

# Evaluation of Periodontal Ligament Cell Viability in Three Different Storage Media: An in Vitro Study

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## Abstract

**Objectives:** This study was undertaken to evaluate the viability of periodontal ligament (PDL) cells of avulsed teeth in three different storage media.

**Materials and Methods:** Forty-five premolars extracted for orthodontic therapeutic purposes were randomly and equally divided into three groups based on storage media used [Group I: milk (control); Group II: aloe vera (experimental); Group III: egg white (experimental)]. Following extractions, the teeth were placed in one of the three different storage media for 30 minutes, following which the scrapings of the PDL from these teeth were collected in Falcon tubes containing collagenase enzyme in 2.5 mL of phosphate buffered saline. The tubes were subsequently incubated for 30 minutes and centrifuged for five minutes at 800 rpm. The obtained PDL cells were stained with Trypan Blue and were observed under optical microscope. The percentage of viable cells was calculated.

**Results:** Aloe vera showed the highest percentage of viable cells ( $114.3 \pm 8.0$ ), followed by egg white ( $100.9 \pm 6.3$ ) and milk ( $101.1 \pm 7.3$ ).

**Conclusion:** Within the limitations of this study, it appears that aloe vera maintains PDL cell viability better than egg white or milk.

**Keywords:** Tooth Avulsion; Periodontal Ligament; Aloe vera gel

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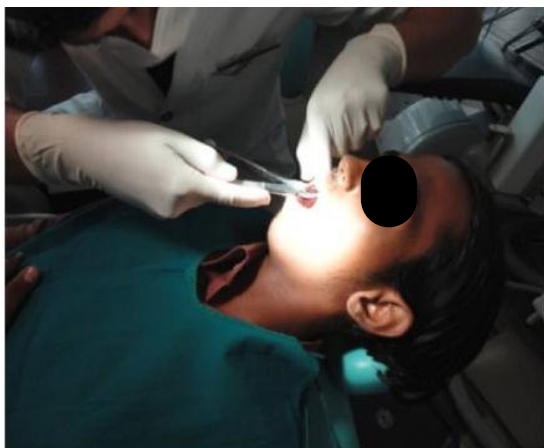
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## INTRODUCTION

Dental trauma is one of the most painful and stressful injuries in young patients and requires both expedient and expert management by a dental practitioner. Children are always keen to explore their surroundings without realizing the

risks they are exposed to. Exarticulation is one of the most complex forms of dental injury. The World Health Organization has defined exarticulation as the complete displacement of a tooth from its alveolar socket due to traumatic injury [1].



**Fig. 1.** Non-carious mature premolar teeth were extracted



**Fig. 2.** Preparation of aloe vera gel

In permanent dentition, it may occur as the result of fights or sport injuries, while falls against hard objects are among the frequent causes in the primary dentition [2]. Research has shown that an exarticulated tooth can be replanted without complications if it is reinserted into the socket within 20 minutes when stored dry, and within one to three hours if placed in a suitable storage medium. When the tooth is kept dry for more than 20 minutes, its PDL cells begin to necrotize, and upon replantation, inflammation and resorption develop in proportion to the extra-oral dry time [3]. Many storage media such as milk, saliva, Hank's balanced salt solution (HBSS),  $\alpha$  Minimum Essential Medium (MEM), propolis, Viaspan, etc. have been previously proposed as transport media. Amongst these, HBSS has been proposed as the storage medium of choice for treatment of avulsed teeth by the American Associations of Endodontists [4]. However, the major disadvantage of HBSS and many of the other aforementioned media is that they are not easily available at places where these traumatic injuries occur [5]. Hence, there is a need to identify a storage medium that will be readily available, yet effective. Aloe vera has recently gained popularity in the medicinal field because of its antidiabetic, anticancer, and antibiotic properties.

Its anti-inflammatory properties enhance wound healing whether it is used topically or orally.

Because of its increased use, the plant can be found commonly in most households, nurseries, parks and recreation areas. Also, several reputable suppliers have produced a stabilized aloe vera gel for use alone or in combined formulations [6-8].

The purpose of this study was to assess the efficacy of three easily available storage media, milk, aloe vera, and egg white, in preserving the viability of PDL cells of avulsed teeth in vitro.

## MATERIALS AND METHODS

Forty-five non-carious human premolar teeth with normal periodontium and closed apices, undergoing extraction for orthodontic therapeutic purposes, were included in this study (Fig. 1). They were divided into three equal groups using simple random allocation (using a chit-pull system).

Group I: Stored in milk (control group)

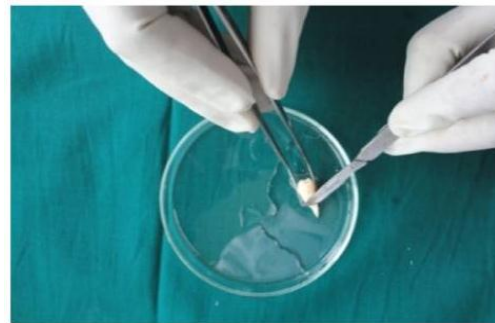
Group II: Stored in aloe vera (experimental group)

Group III: Stored in egg white (experimental group).

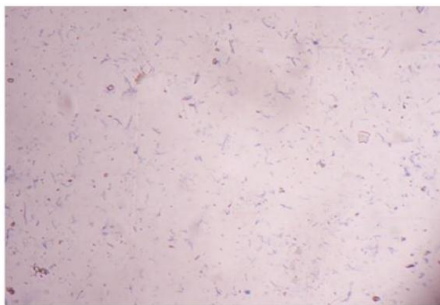
Pasteurized, homogenized milk with fat content of 3gm/100mL (Saras Dairy) at room temperature was used as the control group.



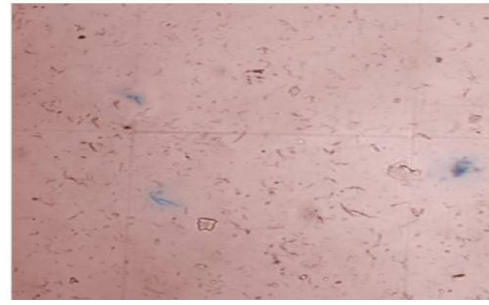
**Fig. 3.** Mud for 15 minutes to simulate avulsion injury



**Fig. 4.** PDL cells (from apical two-third of the root surface) were scraped in a petri dish



**Fig. 5.** Microscopic view of viable and non-viable cells in milk



**Fig. 6.** Microscopic view of viable and non-viable cells in aloe vera

To prepare aloe vera gel, aloe vera leaves were collected and washed. A kitchen knife was used to cut off the outer spiked edges of the leaves. After removing the outer skin of the leaves, the inner gel was extracted and transferred into a blender. The contents were blended thoroughly and filtered through a piece of muslin cloth and were placed into a glass jar with a tight fitting lid [9] (Fig. 2). Egg white was separated from the yolk and collected in a bowl using the half shelves [10]. Following extractions, the coronal third of PDL was scraped with a curette to remove cells likely to be damaged due to instrumentation [11]. To simulate avulsion injury, teeth were kept in mud for 15 minutes immediately after removal of the damaged cells from the coronal one-third [12] (Fig. 3). Teeth were then placed in three different storage media for 30 minutes. After 30 minutes, the root surface was irrigated twice with sterile isotonic saline to remove the residual storage media. The apical two-third of the root surfaces (as measured from epithelial attachment) were scraped in a petri dish to obtain the PDL cells.

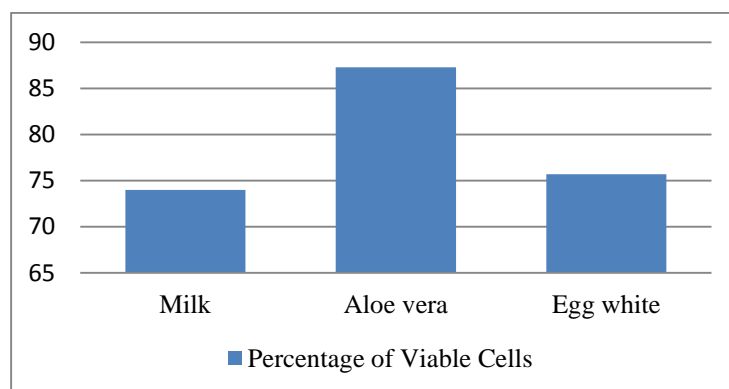
The obtained scrapings were subsequently added to a Falcon tube containing 2.5 mL of phosphate buffer [11] (Fig. 4). To the above mixture, 0.5 mg of type I collagenase was added, and this mixture was incubated for 30 minutes. Following incubation, the Falcon tubes were centrifuged for five minutes at 800 rpm [11].

The supernatant was discarded and the centrifuged residue was collected. To this, an equal volume of 0.4% Trypan Blue stain was added, and it was mixed well. Trypan Blue stains non-viable cells blue and viable cells appear colorless or pink [13].

Following staining, the cells were observed with the help of hemocytometer with an optical microscope [14].

#### ***Determination of the number of cells (total and viable):***

The cells were viewed under a microscope at  $\times 100$  magnification. The number of cells (total and non-viable) was determined by counting the cells overlying a  $4 \times 1 \text{ mm}^2$  area of the counting chamber (Figs. 5 and 6).



**Graph 1.** Percentage of viable cells

The viable cell percentage was calculated as:  $[(\text{Total cells} - \text{Stained cells}) / \text{Total Cells}] \times 100$ . The mean and standard deviation of the number of viable cells from different storage media were calculated and statistical analysis was performed. Inter-group comparison was done using one-way analysis of variance (ANOVA) complemented by the Tukey's test.

## RESULTS

The teeth stored in aloe vera demonstrated significantly higher percentage of viable PDL cells (87.3%) compared to those stored in egg white (75.7%) or milk (74.0%) (Table 1, Graph 1). Aloe vera showed higher number of viable cells when compared to milk and egg white ( $P=0.0001$  between groups I-II and II-III,  $P=0.4757$  between groups I-III, Table 2).

Post hoc power analysis was done using G power software 3.0.10. By fixing the alpha at 0.05, the calculated effect size from the given values was 0.55 and the power of the study was 0.9 or 90%.

## DISCUSSION

Recent clinical studies have shown that avulsed teeth replanted within five minutes have the best prognosis, resulting in much higher reattachment success [15].

To avoid root resorption and maintain PDL cell viability, various transport media have been proposed to store the tooth in, if immediate replantation is not possible. The basic principle behind the use of these media is to transport the tooth to an environment similar to that of the oral cavity.

Various studies have found HBSS, Viaspan, and Eagle's medium to be suitable for transportation of avulsed teeth as they preserved PDL cell viability. However, the main disadvantages of these media are high cost and lack of availability. Therefore, there should be an effort to find a storage medium that is easily available and cheap. Hence, the present study was carried out to compare the effectiveness of two of the most commonly available and cost effective storage media, aloe vera and egg white.

**Table 1.** The mean values of viable and nonviable and percentage of viable PDL cell count in the study groups and control group

Groups	Ligament cells (Mean $\pm$ SD)			
	Total	Viable	Non viable	%of viable cells
<b>I. Milk</b>	135.2 $\pm$ 7.8 (119-165)	100.1 $\pm$ 7.3 ( 90-112)	35.1 $\pm$ 6.7 ( 29-48)	74.0%
<b>II. Aloe vera</b>	130.9 $\pm$ 7.8 (113-141)	114.3 $\pm$ 8.0 ( 103-130)	16.6 $\pm$ 4.0 ( 10-20)	87.3%
<b>III. Egg white</b>	134.5 $\pm$ 11.8 (116-155)	101.9 $\pm$ 6.3 (92-112)	31.5 $\pm$ 6.3 (24-43)	75.7%

Fibroblast function is known to be affected by age, trauma and inflammation. Therefore, non-carious mature human premolar teeth undergoing extraction for orthodontic therapeutic purposes were selected. For the same reason, teeth from young healthy individuals without periodontal disease were included in the study [16]. On average it takes about 10 to 15 minutes for the victim and the person caring for him/her to recover from the traumatic event and act appropriately. According to Pohl et al, PDL cells remain in a non-compromised state with up to 15 minutes of dry time [17].

Other investigators have shown that at two hours of dry time, no vital PDL cells remain [18].

In the present study, it was decided to keep the extracted tooth in mud for at least 15 minutes to simulate avulsion injury, and to ensure that sufficient viable PDL cells are available for assessment [12].

Following a 15-minute dry time, the teeth were placed in different storage media for 30 minutes. This period was very important because the PDL cells were most susceptible to damage during this time. Hence, preservation of teeth in storage media might reduce the damage [19].

Blomlof et al. considered milk as a gold standard for transporting avulsed teeth [20].

It is a physiological medium, which contains fewer bacteria compared to saliva; therefore it is one of the most frequently used transport media. Due to these advantages, in the present study, milk was chosen as the positive control. In the present study, aloe vera gel was chosen as a transport medium because it contains 99% water and over 75 nutrients, which include 20 minerals, 19 amino acids and 12 vitamins. The human body requires 22 amino acids to maintain good health, eight of which are essential, as the body cannot synthesize them. All of these eight essential amino acids and 11 of 14 secondary amino acids are found in aloe vera. It is thought to promote healing; therefore it can be used in surgical wounds, in root canal treatment as an analgesic dressing, and around dental implants to control inflammation. In a recent study, human kidney cell death rate was found to be reduced by two-thirds when cultured in aloe gel [21,22].

The egg white was chosen due to its nutritious constituents. Egg white from a single egg contains 4.7 grams of 40 different proteins, 0.3 grams of carbohydrate, 62 milligrams of sodium, and the remainder being water [23].

**Table 2.** Inter-group comparison using one-way ANOVA complemented by the Tukey's test.

Groups	Viable cells	
	Mean $\pm$ SD	
I. Milk (control)	100.1 $\pm$ 7.3	
II. Aloe vera	114.3 $\pm$ 8.0	
III. Egg white	101.9 $\pm$ 6.3	
ANOVA		
F Statistics	17.13	
P value	< 0.01	
	<b>P value</b>	<b>Significancy</b>
I-II	0.0001	S*
I-III	0.4757	NS**
II-III	0.0001	S

\*: Significant

\*\*: Non Significant

A method suggested by Ragnarsson et al. has been quoted in the literature to evaluate the efficacy of different storage media in preserving the viability of dental fibroblasts. Ragnarsson et al. removed fibroblasts from the root surfaces and cultured them after adding them to a storage medium. The viability of cells was evaluated at different time intervals [24]. However, Doyle et al. placed the extracted teeth directly in a storage medium. At a pre-determined time, the teeth were taken out of the medium and PDL cells were isolated to evaluate cell viability. Since the method by Doyle et al. was found to closely replicate the clinical scenario, it was followed in this study [25].

To quantitate the number of viable PDL cells in the current study and to preserve maximum cell viability, the root surfaces were treated with type I collagenase for rapid cell retrieval and cellular integrity [19,26].

Trypan Blue exclusion assay quickly and easily differentiates non-viable cells from viable ones. As chromophore present on cell membranes is negatively charged, it does not take up Trypan Blue stain unless the membrane is damaged; consequently all the viable cells are excluded as they do not pick up the dye [16,27].

Trypan Blue stain used in this study assessed only viability of the cells and not the actual physiologic health and metabolic capabilities of them [11]. According to our results, maximum percentage of viable cells was found in aloe vera (87.3%), followed by egg white (75.7%) and milk (74.07%). Aloe vera maintained the highest number of viable cells, which could be attributed to its composition. It contains a glycoprotein with cell proliferating activity [28]. Aloe vera also contains allantoin, which has been found to stimulate fibroblast activity and collagen proliferation [29]. In a recent study, green tea extract also showed higher number of viable cells compared to milk, which was used as a positive control group [30]. Egg white maintained less viable cells than aloe vera, which may be attributed to its high pH (9.38), and also to the large amount of proteins in egg

white, which might act as foreign bodies. In our study, milk showed the least number of viable cells (74.0%), which could be attributed to the presence of various enzymes in the milk, which could be harmful to the fibroblasts of the PDL. Gamsen et al. found that milk was unable to regenerate depleted cell metabolites and restore the viability of PDL cells. Also, because of the lack of a cell energy source and ions in milk, repopulation of PDL was not permitted [31]. Milk was found to be a compatible storage medium only when it was cold and fresh [32].

## CONCLUSION

Immediate re-implantation is the best treatment for exarticulated teeth, provided that the teeth have viable PDL cells at the time of re-implantation. If that is not possible, storing the tooth in a storage medium would be the next best option to preserve its viability until re-implantation is possible. Within the limitations of this study, it appears that aloe vera maintains PDL cell viability better than egg white or milk. Because of its superiority as a storage medium, and other medicinal properties, it is recommended that it should be made easily available in the form of gel or other formulations over the counter, and be added to first aid kits at schools and sport clubs, so that it can be easily accessed at the time of trauma/injury to teeth. Further studies, including in vivo studies, are needed to investigate whether aloe vera can maintain PDL cell viability if maintained at lower temperatures or at extended extra oral dry time.

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