Journal of Research in Medical and Dental Science
2022, Volume 10, Issue 4, Page No: 92-97

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Efficacy of Several Forms of Storage Medium on Avulsed Teeth's Enamel Surface Roughness (An *in Vitro* Study)

Rawaa Sadiq Obeid[1*], Muna Saleem Khalaf[2]

[1]Iraqi Ministry of Health, Babil Health Directorate, Iraq
[2]Department of Pedodontic and Preventive Dentistry, College of Dentistry, University of Baghdad, Iraq

ABSTRACT

Introduction: Avulsion is the full displacement of a tooth from its socket, resulting in periodontal ligament damage. Replantation of the tooth is the preferred therapeutic option.

Aim: To evaluate the effect of different types of storage media on enamel surface roughness of avulsed teeth by using atomic force microscope.

Materials and Methods: twenty four teeth blocks from freshly extracted premolars for orthodontic treatment. The study samples were divided into three groups according to type of storage media: A-egg white, B- probiotic yogurt, and C-bovine milk. All the samples were examined for changes in surface roughness using atomic force microscope, at three periods: baseline, after 1 hour, and 4 hours of immersing in the three types of storage media.

Results and discussion: Milk group has showed a significant increase in mean roughness values at the test periods. Egg white and probiotic yogurt groups have showed decrease in mean roughness value at the test periods ,except probiotic yogurt at four hours slight increase in roughness value , but the result was not statistically significant.The use of egg white and probiotic yogurt to store samples may be beneficial in that they contain various ions and proteins that fill up enamel valleys, while longer periods of milk exposure encourage bacteria to continue fermenting lactose, resulting in continual acid generation and increased demineralization.

Conclusion: Milk group demonstrated the highest roughness values while egg white group demonstrated the lowest roughness values of the teeth.

Key words: Egg white, Probiotic yogurt, Bovine milk, Roughness, Atomic force microscope

HOW TO CITE THIS ARTICLE: Rawaa Sadiq Obeid, Muna Saleem Khalaf, Efficacy of Several Forms of Storage Medium on Avulsed Teeth's Enamel Surface Roughness (An *in Vitro* Study), J Res Med Dent Sci, 2022, 10 (4):92-97.

Corresponding author: Rawaa Sadiq Obeid
e-mail✉**:** ali.mario28@yahoo.com
Received: 07-Mar-2022, Manuscript No. JRMDS-22-47128;
Editor assigned: 09-Mar-2022, Pre QC No. JRMDS-22-47128 (PQ);
Reviewed: 23-Mar-2022, QC No. JRMDS-22-47128;
Revised: 28-Mar-2022, Manuscript No. JRMDS-22-47128 (R);
Published: 04-April-2022

INTRODUCTION

Permanent tooth avulsion is the most severe of all traumatic tooth injuries because the tooth is completely dislodged from its socket, causing extensive damage to the supporting tissues, vascular, and nerve structures [1]. The viability of the periodontal ligament (PDL) cells remaining on the root surface, the integrity of the root cementum, and minimal bacterial contamination, all of which are conditions directly related to the extra-alveolar time, type of storage after avulsion, and root surface alterations, determine the prognosis of a replanted tooth and its maintenance on the dental arch for the longest possible time. An avulsed permanent tooth should be replanted into the socket as soon as possible. Despite its recognized therapeutic value, clinical experience has shown that immediate replantation is uncommon due to factors related to the accident itself, such as the presence of extensive life-threatening injuries, complex damage to the recipient site, the patient's emotional state at the time of trauma, or simply a lack of knowledge or confidence in replantation procedures among the general public and even professionals [2].

Enamel is the tooth's protective outer covering. An acidic environment increases tooth demineralization, which leads to cavity formation [3]. Acidic solutions can dissolve inorganic minerals and organic residues, resulting in a roughened and weaker enamel surface , due to easier bacterial adhesion on a rougher surface, a high surface roughness of enamel will result in a higher risk of cavities, while a significant loss of the mechanical characteristics of the enamel will decrease its protection property [4].

Surface texture includes roughness. The deviations in the direction of an actual surface's normal vector from its ideal form are used to calculate it. The surface is rough if the deviations are considerable; smooth if the variances are minimal [5].

When periodontal ligament cells are out of their alveolar socket, there are solutions that can prolong their lives. These options must be employed if immediate reimplantation is not possible [6]. A number of factors, including the patient's age, root canal width and length , stage of root development, mechanical damage during trauma and reimplantation , form of splinting used , mastication, socket cure, endodontic treatment, antibiotics, reimplantation period, macroscopic contamination, storage media, and storage period, all play a role in the clinical performance of the reimplantation. In order to obtain a successful functional result, in cases of delayed reimplantation, it is recommended that the avulsed teeth be stored in an interim storage medium [7].

In the current study, the experimental teeth groups were stored in the experimental storage media for 1 hour, and 4 hours. These test periods were chosen as it allows for comparison with previous studies. Egg white, probiotic yogurt and bovine milk used in this study because they are among the most popular natural storage media of avulsed tooth.

Natural products have been considered as possible pharmacological replacements, and as interest in complementary and alternative medicine has developed, numerous research on the use of natural products for tissue repair have been conducted [8]. Because natural ingredients are easily available and have the ability to keep periodontal ligament cells alive for longer periods of time, they can be employed as storage media [9].

Egg white is a viable alternative as a storage medium for teeth undergoing delayed replantation because of its high protein, vitamin, and water content, lack of microbial contamination, and ease of access [10].

Bifidobacterium can also contribute to good health by creating a microbiological balance in the oral and decreasing bacteria's acidogenicity via oral defense systems like the peroxidase system, because Bifidibacterium animalis DN 173010 appears to be an alternative for temporary preservation of avulsed teeth due to the high number of viable PDL cells, probiotics may be a potential transport medium for avulsed teeth [11].

Milk offers a number of advantages as a storage medium for avulsed teeth, including being an isotonic liquid with physiological osmolality and pH, carrying growth factors and critical nutrients for cells, and being readily available and inexpensive [10].

Aims: According to the authors knowledge on the effect of different types of storage media on surface roughness of the enamel of avulsed teeth have not been conducted. In this study the aim was to evaluate the effect of different types of storage media on enamel surface roughness of avulsed teeth by using atomic force microscope.

Research Question

Is there a difference in effect of different storage media on the enamel surface roughness of avulsed teeth?

Hypothesis

The null hypothesis is that there will be no differences between the effect of different types of storage media on enamel surface roughness of avulsed teeth.

The alternative hypothesis is that there will be differences between the effect of different types of storage media on enamel surface roughness of avulsed teeth.

MATERIALS AND METHODS

Teeth were selected that had recently been extracted as part of an orthodontic treatment plan. The teeth were collected fresh within the date of extraction and maintained wet at all times without any additional disinfection. The coronal portion of each tooth was held with dental forceps following extraction, and the periodontal ligament was scraped using a dental curette [11]. The procedure began with visual examination of the teeth under good light and with the naked eye. Any visible broken or cracked teeth were discarded. The teeth were then placed in universal glass tubes and preserved in distilled water as soon as they were extracted. The teeth were then placed in universal glass tubes (Ningbo, China) and immediately placed in distilled water following extraction [12], the distilled water was changed weekly until the experiment was completed to avoid bacterial growth [13]. The teeth chosen for sample preparation were polished with non-fluoridated pumice (Pumice Powder, I Dental Company, Lithuanian) using a rubber cup. And a low-speed handpiece (STRONG 90-Saeshin Precision Co., Ltd., Korea) to remove any remaining debris on the tooth surface prior to use. After that, they were washed with distilled water and dried with cotton pads [14]. The samples were prepared using a double-sided diamond disc (Dental lab diamond disc, Guangdong, China) at 4x4 mm width and 2 mm thickness, and a low-speed handpiece with water cooling. The crowns of premolar teeth were separated from their roots at cemento- enamel junction. To obtain correct dimensions, they were determined with an electronic digital caliper (Carbon Fiber Composite Digital Caliper, SL01-1/-2, China). All of the sample surfaces were painted with nail varnish all over except the outer enamel surface that was to be evaluated, such that changes in enamel texture could only be identified from the exposed surface. The prepared samples were exposed to three types of storage media egg white (Fresh Iraqi egg, Mazarie Albalad), probiotic yogurt (Al Safi-Danone Iraq LLC Erbil-Iraq), and bovine milk (Qirat Iraq). The samples were divided and subdivided as mentioned later. They were measured for surface roughness of each group by using atomic force microscope (SPM-AA3000 Angstrom Advanced Inc.,USA AFM Contact Mode).

Twenty four teeth blocks from freshly extracted premolars for orthodontic treatment with closed apices

and no caries were included in this study.

The study samples were divided into three groups:-

Egg white group; Group A consist of 8 teeth blocks stored in green -colored universal tubes and were subdivided into two subgroups according to immersing time with 4 teeth blocks for each one; group A1 for 1 hour; and group A 2 for 4 hours.

Probiotic yogurt group; Group B consist of 8 teeth blocks stored in pink-colored universal tubes and were subdivided into two subgroups according to immersing time with 4 teeth blocks for each one; group B 1 for 1 hour; and group B 2 for 4 hours.

Bovine milk group; Group C consist of 8 teeth blocks stored in orange-colored universal tubes and were subdivided into two subgroups according to immersing time with 4 teeth blocks for each one; group C 1 for 1 hour , and C 2 for 4 hours. These divisions and subdivisions have been summarized in the following (Figure 1).

Determination of the pH values of the storage media

The physico-chemical parameters (pH, salinity) were calculated using a WTW multi-parameter inolab720, but only after calibrating the tool with standard buffer solutions for pH and electric conductivity. WTW inolab 720 (WTW Gmbh, Weilheim, Germany) is powered by two sensors: one that measures pH and the other that measures salinity [15]. The glass electrode was inserted in it and the pH level was displayed on the meter is shown in (Table1). Between readings the electrode was washed with distilled water and wiped with cotton pads to avoid of mixture materials [15,16].

In the present study, the enamel surface of the study samples was analyzed to determine the changes in surface topography; which was measured before the experiment, after exposure to three types of natural storage media (egg white , probiotic yogurt and bovine milk) at two different times intervals (1 hour , and 4 hours). 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 [14].

Root mean square roughness (Sq): The distribution of heights in relation to the mean line [14].

Average maximum height (Sz): Within the sampling length, reflects the profile's average maximum height,

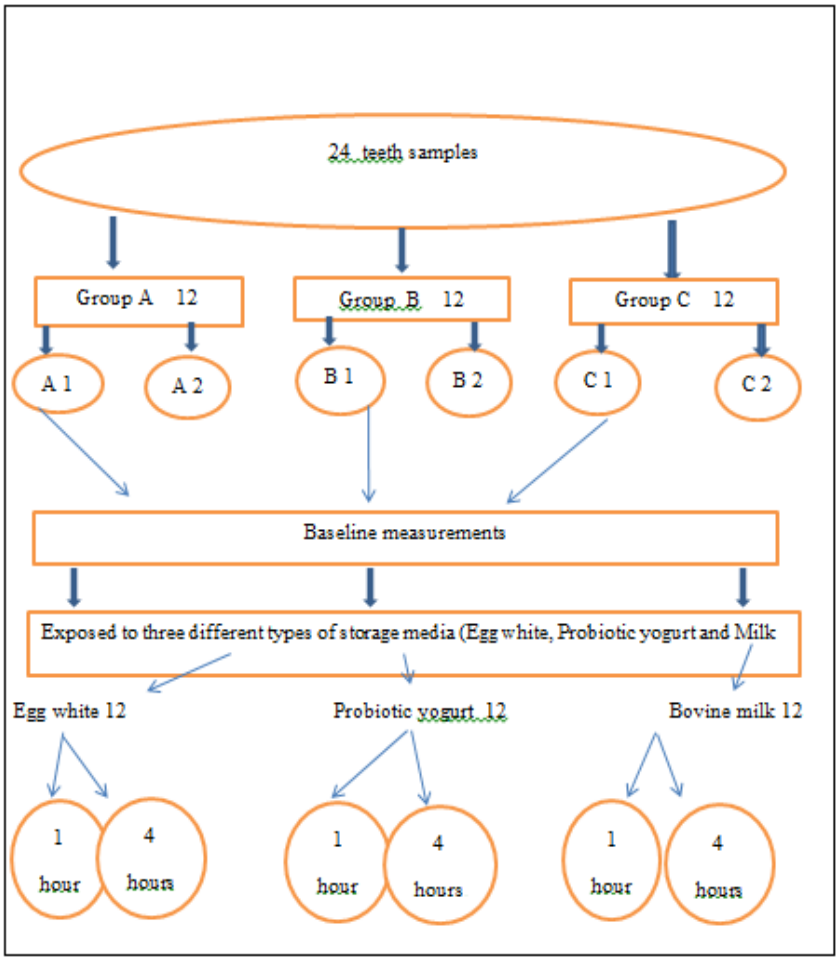

Figure 1: Sample grouping diagram.

taken over five highest peaks Zp and five deepest valleys Zv [17].

Measurement periods

Baseline measurements, which were performed before starting the experiment, and considered the control group.

One hours' measurements, which were made after exposure to the three types of storage media for one hour.

Four hours' measurements, which were made after exposure to the three types of storage media for four hours.

According to these periods, this study had a total of forty eight measurements for the twenty four samples blocks used; divided into twenty four measurements as baseline measurements for control group, and twenty four measurements for experimental groups.

Table 1: pH values of the each type of storage media

Storage media	pH
Egg white	9.5
Probiotic yogurt	5.5
Bovine milk	7.5

RESULT AND DISCUSSION

Data description, analysis and presentation were performed using Statistical Package for Social Science (SPSS version 21). For all statistical analysis, a P value 0.05 was considered significant.

Difference in surface roughness between storage media before and after immersion and within each media.

Tables 2 to Table 4 show description of sample of surface roughness values for egg white , probiotic yogurt and bovine milk at two time interval for one hour, and four hours.

Statistical analysis of results using (ANOVA) test demonstrate that no significant difference was found among egg white and probiotic yogurt groups in average roughness (Sa) parameter at all times interval while significant difference was found among in average roughness (Sa) parameter in milk group at all-time interval. statistical test of root mean square roughness (Sq) and average maximum height (Sz) parameters demonstrate that no significant difference was found among egg white and probiotic yogurt groups at all-time except egg white in 4 hours group in which there was a significant difference and milk group demonstrate significant difference in root mean square roughness (Sq) and average maximum height (Sz) parameters at all-time interval.

Table 2: Descriptive and statistical test of roughness (Sa) among period and storage.

Periods		Egg		Probiotic		Milk		F	P value	R2
		Mean	±SD	Mean	±SD	Mean	±SD			
1hr	Before	36.854	4.796	50.5	36.452	33.938	21.29	0.52	0.612	
	After	33.164	16.16	45.102	12.714	57.825	23.104	1.908	0.204	0.298
	P value	0.647		0.761		0.004*				
4hr	Before	70.376	25.016	40.835	11.809	37.364	10.227	3.746	0.09	
	After	41.73	18.356	64.112	31.225	69.243	21.471	1.449	0.285	0.244
	P value	0.062		0.352		0.025*				
significant at p<0.05										

Table 3: Descriptive and statistical test of roughness (Sq) among period and storage.

Periods		Egg		Probiotic		Milk		F	P value ^	R2
		Mean	±SD	Mean	±SD	Mean	±SD			
1 hr	Before	42.758	4.984	61.425	45.454	41.125	26.827	0.543	0.599	
	After	39.879	19.087	53.319	15.364	68.975	30.24	1.68	0.24	0.272
	P value	0.763		0.702		0.006*				
4 hr	Before	85.103	24.583	48.243	13.116	45.88	13.497	4.54	0.07	
	After	50.308	20.257	74.794	36.282	82.216	24.923	1.425	0.29	0.24
	P value	0.028*		0.353		0.023*				
significant at p<0.05										

Table 4: Descriptive and statistical test of roughness (Sz) among period and storage.

Periods		Egg		Probiotic		Milk		F	P value	R2
		Mean	±SD	Mean	±SD	Mean	±SD			
1 hr	Before	147.005	22.517	225.2	127.198	126.235	79.745	1.418	0.292	
	After	140.046	32.995	176.595	42.526	200	88.683	1.018	0.399	0.184
	P value	0.535		0.482		0.007*				
4 hr	Before	292.617	87.631	180.679	58.383	154.538	30.334	3.977	0.091	
	After	189.735	57.433	208.992	131.29	275.254	85.951	0.865	0.453	0.161
	P value	0.025*		0.78		0.034*				
significant at p<0.05										

Roughness parameters (Sa, Sq and Sz) assessment

The surface topography of each storage media of avulsed teeth was quantitatively evaluated using the three roughness parameters.

Egg white group

A decrease in surface roughness parameters (represented by mean Sa, Sq and Sz values) from baseline periods was observed following immersing of avulsed tooth in egg white.

This result came in agreement with Hemingway et al., (2008) who reported that ovalbumin lowers the rate of hydroxyapatite dissolution under conditions that simulate tooth erosion caused by citrus-based soft beverages with a range of pH and calcium concentrations [18].

This can be explained in that egg white contains various ions that fill up enamel valleys; creating a smoother surface and also alkaline pH and stability of pH in the all times of preserving of avulsed teeth. The primary protein contained in the white of hens' eggs is ovalbumin. Bovine enamel adsorbs ovalbumin [19].

Probiotic yogurt group

Probiotic yogurt decrease in surface roughness parameters in terms Sa, Sq and Sz from baseline period was observed after immersing of avulsed tooth in its. But the result was not statistically significant.

This result was in agreement with Ferrazzano et al., (2008) who demonstrated that yogurt is a good source of calcium and phosphorus and has a greater protein level than milk [20]. The advantageous ionic form of calcium is due to yogurt's lower pH as compared to milk. Furthermore, because of the proteolytic activity of the microbe found in yogurt, the concentration of casein phosphopeptide is greater than in milk [20]. The ionic form of calcium keeps the calcium in the tooth structure and the fluids around it in equilibrium [21].

Due to their natural presence of casein, calcium, and phosphorus, they are also thought to be safe for teeth, with possible favorable effects on both salivary microbial composition and caries development [22].

In spite of the low PH of yogurt but it has high calcium and phosphate content which decrease the rate of erosion by remineralization of the tooth. Nevertheless; the results of the current study were in disagreement with Shen et al., (2020) who reported that the high concentration of lactic acid, sugar, and live lactic acid generating bacteria in the processed yogurt causes considerable enamel subsurface demineralization [23].

Bovine milk group

A significant increase in surface roughness parameters (represented by mean Sa, Sq and Sz values) from baseline period was noticed following immersing of avulsed tooth in bovine milk. The roughness values showed increase which suggests that more erosion occurs with increased exposure time to bovine milk.

The results of this study were in agreement with Lee et al., (2011) who reported that micro-hardness was lower in the human and formula milk groups than in the control. In saliva and water, scan electron microscope (SEM) observation demonstrated higher surface roughness and loss of inorganic substance in the formula milk group than in human milk [24].

Longer periods of milk exposure encourage bacteria to continue fermenting lactose, resulting in continual acid generation and increased demineralization. As a result, the amount of time the biofilm is immersed in milk is crucial [25].

CONCLUSION

Depending on the findings of this 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. Egg white group demonstrated the lowest roughness values of the teeth at all times intervals of the test periods and probiotic yogurt group showed the next lower roughness values.

Fresh egg whites and probiotic yogurt were a suitable storage medium that maintain viable periodontal ligament cells and demonstrated the lowest surface roughness values among the three groups throughout the test period. There was no erosion effect on enamel surface of avulsed teeth. Because chicken eggs and probiotic yogurt are readily available in markets, are inexpensive, and can be obtained in almost every home, individuals can be taught how to preserve avulsed teeth by immersing them in these storage medium as soon as possible after an accident that results in tooth avulsion.

REFERENCES

1. Gopikrishna V, Baweja PS, Venkateshbabu N, et al. Comparison of Coconut Water, Propolis, HBSS, and Milk on PDL Cell Survival. Elsevier 2008.
2. de Carvalho Cardoso L, Poi WR, Panzarini SR, et al. Knowledge of firefighters with special paramedic training of the emergency management of avulsed teeth. Dent Traumatol 2009; 25:58-63.
3. Tsai MT, Wang YL, Yeh TW, et al. Early detection of enamel demineralization by optical coherence tomography. Sci Rep 2019; 9:1-9.
4. Li P, Oh C, Kim H, et al. Nanoscale effects of beverages on enamel surface of human teeth: An atomic force microscopy study. J Mech Behav Biomed Mater 2020; 110:103930.
5. Zhai C, Gan Y, Hanaor D, et al. The role of surface structure in normal contact stiffness. Exp Mech 2016; 56:359-68.
6. Lin S, Zuckerman O, Fuss Z, et al. New emphasis in the

treatment of dental trauma: avulsion and luxation. Dent Traumatol 2007;23:297-303.

7. Marwah N. Textbook of pediatric dentistry. JP Medical Ltd 2018.
8. Costa CR, Amorim BR, Silva SM, et al. In vitro evaluation of Eugenia dysenterica in primary culture of human gingival fibroblast cells. Braz Oral Res 2019; 33.
9. Jain D, Dasar PL, Nagarajappa S. Natural products as storage media for avulsed tooth. Saudi Endod J 2015; 5:107.
10. Goswami M, Chaitra TR, Chaudhary S, et al. Strategies for periodontal ligament cell viability: An overview. J Conserv Dent 2011; 14:215.
11. Esber C, Sertac P, Pınar K, et al. Kvantitativna analiza probiotskih medija za pohranu izbijenih zuba. Acta Stomatol Croat 2015; 49.21-6.
12. Prabhakar AR, Yavagal CM, Limaye NS, et al. Effect of storage media on fracture resistance of reattached tooth fragments using G-aenial Universal Flo. J Conserv Dent 2016; 19:250.
13. Özer T, Başaran G, Kama JD. Surface roughness of the restored enamel after orthodontic treatment. Am J Orthod Dentofacial Orthop 2010; 137:368-74.
14. Karan S, Kircelli BH, Tasdelen B. Enamel surface roughness after debonding: comparison of two different burs. Angle Orthod 2010; 80:1081-8.
15. Călin DI, Roşu C. Drinking water quality assessment of rural wells from Aiud Area. Adv Environ Sci 2011; 3:108-22.
16. Jain AA, Bhat M, Killada J, et al. Dental implications and laboratory evaluation of tooth dissolution in medicated liquid syrups. J Adv Med Dent Scie Res 2016; 4:26.
17. Świetlicka I, Muszyński S, Tomaszewska E, et al. Prenatally administered HMB modifies the enamel surface roughness in spiny mice offspring: An atomic force microscopy study. Arch Oral Biol 2016; 70:24-31.
18. Hemingway CA, Shellis RP, Parker DM, et al. Inhibition of hydroxyapatite dissolution by ovalbumin as a function of pH, calcium concentration, protein concentration and acid type. Caries Res 2008; 42:348-53.
19. Pearce EI, Bibby BG. Protein adsorption on bovine enamel. Arch Oral Biol 1966; 11:329-36.
20. Ferrazzano GF, Cantile T, Quarto M, et al. Protective effect of yogurt extract on dental enamel demineralization in vitro. Aust Dent J 2008; 53:314-9.
21. Singh C, Doley S. Invitro Evaluation of the Inhibitory Effect of Probiotic Enriched and Traditional Yogurt Extracts on Dental Enamel Demineralization-Comparative Study. Int J Oral Health Med Res 2016; 3:31-5.
22. Levine RS. Milk, flavoured milk products and caries. Br Dent J 2001;191:20-.
23. Shen P, Fernando JR, Walker GD, et al. Addition of CPP-ACP to yogurt inhibits enamel subsurface demineralization. J Dent 2020; 103:103506.
24. Lee MR, Lee CJ, Park JH. The roughness & micro-hardness on the deciduous teeth according to formula milk or human milk. Int J Clin Prev Dent 2011; 7:179-88.
25. Ricomini Filho AP, De Assis AC, Oliveira BE, et al. Cariogenic potential of human and bovine milk on enamel demineralization. Caries Res 2021; 55:260-7.