Oral Rehydration Salt-Liquid as an Alternative Storage Medium - A Preliminary Study

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***Objective**: To assess the efficacy of Oral Rehydration Salt-Liquid (ORS-L) in the maintenance of the viability of Periodontal ligament cells (PDL). **Materials and Method**: Twenty freshly extracted teeth were used for this study. They were then randomly divided into 3 groups: Positive control group - 5 teeth which were immediately subjected to collagenase assay, without immersing in ORS-L; Negative control group - 5 teeth with an extra oral dry time of 24 hours, followed by subjecting to collagense assay without immersing in ORS-L and Test group (ORS-L) - 10 teeth with an extra oral dry period of 30 minutes, followed by immersion in ORS-L for a period of 45 minutes and then subjected to collagenase assay. The mean number of viable PDL cells were counted on a hemocytometer under 20X magnification. **Results**: The mean number of viable PDL cells was highest in the positive control group. In comparison to the negative control group, the test group showed a higher number of viable PDL cells. **Conclusion**: The study found that ORS-L was an effective solution in maintaining the viability of PDL cells.*

***Keywords**. Oral Rehydration, Salt-Liquid, viability, periodontal ligament cells,*
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INTRODUCTION

Traumatic dental injuries in children and adults are a common problem. In case of tooth avulsions the primary goal is to preserve the viability of the periodontal ligament (PDL) cells attached to the root surface, until appropriate treatment can be performed. This may bring about a favorable reattachment of the periodontal ligament. The ideal treatment of choice at the time of avulsion should be immediate replantation, in order to reestablish the natural nutrient supply to the periodontal ligament cells. The viability of the periodontal cells decreases very rapidly with an increase in extra-oral dry period.[1] The ability of a storage or transport medium to support cell viability is as important as the extra oral time. The cell membrane of cells stored in saliva were more extensively damaged than the cell membrane of cells stored in a sucrose solution with a hypotonic osmolality similar to saliva.[2] The choice of storage medium for preserving traumatically avulsed teeth is important for the success of future replantation. Various storage media such as tap water, saliva, saline, milk, culture media and Viaspan have been investigated to maintain cell viability.[3]

The American Association of Endodontists (AAE) has recommended Hank's Balanced Salt Solution (HBSS) as the storage medium of choice for avulsed teeth.[4] Oral Rehydration Salt, recommended by the World Health Organization is known to replenish the body's lost electrolytes and hence can be considered to be a storage medium. The objective of this preliminary study was to examine the efficacy of Oral Rehydration Salt- Liquid (ORS-L) as a readily available storage medium for avulsed teeth.

MATERIAL AND METHOD

Twenty caries free human premolars with normal periodontium and closed apices that were extracted for orthodontic purpose were obtained for this study. The extraction was performed as atraumatically as possible. Following extraction, the teeth were held with forceps by the coronal region and the coronal 3mm of PDL was scraped with a curette to remove cells that may have been damaged during extraction. The teeth were then randomly divided into 3 groups:

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Positive control group - 5 teeth which were immediately subjected to collagenase assay, without immersing in ORS-L; Negative control group - 5 teeth with an extra oral dry time of 24 hours, followed by subjecting to collagense assay without immersing in ORS-L and Test group (ORS-L) - 10 teeth with an extra oral dry period of 30 minutes, followed by immersion in ORS-L for a period of 45 minutes and then subjected to collagenase assay. An extra-oral dry period of 30 minutes was selected because under usual circumstances it will take half an hour to reach a dental clinic.

ORS-L is commercially available in sterile tetrapacks (Jagdale Health Care, Health Care Division of Jagdale Industries Ltd., Bangalore). The composition of ORS-L medium is given in Table 1.

Table 1. Composition of ORS-L (per 200ml)

INGREDIENTS	QUANITY
Sodium chloride	250mg
Potassium chloride	300mg
Sodium citrate	580mg
Dextrose	5.4g
Carbohydrate	24g
Purified water	q.s

Collagenase Assay [5,6]

From each group, the teeth were then incubated for 30 minutes in a test tube containing 2.5ml solution of 0.2mg/ml collagenase. After incubation 50µl of fetal bovine serum was added to the test tube and centrifuged for 4 minutes at 1000 rpm. The supernatant fluid was removed with a sterile micropipette and the cells labeled with 0.4% trypan blue for determination of cell liability. The number of viable protective least significant difference PDL cells were counted under a light microscope using a hemocytometer at 20X magnification.

RESULTS

The mean number of viable PDL cells was highest in the positive control group. In comparison to the negative control group the test group showed a higher number of viable PDL cells (Table 2).

Table 2. Mean number of viable PDL cells.

Group	No. of teeth	No. of viable PDL cells Mean±SD
Positive Control	5	1461.2±84.5
Negative Control	5	57.3±16.5
Test Group (ORS-L)	10	513.5±19.2

DISCUSSION

One of the seqeulae following replantation of avulsed teeth includes inflammatory or replacement resorption.[7] An important factor for successful replantation of an avulsed tooth is maintaining the viability of periodontal ligament cells. Fibroblast function is affected by age, trauma and inflammation, hence teeth from healthy individuals without periodontal disease were used in this study.

There are two methods for evaluating the efficacy of different storage media in preserving the viability of dental fibroblasts. In one method the fibroblasts are removed from the root surface and added to a storage medium for culturing. The principle advantage of this method is that a large number of fibroblasts are made available using a fewer number of teeth in the beginning of the study. However, the biggest disadvantage is how this method differs from what actually occurs in the clinical practice because cells in the proliferative phase are placed directly in the medium which is not rich in nutrients.[8]

In the second method, the extracted tooth is placed directly in the storage medium.[9] After a pre-determined time the PDL cells are isolated using enzymes and the tooth is taken out of the medium to evaluate cell viability. This method is identical to primary cell culture. This method closely replicated the actual clinical situation.[8]

The trypan blue dye exclusion staining method was used because it is quick, simple, easily performed, and distinctively differentiates non-viable cells from viable cells. The reactivity of trypan blue, a vital dye, is based on the fact that the chromopore of the dye is negatively charged and does not interact with the cell unless the membrane is damaged. Therefore, all the cells which exclude the dye are viable.

Tap water, saliva, saline and whole cold pasteurized bovine milk have been used as storage media. Blomlof showed that the important factor in maintaining viability is the osmolarity of the transport media.[10] He found that milk was a suitable storage medium only when it was cold and fresh.[10] Teeth stored in sour milk showed a decrease in periodontal cell viability.[10] Tapwater, when used as a storage medium was found to be unsuitable due to its hypotonicity leading to rapid cell lysis.[9] Saliva was found to be more effective than tapwater but has a potential for bacterial contamination. Further, saliva is a hypotonic solution (60-80mOsm/l) that causes periodontal ligament cells to swell and burst.[11] Saline has found to be a short term storage medium because of its physiological osmolality.[12] Both pH and osmolality are more important than chemical composition of the medium in preserving the viability of PDL cells.[2]

A number of studies have shown that Hank's Balanced Salt Solution (HBSS) is a culture medium with excellent capability of maintaining viability of periodontal ligament cells.[2,13,14] It contains essential nutrients and buffering agents capable of maintaining the pH of the solution. HBSS and Viaspan have been shown to preserve avulsed teeth for extended periods at room temperature (24ºC).[3] Within 15 minutes HBSS was found to be the most effective storage medium.[15]

Propolis, a substance produced by honeybees, has also been studied for its use as a storage medium.[5] Recently, a

study concluded that coconut water kept significantly more PDL cells viable compared to Propolis, HBSS or milk.[5] Cellular growth occurs at an osmolality of 230-400mOsm/kg and a pH of 6.6-7.8, optimal growth is seen at an osmolality of 290-300mOsm/kg and a pH of 7.2-7.4. [12,16]

A popular solution used to replace fluids, electrolytes and sugars lost from the body is Oral Rehydration Salt, which has been considered to be a simple medical miracle. It is highly recommended by the WHO for children suffering from dehydration. Since, Oral Rehydration Salt is known to replace the body's lost electrolytes and fluids, it has a potential to maintain the viability of PDL cells. According to the WHO, osmolality of Oral Rehydration Salt is 245mOsm/l,[17] which is comparable to that of Hanks Balanced Salt Solution, milk and coconut water.

In our study, a fairly high number of viable PDL cells was observed with ORS-L, which is comparable to that of other storage media.[4] The composition of ORS-L is similar to that of coconut water with predominance of sodium and potassium cations. An advantage of ORS-L is its neutral pH in comparison to coconut water which is slightly acidic.[4]

Unlike coconut water, Oral Rehydration Salts is available in all countries, with no particular geographical preferences. It is available as a powder (sachets) and as a ready-to-use liquid in tetrapacks. The latter is more convenient to use as a storage medium following avulsion.

Since the objective of this pilot study was to investigate the feasibility of ORS-L as a storage media, only positive and negative control groups were used for comparison. Further investigations are in progress to compare ORS-L with other standard storage media at different extra-oral periods and immersion time intervals.

CONCLUSION

Oral Rehydration Salt - Liquid appears to be effective in maintaining viability of the PDL cells and can be used as a suitable storage medium for avulsed teeth.

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