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A New Storage Medium for an Avulsed Tooth

Abbas Ali Khademi, DDS; Saeed Saei, DDS;
Mohammad Reza Mohajeri, MD; Nooshin Mirkheshti, MD;
Fatima Ghassami, MD; Nakisa Torabi nia, MD; Sayyed Ali Alavi, MD

Aim: The purpose of this study is to determine the efficacy of egg white in maintaining the viability of human periodontal ligament (PDL) cells on avulsed teeth.

Methods and Materials: The experimental media were: egg white, milk, Hanks' Balanced Salt Solution (HBSS) as the positive control, and tap water as the negative control. The storage times were 1, 2, 4, 8, and 12 hours. Extracted premolar teeth of healthy individuals were rinsed in the media. After trypsinization and subsequent treatment in collagenase, cell viability was determined using trypan blue staining. The two-way analysis of variance (ANOVA) statistical test was used to compare the results among different media.

Results: There was no difference in the cell viability between egg white and HBSS media, but there was a statistically significant difference between the viability of PDL cells in egg white medium in comparison with milk ($P<0.05$) and water ($P<0.05$).

Conclusion: Egg white could be suggested as a suitable storage medium. Its principle advantage is its availability.

Keywords: Periodontal ligament cells, PDL, cell culture, tooth avulsion, storage medium, egg white

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Introduction

According to the World Health Organization (WHO) classification for traumatized teeth, avulsion, or exarticulation is the complete displacement of a tooth from its alveolar socket due to traumatic injury.[1] When an injury occurs, the avulsed tooth should be replanted immediately to prevent further injury to periodontal ligament (PDL) cells in the future.

However, rapid replantation rarely occurs since factors such as the emotional stress of parents and lack of knowledge of appropriate first-aid measures to manage the problem at the location of the injury tend to delay definitive care. In these conditions the tooth should be maintained in a suitable medium until it is replanted by a dentist as soon as possible.[2,3] Two factors that have a profound effect on the prognosis of replanted avulsed teeth are extraoral time of the avulsed tooth and the medium used to preserve the tooth before replantation.[4]

Within a short time after avulsion attached PDL cells begin to necrosis. Andreasen[5] showed if replantation is done immediately, the tooth will remain in the dental arch without resorption and with normal function. If the tooth cannot be immediately replanted, the viability of PDL cells on the surface of the root can be maintained in a suitable transport medium which will lead to a decreased incidence of root resorption.[6] However, use of an inappropriate storage medium can increase the rate of cell necrosis and result in ankylosis or resorption of dental root.[5]

Storage media such as milk, saline, water, saliva, culture media, and Hanks' Balanced Salt Solution (HBSS) have been suggested to preserve the viability of PDL cells.[7] The American Association of Endodontists (AAE) recommends the use of HBSS as the storage medium of choice for treatment of avulsed teeth because of its ability to provide long-term preservation of PDL cell viability.[8] However, HBSS is not available in most places where these traumatic events usually occur such as in school, home, camps, and sports field settings where people are physically active.

The objective of this study was to examine the efficacy of an alternative storage medium with suitable properties for transferring an avulsed tooth that is readily available to the public.

Rozenfarb et al.[9] conducted an *in vitro* study to compare milk, egg white, saliva, and MEM (a culture medium) in terms of the maintenance of skin fibroblast viability and found three media (milk/egg white/MEM) to be more suitable than saliva. Despite similar morphology, fibroblasts obtained from other sources (gingival, lips, and skin) behave differently in culture medium than PDL cells.[10,11] This finding lead to the present study of the suitability of egg white on the viability of PDL cells. Therefore, the aim of the present study was to compare the suitability of different media on the viability of PDL cells over several time periods. In this study egg white and milk served as the experimental media while HBSS served as the positive control and tap water as a negative control.

Methods and Materials

Chemicals

The following were obtained from Sigma-Aldrich (St. Louis, MO, USA) for use in this study:

- HBBS (Ca^2+/Mg^2-free)
- Trypsin derived from pig pancreas
- Type IV collagenase
- RPMI 1640 culture medium
- Hydrochloric acid
- Trypan blue

Common cow's milk was obtained from Pegah Milk® (Isfahan, Iran).

Tooth Preparation and Measurements

A total of 100 permanent, periodontal disease-free, premolar teeth atraumatically extracted for orthodontic reasons were used in this *in vitro*

study. The teeth were randomly divided into four groups of 25 teeth each. A total of 25 teeth were used for each medium (milk, egg white, HBSS, and tap water) that were evaluated at 1, 2, 4, 8, and 12 hours (five teeth at a time). Tap water served as the negative control and HBSS served as the positive control.

The method used in this study was the same as the method used by Doyle et al.[12] Following extraction, the PDL was removed from the root area to 3 mm below the cementoenamel junction with a sharp curette to eliminate damaged PDL cells from the extraction forceps then rinsed with Ca^{2}+/Mg^{2}-free HBSS. The specimens were transferred to the laboratory in test tubes containing 10 ml of the HBSS placed inside a flask containing crushed ice.

Every tooth was maintained for the determined time in a tube containing the storage medium at 25ºC. To harvest the PDL cells the tooth specimens were washed with the HBSS, then trypsinized using a 2.5% trypsin solution in normal saline at 37ºC for 10 minutes with occasional shaking then drawing off the supernatant fluid.

After washing again with the HBSS, each tooth was placed in 10 ml of 0.1% collagenase in normal saline at 37ºC for 40 minutes with occasional shaking. Finally, the enzyme activity was halted by the addition of 5 ml of RPMI 1640 culture medium with 10% fetal calf serum. The tooth was then removed and the cellular suspension was centrifuged for 5 minutes at 1200 rpm at 4ºC. The supernatant fluid was drawn off and the precipitate re-suspended in 10 ml of the HBSS (4-6ºC) and filtered through a 100µm-size pore filter.

To assess cell viability, Trypan blue (1 volume per 2.5 volumes of medium) was added to the suspension and cells were examined for staining under 400× magnification by counting an average of 100 cells per field in four different fields per culture.

Measurement of Osmolality and Acidity

Osmolality and acidity of each media was determined twice with a vapor pressure Model 5500 osmometer (Wescor Inc., Logan, UT, USA) and a pH meter (TS Technology, 262, Isfahan, Iran).

Statistical Analysis

Values are expressed as the mean ± SD. The two-way analysis of variance (ANOVA) statistical test was used for comparing the percentages of viable cells among different storage media. A Tukey-HSD post-hoc test was used to determine which group is statistically different from each other. For all tested times, significance was assumed at $p<0.05$.

Results

The viability of PDL cells in egg white medium was significantly more than tap water (as negative control) in all tested times ($P<0.001$). There was also a statistically significant difference between the viability of PDL cells in egg white medium in comparison with milk ($P<0.05$). Results of this study demonstrated no statistically significant difference between egg white and Ca^{2} +/Mg^{2}-free HBSS (as the positive control).

There was no statistical difference in the viability of PDL cells between tap water (as negative control) and milk. Table 1 shows pH and osmolality values of different tested media.

The mean and standard deviation of viability of PDL cells in different tested times are summarized in Table 2.

Table 1. pH and osmolality (mosmol/kg) mean values of different tested media

Variation	HBSS	Tap Water	Milk	Egg White
pH	7.4	7.3	6.7	8.6
osmolality	280	3	286	258

Table 2. Mean of percentage viable cells to total cells ratio in different times in each medium.

Storage Medium	Mean ± SD of Percentage of Viable Cells to Total Cells				
	1 hour	2 hour	4 hour	8 hour	12 hour
Tap Water	0.00	0.00	0.00	0.00	0.00
Milk	1.6±0.89	0.80±0.83	0.00	0.00	0.00
HBSS	95±1.58	90.6±1.94	90.2±1.92	90.4±2.5	87±1.22
Egg White	93.4±1.14	90.8±2.28	90.2±1.64	86.8±1.48	87±2.91

Discussion

Because fibroblast function is affected by age, trauma, and inflammation,[13] teeth from young healthy individuals (21±5 years old) without periodontal disease were used. The teeth were extracted with great care to minimize injury to the PDL cells.

There are two methods for evaluating the efficacy of different storage media in preserving the viability of dental fibroblasts. The most common method is to first remove fibroblasts from the root surfaces and add them to a storage medium for culturing. The viability of cells is evaluated at different times and the cell line is used for the test in this method.[14]

In another method the extracted tooth is placed directly in the storage medium.[12] After a pre-determined time, the PDL cells are isolated using enzymes and the tooth is taken out of medium to evaluate cell viability. This method is identical to the primary cell culture.

Both methods have advantages and disadvantages. The principle advantage of the first method is the large number of fibroblasts made available using a fewer number of teeth in the beginning of a study. However, the biggest disadvantage is how this method differs from what actually occurs in clinical practice because cells in the proliferative phase are placed directly in the medium which is not rich in nutrients. The cellular reaction in this condition may be different from what happens in reality. The method used in the present study more closely replicated the actual clinical scenario, and the results confirmed the finding of previous studies with regard to HBSS and tap water as storage media. Unlike water, HBSS is a suitable medium for preserving the viability of PDL cells of avulsed tooth.

Both pH and osmolality are more important than chemical composition of the medium in preserving the viability of PDL cells.[15] Cellular growth occurs at an osmolality of 230-400 mosmol/kg and a pH of 6.6-7.8, but its optimal growth happens at an osmolality of 290-300 mosmol/kg and pH of 7.2-7.4.[15,16] Water osmolality and its pH are 3 mosmol/kg and 7.40-7.79, respectively. Since water is hypotonic, its use results in rapid cellular lysis.[17] HBSS osmolality and pH are 270-290 mosmol/kg and 7.2, respectively.

As mentioned previously, Rozenfarb et al. proved egg white to be a suitable medium for preserving skin fibroblasts, but differences between their study and the present study were their use of skin fibroblasts and their storage times were only 15/45/90 minutes.

Based on the results of the present study, there was no significant difference between HBSS and egg white at storage times of 1, 2, 4, 8, and 12

hours. Both were more suitable than water or milk as storage media. Differences found in the results related to used in this study compared with other studies may be due to the acidity of the milk used (Pegah Milk®,pH=6.7). Therefore, the benefit of other brands of milk as a suitable storage media for avulsed teeth cannot be ruled out.

Conclusion

Egg white is a suitable storage medium for an avulsed tooth. It is as good as using HBSS and may be preferable because it is more likely to be available at the site of a traumatic event than HBSS.

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About the Authors

Abbas Ali Khademi, DDS

Dr. Khademi is a Professor in the Department of Endodontics of the Faculty of Dentistry at Isfahan University of Medical Sciences in Isfahan, and a researcher of Torabinejad Dental Research Center, Iran.

Saeed Saei, DDS

Dr. Saei is an endodontist, Tehran, Iran.

Mohammad Reza Mohajeri, MD

Dr. Mohajeri is a Pathologist at the Mohajeri Laboratory in Isfahan, Iran.

Nooshin Mirkheshti, MD

Dr. Mirkheshti is a researcher for the East Sage Investigative Corporation, Isfahan Science & Technology Town in Isfahan, Iran.

Fatima Ghassami, MD

Dr. Ghassami is a researcher for the East Sage Investigative Corporation, Isfahan Science & Technology Town in Isfahan, Iran.

Nakisa Torabi nia, MD

Dr. Torabi nia is a Pathologist in the Department of Pathology of the Faculty of Dentistry at Isfahan University of Medical Sciences in Isfahan, Iran.

Sayyed Ali Alavi, MD

Dr. Ali Alavi is a researcher for the East Sage Investigative Corporation, Isfahan Science & Technology Town in Isfahan, Iran.

e-mail: sayyedalialavi@gmail.com