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Evaluation Of Efficacy Of Fluoride On Enamel Surface Microstructure Of Avulsed Tooth After Storage On The bovine Milk (An in Vitro Study)

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Abstract :

Background: Dental avulsion is a severe traumatic dental injury involving complete displacement of the tooth from its socket. Immediate replantation is preferred, but when this is not possible, proper temporary storage is essential to preserve periodontal ligament cell viability and improve prognosis. The type and characteristics of the storage medium can influence complications such as root resorption and ankylosis. An ideal medium should maintain suitable moisture, pH, osmolality, and nutrients. Since commercial storage media may not always be available during emergencies, evaluating readily accessible alternatives is clinically important. The aim of the present study to evaluate the effect of 5% sodium fluoride on the surface characteristics of avulsed teeth following immersion in bovine milk. **Materials and Methods** :Sixteen tooth blocks obtained from freshly extracted premolars indicated for orthodontic treatment were included in this in vitro study. The specimens were divided into two groups according to the duration of storage in bovine milk: a 1-hour storage group and an 8-hour storage group, which served as the control groups. Following the designated storage periods, the specimens were treated with 5% sodium fluoride varnish as the experimental intervention. Surface characteristics were subsequently evaluated and compared statistically. **Results** :Statistical analysis using one-way analysis of variance (ANOVA) showed that fluoride application did not produce a statistically significant difference in surface roughness as measured by the Sa (arithmetical mean height) parameter. In contrast, statistically significant differences were observed in the Sq (root mean square height) and Sz (maximum height) parameters following fluoride application in the 4-

hour storage group. **Conclusion** :Under the conditions of this in vitro study, application of 5% sodium fluoride varnish demonstrated potential for modifying and remineralizing early enamel demineralization. Further studies with larger sample sizes and clinically relevant experimental conditions are recommended to confirm these findings and determine their potential clinical significance.

Keywords:
Dental avulsion; Tooth replantation; Storage medium; Bovine milk; Sodium fluoride; Fluoride varnish; Enamel surface; Surface roughness; Periodontal ligament cells; Root resorption.

Introduction :

Avulsion of permanent teeth is the most serious of all types of traumatic tooth injuries because the complete dislodgement of the tooth from its socket causes severe damage to the supporting tissues and vascular and nerve structures (Gopikrishna *et al*., 2008). It corresponds to 1% to 16% of all types of tooth injuries involving the permanent dentition (Andreasen, 1981), (Panzarini *et al*., 2008).This wide variation can be explained by the differences in the evaluated populations, including the levels of interpersonal violence, involvement in motorcycle and bicycle road and traffic accidents, especially without use of helmets, and practice of contact sports, especially without use of mouth guards (Casaroto *et al.,* 2010).Fluoride, apart from its antimicrobial property, acts on cementum and dentin converting hydroxyapatite into fluoroapatite which is more resistant to resorption, or even inhibits clastic cell formation (Shulman LB *et al*.,1973).As per guidelines for avulsed teeth with completely formed roots that are replanted after an extraoral time beyond 60 minutes, the International Dental Traumatology Association has suggested that these teeth, after much removal of PDL remnants, should be immersed in 2.4% APSF (pH 5.5) for 5 minutes (Flores MT et al.,2001).Endodontic treatment is required for replanted teeth because the necrotic pulp and its toxins affect the PDL through dentinal tubules causing resorption (Andreasen JO *et al*.,1995).

Andreasen claimed that replacement resorption occurred if the extraoral dry time was more than 60 minutes and no cell remained vital beyond 120 minutes of dry time on the root (Lekic and Mcculloh demonstrated in vitro that beyond 15 minutes, progenitor periodontal ligament cells exhibited limited proliferative ability, which became impossible after 30 minutes (Lekic P, McCulloch CA , 1996) .Recently, the time period for immediate replantation has changed from 30 minutes to less than 5 minutes of extraoral time (Kenny DJ *et al*., 2001).Depending on the previous study, one can conclude that bovine milk group demonstrated the highest roughness values of the teeth at all times intervals of the test periods , milk was highly erosive in comparison with remaining types of storage media (RS Obeid, and MS Khalaf, 2022).In the present study, the enamel

surface of the study samples was analyzed to determine the effect of fluoride varnish to remineralizer the avulsed teeth after storage in bovine milk .
To create a topographic image from the surface, the atomic force microscope analysis was performed in contact mode. The images were taken at 3080 x 3080 nm to form two-dimensional (2-D) and three dimensional (3-D) images for each sample. Roughness parameters measured in nanometers were used in these assessments:
Average roughness (Sa): The arithmetic mean of peak heights and valley depths from a mean line. (Karan S, *et al*,.2010)
Root mean square roughness (Sq): The distribution of heights in relation to the mean line (Karan S, *et al*,.2010).
Average maximum height (Sz): Within the sampling length, reflects the profile's average maximum height.
Topical fluorides promote the formation of flourapatite in the presence of calcium and phosphate ions produced during enamel demineralization. However, for every 2 fluoride ions, 10 calcium ions, and 6 phosphate ions are required to form one-unit cell of fluorapatite [Ca10 (PO4)6 F2].[4] Hence, the availability of calcium and phosphate ions can be the limiting factor for net remineralization to occur. Also given the mechanism of remineralization by topical fluorides they attempt only to reduce apatite dissolution rather than aiming to promote mineralization of apatite crystal or replacement of the lost minerals. Roveri N, *et al*,.2009)

Materials and Methods

Study Design

This study was designed as a comparative experimental in vitro study to evaluate the effect of fluoride application on the surface characteristics, particularly surface roughness, of avulsed teeth following immersion in bovine milk.

Sample Selection and Preparation

Freshly extracted premolars indicated for orthodontic treatment were selected for the study. Only teeth with closed apices, intact crowns, and no evidence of caries, cracks, or fractures were included. The teeth were collected immediately after extraction and maintained in a moist condition throughout the initial handling and preparation procedures. No additional disinfection procedure was performed. Immediately after extraction, the coronal portion of each tooth was grasped with dental forceps, and the remaining periodontal ligament tissue was carefully removed from the root surface using a dental curette, following the procedure described by Esber *et al.* (2015).The teeth were initially examined visually under adequate illumination and with the naked eye. Teeth showing any visible cracks, fractures, or other structural defects were excluded. The

selected teeth were then placed individually in universal glass tubes (Ningbo, China) containing distilled water immediately after extraction. The distilled water was replaced weekly throughout the storage period to minimize bacterial growth, as previously described by Özer *et al*. (2010) and Prabhakar *et al*. (2016).

Tooth Surface Cleaning

Before specimen preparation, the teeth were polished using non-fluoridated pumice powder (Pumice Powder, I Dental Company, Lithuania) with a rubber cup attached to a low-speed handpiece (STRONG 90, Saeshin Precision Co., Ltd., Korea). This procedure was performed to remove residual debris and deposits from the tooth surfaces. The teeth were subsequently rinsed thoroughly with distilled water and dried using cotton pads, according to the method described by Karan *et al.* (2010).

Preparation of Tooth Specimens

The crowns of the premolar teeth were separated from the roots at the cementoenamel junction (CEJ). Tooth specimens were prepared using a double-sided diamond disc (Dental Lab Diamond Disc, Guangdong, China) mounted on a low-speed handpiece under continuous water cooling.

The specimens were prepared to standardized dimensions of approximately 4 × 4 mm in width and 2 mm in thickness. Dimensions were verified using an electronic digital caliper (Carbon Fiber Composite Digital Caliper, SL01-1/-2, China).To ensure that only the designated enamel surface was exposed to the experimental solutions, all surfaces of each specimen were coated with nail varnish except for the external enamel surface selected for evaluation. This procedure ensured that any changes in enamel surface characteristics could be attributed specifically to exposure of the evaluated surface.

Experimental Groups

The prepared specimens were divided into control and experimental groups according to the storage conditions and treatment protocols described below.

The control specimens were immersed in bovine milk (Qirat, Iraq) for predetermined periods of 1 hour and 8 hours.The experimental specimens underwent the corresponding bovine milk immersion protocol followed by treatment with 5% sodium fluoride varnish. Surface characteristics were subsequently compared between the control and fluoride-treated specimens.

Evaluation of Surface Characteristics

Surface roughness was evaluated using an Atomic Force Microscope (AFM) (SPM-AA3000, Angstrom Advanced Inc., USA) operating in contact mode. AFM analysis was used to assess changes in the enamel surface topography following immersion in bovine milk and subsequent fluoride treatment. The primary surface roughness parameters evaluated included Sa (arithmetical mean height), Sq (root mean square height), and Sz (maximum height). The obtained measurements were compared between the control and experimental groups to determine the effect of 5% sodium fluoride on the enamel surface characteristics.

Aim and Objectives

Aim

To evaluate the effect of 5% sodium fluoride on the surface characteristics of avulsed teeth following immersion in bovine milk.

Objectives

1. To investigate changes in the surface characteristics of avulsed teeth following immersion in bovine milk.
2. To evaluate changes in enamel surface roughness following exposure to bovine milk and subsequent application of 5% sodium fluoride.
3. To compare the surface roughness of fluoride-treated specimens with that of untreated control specimens using atomic force microscopy.

Research Hypothesis

Null hypothesis (H_0): There is no statistically significant difference in the surface characteristics of avulsed teeth treated with 5% sodium fluoride following immersion in bovine milk compared with untreated control specimens.

Sample Size

The study included freshly extracted premolars indicated for orthodontic treatment. The teeth had closed apices and were free from caries and visible structural defects. The specimens were divided into control and experimental groups according to the predefined storage and treatment protocols.

The Results

Table 1: Descriptive and statistical test of roughness (Sa) among period

Period		Mean	±SD	t	P-value
4 hr	**Before**	3.451	0.906	2.485	0.089
	After	1.791	0.50		
8 hr	**Before**	3.667	0.376	5.427	0.12
	After	1.229	0.878		
* Significant at P-value ≤ 0.05 using a Paired-t-test					

Table 2: Descriptive and statistical test of roughness (Sq) among period

Period		Mean	±SD	t	P-value
4 hr	**Before**	4.321	0.996	3.451	0.041*
	After	2.512	1.088		
8 hr	**Before**	4.718	0.638	3.758	0.33
	After	2.133	0.905		
* Significant at P-value ≤ 0.05 using a Paired-t-test					

Table 3: Descriptive and statistical test of roughness (Sz) among period

Period		Mean	±SD	t	P-value
4 hr	**Before**	46.619	16.212	3.436	0.041*
	After	39.871	15.346		
8 hr	**Before**	54.730	18.557	1.942	0.147
	After	21.703	15.826		
* Significant at P-value ≤ 0.05 using a Paired-t-test					

Discussion

The present in vitro study evaluated the effect of 5% sodium fluoride varnish on the surface characteristics of enamel following immersion of avulsed teeth in bovine milk. Surface topography and roughness were assessed using atomic force microscopy (AFM), with **Sa, Sq, and Sz** used as the main surface roughness parameters.The statistical analysis demonstrated that fluoride application did not produce a statistically significant difference

in the Sa parameter. However, significant differences were observed in the Sq and Sz parameters following fluoride treatment in the relevant storage group. These findings suggest that the effect of sodium fluoride on enamel surface characteristics may vary according to the specific parameter used to describe surface topography. While Sa represents the average surface height variation, Sq and Sz may be more sensitive to changes involving surface irregularities and extreme height variations.

Fluoride is one of the most widely used agents for the prevention and management of enamel demineralization. Topical fluoride can promote remineralization by enhancing the formation of fluoridated apatite and reducing the solubility of enamel mineral. It may therefore increase the resistance of enamel to subsequent mineral loss. However, fluoride treatment does not necessarily restore the original architecture of enamel once substantial mineral loss has occurred (Nobre-dos-Santos *et al*., 2009).The present findings are also consistent with previous investigations demonstrating that remineralization can produce measurable changes in enamel surface morphology. Atomic force microscopy provides detailed information regarding surface topography and allows subtle alterations in enamel roughness to be detected. Previous studies using AFM have demonstrated that remineralizing agents can promote mineral deposition within surface defects and partially restore the morphology of demineralized enamel. Nanohydroxyapatite, for example, has been reported to deposit within demineralized areas and form a relatively continuous apatite layer over the enamel surface (Roveri *et al*., 2009).

In the present study, the observed changes in **Sq and Sz**, together with the absence of a significant difference in **Sa**, may indicate that sodium fluoride produced localized modifications of the enamel surface rather than a uniform change across the entire evaluated area. This finding highlights the importance of assessing multiple roughness parameters when investigating the effects of remineralizing agents on enamel.Overall, the findings suggest that 5% sodium fluoride varnish may have a beneficial effect on the surface characteristics of enamel following exposure to bovine milk under in vitro conditions. Nevertheless, the interpretation of these findings should consider the experimental nature of the study, the relatively small sample size, and the limitations associated with reproducing the complex oral environment in vitro. Further studies involving larger sample sizes, additional mechanical and chemical assessments, and clinically relevant conditions are recommended to confirm the present findings.

Conclusion

Within the limitations of this in vitro study**,** 5% sodium fluoride varnish demonstrated a potential beneficial effect on the surface characteristics of enamel following immersion in bovine milk. Changes were observed in the Sq and Sz surface roughness parameters, whereas no statistically significant difference was detected in **Sa**. These findings suggest that sodium fluoride may contribute to the modification and remineralization of early enamel surface changes and support its potential use as a remineralizing agent. Further research is required to establish the clinical relevance of these findings.

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Authors' contributions
All authors contributed to the study conception, design, data analysis, and manuscript preparation, and approved the final manuscript.

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Conflicts of interest

The authors declare that they have no conflicts of interest related to this work.

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