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Comparative Evaluation of the Effect of Various Storage Mediums on the Fracture Resistance of Re-Attached Tooth Fragments: An Ex-Vivo Study

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ABSTRACT

Purpose: To comparatively evaluate the fracture resistance of re-attached tooth fragments stored in dry, saline, pasteurised bovine milk, egg white and coconut water for 24 hours. **Material and Methods:** 50 permanent maxillary central and lateral incisors were cut perpendicular to the long axis with a diamond disk. Immediately after fracturing, the fractured fragments were stored in appropriate storage medium for 24 hours and the remaining tooth structure along with blocks were stored again in distilled water until re-attachment. Each fragment was rinsed under distilled water for 5 seconds to remove any remaining storage media. The fragments were re-attached, thermocycled and subjected to fracture strength test using universal testing machine at 0.6 mm min⁻¹. **Results:** The mean value for fracture resistance of teeth samples stored in dry medium was found to be 24.3 N, for teeth samples stored in saline medium was found to be 95.4 N, for teeth samples stored in egg white medium was found to be 50.8 N, for teeth samples stored in milk medium was found to be 53.3 N and for teeth samples stored in coconut water medium was found to be 74 N. **Conclusion:** Within the limitations of this study, it was concluded that storage of tooth fragments in saline demonstrated the highest fracture resistance, followed in the order by coconut water (a new storage medium used in this study), milk and egg white, which showed significantly higher fracture resistance in comparison to the samples kept dry.

KEYWORDS

Fragment Reattachment,
Fracture Resistance,
Crown Fracture.

INTRODUCTION

Attractive appearance is very important in modern society, and mouth is one of the first features people look at, with a pleasant smile being the key. An esthetic smile plays a big role in a person's self-es-

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teem and has become a respectable concept in dentistry. Likewise, A trauma involving anterior teeth fracture, may be the most traumatic incident and demand immediate attention for a young patient.

Crown fractures has been classified by **Andreasen JO** as enamel infractions, uncomplicated crown fractures (enamel-dentin fractures with no pulp involvement), and complicated crown fractures (enamel-dentin fractures with pulp involvement ⁽¹⁾).

The incisors are most often affected, with central incisors 80%, lateral incisors 16% and it has been reported that it is mainly due to the front position of the maxilla and tooth protrusion ⁽²⁻⁴⁾.

Various techniques have been proposed for the restoration of broken crowns including orthodontic bands, stainless steel crowns, resin restorations held with pins, anterior composite restoration, porcelain laminate and jacket crown, each showing a varying degree of success ⁽⁵⁻⁹⁾. Subsequently, a method to maintain the natural characteristics of wear, shape, surface texture and color should be employed whenever possible. Previous studies reported that re-attachment was possible with no additional preparation of the fracture site ⁽²⁻⁴⁾. Also, the advantage of reattaching the incisal edge is that it does not prevent any future treatment, therefore, in cases where the splint is available, it constitutes a viable option for initial treatment ^(2,8,10,11).

Type of storage media is one of the factors that have an important role in the success of fragment re-attachment as a treatment following trauma. If the fragment is outside the mouth, it is also essential to keep appropriate hydration. This is because hydration maintains the initial esthetic appearance of the tooth ^(5,12,14).

Finite studies of environmental types, such as saliva, water, milk or natural saline solution, have been reported to store broken parts of the teeth before referring to the dentist. It is also not uncommon to face a broken part that has been transferred to the clinic in different storage environments ⁽¹⁵⁻¹⁹⁾.

This research therefore focused on evaluating the impact of different storage media on the fracture resistance of re-attached tooth fragments in a comparative way.

MATERIAL AND METHODS

For the current research, 50 permanent maxillary central and lateral incisors, extracted for periodontal purposes, were chosen. All teeth were free from cracks, caries or any other structural defect. Until crown sectioning, the samples were placed in distilled water. All teeth were placed on acrylic blocks and measured with a digital caliper on the labial side from the cervical to the incisal border. The measurement was then divided by three, after which the tooth was marked at 1/3 of the incisal edge. Teeth sliced on the labeled line perpendicular to the long axis, with a diamond disk and micromotor (NSK, Japan).

In the present study, teeth samples were stored in the various mediums namely saline, egg white, milk, coconut water or were kept dry and accordingly assigned into the following groups namely:

Group A-Dry

Group B- Saline

Group C- Egg white

Group D- Pasteurized bovine milk

Group E- Coconut water

Random division of the chosen teeth into 5 groups of 10 each was performed. The fractured fragments were stored immediately following the fracturing for 24 hours on a proper storage medium and the remainder of the tooth structure together with the acrylic block was stored in distilled water again until reattachment. To remove any remaining storage media, each fragment was rinsed under distilled water for 5 seconds.

For 15 seconds, 35% phosphoric acid etchant (3 M ESPE) was added to the tooth fragment and rinsed for 10 seconds, followed by 5 seconds of air drying. Two consecutive coats of bonding agent (Adper single bond 2, 3 M ESPE) were placed.

Air syringe was used to dry the surfaces for 5 secs to enable solvent evaporation and light cured (Litex 680 A, Dentamerica-18688 E, USA) for 20 seconds in the broken fragment and 20 seconds in the tooth rest. In the fractured surface of the fragment and tooth remains the flowable composite (Filtek flowable Z 350, 3 M ESPE) was applied.

The fragment was then placed back to the remaining tooth with sticky wax to carry the fractured part.

After positioning, light curing was proceeded in 4 stages:

- a) 20 secs for the mesio-buccal surface
- b) 20 secs for the disto- buccal surface
- c) 20 secs for the mesio-lingual surface
- d) 20 secs for the disto-lingual surface

Specimens were placed in distilled water again after re-attachment. All specimens were retained in 37°C distilled water and subjected to 100 cycles of 5° C and 55° C thermocycling, with a 15-second dwell time and a 10-second transfer time. The samples were re-stocked in distilled water until testing after thermocycling. All specimens were then subjected to a fracture strength test using a universal test device (UNITEK 94100, Fuel Instruments and Engineers Pvt Ltd, India) at a rate of 0.6 mm min⁻¹.

The direction of application force was 90 degrees with regard to the mouth surface, the chisel width was incisal to the fitting line, and Newton's forces are registered to divide every tooth. The gathered information's were then tabulated and analyzed statistically. Statistical analyzes have been conducted using the Social Sciences version 16 statistical package⁽²¹⁻²³⁾.

RESULTS

Fragments stored in Group B (saline) gave the highest mean fracture resistance values among the tested groups which was found to be **(95.4±10.741)**, followed by Group E (coconut water) which was found to be **(74 N ± 4.269 N)**, Group D (milk) which was **(53.3 N ±6.482 N)**, Group C (egg white) which was found to be **(50.8 N ±4.417 N)**. and the least fracture resistance values was found to be among the samples in Group A (dry) which was **(24.3 N ±2.83 N)**. As per statistical results by One-way ANOVAanalysis, P value was found to be significant among the five storage mediums. Hence this shows that, there was statistically significant difference in fracture resistance among the teeth samples stored in the five storage mediums. Group B (Saline) demonstrated the highest fracture resistance, followed by Group E (Coconut water), Group D (Milk), Group C (Egg white) and Group A (Dry).

Table (1): Showing the comparison of mean and standard deviation (SD) for fracture resistance of teeth samples among the five groups by one- way ANOVAanalysis.

Groups	N	Mean (Newton)	SD	F value	P value
Dry	10	24.3	2.83	175.5	0.00 (S)*
Saline	10	95.4	10.741		
Egg White	10	50.8	4.417		
Milk	10	53.3	6.482		
Coconut Water	10	74	4.269		

*: Significant (S) at $P \leq 0.05$

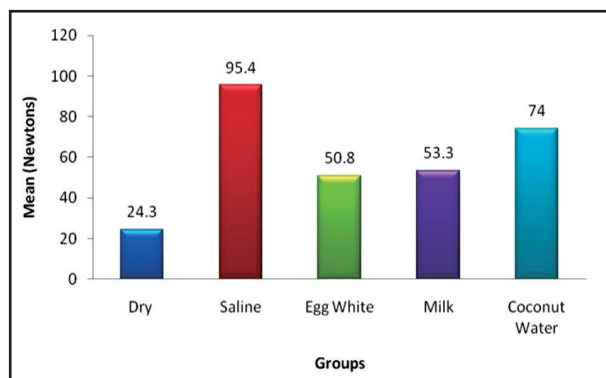


Figure (1): A bar chart showing comparison of mean fracture resistance for teeth samples among the five storage medium.

Table (2): Comparison of mean difference in the fracture resistance of teeth samples stored in various storage medium by independent student *t* test.

GROUPS	INTER-GROUPS	MEAN DIFFERENCE (NEWTONS)	P VALUE
SALINE	DRY	71.10	0.000 (S)
	EGG WHITE	44.6	0.000 (S)
	MILK	42.10	0.000 (S)
	COCONUT WATER	21.40	0.000 (S)
DRY	EGG WHITE	26.50	0.000 (S)
	MILK	29.00	0.000 (S)
	COCONUT WATER	49.70	0.000 (S)
EGG WHITE	MILK	2.50	0.904 (NS)*
	COCONUT WATER	23.20	0.000 (S)
COCONUT WATER	MILK	20.70	0.000 (S)

Significance level $p \leq 0.05$, *S = significant, NS=non-significant

DISCUSSION

Human maxillary incisors were used in this study, because the prevalence surveys disclosed the extent to which maxillary central incisors were involved in uncomplicated anterior teeth fractures followed by maxillary lateral incisors^(3,4). The success of re-attachment will depend on how dehydrated the tooth fragment is, as the longer it remains dehydrated, the lesser will be the fracture strength⁽⁸⁾. Whenever the fracture fragment is available, reattachment should be the first choice of treatment⁽¹¹⁾.

Previous studies have suggested that saline, milk and egg white are effective storage media in fragment re-attachment^(15,17,18). Hence in the present study saline, milk and egg white was used.

Research conducted on quantitative analysis of coconut water as storage medium indicated that coconut water could be a better alternative to Hank's balanced salt solution or milk for use as a fresh storage medium for avulsed teeth⁽¹⁶⁾. Distilled water has been used as a universal storage medium in this research because it has a neutral pH and contains no contaminants⁽¹⁷⁾.

There has been a paucity of studies regarding the use of different materials and storage media for re-attachment procedure. Thus, the aim of the study was to compare the effect of various storage media on the bond strength of the re-attached tooth fragment. In this study, the incisal edge of specimens was sectioned using low speed diamond disk. With the cut, a smear layer is produced that is otherwise not found on a fractured surface. However, a sectioned fragment creates a standardized and repeatable condition which is indispensable to an in vitro research⁽⁶⁾.

In this study, dentin conditioning was done with Adper Single bond (5th Generation) which has been revealed to have better bond strengths as compared to Scotch bond multipurpose (4th generation) and Prompt-LPop (6th generation)⁽⁷⁾. Filtek Z – 350, 3M ESPE was used in the re-attachment and the fit was

re-verified. Therefore, due to its thixotropic quality, penetration and relative fluidity, the flowable resin composite was chosen ⁽¹³⁾. The specimens were mounted in a universal testing machine.

To apply force, a 1 mm wide chisel-shaped device was used, as smaller the contact interface, the greater is the bond strength values ⁽²²⁾. The attached device was at 90° to the labial surface of the tooth, adjacent to the re-attachment line⁽¹¹⁾. The specimens were loaded at a cross-head speed of 0.6 mm min⁻¹ as suggested, that a cross-head speed of 0.5mm min⁻¹ or 0.75mm min⁻¹ may be capable of adequately measuring the bond strength⁽¹⁷⁾. And in Newton's the force needed to fracture each tooth was recorded (N). Each test specimen was held securely in a stainless steel jig in order to avoid any change in position at the time of testing block ^(21,23).

This result was in accordance to the previous study where the fragments stored in saline, milk and kept dry were compared and concluded that the hydrated medium's fracture resistance was greater than those kept dry ^(17,18). This study was also consistent with the study conducted to test the influence of storage media and fragment duration in the media on the bond strength of the tooth fragment reattached, the results of which showed that the strength of the hydrated and rehydrated bonded fragments was greater than that of the dehydrated fragments. The correct dentin fragment hydration affects the strength of the final restoration ⁽¹²⁾. Crown dried fragments for more than 1 hour had lesser bond strength when attached to the remaining tooth structure compared to the power of non-dried bonded fragments ⁽²²⁾. In this study, the mean fracture resistance of the samples stored in saline (95.4 N $\pm$ 10.741 N) and coconut water (74 N $\pm$ 4.269 N) was higher compared with those stored in milk (53.3 N $\pm$ 6.482 N). This may be due to the quantity of milk water content that is less than saline and coconut water. Collagen fibers hydration could have been better accomplished resulting in stronger resistance to fracture ⁽¹⁷⁾.

In the present study, the mean fracture resistance of milk (53.3 N $\pm$ 6.482 N) was slightly higher than the mean fracture resistance of egg white (50.8 N $\pm$ 4.417 N). The results of this study was in contradiction to the study conducted in which teeth stored in egg white showed higher bond strength compared to milk ⁽¹⁸⁾. The fracture resistance of the samples stored in milk (53.3 N $\pm$ 6.482 N) and egg white (50.8 N $\pm$ 4.417 N) showed statistically significant higher values compared to the samples in dry medium (24.3 N $\pm$ 2.83 N), But between them there was no significant difference ($P > 0.05$). This may be due to the fact that by permeating the surface, milk components such as calcium and phosphate can harden and stabilize both demineralized and healthy dentin. This is likely the reason why in the milk, which was rich in calcium and phosphates, there was an increase in bond strength. The surface topography of dentin and its dissolution during acid etching may also be affected by calcium and phosphate sedimentation, which may also explain improves bond strength within a milk group ⁽¹⁵⁾.

In this study, fragments of the tooth were kept for 24 hours in the medium. Even samples stored in milk would have obtained the same fraction resistance as stored in saline if their storage time was more than 48 hours ⁽¹⁶⁾.

The broken portion that was held in a dry atmosphere prior to reattachment showed the lowest fracture strength (**24.3 N $\pm$ 2.83 N**) and this was consistent with the research in which there is drying and rewetting anterior crown fragments prior to bonding which indicated that intact sound dentin, when stored in a dry environment for 24 hours, only maintains about 25 percent of its complete moisture content. The partial loss of dentin moisture and its shrinking result in reduced contact with dentin on the composite surface ⁽²²⁾. Moreover, over-acid etching due to loss of moisture in the dentine of the fractured part may happen and may have unfavorable impacts on the bonding status ⁽¹⁷⁾.

However, with the ongoing rise in dental health, more conservative dental therapy, and restrictions on access to human components, sound human incisors for research have been hard to obtain. It was also hard for the research to obtain non-carious human sound incisors of similar size. In addition, obtaining non-carious human sound incisors from the same age group of patients to eliminate the bias was complex.

The fitting between the tooth and the fragment in this research was not ideal and even posed a gap sometimes. Consequently, the findings acquired in this research may be a restriction on what clinically could be accomplished using these techniques. In the scenario of re-attachment, many researchers maintain the need to use extra preparations to increase the retention of the re-attached fragment, while others rely on improving consolidated dental bonding methods that give strength equivalent to that provided by the enamel. Preparation-less incisal edge re-attachment with a modern adhesive approach is compelling, because most incisal fractures occur with minimal or no net loss of tooth structure. Ideally, in order to reduce manipulative trauma to the tooth and chair, it would seem that the restorative operation should involve minimal tooth preparation ⁽⁹⁾.

The loss of tooth structure during sectioning with diamond disk, the speed at which repeat trauma actually occurs in vivo, and the inability to adequately reproduce actual intraoral forces in a laboratory simulation are notable limitations to the external validity of the present findings. Clinical trials should be conducted to confirm the hypothesis presented by laboratory.

CONCLUSION

Hence in the light of the present study and within its parametric limits, it was found that the storage in saline of tooth fragments showed the greatest resistance to fracture and was suggested as the most efficient storage medium for fragment reattachment.

This was followed in the order by coconut water (a new storage medium used in this study), milk and egg white, which showed significantly higher fracture resistance in comparison to the samples kept dry. Also the result of the present study, indicate the significance of the role of the broken fragment hydration as the force needed to fracture the restored teeth is influenced by the environment in which the broken portion is held before bonding.

A unified protocol for managing the traumatized tooth (tooth avulsion and crown fractures) can assist to maintain the protocols as straightforward as possible, which can ultimately lead to easier and faster data dissemination. Further in-vitro investigation must be extrapolated on more number of samples with these storage mediums to confirm the validity of these recommendations.

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