tea extract	10	268.4800	4.88985
Group 4 - Coconut water	10	282.4500	40.25067
Group 5 - Oral rehydration salt	10	271.6200	6.70403

Table 2: Level of significance on intergroup comparison using Tukey HSD post hoc test

(I) Group	(J) Group	Mean Difference (I-J)	p
Group 1- Dry environment	Group 2 - Milk	-105.68000*	0.000
	Group 3 - Green tea extract	-59.32000*	0.000
	Group 4 - Coconut water	-73.29000*	0.000
	Group 5 - Oral rehydration salt	-62.46000*	0.000
Group 2 - Milk	Group 1- Dry environment	105.68000*	0.000
	Group 3 - Green tea extract	46.36000*	0.000
	Group 4 - Coconut water	32.39000*	0.003
	Group 5 - Oral rehydration salt	43.22000*	0.000
Group 3 - Green tea extract	Group 1- Dry environment	59.32000*	0.000
	Group 2 - Milk	- 46.36000*	0.000
	Group 4 - Coconut water	-13.97000	0.453
	Group - 5 Oral rehydration salt	-3.14000	0.995
Group 4 - Coconut water	Group 1- Dry environment	10.83000	0.689
	Group 2 - Milk	-32.39000*	.003
	Group 3 - Green tea extract	13.97000	.453
	Group 5 - Oral rehydration salt	10.83000	.689
Group 5 - Oral rehydration salt	Group 1- Dry environment	62.46000*	.000
	Group 2 - Milk	-43.22000*	.000
	Group 3 - Green tea extract	3.14000	.995
	Group 4 - Coconut water	-10.83000	.689

* The mean difference is significant at the 0.05 level.

DISCUSSION:

Due to the changing lifestyle in the modern era, episodes of dental trauma of anterior teeth more commonly coronal fractures are being increasingly reported¹¹. The current generation wants an esthetically very pleasing and natural remedy for such traumatic injuries. Fragment reattachment can be considered a biologically viable option which not just meets the current expectations but is minimally invasive as well¹⁶. The philosophy behind this approach dates back to the early 1960's¹⁷. However, the interest in this concept got renewed after it was found that, the mode of fragment storage following trauma was a key prognostic determinant.¹⁸

According to Farik et al.¹⁹ additional drying of fractured fragment beyond 1 hour decreases the fracture resistance significantly, thus it is important to keep the fragment in some storage medium. Hence, the focus of the present in - vitro study was to evaluate the effect of storage media on fracture resistance of the reattached tooth fragments stored in five different storage medium viz., dry environment, milk, green tea extract, coconut water and oral rehydration salt.

In the present study, permanent maxillary central incisors extracted for periodontal reasons were selected because of the high incidence and prevalence of trauma in this region in natural conditions.^{20,21}

Commonly used storage media for extracted and sectioned teeth are 0.9% saline, 5.25% sodium hypochlorite solution, distilled water, and formalin¹⁸. According to Lee et al.,²² residual chlorine from normal saline and sodium hypochlorite can negatively influence the bond strengths when used as storage medium. Hence in this study, distilled water was used as the universal storage medium for freshly extracted teeth because it has a neutral pH and does not contain any contaminants.

In this study, all the teeth were sectioned in a standardized manner with a diamond disc rather than fractured, as the aim was to evaluate the storage media. The fitting between the fragment and the tooth was not always perfect. However, fracturing a tooth in-vitro has its own disadvantages as the fractured fragments produced could be of uneven dimensions. As a result, the amount of material required for reattachment will vary and give inconclusive results. Hence, with this limitation to simulate the natural fracture forces, this procedure of sectioning using a diamond disk was followed as it allows the standardization of the fragment size. Similar standardization protocol was adapted by Sharmin et al in their study¹⁸.

The success of fragment reattachment depends on the type of storage media used after trauma. Storing the fragment in a moist environment prevents the drying of the fragment, ensured no or minimal collapse of the collagen fibers in the dentin and preserves the esthetics¹⁸. Hence in the present study, storage medium were selected based on publicly accessible materials and cost effective like milk, green tea extract, coconut water and oral rehydration salt for restoring the fractured fragment.

According to Shirani et al¹³, milk is rich in calcium and phosphate which harden and stiffen both demineralized and healthy dentin by permeating the surface and results in enhanced bond strength of reattached fragment. Calcium and phosphate sedimentation affects the surface topography of dentin and the degree of its dissolution during acid etching results in better bond strength. Hence the best storage environments, as observed in this study is milk as it is isotonic with high water content which allowed adequate rewetting of the dentinal tubules, little osmotic and dimensional changes happen in the dentin surface and a stronger bond strength is achieved. This is in accordance to the previous studies²⁴.

Followed by milk, coconut water showed better result as compared to oral rehydration salt, green tea extract and dry environment. It can be hypothesized that water content of coconut water being greater, allowed better wetting of the dentin preventing the collapse of the collagen fibres and helps in resin tag formation¹⁶. Similarly, Gopikrishna et al²⁵ found that, coconut water kept PDL cells significantly more viable than HBSS or milk in simulated avulsed teeth.

Oral rehydration salt is a glucose and electrolyte solution consists of essential cell nutrients which maintains the optimal osmolality as well as pH.²⁶ Green tea extract has been reported to have remarkable

antibacterial, anti-inflammatory, and anticariogenic effects and it prolong allograft survivals¹⁴. In the present study both these storage medium showed better results after coconut water and milk, and can be used as an alternative storage medium for fractured tooth.

The storage time was taken as 2 hours to simulate a clinical scenario wherein the reattachment procedure is carried out in the same appointment. The technique of simple reattachment was followed as the point of contention here was the hydration media. Dehydration of human dentin has demonstrated a brittle behavior²⁷. Hence, at no point in the entire study was the samples allowed to dry except for the fragments of Group 1.

Various techniques can be used for fragment reattachment, out of which a simple reattachment technique was used in this study. An adhesive agent is used as it keeps the union of tooth and the fragment intact, has enough fracture toughness to bear the masticatory load, less time-consuming and simple procedure¹⁸.

All of the samples were tested within 48–72 hours of their extraction to prevent from any major variation occurring in the values between the samples. The direction of load application for fracturing the reattached teeth simulated a clinical scenario wherein a tooth restored using fragment reattachment encounters the second episode of trauma. However, one potential drawback of this study was the amount of load which was applied using the universal testing machine at a crosshead speed of 1 mm/min did not simulate a natural traumatic scenario. Similar settings were adopted by Shirani et al in their study¹³.

The overall knowledge of dental emergencies and their management seems to be unsatisfactory both among lay people and care giver²⁸. Despite crown fractures, higher frequency, knowledge regarding tooth fracture management seems to be relatively lower than tooth avulsion²⁹. Education regarding the management of dental injuries is usually based on tooth avulsions and indicates the proper storage media and condition for keeping the avulsed tooth¹³. However, it may be possible to advise keeping other dental tissues obtained at an accident (such as tooth fragments) in a storage medium until reaching a dentist. Having a unified protocol for the management of the traumatized tooth (in case of both tooth avulsion and crown fractures) would help keep the protocols as simple as possible, which could eventually lead to an easier and more rapid spread of information.

CONCLUSIONS:

From the present study it can be concluded that, fractured fragments stored in milk showed maximum fracture resistance and dry environment showed least fracture resistance. Oral rehydration salt, coconut water and green tea extract can be considered a viable alternative for storage of fractured tooth fragment.

REFERENCES

1. Jackson NG, Waterhouse PJ, Maguire A. Factors affecting treatment outcomes following complicated crown fractures managed in primary and secondary care. *Dent Traumatol* 2006; 22:179-185.
2. Farik B, Munksgaard EC, Andreasen JO, Kreiborg S. Fractured teeth bonded with dentin adhesives with and without unfilled resin. *Dent Traumatol* 2002; 18:66-69.
3. Andreasen JO, Ravn JJ. Epidemiology of traumatic dental injury to primary and permanent teeth. *Int J Oral Surg* 1972; 1(5):235-239.
4. Andreasen JO. Etiology and pathogenesis of traumatic dental injuries. A clinical study of 1,298 cases. *Scand J Dent Res* 1970; 78:329-342.
5. Andreasen JO, Andreasen FM, Andersson L. *Textbook and Color Atlas of Traumatic Injuries to the Teeth*. Oxford, UK: Blackwell; 2007.
6. Sengun A, Ozer F, Unlu N, Ozturk B. Shear bond strengths of tooth fragments reattached or restored. *J Oral Rehabil* 2003; 30:82-86.
7. Capp CI, Roda MI, Tamaki R, Castanho GM, Camargo MA, de Cara AA. Reattachment of rehydrated dental fragment using two techniques. *Dent Traumatol* 2009 Feb; 25(1):95-99.
8. Thapak G, Arya A, Arora A. Fractured tooth reattachment: A series of two case reports. *Endodontology*. 2019 Jan 1;31(1):117-120.
9. Terry DA. Adhesive reattachment of a tooth fragment: The biological restoration. *Pract Proced Aesthet Dent* 2003; 15:403-409.
10. Wiegand A, Rodig T, Attin T. Treatment of crown fractured incisors: Reattachment instead of restoration? *Schweiz Monatsschr Zahnmed* 2005; 115:1172-1181.
11. Reis A, Loguercio AD, Kraul A, Matson E. Reattachment of fractured teeth: A review of literature regarding techniques and materials. *Oper Dent* 2004; 29:226-233.
12. Jalannavar P, Tavargeri A. Influence of storage media and duration of fragment in the media on the bond strength of the reattached tooth fragment. *International journal of clinical pediatric dentistry*. 2018 Mar; 11(2):83-88.
13. Shirani F, Sakhaei Manesh V, Malekipour MR. Preservation of coronal tooth fragments prior to reattachment. *Aust Dent J* 2013 Sep; 58(3):321-325.
14. Khinda VI, Kaur G, Brar GS, Kallar S, Khurana H. Clinical and practical implications of storage media used for tooth avulsion. *International journal of clinical pediatric dentistry*. 2017 Apr; 10(2):158-165.
15. Ashkenazi M, Marouni M, Sarnat H. In vitro viability, mitogenic and clonogenic capacities of periodontal ligament fibroblasts after storage in four media supplemented with growth factors. *Dent Traumatol* 2001; 17:27-35.
16. Prabhakar AR, Yavagal CM, Limaye NS, Nadig B. Effect of storage media on fracture

- resistance of reattached tooth fragments using G-aenial Universal Flo. Journal of conservative dentistry: JCD. 2016 May;19(3):250-253.
17. Chosack A, Eidelman E. Rehabilitation of a fractured incisor using the patient's natural crown: Case report. J Dent Child 1964; 31:19-21.
 18. Sharmin DD, Thomas E. Evaluation of the effect of storage medium on fragment reattachment. Dent Traumatol 2013; 29:99-102.
 19. Farik B, Munksgaard EC, Andreasen JO, Kreiborg S. Drying and rewetting anterior crown fragments prior to bonding. Endod Dent Traumatol 1999; 15:113-116.
 20. Tovo MF, dos Santos PR, Kramer PF, Feldens CA, Sari GT. Prevalence of crown fractures in 8-10 years old schoolchildren in Canoas, Brazil. Dent Traumatol 2004 Oct; 20(5):251-254.
 21. Zuhail K, Semra OE, Huseyin K. Traumatic injuries of the permanent incisors in children in southern Turkey: a retrospective study. Dent Traumatol 2005 Feb; 21(1):20-25.
 22. Lee JJ, Nettey-Marbell A, Cook A Jr, Pimenta LAF, Leonard R, Ritter AV. Using extracted teeth for research: the effect of storage medium and sterilization on dentin bond strength. J Am Dent Assoc 2007; 138:1599-1603.
 23. Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, et al. Effect of CPP-ACP paste on tooth mineralization: An FE-SEM study. J Oral Sci 2007; 49:115-120.
 24. Shirani F, Malekipour MR, Tahririan D, Sakhaei Manesh V. Effect of storage environment on the bond strength of reattachment of crown fragments to fractured teeth. J Conserv Dent 2011; 14:269-272.
 25. Gopikrishna V, Thomas T, Kandaswamy D. A quantitative analysis of coconut water: A new storage media for avulsed teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105:e61-65.
 26. Eskandarian T, Badakhsh S, Esmailpour T. The effectiveness of oral rehydration solution at various concentrations as a storage media for avulsed teeth. Iranian endodontic journal. 2013;8(1):22.
 27. Jameson MW, Hood JA, Tidmarsh BG. The effects of dehydration and rehydration on some mechanical properties of human dentine. J Biomech 1993; 26:1055-1065.
 28. Al-Asfour A, Andersson L. The effect of a leaflet given to parents for first aid measures after tooth avulsion. Dent Traumatol 2008; 24:515-521.
 29. Sae-Lim V, Chulaluk K, Lim LP. Patient and parental awareness of the importance of immediate management of traumatized teeth. Endod Dent Traumatol 1999; 15:37-41.