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Comparative Evaluation of Fracture Resistance of Reattached Teeth Fragments Stored in Cornisol and Hanks Balanced Salt Solution: An In Vitro Study

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This study evaluates the fracture resistance of reattached tooth fragments stored in Cornisol, HBSS (Hanks Balanced Salt Solution), and dry conditions for 60 minutes and 24 hours using a Universal Testing Machine. Sixty freshly extracted human maxillary central incisors were divided into three groups: Dry storage (Group 1), HBSS (Group 2), and Cornisol (Group 3), with 20 teeth per group. Each group was further subdivided for storage durations of 60 minutes and 24 hours. Teeth fragments underwent simulated Elli's class II fractures, etching, bonding, and were approximated with flowable composite. Fracture resistance testing was conducted after thermocycling. Statistical analysis revealed that for the 60-minute period, Cornisol showed the highest fracture resistance, followed by HBSS and dry storage. For the 24-hour period, HBSS had the highest fracture resistance, with significant differences noted between HBSS and Cornisol compared to dry storage. Cornisol and HBSS are preferable storage media for fractured tooth fragments prior to reattachment, with Cornisol being optimal for shorter durations.

Keywords: Cornisol, Fracture resistance, Storage Media, Hanks Balanced Salt Solution, Viability

1. Introduction

Trauma to oral and maxillofacial structures constitutes 5% of total injuries, with coronal tooth fractures representing 18–22% of dental hard tissue fractures, predominantly affecting maxillary incisors [1,2]. Immediate management of these fractures is crucial to prevent further damage and address potential psychological impacts [3, 4].Various treatment options include composite resin restorations, reattachment of fragments, and various crowns [5]. Reattachment, first documented in 1964, preserves aesthetics and function, providing psychological comfort and reducing chair time for patients [6].

Key factors influencing reattachment success include fragment hydration and storage media. Studies show that rehydrated fragments exhibit greater bond strength than dehydrated ones, with Hank's Balanced Salt Solution (HBSS) emerging as the most effective medium for maintaining periodontal ligament cell viability [7, 8]. Other media, including milk and saline, also demonstrate varying effectiveness [9].Capp et al. demonstrated that the strength of hydrated and rehydrated bonded fragments surpassed that of dehydrated fragments [10]. Maintaining fragments in a moist environment helps prevent or minimize collagen fibre collapse in dentin, thereby enhancing bond strength [11]. This finding is consistent with Shirani et al., who observed that loss of dentin moisture and subsequent shrinkage reduce composite surface contact with dentin [12]. Overall, these studies emphasize the importance of moisture preservation in achieving optimal outcomes in tooth fragment reattachment procedures [13].

Dry storage reflects a common scenario where a tooth fragment may be exposed to air or left untreated before reattachment [14]. Dry storage has a negative impact on the fracture resistance of reattached tooth fragments [12]. Hence dry storage as a negative control allows the researchers to assess fracture strength with that of rehydrated reattached tooth fragments [11, 12].

Hank's Balanced Salt Solution (HBSS) is a sterile, physiologically balanced isotonic solution commonly employed in biomedical research to facilitate the growth of various cell types [15]. Notably, HBSS is non-toxic and biocompatible with periodontal ligament (PDL) cells [16]. It boasts a pH level balanced at 7.2 and an osmolality of 320 mOsm/kg, ensuring optimal conditions for cell viability and function and has a has a higher water content, which helps in maintaining hydration [12, 13].

Cornisol, a corneal cell preservation medium patented by Aurolab Pvt Ltd. in 2014, maintains corneal cell viability for up to 14 days [17,18].Its formulation includes chondroitin sulfate to stabilize cell membranes, recombinant human insulin to support metabolism, dextran for osmotic balance, and stabilized L-glutamine for cellular metabolism [19]. ATP precursors aid in ATP synthesis, while vitamins and trace elements promote cell growth and function. Gentamicin and streptomycin prevent bacterial contamination [20, 21].

Studies highlight the similarities between dental fibroblasts and corneal cells, including their common neural crest origin and similar proteoglycan secretion [18, 21]. Cornisol also contains HEPES, which effectively buffers pH fluctuations, ensuring a stable environment for cell preservation during storage [22].

The choice of adhesive technique—self-etch, total etch, or selective etch—affects bond strength and sensitivity [23]. Flowable composite resins are recommended for their superior adaptation and mechanical properties in fragment reattachment [24]. Despite existing studies on various media and techniques, no research has specifically evaluated the fracture resistance of reattached fragments stored in Cornisol. This study aims to assess and compare the fracture resistance of reattached tooth fragments stored in Cornisol, HBSS, and dry conditions

2. Materials and Methods:

Sample Size Calculation:

The sample size was determined using the formula:

$$n = \frac{2 (Z_\alpha + Z_\beta)^2 * S^2}{d^2}$$

Where Z_α =1.96 at 95% confidence level And Z_β =1.28 at 90% power S= combined standard deviation and d= Mean difference, d=34.72 s=24.67

With 95% confidence level and 90% power, sample size comes to be minimum of 10 in each group. A total of 60 permanent maxillary central incisors were included, sourced from periodontal extractions. Teeth with cracks, caries, or structural defects were excluded.

Methodology:

Preparation of Samples:

Teeth were cleaned with ultrasonic scaler tips, disinfected in 0.2% thymol, and stored in distilled water until intentional fracture. Samples were mounted in acrylic blocks. The cervico-incisal distance was measured, and the labial surface divided into three equal sections (incisal, middle, and cervical) [Figure 1 and Figure 2].

Figure 1: Armamentarium for the study

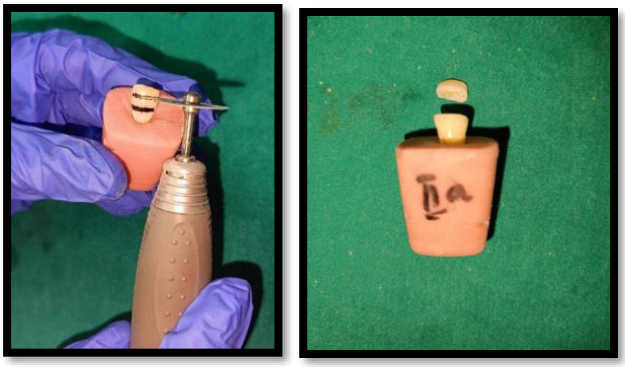

Figure 2: Sample sectioned using double sided diamond disk

Teeth were randomly divided into three groups of 20:

Group I: Dry storage

Group II: Hank's Balanced Salt Solution (HBSS) (Sigma-Aldrich)

Group III: Cornisol (Aurolab)

Each group was further subdivided into two time periods (60 minutes and 24 hours), resulting in six subgroups.

Fracture Simulation and Storage:

Incisal thirds were sectioned using a low-speed diamond disk to simulate a Class II fracture. Fragments were stored as follows:

Group IA: Dry storage for 60 minutes

Group IB: Dry storage for 24 hours

Group IIA: HBSS for 60 minutes

Group IIB: HBSS for 24 hours

Group IIIA: Cornisol for 60 minutes

Group IIIB: Cornisol for 24 hours

Reattachment Procedure:

Fragments were rinsed in distilled water, dried, and etched with 37% phosphoric acid for 15 seconds, then rinsed and air-dried. Bonding agent (Adper Single Bond 2) was applied and air-dried for 5 seconds before curing for 20 seconds using an LED light (3M, ESPE). A layer of flowable composite (Filtek Z350) was applied, and fragments were reattached. Light curing (3M, ESPE) was performed for 20 seconds on all sides [Figure 3].

Figure 3: Fragment reattached with respective specimen using flowable composite

Thermocycling and Testing:

Samples underwent 100 cycles of thermocycling (5°C–55°C, 15 seconds dwell time, 10 seconds transfer time). Finally, fracture resistance was tested using a universal testing machine (Instron, USA) at a rate of 1 mm/min.

Results: Statistical analysis was done using SPSS version 25.0. Inter group comparison was done by ANOVA and Tukey's test. Chisquare test was used for qualitative data. p<0.05 was considered as significant statistically (95% confidence interval). As per the Kolmogrov Smirnov test the data is found to be normally distributed [Table 1].

Table 1: Tests for normality

	Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Fracture resistance-60 minutes	Dry storage	.149	10	.200*	.944	10	.599
	Hanks BalancedSalt Solution	.177	10	.200*	.893	10	.185
	Cornisol	.240	10	.108	.856	10	.069
Fracture resistance-24 hr	Dry storage	.141	10	.200*	.980	10	.968
	Hanks Balanced Salt Solution	.212	10	.200*	.883	10	.143
	Cornisol	.211	10	.200*	.917	10	.336

There was significant difference among the three groups at 60 minutes. The fracture resistance in Cornisol was high and the least in Dry storage (p<0.001) [Table 2] [Figure 4].

Table 2: Fracture resistance - 60 minutes

	N[a]	Mean	Std. deviation	Minimum	Maximum
Dry storage	10	229.1310	66.3876	122.12	316.47
Hanks balanced salt solution	10	433.0510	76.42496	321.76	518.19
Cornisol	10	816.0410	222.88672	453.51	1043.90

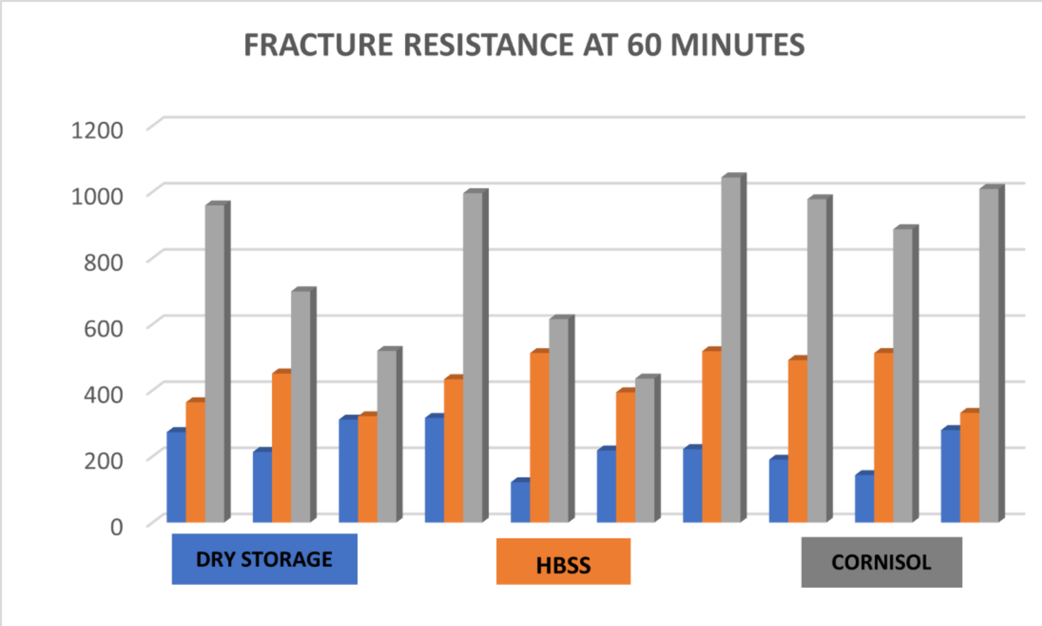

Figure 4: Fracture resistance at 60 minutes

The inter comparison was done by Tukey test. It was found that very high statistically significant difference (p<0.001) was seen between Cornisol and dry storage and Cornisol & HBSS and between dry storage and HBBS it's highly significant (p=0.009) [Table 3].

Table 3: Intergroup comparison

(I) Group	(J) Group	Mean difference (I-J)	P
Dry storage	Hanks balanced salt solution	-203.920	.009hs
	Cornisol	-586.910	p<0.001vhs
Hanks balanced salt solution	Cornisol	-382.990	p<0.001 vhs

There was significant difference among the three groups at 24 hours also. The fracture resistance is high in HBSS and the least in Dry storage. The dereference was statistically significant (p=0.031) [Table 4] [Figure 5].

Table 4: Fracture resistance -24 hours

	N[a]	Mean	Std. deviation	Minimum	Maximum
Dry storage	10	208.7360	46.02442	129.97	281.79
Hanks balanced salt solution	10	307.2310	102.8686	156.94	421.21
Cornisol	10	277.9340	81.54457	167.36	402.20

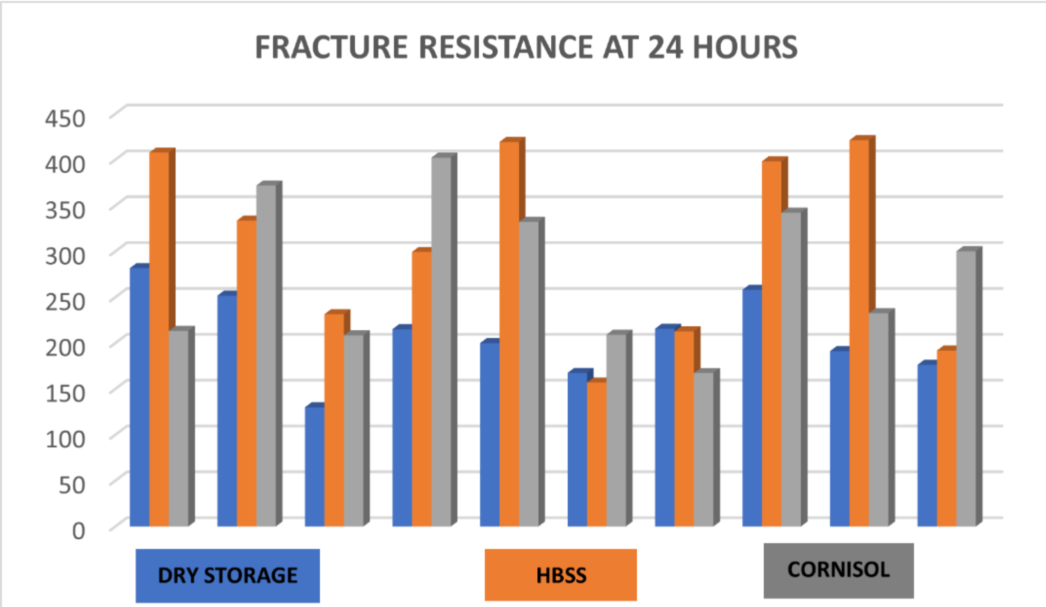

Figure 5: Fracture resistance at 24 hours

Since group comparison was significant there was significant difference between the Dry storage and HBBS only at 24 hours (p=0.031) [Table 5].

Table 5: Multiple comparisons- fracture resistance-24 hrs

Dependent variable	I Group	(J) Group	Mean difference (I-J)	P
Fracture resistance-24hrs	Dry storage	Hanks balanced salt solution	-98.495	.028
		Cornisol	-69.198	.151
	Hanks balanced salt solution	Cornisol	29.297	.697

Between 24 hours and 60 minutes the difference was seen in HBSS and Cornisol (p<0.001). There was no statistical change in dry storage (p=0.476) [Table 6].

Table 6: Paired samples test

		Mean	Std. Deviation		
Dry storage	Fracture resistance-60 minutes-Fracture resistance-24 hrs	20.395	86.190	.748	0.476
Hanks balanced salt solution	Fracture resistance-60 minutes-Fracture resistance-24 hrs	125.820	93.317	4.264	0.002 HS
Cornisol	Fracture resistance-60 minutes-Fracture resistance-24 hrs	538.107	226.252	7.521	<0.001 vhs

3. Discussion:

Traumatic dental injuries are significant emergencies in dental practice, where immediate reattachment of fractured tooth fragments is crucial [25,26]. This approach offers benefits including conservation of natural tooth structure, biological compatibility, and cost-effectiveness [27]. Studies demonstrate that when the fractured segment is available, reattachment provides superior outcomes compared to composite restorations, particularly regarding wear rates and aesthetics [29, 30]. The choice of storage medium for tooth fragments post-trauma significantly affects reattachment success [31, 32]. Maintaining moisture helps prevent collagen fiber collapse and enhances bond strength. Research indicates that fragments stored in moist environments maintain superior integrity and mechanical properties [33,34]. HBSS (Hank's Balanced Salt Solution) and whole milk are often recommended due to their optimal osmolality and nutrient composition [35]. Recently, Cornisol has emerged as a promising storage medium, containing chondroitin sulfate and dextran to maintain cell viability effectively [21].

Dehydration of dentin results in reduced tensile strength and increased brittleness, emphasizing the need to avoid dry storage of fragments [36, 37]. Research indicates that even short periods in dry conditions can compromise viability [6, 28]. Conversely, rehydration can partially restore the mechanical properties of dentin [38].

In comparative studies, HBSS demonstrated superior fracture resistance compared to dry storage, underscoring the necessity of moisture for maintaining bond strength [39]. Cornisol has shown even greater efficacy in preserving periodontal cell viability than traditional options like HBSS, highlighting the need for optimal storage solutions to improve clinical outcomes for reattached fragments [40].

Long-term monitoring of reattached fragments is vital for assessing treatment success [41]. Andreasen et al. found that reattached fragments can retain satisfactory aesthetics and pulp vitality over extended periods, affirming the clinical viability of this treatment approach [42, 43]. However, challenges persist due to the limited duration of existing clinical studies and the variability inherent in in vitro conditions [44].

4. Conclusion

In summary, reattachment of fractured tooth fragments is a valuable treatment modality that preserves natural tooth structure and function. The choice of storage medium plays a critical role in maintaining the viability of the fragment, with Cornisol and HBSS emerging as superior options compared to dry storage. Further research and clinical studies are essential to enhance our understanding of long-term outcomes for this treatment and refine techniques to improve bond strength and durability.

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