

# Comparative Evaluation of the Effect of Various Storage Media on the Fracture Resistance of the Reattached Tooth Fragment: An Observational Study

Sathyajith Naik<sup>1</sup>, Jean N Murry<sup>2</sup>, Shivangi Sharma<sup>3</sup>, Pallavi Vashisth<sup>4</sup>, Puru Abbey<sup>5</sup>, Vaishnavi Singh<sup>6</sup>

## ABSTRACT

**Background:** Traumatic injuries are the most disruptive and distressing emergencies and pose a challenge for dental professionals because many different treatment protocols are currently available. They also affect the social and psychological well-being of children. A multidisciplinary approach is involved in the management of traumatic dental injuries to maintain function and esthetics. The aim of this study was to evaluate the fracture resistance of a reattached tooth fragment kept in four storage media, namely Hank's balanced salt solution (HBSS), Tooth Mousse, ginger honey, and probiotic yogurt, for 1, 6, and 24 hours.

**Materials and methods:** The study consisted of 84 intact maxillary central incisors. All the teeth were stored in distilled water until used and were embedded in acrylic molds, 1 mm below the cervical end. The teeth were then sectioned to simulate an uncomplicated crown fracture (Ellis class II fracture), and all the teeth were randomly divided into four groups of 21 each. The teeth were then reattached using Tetric N Universal bonding agent and Tetric N Flow flowable composite. Fracture resistance was assessed using a universal testing machine. Statistical analysis was done using a one-way analysis of variance (ANOVA) test and a *post hoc* Tukey test.

**Results:** The results showed that fracture resistance was highest in the Tooth Mousse group at 6 and 24 hours. Ginger honey exhibited the highest fracture resistance at 1 hour, whereas the probiotic yogurt group showed the lowest values.

**Conclusion:** It was concluded that Tooth Mousse was the best storage medium for storing tooth fragments. Ginger honey can also be used as an alternative storage medium as it is easily available and inexpensive.

**Keywords:** Fracture resistance, Storage media, Trauma.

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## INTRODUCTION

A traumatic fracture of an anterior tooth is the most common problem among different age-groups and genders, which impedes the esthetics and psychology of the individual.<sup>1,2</sup> Of all the trauma affecting the dental hard tissues, the percentage of coronal fractures of permanent incisors is 18–22%; of these, maxillary incisors account for 96%.<sup>3</sup> Injuries may be caused by falls, contact sports, automobile accidents, and the striking of foreign bodies.<sup>4,5</sup>

Management of coronal tooth fractures is influenced by several factors, such as violation of biological width, fracture pattern, involvement of the pulp, esthetics, occlusion, restorability of the tooth, and the presence or absence of the fractured fragment.<sup>6</sup> Hence, the preservation of dental tissue, reestablishment of natural esthetics, and maintenance of the integrity of the dental arch must be the primary goals of treatment.<sup>7</sup>

In the pre-adhesive era, traumatized fractured teeth were restored with resin crowns, ceramic crowns, steel crowns, orthodontic bands, and pin-retained inlays. These restorative techniques, however, were not an immediate solution to an esthetic emergency, did not promote adequate long-term esthetics, and required significant tooth reduction during preparation. Other treatment options include the use of composite restorations with or without pins, laminate veneers, porcelain onlays, and resin-based bridges.<sup>8</sup>

The development in adhesive dentistry has allowed for the reattachment of the patient's own fragment to restore the

<sup>1–4,6</sup>Department of Pedodontics and Preventive Dentistry, Institute of Dental Sciences, Bareilly, Uttar Pradesh, India

<sup>5</sup>Department of Periodontology and Implantology, Institute of Dental Sciences, Bareilly, Uttar Pradesh, India

**Corresponding Author:** Sathyajith Naik, Department of Pedodontics and Preventive Dentistry, Institute of Dental Sciences, Bareilly, Uttar Pradesh, India, Phone: +91 9845398403, e-mail: sathyajithnaik@gmail.com

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fractured tooth. Reattachment of tooth fragments became a more common procedure with the acceptance of flowable composite due to the excellent retention obtained with the fluid resin on the etched enamel. The success of fragment reattachment depends on several factors, such as the time lapse between trauma and restoration and the storage of the fragment before reattachment. The patient's understanding of the significance of fragment storage and the interval between trauma and restoration are both critical factors in the success of the fragment

reattachment approach. After some time, the fragmented portion may lose its moisture.<sup>9</sup> As dentin moisture is necessary for attaining a high bond strength between composite resins and dentin, the length of time taken for the restoration process can impair the bond strength of these restorations.<sup>10</sup> Storing the fragment in storage media increases bond strength and fracture resistance by preventing dehydration and dimensional changes.<sup>5,11,12</sup> It also improves esthetics.<sup>13</sup>

There are many storage media available, such as milk, saline, coconut water, egg white, contact lens solution, Hank's balanced salt solution (HBSS), distilled water, green tea, oral rehydration solution (ORS), and bovine milk. Among these, HBSS is the gold standard storage medium, as it demonstrates a good amount of hydration and stability of collagen structure.<sup>14</sup> However, its use is restricted to laboratory environments and is not available at an accident site, which makes it impracticable as a storage medium. Moreover, its biggest disadvantage is that it is quite expensive.

Many newer storage media have been proposed and/or tested for fractured teeth that are readily and affordably accessible, such as Tooth Mousse, ginger and honey, and probiotic yogurt. These recent storage media have several advantageous qualities.

Hank's balanced salt solution has been widely employed as a reference solution in studies on dental avulsion, as it has the ideal osmolality and pH for preserving the vitality of cells.<sup>15</sup>

*Zingiber officinale* (or ginger, ginger rhizome) is one of the most traditionally used herbs in India. This natural food source possesses antimicrobial and antifungal activities. Another traditionally used substance in herbal medicine in India is honey. Its pH level, which is around 3.9, has a bacteriostatic impact on infections.

Tooth Mousse contains a high percentage of essential elements like calcium and phosphate, which enhance the bond strength of the reattached fragment.

Probiotic yogurt is a good source of calcium and phosphorus and has a greater protein level than milk. The advantageous ionic form of calcium is due to yogurt's lower pH compared to milk. Furthermore, due to the proteolytic activity of the microbes found in yogurt, the concentration of casein phosphopeptide is greater than in milk.<sup>16</sup> The ionic form of calcium keeps the calcium in the tooth structure and the fluids around it in equilibrium.<sup>17</sup>

Since there is a lack of literature available on these newer storage media, the present study was carried out with the aim of evaluating the effect of various newer storage media compared to the gold standard HBSS on the fracture resistance of reattached incisor tooth fragments at different time intervals.

## MATERIALS AND METHODS

The study samples comprised 84 intact human maxillary central incisors, which were extracted due to periodontal reasons (Fig. 1). All the teeth were cleaned of debris and soft tissue remnants, disinfected using 0.2% thymol, and stored in distilled water until the experimentation.

To simulate the periodontium, the root surfaces of the selected teeth were dipped into melted wax to a depth of 2 mm below the cemento-enamel junction to produce a thin layer and were vertically embedded in self-curing acrylic polyvinyl cylinders (to represent the alveolar bone) (Fig. 2). The samples were then divided into four groups of 21 each and further divided into three subgroups, with seven teeth in each subgroup.

Group A (21 teeth): The fractured fragments were stored in HBSS.

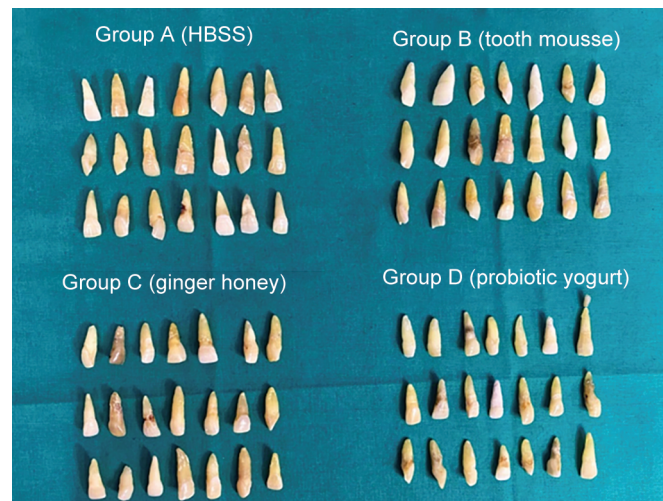


Fig. 1: Sample teeth

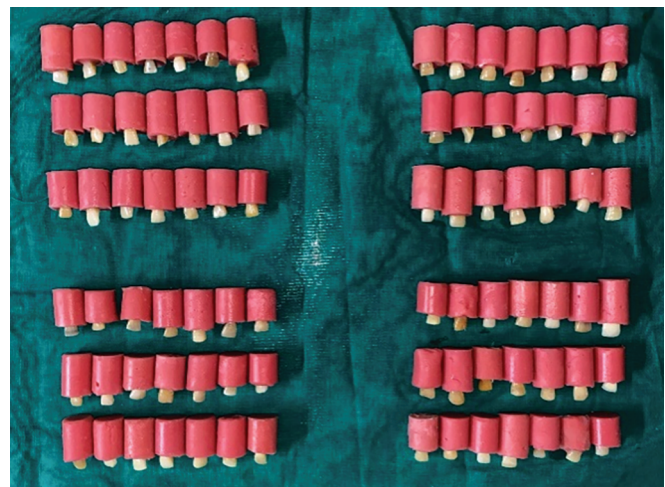


Fig. 2: Samples embedded in acrylic blocks

• Subgroups A1 (7 teeth), A2 (7 teeth), and A3 (7 teeth) had fragments stored for 1, 6, and 24 hours, respectively.

Group B (21 teeth): The fractured fragments were stored in Tooth Mousse.

• Subgroups B1 (7 teeth), B2 (7 teeth), and B3 (7 teeth) had fragments stored for 1, 6, and 24 hours, respectively.

Group C (21 teeth): The fractured fragments were stored in ginger and honey.

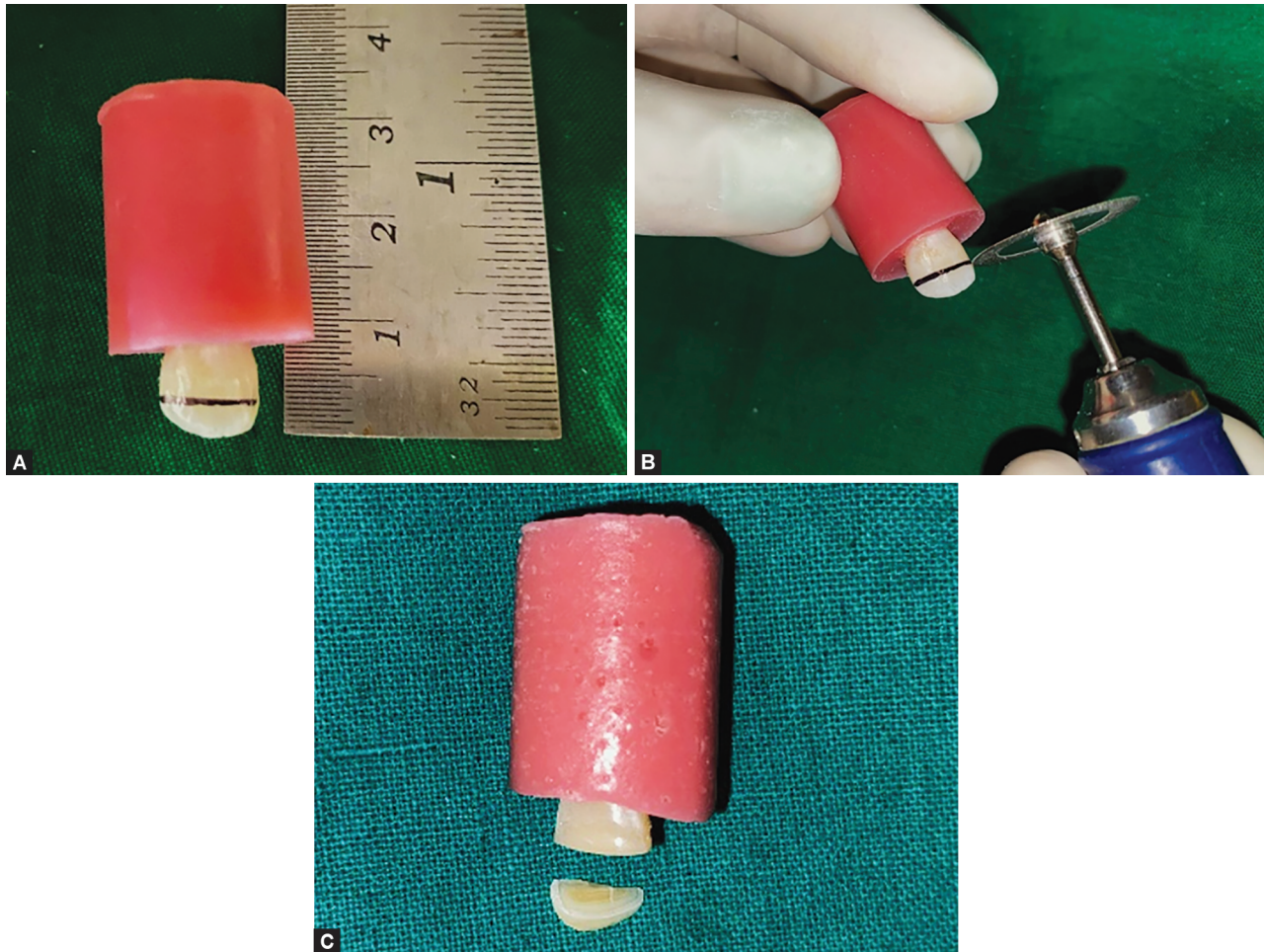
• Subgroups C1 (7 teeth), C2 (7 teeth), and C3 (7 teeth) had fragments stored for 1, 6, and 24 hours, respectively.

Group D (21 teeth): The fractured fragments were stored in probiotic yogurt.

• Subgroups D1 (7 teeth), D2 (7 teeth), and D3 (7 teeth) had fragments stored for 1, 6, and 24 hours, respectively.

Intentional fractures were created by dividing the tooth 3 mm from the incisal edge using a low-speed diamond disk perpendicular to the long axis (Fig. 3) with saline as a coolant. The fractured fragments were then stored separately with the required storage media (HBSS, Tooth Mousse, ginger and honey, and probiotic yogurt) for the time intervals of 1, 6, and 24 hours (Fig. 4). Fragments





Figs 3A to C: Tooth sectioned 3 mm from the incisal edge using diamond disk

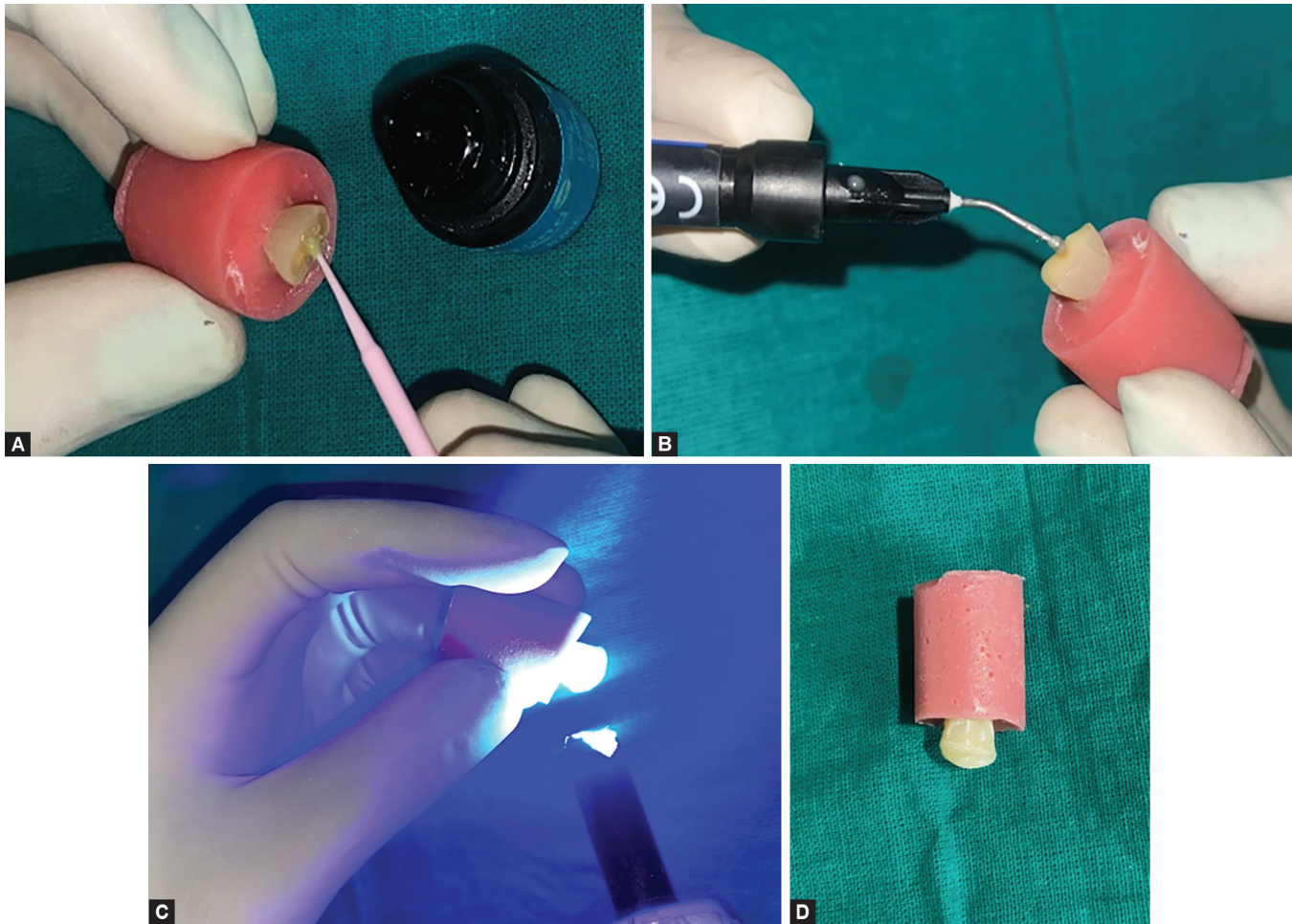


Fig. 4: Tooth fragments of the specimens in respective storage media for 1, 6, and 24 hours

were reattached using a simple reattachment technique without any additional preparation. Before reattachment, the fragments were removed from their respective storage media, rinsed with distilled water for 10 seconds, and dried with a paper towel. Both surfaces of the tooth remnant and the tooth fragment were etched

using 37% phosphoric acid for 15 seconds, then rinsed with distilled water for 10 seconds, followed by air drying for 5 seconds. Two consecutive coats of 3M ESPE Adper Single Bond Plus bonding agent were applied to the etched enamel and dentin for 15 seconds with gentle agitation using a fully saturated applicator. The bonding agent was air-thinned for 5 seconds to evaporate solvents, followed by light curing for 10 seconds. The fragments were held with a gutta-percha stick and approximated with their respective apical portions using a flowable composite by pressing both parts together. Excess composite material was removed, and the composite was cured for 10 seconds on the mesial, distal, labial, and lingual sides. The tooth surface was polished using composite finishing burs (Fig. 5). Reattached samples were kept in distilled water until further experimentation (Fig. 6). In a universal testing machine, all samples were subjected to a compressive load with a crosshead speed of 1 mm/minute (Fig. 7). The force was applied to the labial surface at the incisal third of each tooth, perpendicular to the long axis of the tooth. The peak load to fracture was recorded in Newtons (N) for each specimen. All data were then subjected to statistical analysis. Groups were compared using one-factor analysis of variance (ANOVA), and the significance of mean differences between groups was determined by Tukey's honestly significant difference (HSD) *post hoc* test. Analysis was performed using Statistical Package for the Social Sciences (SPSS) software (Windows version 22.0).





Figs 5A to D: Fragment reattachment with respective tooth specimens using flowable composite



Fig. 6: Reattached tooth sample stored in distilled water



Fig. 7: Sample loaded under universal testing machine

## RESULTS

The fracture resistance of the reattached tooth fragment kept in various storage media at 1, 6, and 24 hours was observed in this study.

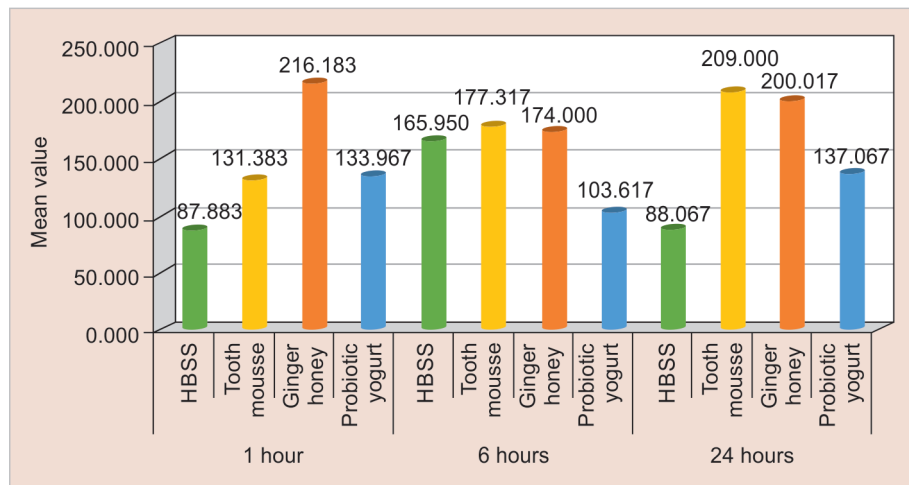
The fracture resistance of the four groups (group A, group B, group C, group D) is summarized in Table 1 and depicted in Figure 8. The mean fracture resistance was highest in

group B, followed by group C, group D, and group A, with group A showing the least resistance (group A < group D < group C < group B). It was observed that a minimum fracture resistance of  $87.883 \pm 21.119$  N was required for fragments stored in HBSS at 1 hour, while the highest fracture resistance of  $216.183 \pm 60.467$  N was observed for fragments stored in ginger honey at 1 hour (Table 2).

**Table 1:** Descriptive analysis of fracture resistance

Storage duration	Group	N	Mean	±SD	SEM	95% CI		Range		p-value
						Lower bound	Upper bound	Minimum	Maximum	
1 hour	HBSS	6	87.883	21.119	8.622	65.721	110.046	52.10	111.30	<0.001**
	Tooth Mousse	6	131.383	17.554	7.167	112.961	149.806	102.50	155.00	
	Ginger honey	6	216.183	60.467	24.686	152.727	279.640	167.30	332.00	
	Probiotic yogurt	6	133.967	16.366	6.681	116.792	151.142	118.00	156.00	
6 hours	HBSS	6	165.950	13.864	5.660	151.401	180.499	144.60	182.30	<0.001**
	Tooth Mousse	6	177.317	28.107	11.475	147.820	206.813	156.90	230.00	
	Ginger honey	6	174.000	22.715	9.273	150.162	197.838	153.00	216.00	
	Probiotic yogurt	6	103.617	30.733	12.547	71.364	135.869	59.20	140.70	
24 hours	HBSS	6	88.067	36.263	14.804	50.011	126.123	45.70	138.80	<0.001**
	Tooth Mousse	6	209.000	50.849	20.759	155.637	262.363	117.00	258.00	
	Ginger honey	6	200.017	20.290	8.284	178.723	221.310	176.30	233.50	
	Probiotic yogurt	6	137.067	18.715	7.640	117.427	156.706	116.00	165.00	

CI, confidence interval; SD, standard deviation; SEM, standard error of mean; one-way ANOVA; \*\* $p < 0.001$ —highly significant

**Fig. 8:** The graphical representation of analysis of fracture resistance

Comparing the mean fracture resistance of the four groups, ANOVA showed significantly different fracture resistance among the groups ( $p < 0.001$ ) (Table 1). Table 2 shows multiple comparisons using the least significant difference (LSD) test at 1 hour. The results indicate no statistically significant difference between HBSS and Tooth Mousse ( $p = 0.157$ ), probiotic yogurt and HBSS ( $p = 0.124$ ), and Tooth Mousse and probiotic yogurt ( $p = 0.999$ ). However, a highly significant difference was observed between HBSS and ginger honey ( $p < 0.001$ ).

Table 3 shows multiple comparisons using the LSD test at 6 hours. The results indicate no significant difference between HBSS and Tooth Mousse ( $p = 0.855$ ), HBSS and ginger honey ( $p = 0.942$ ), and Tooth Mousse and ginger honey ( $p = 0.995$ ). However, a highly statistically significant difference was observed between Tooth Mousse and probiotic yogurt ( $p < 0.001$ ) and ginger honey and probiotic yogurt ( $p < 0.001$ ).

Table 4 shows multiple comparisons using the LSD test at 24 hours. The results indicate no statistically significant difference between HBSS and Tooth Mousse ( $p = 0.000$ ), HBSS and ginger honey ( $p = 0.000$ ), and HBSS and probiotic yogurt ( $p = 0.093$ ). There was no significant difference between Tooth Mousse and ginger honey ( $p = 0.968$ ), but a statistically significant difference was

observed between Tooth Mousse and probiotic yogurt ( $p = 0.008$ ) and between ginger honey and probiotic yogurt ( $p = 0.022$ ).

## DISCUSSION

The major objective of restorative dentistry is to restore teeth in a way that conserves healthy dental tissues, and ensures esthetics, function, and durability. In many clinical situations, the best option for fractured anterior teeth is the reattachment of the tooth fragment, as it is a superior method for restoring the natural shape, contour, surface texture, occlusal alignment, and color of the teeth.<sup>18</sup>

Though there are several alternatives for restoring fractured teeth, reattachment is considered one of the best methods to restore function and esthetics when the fragment is available. According to Shirani et al.,<sup>19</sup> reattachment with hydration or without dehydrating the surfaces using a storage medium, and without any additional preparation, restores approximately 50% of the fracture strength of the original tooth.

Fragment reattachment is a simple, easy, minimally invasive, fast, economical, and effective procedure, but the prognosis may not always be ideal. Fragment de-bonding can occur due to repeated trauma, nonphysiological use of the tooth, or horizontal



**Table 2:** Multiple comparison at 1 hour using *post hoc* Tukey HSD

Storage media		Mean difference	Standard error	p-value	95% confidence interval	
					Lower bound	Upper bound
HBSS	Tooth Mousse	-43.500	19.745	0.157	-98.765	11.765
	Ginger honey	-128.300	19.745	<0.001**	-183.565	-73.035
	Probiotic yogurt	-46.083	19.745	0.124	-101.348	9.181
Tooth Mousse	Ginger honey	-84.800	19.745	0.002*	-140.065	-29.535
	Probiotic yogurt	-2.583	19.745	0.999	-57.848	52.681
Ginger honey	Probiotic yogurt	82.217	19.745	0.002*	26.952	137.481

\* $p < 0.05$ —significant; \*\* $p < 0.001$ —highly significant

**Table 3:** Multiple comparison at 6 hours using *post hoc* Tukey HSD

Storage media		Mean difference	Standard error	p-value	95% confidence interval	
					Lower bound	Upper bound
HBSS	Tooth Mousse	-11.367	14.267	0.855	-51.300	28.567
	Ginger honey	-8.050	14.267	0.942	-47.984	31.884
	Probiotic yogurt	62.333	14.267	0.002*	22.400	102.267
Tooth Mousse	Ginger honey	3.317	14.267	0.995	-36.617	43.250
	Probiotic yogurt	73.700	14.267	<0.001**	33.766	113.634
Ginger honey	Probiotic yogurt	70.383	14.267	<0.001**	30.450	110.317

\* $p < 0.05$ —significant; \*\* $p < 0.001$ —highly significant

**Table 4:** Multiple comparison at 24 hours using *post hoc* Tukey HSD

Storage media		Mean difference	Standard error	p-value	95% confidence interval	
					Lower bound	Upper bound
HBSS	Tooth Mousse	-120.933	19.712	0.000	-176.105	-65.762
	Ginger honey	-111.950	19.712	0.000	-167.121	-56.779
	Probiotic yogurt	-49.000	19.712	0.093	-104.171	6.171
Tooth Mousse	Ginger honey	8.983	19.712	0.968	-46.188	64.155
	Probiotic yogurt	71.933	19.712	0.008*	16.762	127.105
Ginger honey	Probiotic yogurt	62.950	19.712	0.022*	7.779	118.121

\* $p < 0.05$ —significant; \*\* $p < 0.001$ —highly significant

pulling of the tooth.<sup>20</sup> The risk of debonding is higher for children, as they are more exposed to traumatic situations due to increased physical activity.

A plethora of studies have reported that the fracture resistance of reattached tooth fragments can be improved with the use of new adhesive agents, bonding materials, and tooth preparation techniques.<sup>2,21–26</sup> Apart from all these improvements, hydration of the fragment also plays an important role in enhancing fracture resistance.<sup>27</sup> The storage medium acts as a key determinant since hydration helps maintain vitality, esthetic appearance, and improves bond strength. This is based on the ability of the storage media to retain the collagen framework and keep intertubular porosity open for subsequent infiltration of monomers.<sup>12</sup> Bond strength may increase as the resin penetrates into the intact dentinal tubules.

The assessment of the reattached fragment's fracture resistance is important because repeated trauma is a main cause of reattachment failures. Keeping the fragment in a variety of storage media can increase its resistance to fracture.

The type of storage media used to store fragments after trauma significantly influences the success of fragment reattachment. If the coronal fragment is allowed to dry out before reattachment, it will desiccate, and *in vitro* testing has revealed a lower bond strength

for such reattached fragments. Therefore, the fragment needs to be kept moist between retrieval and reattachment.

In this study, the teeth were sectioned using a low-speed diamond disk in a standardized manner. According to Prabhakar et al.,<sup>28</sup> it has been hypothesized that breaking a tooth *in vitro* will result in an uneven fracture line and subsequent fragments of varying sizes. Variations in the amount of material used for reattachment can produce inconsistent results.

Hank's balanced salt solution, the gold standard for storage media, was used in this study. The International Association of Dental Traumatology recommends HBSS as a storage medium because it is frequently used as a standard in research on dental avulsion cases. It has a pH and osmolality close to ideal, provides sufficient hydration, and preserves the stability of the collagen framework.<sup>29,30</sup>

The only disadvantage of HBSS is that it is expensive and not widely accessible. Therefore, this study was conducted to find more accessible and relatively affordable storage media for storing fractured tooth fragments.

Tooth Mousse, a water-based, sugar-free topical cream containing Recaladent, a milk protein derived from casein, was used in this study. According to a study by Jalannavar and

Tavargeri,<sup>31</sup> reattached teeth preserved in Tooth Mousse exhibited the maximum fracture resistance and demonstrated superior performance as a storage medium compared to other more recent storage media.

Ginger honey and probiotic yogurt were other storage media used in this study. The reason ginger honey and probiotic yogurt were chosen for this study was mostly that people have access to these media at home, whereas HBSS and Tooth Mousse might not be present where the incidence occurred.

The best storage medium, according to this study's findings, was Tooth Mousse, which had the highest fracture resistance at 6 and 24 hours in comparison with the other experimental groups. The mean fracture resistance of Tooth Mousse was  $177.317 \pm 28.107$  N at 6 hours (B2) and  $209 \pm 5.849$  N at 24 hours (B3); this can be attributed to the high concentration of vital nutrients like calcium and phosphate found in Tooth Mousse. These findings are in accordance with the study reported by Shirani et al.,<sup>12</sup> who demonstrated that milk components like calcium and phosphate can permeate the surface of both healthy and demineralized dentin to harden and stiffen it. This is likely the cause of the increased fracture resistance seen in the calcium and phosphate-rich groups.

Borges et al.<sup>32</sup> also reported that fragments treated with Tooth Mousse paste prior to the application of Adper SE Plus presented statistically improved bond strength compared with nontreated samples, as confirmed by the lower frequency of adhesive failures and the higher frequency of cohesive failures in composite. Adper SE Plus presents phosphoric acid esters that are capable of bonding chemically to hydroxyapatite. Thus, this adhesion potential could have been improved by increased calcium availability after dentin treatment with Tooth Mousse paste.

In this study, ginger honey was found to have greater fracture resistance at 1 hour and nearly identical results at 6 and 24 hours compared to Tooth Mousse. Due to its antibacterial qualities and high fluoride and calcium levels, ginger may have some remineralization capacity. Ginger is also rich in iron, magnesium, phosphorus, and potassium, according to Namir and Wesal<sup>33</sup> which may be another factor influencing its remineralization process and resulting improvement in bond strength. Celik et al.<sup>34</sup> in their investigation on the effectiveness of ginger honey in the remineralization process, found a high potential for remineralization and an increase in the surface microhardness of enamel. The anticaries considerable synergistic effect of antibacterial activity in terms of surface remineralization has also been demonstrated for the ginger and honey combination.<sup>35</sup>

Probiotic yogurt, a milk product, demonstrated decreased fracture resistance at 1, 6, and 24 hours in this study. This might be a result of low pH, which may affect the mechanical properties of the composite resin by accelerating biodegradation through a complex process that ultimately results in the breakdown of the polymer matrix.<sup>27</sup> This could result in the debonding of the restorative material, release of residual monomers, decrease in hardness, change in topography, increase in roughness, accumulation of biofilm, and more.<sup>36</sup> Additionally, this might be caused by the composition of these probiotics and how they interact with composite resins. However, manufacturers do not specify the exact ingredients in their probiotic fermented milks, making it challenging to discuss how they interact.

Hank's balanced salt solution, on the other hand, showed the least fracture resistance despite being the gold standard for storage media at 24 hours, which was  $88.067 \pm 36.263$  N when compared with Tooth Mousse, ginger honey, and probiotic yogurt.

This might be a result of being unstable when exposed to light for brief periods of time.<sup>31</sup> Additionally, HBSS should be used at 37 °C in a controlled incubator, which could account for this solution's lack of effectiveness when compared to alternative media in some research.<sup>37-40</sup>

The Tooth Mousse group in particular showed improved fracture resistance after 24 hours, which supported the findings of Borges et al.<sup>32</sup> and had a significant effect on bond strength.

## CONCLUSION

The outcomes of the studies mentioned above confirm the notion that storing the fragment in a medium before reattachment would be advantageous, particularly a calcium-rich medium that can further strengthen the bond of the reattached fragment. Also, ginger honey, despite having the second-highest mean in this study and being easily accessible at the trauma site, highlights the need to raise public awareness of the best ways to store such fragments inexpensively and with a better prognosis.

## Limitations

When the reattached teeth were fractured, the direction of load application simulated a clinical scenario in which a tooth that had been reattached using fragment reattachment encountered a second episode of trauma. The amount of load delivered using a universal testing machine at a crosshead speed of 1 mm/minute, which did not accurately represent a natural traumatic scenario, was one major drawback in this study.

## ORCID

Jean N Murry  <https://orcid.org/0000-0001-9483-0985>

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