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COMPARATIVE ASSESSMENT OF PERIODONTAL LIGAMENT CELL VIABILITY USING THREE DIFFERENT STORAGE MEDIA FOR AVULSED TEETH

Clinical Research

Dr.Viddyasagar mopagar prof. HOD (Department of pediatric and preventive dentistry)

Dr.Priyenka Patil* MDS POST GRADUATE (Department of pediatric and preventive dentistry) *Corresponding Author

Dr.Neeta Padmawar snr.lecturer (Department of pediatric and preventive dentistry)

Dr.Sourabh joshi Reader (Department of pediatric and preventive dentistry)

Dr. Asha nara Reader (Department of pediatric and preventive dentistry)

Dr. Prajakta joshi snr.lecturer (Department of pediatric and preventive dentistry)

ABSTRACT

Aim: To compare and evaluate the efficacy of curcumin extract, cow milk and artificial saliva as storage media for avulsed teeth

Methods: In this study,60 premolars undergoing extraction for orthodontic purpose were selected and divided into three experimental groups, each having 20 teeth as Group A:curcumin extract Group B:cow milk,Group C:Artificial saliva. Teeth were stored for period of 4 and 24 hrs. The cell viability was evaluated using DMEM, under light microscopy at 45x magnification.

Results: Teeth stored in curcumin extract showed 68%,55% that of Periodontal liagament cells were viable, while cow milk reported 83%, 45% viable cells and artificial saliva showed 39%, 29% viable cells after 4 & 24 hours of storage time respectively.

Conclusion: Curcumin extract has the ability of replenishment of metabolites in depleted cells. Moreover it has significant superior properties than the approved evidences of cow milk especially for teeth stored for more than 4 hours.

KEYWORDS

Periodontal ligament cells, Curcumin, cow's milk, Artificial saliva.

Introduction

Dental avulsion is the most severe type of traumatic tooth injuries as it results in the complete displacement of the tooth from its socket. The ideal situation is to replant an exarticulated tooth immediately after avulsion because the extraoral time is the most important determinant factor for treatment success and for a good prognosis.[1,2]

In tooth avulsions, preservation of the vitality of the periodontal ligament (PDL) cells attached to the root surface is the primary goal till the time appropriate treatment can be obtained. This may bring about a favorable reattachment of the periodontal ligament. Soder *et al.*[3] stated that the number of viable cells on the root surface decreased with increased drying time and that after two hours, it would not be possible to demonstrate cell viability after avulsion, Therefore, the ideal treatment of choice at the time of avulsion should be immediate reimplantation, in order to reestablish the natural nutrient supply to the periodontal ligament cells. This would also minimize further damage and enhance the healing process. In situations where delayed reimpalntation takes place, prognosis and treatment outcome is compromised. During such circumstances, until a definitive dental treatment can be accomplished the tooth should be stored in a medium that maintains periodontal ligament cell viability.

The ability of a storage/transport medium to support cell viability can be more important than the extra oral time for preventing ankylosis and replacement resorption.[3,6]Various storage media such as tap water, saliva, saline, milk, culture media and Viaspan have been investigated for their ability to maintain cell viability.

The types of healing that take place after the avulsion injury may be favorable healing. (a)Healing with a normal Periodontal ligament (without root resorption) and (b) Healing with surface resorption (repair-related resorption)] and unfavorable healing. Healing with ankylosis (replacement)and (b) Healing with inflammatory resorption (infection related resorption)]. The progression of osseous replacement resorption is dependent on the age of the patient and the turnover rate of bone. It is very aggressive in young individuals and runs a protracted course in older patients. The life span of such an ankylosed tooth can vary from 2 to 20 years.[7]

It is desirable to replant the avulsed tooth as quickly as possible to ensure the maximal viability of Periodontal ligament cells attached to the root surface because, teeth are usually subjected to a period of desiccation between their avulsion and reimplantation. Therefore, as dry storage is detrimental to the preservation of the Periodontal ligament, the avulsed tooth must be prevented from drying by the use of storage media having ideal osmolarity and pH. Numbers of clinical studies have indicated a dependence of periodontal and pulpal healing on storage period and media. Therefore, in cases where an immediate reimplantation is not feasible, use of a storage medium is prudent to enhance and preserve the vitality of Periodontal ligament fibroblasts of an avulsed tooth. In a clinical setup, a medium should possess certain properties to make it an acceptable storage medium for avulsed teeth.

Krasner and Person[8] introduced a commercially available form of Hank's Balanced Salt Solution (HBSS), marketed as Save-A-Tooth, which was proven to maintain periodontal ligament cell viability. As HBBS is not readily available across the world and not commonly used. Hence, an alternative medium that is not only comparable in performance to HBBS, but is also readily available and should be inexpensive, thus considering this curcumin was included in the study.

Ideal storage medium should have antimicrobial characteristics. It should be capable of preserving the feasibility of cellular Periodontal ligament, be able to maintain the viability of periodontal fibers for an acceptable period of time, favor proliferative capacity of cells and should have the same osmolarity as that of body fluids should not react with body fluids not produce any antigen antibody reactions, reduce the risk of post-reimplantation root resorption or ankylosis have a good shelf life. It should be effective in different climate and under different conditions, wash off extraneous materials and toxic waste products and most importantly aid in the reconstitution of depleted cellular metabolites.[9]Considering the search for newer storage media and their effectiveness to keep Periodontal cells viable this study was carried out to search the best possible storage media available to keep the periodontal cells viable for t longer duration of time

Materials and methods

The study was carried out in the Department of Pediatric and

Preventive Dentistry. Ethical clearance for the study was obtained from institutional Ethical Board for carrying out the study. Sixty premolars extracted for orthodontic treatment purpose were selected for the study. Teeth with complete root formation having no root caries were included in the study. Teeth with periodontal diseases, patients with any systemic diseases were excluded from the study. Teeth which were not transferred to storage media (due to any reason) within five minutes after extraction were also excluded from the study.

Storage media used in the study included Curcumin extract (G2 gold company) ;Cold, low fat pasturized cow's milk and Artificial saliva. **(figure 1)**

Figure 1: (a) curcumin extract(G2 Gold) (b) cow's milk (c) artificial saliva

As per the formula used by Namrata et al artificial saliva was prepared that included following ingredients[10]

- 2.200 g/L gastric mucin,
- 0.381g/L sodium chloride (NaCl)
- 0.213 g/L calcium chloride ($CaCl2.2H_2O$)
- 0.738 g/L potassium hydrogen phosphate ($K_2HPO_4.3H_2O$)
- 1.114 g/L potassium chloride (KCl).

After applying inclusion and exclusion criteria teeth were randomly divided into three groups as follows:

Group 1: Curcumin extract
Group 1A: 10 premolars kept in storage medium for 4 hrs.
Group 1B: 10 premolars kept in storage medium for 24 hrs.

Group 2: Cow's milk
Group 2A: 10 premolars kept in storage medium for 4hrs
Group 2B: 10 premolars kept in storage medium for 24hrs.

Group 3: Artificial saliva
Group 3A: 10 premolars kept in storage medium for 4 hrs.
Group 3B: 10 premolars kept in storage medium for 24 hrs

Immediately after extraction the premolars under the study were placed directly into 2.5ml of the desired media under the study in a sterile container. Maximum care was taken to maintain sterilization and to avoid any type of contaminations. Teeth which could not be stored in media within five minutes after extraction were rejected from including in the study. The teeth were kept in the media for 4 and 24 hrs and at the end of desired time period were evaluated for the viability of the periodontal cells. For assessment of cells the premolars were washed with Dulbecco's Modified Eagles' Medium. The cells were obtained by carefully scraping of roots of the premolars with the help of #15 BP blade (figure 2a), then the cells were carried to micro-centrifuge tubes.

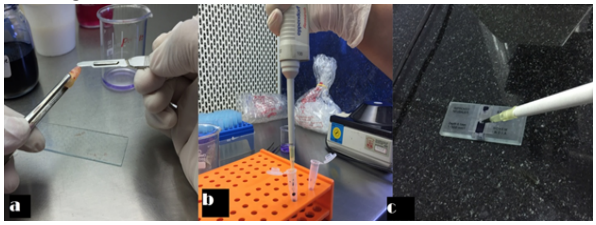


Figure 2: (a) cells were obtained by scrapping of the roots of premolars (b) Trypan blue staining was done for cell suspension (c) charging of Neubauers chamber with hemocytometer

100 µl of the cell suspension was obtained with the help of a micropipette to which 100 µl of Trypan blue stain was added (Figure 2b). The solution was transferred to Neubauers chamber.

The numbers of viable and non viable cells were counted on Neubauers chamber under the light microscope with Hemocytometer at 45X magnification. (Figure 2c)

For all the groups same procedure was followed excepting the variation in type of medium under the study. The percentage of the viable cell population of each sample was obtained by applying the following mathematical equation;

(viable cells / sum of viable and dead cells) X 100 = %
The cells were calculated and tabulated, as per the given formula.

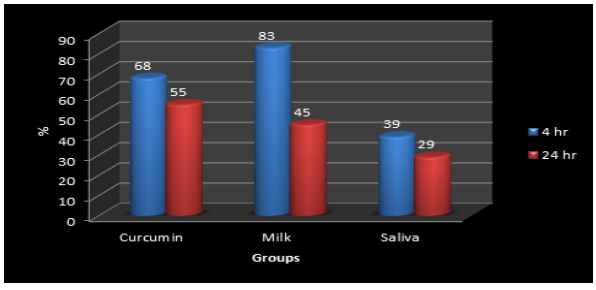
Analysis of the data was accomplished by using One-way Anova, Mann Whitney u test and paired t-test using SPPS version 21 software (IBM SPSS Statistics Inc., Chicago, Illinois, USA)

Results

The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA) Windows software program. The variables were assessed for normality using the Kolmogorov Smirnov test. Descriptive statistics included computation of percentages, means and standard deviations. Analysis of variance (ANOVA) [for quantitative data within three groups]

Present study showed 68%, 83% and 39% of the viable periodontal ligament cells in group A, Group B and Group C respectively after 4 hours of storage period . In 24 hrs of storage period study showed 55%, 45% and 29% of the viable periodontal ligament cells in Group A, group B and Group C respectively.

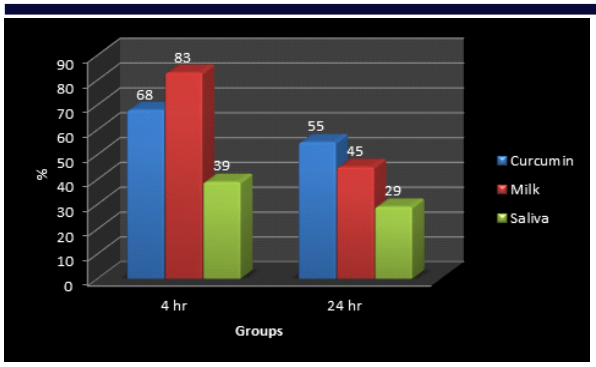
Mean values for the intragroup comparison of Group A (curcumin) at 4hrs and 24hrs were 0.68(SD± 0.05) and 0.55 (SD± 0.13) respectively. For Group B (milk) at 4hrs and 24hrs values were 0.83 (SD 0.03) and 0.45 (SD ±0.05) respectively and the mean values for Group C (saliva) at 4hrs and 24hrs were 0.39 (SD ±0.03) and 0.29 (SD± 0.02) respectively. These values when subjected for statistical analysis for intragroup comparison by using One-way anova , Mann Whitney u test and paired t-test using SPPS version 21 software (IBM SPSS Statistics Inc., Chicago, Illinois, USA) At the interval of 4 and 24 hours showed significant differences in all three groups [Group A ($p<0.003$), Group B and Group C ($p<0.001$)]. (graph 1)



Graph 1:The intragroup comparison of curcumin, cow's milk , and artificial saliva at the interval of 4 and 24hrs respectively which were statistically significant

The intergroup comparison of values of storage mediums in maintaining the viability of the cells at 4hrs between all the three Groups showed statistically significant differences. The values were highest for Group B (0.83) and lowest for Group C (0.39).

After 24 hours the values of Group A exceeded remaining two Groups (0.55 compared to 0.45 and 0.29). The intergroup comparison of values of storage mediums in maintaining the viability of the cells at 24hrs between all the three Groups also showed statistically significant differences. **(graph 2)**



Graph 2 : The intergroup comparison at the interval of 4 and 24hrs of curcumin extract, cow's milk, and artificial saliva which were statistically significant

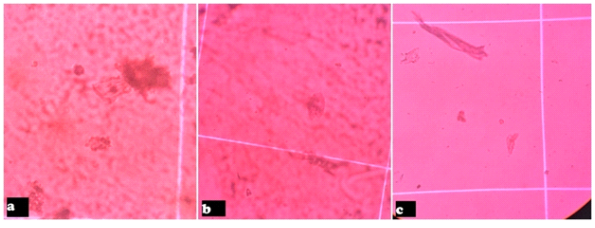


Figure 3: Histological picture showing viable periodontal ligament cells when avulsed tooth was stored (a) in curcumin extract for 24hrs (b) in cow's milk for 24hrs (c) in artificial saliva for 24hrs

Discussion

According to the World Health Organization avulsion is defined as displacement of a tooth from its alveolar socket due to trauma causing severe damage to the periodontal tissues and recent clinical studies have shown that reimplanatation should be done of permanent Avulsed tooth as early as possible11. Immediate reimplantation 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 reimplantation 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 capacity12 and should be readily available at the site of accident or be easily accessible. 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 7.2.13Optimal 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 7.8.14 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 injury15.

Dulbecco's Modified Eagles' Medium (DMEM) is a modification of Basal Medium Eagle that contains a four-fold higher concentration of amino acids and vitamins, as well as additional supplementary components. The original formula of (DMEM) contains 1000 mg/L of glucose and was first reported for culturing embryonic mouse cells. Eagle's medium at 37°C is a recommended storage medium as it can preserve the PDL for extended periods before replantation.16

Tryphan blue used in the present study 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 damaged17

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 Several investigators namely Blomlof and Otteskog 1985, Marino et al. 2009, compared milk with several other storage media and found that milk was gold standard to the others in maintaining the viability. The protein fraction of milk contains many valuable components and biologically active substances. Moreover, milk proteins are precursors of many different biologically active peptides which are inactive within the sequence of the precursor protein but can be released by enzymatic proteolysis. Many milk protein-derived peptides, such as casein phosphopeptides, reveal multifunctional bioactivities. Casein phosphopeptides can form soluble organophosphate salts and may function as carriers for different minerals, especially calcium. Furthermore, they have been shown to exert cytomodulatory effects. Cytomodulatory peptides inhibit cancer cell growth, or they stimulate the activity of immunocompetent cells and neonatal intestinal cells. Marino et al. 200018showed 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. 19825found 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 4 and 24 hrs were 83% and 45% respectively.

Curcumin has powerful natural inflammatory effects and is a very strong antioxidant properties also easily available and cost effective .The percentage of mean viable periodontal ligament cells for Curcumin at the different time intervals of 4 and 24 hrs were 68% and 55% respectively.

Saliva is one of the suitable storage medium for protection against root resorption. Saliva may be used as an immediate interim storage medium. In the present study the percentage of mean viable periodontal ligament cells for artificial saliva at the different time intervals of 4 and 24 hrs were 39% and 29% respectively.

Since curcumin extract has enough antioxidant properties, it is thought to be useful in preserving the PDL cell viability. Moreover, the high success rate of curcumin extract in protecting the cell viability might be due to its antibacterial and antifungal properties. Although this in vitro study revealed the effectiveness of the curcumin extract in keeping more viable cells, further research is essential to determine its capacity in reducing or preventing the sequelae which frequently occur following reimplantation; i.e. root resorptions.

Conclusion

This study evaluated the post-traumatic periodontal ligament cells viability following storage in curcumin extract, cow's milk and artificial saliva as storage media at different time intervals. From the findings of this study, it can be concluded that Curcumin is a better storage media as compared to milk and saliva. Curcumin can be considered as storage media to prevent desiccation of PDL cells upto the duration of 24 hrs, but for a duration upto 4hrs, milk can also be considered as a better storage media.

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