

Original Article**Efficiency of Castor Oil as a Storage Medium for Avulsed Teeth in Maintaining the Viability of Periodontal Ligament Cells**Mohammadreza Nabavizadeh ¹, Abbas Abbaszadegan ², Afroz Khodabakhsi ³, Shamseddin Ahzan ⁴, Davood Mehrabani ⁵¹ Oral and Dental Diseases Research Center, Dept. of Endodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.² Dept. of Endodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.³ Undergraduate Student, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.⁴ Postgraduate Student, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.⁵ Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.**KEY WORDS**Cell viability;
Castor oil;
Tooth avulsion;
Periodontal ligament;**ABSTRACT****Statement of the Problem:** Researchers always seek a new storage medium for avulsed teeth. Castor oil is a vegetable oil with several advantages such as antimicrobial and antioxidant properties, low toxicity, and glutathione preservation capability, low cost, and high availability.**Purpose:** The purpose of this study was to evaluate and compare the capacity of castor oil as a new storage medium in preserving the viability of periodontal ligament (PDL) cells compared to Hank's balanced salt solution (HBSS) and milk.**Materials and Method:** Forty freshly extracted human teeth were divided into 3 experimental and 2 control groups. The experimental teeth were stored dry for 30 min and then immersed for 45 min in one of the following media; castor oil, HBSS, and milk. The positive and negative control groups were exposed to 0 min and 2 h of dry time respectively with no immersion in any storage medium. The teeth were then treated with dispase grade II and collagenase and the number of viable PDL cells were counted. Data were analyzed using Kruskal- Wallis test.**Results:** The percentage of viable cells treated with castor oil, HBSS and milk counted immediately after removal from these media were 46.93, 51.02 and 55.10 % respectively. The statistical analysis revealed that the value for castor oil was significantly lower than HBSS and milk ($p > 0.05$).**Conclusion:** Within the parameters of this study, it appears that castor oil cannot be served as an ideal medium for storage of avulsed tooth. More investigations under *in vivo* conditions are required to justify the results of this study.Received February 2017;
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Accepted July 2017;**Corresponding Author:** Abbaszadegan A., Dept. of Endodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran. Email: abbaszade@sums.ac.ir Tel: +98-71-36263192 Fax: +98-71-36263194

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Introduction

Avulsion is considered as the most important and serious dental injury with an incidence rate of up to 16%. [1] The best treatment protocol is the immediate replantation of the involved tooth at the accident site however; this may not be possible to occur. In case of inability to quick repositioning, the best way to ensure a high suc-

cess rate is to keep the tooth in a proper storage medium during transportation and before initiation of replantation procedure. [2]

An ideal storage medium for an avulsed tooth should have a proper pH and osmolality and be able to maintain the pulp and periodontal ligament (PDL) cells viable in an uncontaminated, physiologic condition. In

addition, it should have an accessibility and low cost. [3] Based on the literature, a wide variety of storage media have been evaluated *in vitro* and *in vivo* including water, saliva, sterile saline, culture media, milk, Hank's Balanced Salt Solution (HBSS), propolis, viaspan, coconut water, egg white, soymilk and green tea. [1, 3-7]

Among the different media tested, HBSS and milk are usually considered as standard media. [1, 4-5] In 1994, the American Association of Endodontists considered HBSS as storage medium of choice for the avulsed teeth. [8] However, this medium is not usually available at the accident sites and the environmental temperature can alter its physical properties. [9-10] As the second acceptable medium for storage of the avulsed teeth, milk is able to preserve the viability of PDL cells with a success rate of 70-90%. However, some studies have questioned the supremacy of milk compared to the other media with similar availability. [1, 11-12] Therefore, studies are continually attempting to investigate and find other alternatives.

Castor oil is a colorless to very-pale-yellow liquid with a distinct taste, obtained from the seeds of castor oil plant (*Ricinus communis*). [13] Castor oil as a vegetable oil has several advantages such as antimicrobial and antioxidant properties, low toxicity, and glutathione preservation capability, low cost, and high availability. [14-17] It also has the capacity to repair bone defects. [18] Furthermore, this substance has been previously suggested as a suitable medium for storage of rat lenses. [17] Hence, these properties may make this substance a suitable storage medium for the avulsed teeth. Therefore, this study was designed to investigate the potency of castor oil as a new storage medium in preserving the PDL cells viability compared to HBSS and milk. The null hypothesis tested was there is no difference in capability of HBSS, milk and castor oil to maintain the viability of PDL cells.

Materials and Method

The Ethics Committee of Shiraz University of Medical Sciences (Grant no. 8794124) approved this study. Forty human single-rooted teeth with closed apices and without any caries or periodontal diseases were selected. These teeth were extracted for orthodontic purposes from the patients with average age of 22.5 years. Extractions were performed atraumatically and the teeth

were washed immediately with sterile saline. The extracted teeth were held with forceps at the coronal region, and the coronal 3 mm of PDL was scraped with a surgical scalpel in an aseptic condition to remove damaged cells.

The teeth were then randomly divided into 3 experimental groups (n=10) and two control groups (n=5). In the group 1, castor oil (Sigma, USA), in the group 2, HBSS (Biosera-xc-s 2065, Biosera Ltd, Ringmer, East group Sussex, UK) and in the group 3, Milk with 2.5% fat were used as storage media. The teeth in experimental groups were dried for 30 min followed by a 45-min immersion in one of the above-mentioned solution at room temperature. Teeth in positive and negative control groups were exposed to 0 min and 2 h of dry time respectively with no immersion in any storage medium.

After transferring samples to the laboratory, they were washed two times with phosphate buffer serum, which contained 2% antibiotic (Pen/St, Fun 0.5%, Gen 0.5%, Gibco, USA).

After that, each tooth was incubated for 30 min at 37°C in 15 mL falcon tubes containing 2.5 mL of 0.2 mg mL⁻¹ collagenase II (Sigma-St. Louis, Mo, USA) in phosphate-buffered saline and a 2.4 mg mL⁻¹ dispase grade II (Roch, Mannheim, Germany) in phosphate-buffered saline.

After incubation, 50 µL of fetal bovine serum (Gibco, USA) was added to each tube using a micropipette. All tubes were then centrifuged for 4 min at 1200 rpm, 1 mL of cell culture was added to the plates, and supernatant was then removed with sterile micropipettes. The cells were labeled with 0.4% trypan blue (Merck, Germany) and examined under the light microscope (Nikon, Tokyo, Japan) with magnification 20×. Immediately after the above procedure, the numbers of viable and nonviable cells were counted using a haemocytometer. The percentage of viable cells was calculated using the following mathematical equation according to Ozan *et al.*, [10] total number of viable cells/total number of cells (viable + nonviable) × 100.

After counting the number of viable cells, data were analyzed with Kruskal-Wallis test with SPSS software version 15.0 (SPSS, Inc., Chicago, IL).

For examining of the PDL cells' morphology, isolated PDL cells were transferred to 25 cm cell culturing

flask containing 1% DMEM (Dulbecco's Modified Eagle's Medium, Bio-Shield, USA).

Results

Microscopic analysis showed that before culturing, the cells were spherical and floated but after that, in all experimental groups, the cells were attached to the bottom of the dishes and they were spindle like. The positive control teeth demonstrated the highest percentage of viable PDL cells followed in rank order by milk, HBSS, castor oil and the negative control. There was a significant difference between control groups and experimental groups in the number of viable cells ($p < 0.05$). The percentage of viable cells in castor oil group was significantly lower than HBSS and milk group ($p < 0.05$). There was no significant difference between HBSS and milk group ($p > 0.05$). The results are summarized in Table 1.

Table 1: Percentage of viable cells for the various test groups

	Median	Min ± SD
Castor oil	46.93 ^{A*}	(46.93±3.24)
HBSS	51.02 ^{B*}	(52.85±4.04)
Milk	55.10 ^{B*}	(61.02±2.55)
Negative control	12.36 ^{C*}	(8.67±1.95)
Positive control	89.79 ^{D*}	(90.30±4.79)

* Different superscript letters indicate statistically significant difference

Discussion

Inflammatory root resorption and replacement resorption are the most common and important complications after replantation of the avulsed teeth. [19] Prognosis of the avulsed tooth depends on critical factors such as extra oral time and the quality of storage medium. [3, 20] Hence, to avoid detrimental damages to the PDL cells, a suitable storage medium capable of preserving cells vitality should be available at the time of avulsion. [21-22] We found that the ability of castor oil in maintaining the viability of PDL cells was lower than other experimental group. The result of this study also demonstrated that HBSS and milk had similar efficiency in preserving the viability of PDL cells. Nutritional substances such as amino acids, carbohydrates and vitamins in milk probably provide these favorable results. [23-28]

In this study, we used the primary cell culture method suggested by Doyle *et al.* [20] In this method, viability of fibroblast cells isolated from the teeth surfaces are directly used to assess the potency of storage

media. This method is identical to what really happens to PDL cells following avulsion. It should be noted that there are two ways for evaluating the capacity of each media for maintaining the viability of fibroblast cells. The more commonly used method is called cell line method in which the fibroblast cells will be first isolated from the root surfaces and will be cultured, then, the viability will be assessed following their immersion in the experimental media. [29] In this method, cells isolated from the culture medium contain more nutrients in contrast with what in really happens before replantation.

According to Andreasen *et al.*, [2] replantation of the avulsed teeth before 30 minutes has better predictable results to survive more PDL cells. In this study, teeth were remained dry for 30 min before storing them in the experimental media in order to close the condition of this study to clinical situation.

Evaluation of the viability and counting the PDL cells was performed according to methodology suggested by Pileggi *et al.* [30] In this method, cells exposure to active trypsin decrease in order to preserve maximum cell viability. By this method, both collagenase and disperse enzymes were used to allow rapid cell retrieval and maintain maximum cellular integrity. This method is because the color of cells with damaged membrane will be changed by allowing the trypan blue dye pass through membrane into cytoplasm. This method is in contrast with stepwise trypsinization technique recommended by Reinholt *et al.* [31] in which each sample is in contact with trypsin for 30 min.

In a recent investigation by Holm *et al.*, [17] the efficiency of castor oil as a medium for transplantation of rat lens was evaluated and revealed that castor oil is a promising storing solution due to its glutathione preservation capability and antioxidant properties. Given that the content of glutathione inside the cells helps to preserve proteins and prevents damage to the cellular membrane, [32-33] other storage media for avulsed teeth such as viaspan also contains glutathione. [34] Therefore, it is likely that the presence of this element in chemical constituent of these media might improve their potency to preserve cell vitality and protection against oxygen radicals. It is notable that Martinez *et al.* [35] who used mineral oils for preserving the water and osmolality of ovule previously suggested the use of oil as a maturation medium.

In the present study, HBSS was considered as a standard solution however, Marino *et al.* [36] and Thomas *et al.* [37] demonstrated that the milk has better results in maintaining the viability of PDL cells over HBSS. We also observed that milk could be able to preserve more viability for PDL cells than HBSS; however, this difference was not significant.

In our study, milk with low fat was used because it was more proper for maintaining the viability of PDL cells [38] and probably more available in the accident sites than other storage media. Furthermore, it has also compatible physiologic pH and osmolality. [21-22] It is notable that the storage of PDL cells in solutions with improper osmolality may cause damage due to alteration in the fluid content of the cells. [39]

One limitation for this study, which is in common in all cell coloration methods, is that trypan blue can only illustrate the viability of the cells not their exact healthy condition. Therefore, further *in vivo* investigations should be performed to validate the results of this study.

The null hypothesis of this study was rejected and castor oil was not able to preserve the viability of PDL cells efficiently comparable to HBSS and milk. Our results for castor oil in maintain the viability of PDL cells was also in contrast with the previous results reported for contact lenses. This discrepancy may be interpreted by different nature of lens and PDL cells in terms of blood circulation and their need for oxygen consumptions.

Conclusion

Under the experimental condition of this study, the ability of castor oil to maintain the viability of PDL cells was lower than milk and HBSS. More investigations under *in vivo* conditions should be performed to confirm the results of this study.

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Conflict of Interest

The authors of this manuscript certify that they have no conflict of interest.

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