Assessment of Viability of Human Periodontal Ligament Cells in Different Fat Content of Milk at Different Time Intervals

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ABSTRACT

Introduction: Traumatic injuries are a common occurrence that require both expedient and informed management by Pediatric dentist. The greatest success of a replanted exarticulated tooth occurs when it is immediately replanted, which is not always practical. The purpose of this present study was to identifying a storage medium which is effective, economically favourable and readily available for the general population.

Material and Methods: In this study, 60 human premolars undergoing extraction for orthodontic purpose were selected. The teeth were kept in the test tube containing the 3 experimental storage media for 1,2,4,6 and 24 hours intervals. The teeth were then treated with collagenase and were incubated for 60 min after the addition of fetal bovine serum. The apical two third of the roots were scraped to obtain periodontal tissue and slides were prepared using trypan blue stain and cells were counted and statistically analyzed.

Results: Statistical analysis showed that Low fat milk preserved significantly more viable PDL cells ($p<0.05$) compared with Medium and High fat milk.

Conclusion: Low fat milk appeared to be a superior storage media in maintaining PDL cell viability when compared to Medium and High fat milk solution.

Keywords: Trauma, Exarticulated teeth, Tooth replantation, Storage media, PDL cells

INTRODUCTION

Avulsion is complete displacement of the tooth from its alveolar socket. It is characterized compromised neurovascular supply, loss of periodontal ligament cell and pulp vitality. The treatment for an avulsed permanent tooth is immediate replantation.[1,2]

Replantation is widely accepted as an effective treatment option for an avulsed tooth. However, it is dependent on various factors such as the time interval between avulsion and replantation, method of storing the avulsed tooth, the vitality status of pulp or periodontal tissues, and method of splinting. The appropriate selection of storage media is an important clinical factor affecting the postoperative prognosis of avulsed tooth following replantation.

Research has shown that exarticulated teeth can be replanted without complications if the tooth is re-inserted into the socket as soon as possible. When the tooth is dry for more than 20 mins, its periodontal ligament cell begins to necrose and on replantation, inflammation and resorption in proportion to the extra-oral dry time develops. The maintenance of viability of the cells of the periodontal ligament and cementum is essential for long term success of replanted teeth. An appropriate storage medium could maintain or improve the vitality of the cells during extra-alveolar period by preventing cell dessication.

Presently, several medias like Milk, Viaspan, H.B.S.S, Saliva, Water and many others are recommended as storage medias. Nevertheless, synthetic media are seldom available near the site of an accident rendering their use rather impractical and only of academic interest. Therefore, it would be useful to find an easily effective, accessible, readily available and economically favourable storage media for the general public which is ideal to maintain the PDL cell viability.

MATERIAL AND METHODS

The study was carried out in the Department of Pediatric Dentistry, S.D.M. College of Dental sciences, Sattur, Dharwad, Karnataka, India to assess the viability of the Periodontal ligament cells in milk of varying fat content at five different time intervals.

60 caries free human premolars with apparently normal periodontium and closed apices undergoing extraction for orthodontic treatment were selected for the study. The extractions were performed atraumatically with utmost care taken to prevent damage to periodontal ligament cells. Following extraction, the teeth were held with forceps at the coronal region and coronal 3mm of periodontal ligament cells on the root surface was scraped from the cervical margin using BP blade no.15 to remove the cells that might have been damaged during extraction. The teeth were then randomly divided into 3 groups of twenty teeth and were transferred in each storage medium namely low fat milk, medium fat milk, high fat milk which were stored in sterile test tubes. Every tooth was maintained for 1, 2, 4, 6 and 24 hours at room temperature.

Harvesting of PDL cells: After the stipulated time interval, the teeth were taken to the clinical laboratory where further procedures were carried out. The teeth were handled by the anatomical crown during the procedure to prevent damage to the periodontal cells. All teeth were then held with tweezer by grasping the coronal portion and cleansed by irrigating with phosphate buffered saline (PBS) to remove the debris, storage media etc. The teeth were incubated for 60 minutes in 10 ml test tubes with a 2.5 ml solution of 0.2 mg/ml of

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collagenase CLS 2 in PBS to minimize the exposure of cells to active trypsin and to preserve maximum cell viability. After incubation, 50µl of fetal bovine serum was added to each tube to halt the enzymatic activity of collagenase. The tube was then centrifuged for 4 minutes at 1000rpm. The supernatant was then removed with sterile micropipettes. The apical two third of the roots were scraped using number 15 BP blade to obtain periodontal tissue. These scrapings were then transferred onto the sterile slide and the cells were labelled with 0.4% trypan blue for determination of viability of the cells.

The number of viable and non viable cells were counted under light microscope with a Hemocytometer at 20X magnification (Figure 1).

The viability percentage of the cell population of each sample was obtained by applying the following mathematical equation;

(UC/ TC) X 100 = %

Where,

UC- unstained cell count (viable cells), TC- total cell count (stained + unstained cells).

There are two methods for evaluating the efficacy of different storage media in preserving the viability of dental fibroblasts.

STATISTICAL ANALYSIS

Analysis of the data was accomplished by using One-way anova and Tukey post hoc test. (Table. 1)

RESULTS

The difference was evident at the early time points when cell viability remained greater than 90% in the cells maintained in group 1 during the first 1 hour, while viability decreased to approximately 77% for group 3. The mean values of viable cell count at 1, 2, 4, 6 hours showed a higher significant values in Group 1 when compared to other groups. ($p< 0.05$). After a period of 4 hours it was seen that group 2 and 3 had no statistical significant difference. Beyond 6 hours, cell viability was significantly reduced when compared to initial values regardless of the fat content of the milk. After a period of 6 hours it was seen that group 1,2 and 3 had no statistical significant difference, therefore low fat milk is maintained cell viability at a significantly greater levels than medium and high fat milk (Figure-2).

DISCUSSION

According to the World Health Organization classification

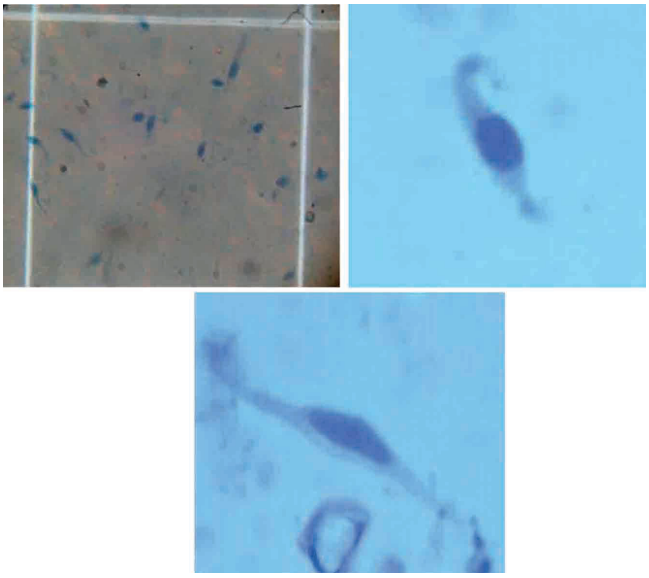

Figure-1: Non viable PDL cells under low and high power

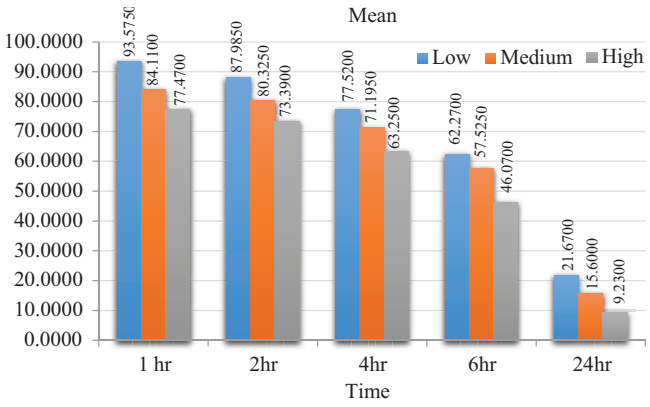

Figure-2: Percentage of viable cell count in the different study groups at different time intervals.

Variables	Source	Sum of Squares	df	Mean Square	F-value	Sig.
1hr	Between Groups	2620.3120	2	1310.1560	187.9390	0.0000
	Within Groups	397.3580	57	6.9710		
	Total	3017.6700	59			
2hr	Between Groups	2131.8920	2	1065.9460	144.5400	0.0000
	Within Groups	420.3610	57	7.3750		
	Total	2552.2530	59			
4hr	Between Groups	2045.0770	2	1022.5390	104.8910	0.0000
	Within Groups	555.6720	57	9.7490		
	Total	2600.7490	59			
6hr	Between Groups	2774.4800	2	1387.2400	109.2010	0.0000
	Within Groups	724.1010	57	12.7040		
	Total	3498.5820	59			
24hr	Between Groups	1547.8360	2	773.9180	77.1610	0.0000
	Within Groups	571.7040	57	10.0300		
	Total	2119.5400	59			

Table-1: Intra and inter comparison of study groups at time interval by one way anova

for traumatized teeth, exarticulation is the complete displacement of a tooth from its alveolar socket due to trauma. This causes severe insult to the periodontal tissues. Recent clinical studies have shown that avulsed permanent teeth should be replanted as soon as possible.[3] Immediate replantation is the ideal treatment of choice as it re-establishes the natural nutrient supply to periodontal ligament cells on the root surface, minimizing further damage, and expedites the healing process. Unfortunately, immediate replantation is not always possible. When such conditions exist, the tooth should be stored in a medium that maintains periodontal ligament cell viability until definitive dental treatment can be accomplished.

The ideal storage medium should be able to preserve cell vitality, adherence and mitogenic and clonogenic capacity[4] and should be readily available at the site of accident or be easily accessible.[5] With non-physiologic storage (e.g. prolonged tap water storage, chloramines, chlorhexidine and alcohol) the chances of pulp revascularization are minimal. With storage in physiologic media (e.g. saline, milk or saliva), there is only a weak relationship between the duration of storage and chances of pulp revascularization. The factors that play an important role in the healing of periodontal ligament after avulsion injuries are primarily the amount of physical damage to the root surface and the type of medium in which the avulsed tooth is stored. Periodontal ligament cells with normal anatomy and physiology present osmolality of 320 mOsm/Kg and pH of 7.2.[6] Optimal growth of cells is obtained between pH 7.2 to 7.4, but they can survive for long periods of time between pH 6.6 to 7.8.[7] The way which the tooth is transported also affects significantly the degree of success. The periodontal ligament fluid supplies the tooth with the nutrition necessary for the periodontal ligament cells to survive. The periodontal ligament remaining on the root after injury is dependent on a supply of vital metabolites. Cell destruction begins when these metabolites are withheld. If these cells survive, they will catalyse the reproduction of new cells, which can differentiate and reinstate the supporting tissues. The main philosophy of this survival may involve prevention of protein synthesis in the bacterial cell, encouraging the action of fibroblasts and healing of connective tissue, which contributes to the recovery of periodontal ligament after injury.[8]

Trypan blue differentiates nonviable cells from viable cells. The cells which exclude the dye were viable as the chromopore present on the cell membrane is negatively charged and take up the stain unless the membrane is not damaged.

Milk is one of the most commonly consumed dairy products. Milk is economical, simple to use and easily available to the general population and has osmolality of 230 – 270 mOsm/kg and a pH of 6.5 – 6.8 required for optimum growth of cells.

The present study used milk of varying fat content included with low fat milk containing 3% fat, medium fat milk containing 3.5% fat and high fat milk containing 4.5% fat. The mean values of viable cell count at 1, 2, 4, 6 hours showed a higher significant values in low fat when compared to medium and high fat milk. The results revealed that low fat milk maintained cell viability at a significantly greater levels than medium and high fat milk. Beyond 6 hours, cell viability was significantly reduced when compared to initial values regardless of the fat content of the milk.

Several investigators namely Blomlof and Otteskog 1980[5], Marino et al. 2000[9], compared milk with several other storage media and found that milk was gold standard to the others in maintaining the viability. Marino et al. 2000[9] showed that both regular pasteurized milk and long shelf life milk were more effective in maintaining human periodontal ligament cell viability than other storage medias. Blomlof et al. 1982[5] found that milk was capable of preserving 50% of the periodontal ligament cells from culture for up to 12 hours. In the present study the percentage of mean viable periodontal ligament cells for Milk at the different time intervals of 1,2,4,6 and 24 hours were 86, 79 74, 59 and 42 respectively. The osmolality of milk being within physiologic limits makes it a more suitable medium than hypotonic solutions. Milk contains important nutrients such as amino acids, carbohydrates and vitamins which provide a suitable environment for the survival of the cells. Another possible explanation for milk performing better in this study can also be attributed to the growth factors that are present in milk.

Belford 1997[10] in an experiment using human skin and embryonic lung fibroblasts, found that the addition of an extract of bovine milk that was rich in naturally occurring growth factor was a source of potent growth promoting activity for all mesodermal-derived cells tested. Vitamin A which is present in milk is a known antioxidant. It has been suggested that storing exarticulated teeth in a medium containing one or more antioxidants might increase replantation success.

Studies have also shown that, in cool conditions, cells have a higher percentage of viability than at room temperature, as cooler temperatures reduce cell swelling, increase cell viability, and improve recovery, all of which promote wound healing. Also, not all types of milk are equally effective as storage media. Some evidence supports the use of cold milk as an interim storage medium for avulsed teeth. Avulsed teeth stored in chilled milk for up to 1 hr can maintain sufficient number of viable periodontal ligament cells to support replantation of the tooth and the possibility of periodontal ligament healing.[11]

Commercially available milk is pasteurized which may inactivate enzymes that are potentially harmful to the periodontal ligament cells. Regular pasteurised milk has a short shelf life and requires refrigeration, which makes it less readily available at the trauma site. Thus long shelf-life milk having identical composition, pH, and osmolarity to regular milk with a storage capability of 6 months without the need for refrigeration has gained more acceptance.[12] Therefore milk, cold or otherwise, can be used as a storage medium of choice for extended extra-alveolar storage (1 to 6 hr).

As with many in-vitro studies, limitations and variability often exist. Milk although superior to water and saliva as storage medium, has not shown to have the capacity to reconstitute lost cellular metabolites. It also doesn't have the ability to maintain morphological integrity of the periodontal ligament cells.

It has been recommended that even if avulsed tooth be soaked in HBSS for 30 min before replantation, be soaked

in HBSS for 30 min before replantation as saline and milk cannot replenish depleted cell metabolites.[13]

Trypan Blue staining technique has been used to assess the cell viability in most of the studies including the present study. The health status of viable periodontal ligament cells are critical in preventing resorptive sequelae of post replantation and the Trypan blue stain used here only assess vitality of the cell and not actual physiologic health or metabolic capabilities of the cell, restraining the study. Thus, more auxiliary studies are required in this regard. There is also the possibility of intra observer bias, by the observer in counting the viable periodontal ligament cells. Despite the in-vitro limitations and variability encountered in this study, Milk demonstrated promising results in terms of maintaining periodontal ligament cell viability for a prolonged period of 6 hours and hence poses to be most reliable and gold standard storage media.

CONCLUSION

Immediate replantation is the best treatment for an avulsed tooth, provided the tooth has viable PDL cells at the time of replantation. Storage media helps in preserving the viability of the periodontal ligament cells when immediate replantation is not possible. This study evaluated the post-traumatic periodontal ligament cells viability following storage in low, medium and high fat content milk as storage media at different time intervals.

From the present study, it is concluded that

Low fat milk stands first in maintaining the PDL cell viability for longer duration than medium and high content milk.

There is steady decline in number of viable cell in all the experimental storage medias as time passes.

Milk can also be used as a storage medium but for a short period of about 6 hours after which its efficacy declines.

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