Research Article

Impact of different types of storage media on enamel surface roughness and granularity distribution of avulsed teeth (In vitro study)

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Received: date: 01-03-2022
Accepted: date: 10-04-2022
Published: date: 15-12-2022

https://doi.org/10.26477/jbcd.v34i4.3275

Abstract: Background: To investigate the effect of different types of storage media on enamel surface microstructure of avulsed teeth by using atomic force microscope.Materials and methods : Twelve teeth blocks from freshly extracted premolars for orthodontic treatment were selected . 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 and surface granularity distribution using atomic force microscope, at two periods: baseline, and after 8 hours of immersing in the three types of storage media. Results: Milk group had showed a significant increase in the mean of the roughness values at the test period, while the egg white and the probiotic yogurt groups showed decrease in the surface roughness at the test period. No significant changes was found in the grain size of enamel surface of the avulsed tooth in any types of three storage media at eight hours interval. The use of egg white and probiotic yogurt to store the samples may be beneficial in that they contain various ions and proteins that fill up enamel valleys, while the longer periods of milk exposure encourage the bacteria to continue fermenting lactose, resulting in continual acid generation and increased demineralization. Conclusion : Milk group demonstrated the highest roughness values, while the egg white group demonstrated the lowest roughness values of the teeth. No significant changes in the grain size of enamel surface of the tested teeth in any types of three storage media at eight hours interval .

Keywords: Atomic force microscope, Avulsion, Bovine milk, Egg white, Granularity distribution , Probiotic yogurt, Storage media , Surface roughness.

Introduction

The most extreme of all sorts of traumatic dental injuries is avulsion of permanent teeth, which occurs when the tooth is completely dislodged from its position, causing extensive harm to the supportive tissue, vascular, and nerve components [(1).] The survival of the periodontal ligament (PDL) that are cells residual on the root surface, the integrity of the root cementum, and limited microbial contamination, all of which are circumstances specifically linked to the extra-alveolar period. Sort of storage after avulsion, and root surface changes, can determine the outcome of a replanted tooth as well as its preservation on the dental arch for the lengthiest probable period , the ideal treatment for an avulsed permanent tooth is its immediate replantation into the socket. Despite its acknowledged therapeutic efficacy, clinical practice has demonstrated that immediate replanting is uncommon owing to injury related circumstances, such as the existence of life-threatening incidents, the recipient location if subjected to significant damage, the patient's emotive state at the time of incident, or easily an insufficient information or trust in replantation

techniques among the common people or even practitioners[2]. Enamel is the tooth's exterior protective layer. Tooth demineralization is accelerated in acidic environment, which leads to cavity formation[3].

Surface texture includes roughness. The variations in the orientation of a practical surface's vector from its perfect form are used to assess it. The surface is rough if the deviations are considerable; smooth if the variances are minimal[4]. 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 [5]. Dry storage (up to 30 minutes) can induce more root resorption and is more detrimental to maintaining a normal PDL as compared to wet storage [6].

Natural products had been investigated as potential pharmacological substitutes, and as interest in complementary and alternative medicine has grown, numerous studies on the use of natural products for tissue healing had been carried out [7]. Because natural ingredients are easily available and have the ability to keep PDL cells alive for longer periods of time, they can be employed as storage media [8]. 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 [9]. Milk has several benefits as a storage medium for the avulsed teeth, including being an isotonic liquid with physiological osmolality and pH, containing growth factors and vital nutrients for cells, and being widely available and inexpensive[9]. Probiotics *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. This is due to the high number of PDL cells that remain viable on the root surface of the avulsed tooth, probiotics may be a potential transport medium for avulsed teeth [10].

There is no available data related to the effect of different types of storage media on surface roughness and surface granularity distribution of the enamel of avulsed teeth. The aim of this study was to evaluate and compare the effect of different types of storage media on enamel microstructure of avulsed teeth by using atomic force microscope at two different time intervals.

Research question Is there a difference in the effect of varies storage media on the enamel surface microstructure of the avulsed teeth?

Materials and Methods

Premolar teeth that had just been extracted as part of a treatment plan for orthodontics were chosen. The teeth were gathered as soon as possible after the extraction, were preserved wet by placement in a universal glass tubes and kept in distilled water. At all times without any additional sanitizing treatment. Following extraction, dental forceps were used to hold the coronal section of each tooth while a dental curette was used to scrape the PDL, rinsing with normal saline was done to remove blood cells [10]. The procedure began with a naked-eye to do a visual examination of the teeth in favorable light. Any teeth that were observed to be damaged or fractured were eliminated[11]. To prevent the bacterial growth, the distilled water was changed once a week until the experiment was finished[12]. Non-fluoridated pumice was used to polish the teeth used for sample preparation (Pumice Powder, I Dental Company ,Lithuanian) using a rubber cup (Denmark), and a low-speed hand piece (STRONG 90- Saeshin Precision Co., Ltd., Korea) prior to experiment to remove any residual material from the tooth surface , then rinsed in distilled water and dried using cotton pads (China)[13]. The crowns of the premolar teeth were separated from their roots at cementoenamel junction . The samples were prepared using a double-sided

diamond disc (Dental lab diamond disc, Guangdong, China) at 4 x 4 mm width, and 2 mm thickness, and a low-speed handpiece with water cooling . To obtain correct dimensions, they were determined with an electronic digital caliper (SL01-1/-2, China). Except for the outer enamel surface that was to be examined, all of the sample surfaces were painted with nail varnish all over, so that variations in texture of enamel could only be determined from the exposed surface. Twelve 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 according to the type of the storage medium:

• Egg white group ; Group A consists of four teeth blocks stored in green -colored universal tubes and immersed in egg white (Fresh Iraqi egg, Mazarie Albalad) for 8 hours.

• Probiotic yogurt group ; Group B consists of four teeth blocks stored in pink-colored universal tubes and immersed in probiotic yogurt (Al Safi-Danone Iraq LLC Erbil-Iraq) for 8 hours.

• Bovine milk group ; Group C consists of four teeth blocks stored in orange-colored universal tubes and immersed in bovine milk (Qirat Iraq) for 8 hours.

Determination of the pH values of the storage media

The physico-chemical parameters (salinity and pH) were calculated using a wissenschaftlich technische werkstatten (WTW) multi-parameter inolab720, but only after standardizing the tool with standard pH and conductivity of electricity. WTW multi-parameter inolab 720 (WTW Gmbh, Weilheim, Germany) is powered by two sensors: one that measures pH and the other that measures salinity[14]. The glass electrode was inserted in it and the pH level was displayed on the meter is shown in Table 1 . Between readings the electrode was washed with distilled water and wiped with cotton pads to avoid of mixture of the materials [15].

Table 1: pH values of the storage media

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

The surface topography properties of the enamel were examined using an atomic force microscope to evaluate the changes in the surface topography, which were determined before the experiment and after eight hours of exposure to three different natural storage media (egg white, probiotic yogurt, and bovine milk). The atomic force microscope examination was set in contact mode to obtain a topographic image from the surface. For each sample, pictures were obtained at 3080 × 3080 nm to create two-dimensional (2-D) and three-dimensional (3-D) images.

These measurements involved roughness parameters expressed in nanometers as follows:

1. Average roughness (Sa) : from a mean line, the arithmetic mean of peak heights and valley depths [13].

2. Root mean square roughness (Sq): the distribution of heights in relative to the mean line [13].

3. Average maximum height (Sz): Within the sampling length, represents the profile's average maximum height, calculated over the five highest peaks Zp and five deepest valleys Zv [16].

Measurement periods:

1-Baseline measurements :- which were performed before starting the experiment, and considered as the control group.

2. eight hours' interval measurements , which were made after exposure to the three types of storage media for eight hours.

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

Granularity cumulation distribution, in addition to determining the average size of the nanoparticles

that made up the surface, the reports of granularity cumulation shows the distribution of particle diameters over volume ratios of the samples [17].

Statistical analysis:

Statistical Package for Social Science (SPSS version 21) was used to describe, analyze, and present the data. Statistical analyses can be categorized into two classes:-

Descriptive statistics:

Minimum, maximum, mean, standard deviation (SD) for quantitative variable

Inferential statistics:

A. Shapiro Wilk test : Test the normality distribution of the quantitative variable.

B. Levene test : Check if the variance is homogeneous between groups.

C. One Way Analysis Of Variance (ANOVA) : Test the difference between k independent groups

D. Paired T test : Test the difference between two measurements on the same subject or two related points.

When the p value was < 0.05 it was considered as significant for all the statistical analysis.

Results

Normality test

The normality of data was tested by Shapiro-Wilk test (Table 2 and 3) which revealed that the samples were normally distributed.

Table 2 : Normality test of roughness among storage media and periods

Periods	Variables	Storage	Shapiro-Wilk	
			Statistic	P value ^
			Periods	Periods
			8hr	8hr
Baseline	Sa	Egg white	0.907	0.468
		Probiotic yogurt	0.763	0.051
		Bovine milk	0.825	0.156
	Sq	Egg white	0.927	0.578
		Probiotic yogurt	0.766	0.054
		Bovine milk	0.867	0.286
	Sz	Egg white	0.956	0.753
		Probiotic yogurt	0.866	0.284
		Bovine milk	0.927	0.578
After 8 hours	Sa	Egg white	0.997	0.991
		Probiotic yogurt	0.866	0.284
		Bovine milk	0.862	0.267
	Sq	Egg white	1.000	0.999
		Probiotic yogurt	0.907	0.467
		Bovine milk	0.820	0.144
	Sz	Egg white	0.927	0.575
		Probiotic yogurt	0.918	0.524
		Bovine milk	0.851	0.229

Table 3: Normality test of granularity diameter among periods and storage media

Variables	Storage media	Shapiro-Wilk	
		Statistic	P value
			0.347
Baseline	Egg white	0.888	0.446
	Probiotic yogurt	0.918	0.056
	Bovine milk	0.775	0.393
After 8 hours	Egg white	0.902	0.892
	Probiotic yogurt	0.997	0.372
	Bovine milk	0.896	

Table 4 shows the minimum, maximum ,mean and standard deviation (SD) of surface roughness values for egg white ,probiotic yogurt and bovine milk at eight hours period. Statistical analysis of the results using (ANOVA) test demonstrates that no significant difference was found among egg white and probiotic yogurt groups in all the roughness parameters (Sa, Sq and Sz) and at the test period. No significant difference was seen in milk group in (Sa and Sq) where as a significant difference was found for Sz parameter (P = 0.025).At the test periods, the lowest recorded minimum , maximum ,mean and standard deviation values at the test periods of surface roughness in egg white group while the highest values were at the test period in surface roughness in milk group.

Table 4 : Descriptive and statistical test of roughness among periods by storage media

Storage	periods		8 hours					
			Min.	**Max.**	**Mean**	**±SD**	**F**	**P alue**
Egg white	**Baseline**	Sa	27.955	115.000	80.414	37.198	3.072	0.096
		Sq	33.630	137.000	94.358	43.713	3.576	0.072
		SZ	88.385	533.000	333.346	183.976	2.740	0.118
	After 8 hours	Sa	28.034	79.800	52.934	21.770	1.100	0.374
		Sq	33.248	92.200	62.437	24.826	1.100	0.374
		SZ	89.149	319.000	209.787	107.410	.971	0.415
Probiotic yogurt	**Baseline**	Sa	51.900	201.000	93.375	71.930	1.413	0.293
		Sq	59.900	260.000	115.675	96.430	1.329	0.312
		SZ	207.000	550.000	335.250	149.107	1.817	0.217
	After 8 hours	Sa	48.000	98.200	66.550	21.956	1.022	0.398
		Sq	56.800	113.000	85.950	26.843	1.452	0.284
		SZ	126.000	393.000	282.000	123.129	1.023	0.398
Bovine milk	**Baseline**	Sa	44.675	81.600	56.969	17.038	2.185	0.168
		Sq	56.700	97.300	71.360	18.060	2.583	0.130
		SZ	189.198	421.000	316.050	107.684	4.048	0.094
	After 8 hours	Sa	83.700	93.119	88.380	4.719	2.812	0.113
		Sq	97.000	130.126	107.957	15.287	2.665	0.123
		SZ	347.000	426.468	376.867	34.395	5.752	**0.025***

***Significant P value = 0.025, Sa- average roughness, Sq- root mean square roughness, Sz-average height**

Difference in surface roughness before and after immersion and within each medium can be seen in Table 5 Comparison of the roughness change for egg white , probiotic yogurt and bovine milk at the test period revealed that there was no statistical significant difference for the egg white and probiotic yogurt groups, where it was a significant difference for the milk group.

Table 5 : Descriptive and statistical test of roughness before and after immersion and within each.

Periods		Egg white		Probiotic yogurt		Bovine milk			
		Mean	±SD	Mean	±SD	Mean	±SD	F	P value
8hr	Presa	80.414	37.198	93.375	71.930	56.969	17.038	0.597	0.571
	postsa	52.934	21.770	66.550	21.956	88.380	4.719	3.922	**0.060**
	p	0.119		0.545		0.031*			
	presq	94.358	43.713	115.675	96.430	71.360	18.060	0.511	0.616
	postsq	62.437	24.826	85.950	26.843	107.957	15.287	3.959	**0.058**
	p	0.119		0.554		0.061			
	presz	333.346	183.976	335.250	149.107	316.050	107.684	0.020	0.980
	postsz	209.787	107.410	282.000	123.129	376.867	34.395	3.022	**0.099**
	p	0.125		0.675		0.446			

***significant at p<0.05 . Pre =Prior(before).**

Post=after

Difference in granularity diameter between storage media before and after immersion and within each media is shown (Table 6) , (Table 7) revealed that there were no significant difference in all groups on the test period.

Table 6 : Descriptive and statistical test of granularity diameter among periods by storage media

Storage	**periods**	**8 hr**				**F**	**P value ^**
		Min.	**Max.**	**Mean**	**±SD**		
Egg	Before	93.340	118.790	103.450	13.507	0.485	0.638
white	After	101.820	151.840	122.077	26.330	2.225	0.189
Probiotic	Before	104.100	150.800	123.423	24.369	0.439	0.664
	After	82.830	192.080	135.673	54.712	1.716	0.257
Milk	Before	101.260	109.630	104.060	4.824	2.122	0.201
	After	96.290	129.610	109.667	17.604	0.736	0.518

Table 7 : Descriptive and statistical test of granularity diameter among storage media and periods **.**

Periods		**Egg white**		**Probiotic yogurt**		**Bovine milk**		**F**	**P**
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**		**value ^**
8hr	**Before**	103.45	13.50	123.42	24.36	104.06	4.824	1.45	0.30
	After	122.07	26.33	135.67	54.71	109.66	17.60	0.38	0.69
	Paired T test	1.035		0.539		0.460			
	P value	0.410		0.644		0.691			

Discussion

The three roughness parameters Roughness (Sa, Sq and Sz) assessment were used to quantitatively evaluate the surface topography of each storage media of avulsed teeth.

A decrease in surface roughness (represented by mean Sa, Sq and Sz values) from baseline periods measured by atomic force microscope was observed following immersing of the avulsed tooth in egg white. But the result were not statistically significant. 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 decreased the surface roughness in terms of Sa, Sq and Sz from baseline period which 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., in 2008 who demonstrated that 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 that the 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., in 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].

A significant increase in surface roughness (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 suggested that more erosion occurs with increased exposure time to bovine milk. The results of this study were in agreement with Lee et al., in 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].

Average diameter of grain size of enamel surface shown no significant changes in grain size of enamel surface of the avulsed tooth in any types of three storage media at eight hours interval . Egg white and probiotic yogurt groups showed slight increase in the average diameter of grain size of enamel surface,

but the results were not significant .This results might be explained in terms of the possibility of surface remineralization of enamel[19].

Conclusion

Depending on the findings of this study, one can conclude that bovine milk group demonstrated the highest roughness values of the teeth at the test period, milk was highly erosive in comparison with remaining types of storage media. Egg white group demonstrated the lowest roughness values of the teeth at the test periods and probiotic yogurt group showed the next lower roughness values.

Because chicken eggs and probiotic yogurt are readily available in markets, 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.

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العنوان: فعالية أنواع مختلفة من وسائط التخزين على خشونة وتوزيع حبيبات سطح المينا للأسنان المخلوعة (دراسة مختبرية)

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المستخلص:

فوائد : تقييم تأثير أنواع مختلفة من وسائط التخزين على خشونة سطح و توزيع حبيبات سطح المينا للأسنان المخلوعة باستخدا م مجهر القوة الذرية
المواد والطرق : لقد تم أختيار اثنا عشر سنًا من الضواحك المقلوعة حديثًا للمعالجة التقويمية للأسنان . تم تقسيم عينات الدراسة إلى ثلاث مجموعات حسب نوع وسائط التخزين- أ-بياض البيض ب- زبادي بروبيوتيك ج- حليب بقري . تم فحص جميع العينات لمعرفة التغيرات في خشونة السطح و توزيع حبيبات السطح المينا للأسنان المخلوعة باستخدام مجهر القوة الذرية: قبل بدء التجربة و ثمانية ساعات من الانغماس في الأنواع الثلاثة لوسائط التخزين النتائج : أظهرت مجموعة الحليب زيادة معنوية في متوسط قيم الخشونة في فترة الاختبار. أظهرت مجموعات بياض البيض و زبادي البروبيوتيك انخفاضًا في متوسط قيمة الخشونة في فترة الاختبار. لا توجد تغيرات كبيرة في حجم حبيبات سطح مينا الأسنان المخلوعة في أي نوع من وسائط تخزين. في مجموعات بياض البيض والزبادي بروبيوتيك تظهر زيادة طفيفة في متوسط قطر حجم الحبوب لسطح المينا. قد يكون استخدام زبادي البيض والبروبيوتيك لتخزين العينات مفيدًا لأنه يحتوي على أيونات وبروتينات مختلفة تملأ وديان المينا, بينما يشجع التعرض للحليب لفترات أطول البكتيريا على الاستمرار في تخمير اللاكتوز, مما يؤدي إلى استمرار توليد الأحماض وزيادة فقدان المعادن
الاستنتاجات : إعتمادا على النتائج المذكورة سابقا تم التوصل الى أن مجموعة الحليب أظهرت أعلى قيم خشونة بينما أظهرت مجموعة بياض البيض أقل قيم خشونة لمينا الأسنان . لم يكن للمجموعات الثلاث أي تأثير على قيم توزيع حبيبات السطح في فترة الاختبار