Oral rehydration salt-liquid as a storage medium for avulsed tooth

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Abstract – Aim: To evaluate the efficacy of oral rehydration salt-liquid as a suitable medium for maintaining the periodontal ligament cell viability over different time periods and to compare its efficacy with that of two other storage media, Hanks’ balanced salt solution and milk.

Materials and Methods: A total of 130 sound- and caries-free premolars extracted atraumatically for orthodontic reasons were selected. Of these, 120 premolars were randomly divided into three experimental groups comprising 40 teeth each, for immersion in three different experimental storage media. Each tooth was subjected to 30 or 60 min of extra oral dry time. Each experimental group was further subdivided into two groups comprising 10 teeth each, based on the immersion time of 45 and 90 min, respectively. Of the remaining 10 premolars, five teeth each formed positive and negative controls. All teeth were subjected to collagenase II and dispase assay. Trypan blue dye exclusion test was used to determine the viability of the periodontal ligament cells. The number of viable cells was counted using Neubauer’s chamber. Data obtained were subjected to statistical analysis using one-way ANOVA and post hoc Tukey’s tests.

Results: There was no statistically significant difference between Hanks’ balanced salt solution and Oral Rehydration Solution-Liquid.

Conclusion: Oral Rehydration Solution-Liquid as a storage medium was found to be as efficient as Hanks balanced salt solution to maintain the viability of periodontal ligament cells, and it was found to be better than milk.

Avulsion (exarticulation) is one of the most severe forms of dental trauma, which is characterized by complete displacement of a tooth from the alveolar socket. It constitutes as much as 0.5 to 16% of all traumatic injuries to permanent anterior teeth (1). It occurs more commonly in children from 7 to 9 years of age, when the permanent incisors are erupting. Due to the complexity of this injury, the neurovascular supply is severely compromised and usually results in a loss of pulpal vitality. Ideally, immediate replantation of the avulsed tooth gives the best prognosis. However, immediate replantation of the tooth is not always feasible.

According to Andreasen, the factors that play a role in healing of the periodontal ligament after replantation of an avulsed tooth are primarily the amount of physical damage to the root surface, type of medium in which the avulsed tooth is stored and also on the extra oral dry time (1).

The fundamental philosophy for the storage of avulsed teeth is that the teeth should be stored in an environment that most closely replicates the oral environment (2). A storage medium may be defined as a physiological solution that closely replicates the oral environment to help preserve the viability of PDL cells, following avulsion. The type of storage medium used following avulsion affects the prognosis of tooth replantation (3). Previous studies have tested a variety of storage media for their ability to maintain periodontal ligament cell viability including water, milk, saline, saliva, Hanks’ balanced salt solution (HBSS), cell culture media, ViaSpan, Eagle’s medium, egg yolk medium, Gatorade, propolis, coconut water and green tea extract (2, 4–12). However, it is important to identify a storage medium that is not only effective but also easily available and economical.

Oral rehydration salt (ORS), which is comprised of salts and sugars, has been recommended by the World Health Organization to be used in conditions of dehydration, following severe diarrhoea or vomiting to replenish the electrolytes lost (13).

A pilot study by the same authors showed oral rehydration salt-liquid (ORS-L) to be a potential storage medium for avulsed teeth (14). Thus, the objective of this study was to evaluate the efficacy of ORS-L as a suitable medium for maintaining the periodontal ligament cell viability over different time periods and to compare its efficacy with that of two other storage media, Hanks’ balanced salt solution (HBSS) and milk.

Materials and method

This study was conducted in the Department of Pedodontics and Preventive Dentistry, The Oxford Dental College, Hospital and Research Centre, Bangalore,
India. The study protocol was approved by the Institutional Ethical Committee. In this study, premolars indicated for orthodontic extraction per se, were collected from healthy children aged 13-18 years. Informed consent was taken from patients and parents for the use of teeth (premolars) that were extracted for orthodontic reasons.

A total of 130 sound- and caries-free premolars which were extracted atraumatically were selected (Fig. 1). Of these teeth, 120 premolars were randomly divided into three experimental groups comprising 40 teeth each, for immersion in three different storage media, namely Hanks’ balanced salt solution (Save-A-Tooth system, Phoenix-Lazerus, Inc. Pottstown, PA, USA), pasteurized, whole cold bovine milk (Nandini, Pasteurised Toned Milk, fat \(0.3\%\)) and ORS-L (ORS-L™, Jagdale Health Care, Health Care Division of Jagdale Industries Ltd., Bangalore, India) (Table 1).

The periodontal ligament (PDL) of the extracted tooth was removed to 3 mm below the cemento-enamel junction on the root surface with a sharp sterile curette while holding the crown of the tooth with sterile forceps. This was performed to remove PDL cells that might have been damaged by the forceps during extraction (15, 16).

Each tooth was then placed in a sterile test tube and was subjected to 30 or 60 min of extra oral dry time. Each experimental group was further subdivided into two groups comprising 10 teeth each, based on the immersion time of 45 and 90 min, respectively. At the end of the immersion time in the specified storage medium, the tooth was placed in a test tube containing 2.5 ml solution of 0.2 mg ml\(^{-1}\) collagenase type II and 2.4 mg ml\(^{-1}\) solution of dispase grade II in phosphate-buffered saline for 30 min, after which 50 l of foetal bovine serum was added to inactivate further collagenase activity. The test tubes were then centrifuged for 4 min at 1000 rpm, and the supernatant was removed using sterile micropipettes. The tooth was removed from the test tube, and the cells were labelled for trypan blue dye exclusion test using trypan blue at 1:1 concentration and incubated for 5 min for the determination of cell viability. Following incubation, 20 l of cell suspension mixed with trypan blue was loaded onto a hemocytometer (Neubauer chamber, Precision Scientific Instruments Corporation, Delhi, India). Cells were examined at \(400\times\) magnification under a light microscope, and the total number of viable cells (cells

![Fig. 1. Distribution of sample.](image-url)
that were not stained) was counted in four different fields (15, 16).

Of the remaining 10 premolars, five teeth were treated with collagenase immediately after extraction (4) and formed the positive control group. The negative control group consisted of five teeth that were dried for 8 h and subjected to collagenase, followed by the similar staining procedure (15, 16).

Data collected were tabulated and subjected to statistical analysis. One-way ANOVA was applied to find the significant difference between the groups. Post hoc Tukey’s test was carried out for intergroup comparison. Significance of all the statistical tests was predetermined at a P value of 0.05 or less.

Results

The mean numbers of viable cells observed were 526.8 ± 4.66 and 1.4 ± 1.34 in the positive and negative controls, respectively (Table 2). With HBSS, a significant difference in the mean number of viable cells was observed, following an extra oral dry time of 60 min. With milk and ORS-L, the difference in mean number of viable cells was significant at both 30 and 60 min. With HBSS, a significant difference in the mean number of viable cells was observed, following an extra oral dry time of 60 min (Table 2). ORS-L was comparable to that of HBSS with regard to cell viability. There was a significant difference between milk and HBSS as well as between milk and ORS-L (Table 3).

Discussion

Damage to the attachment apparatus during an avulsion injury is unavoidable, but maintaining the viability of the PDL that is attached to the avulsed tooth is critical (17). If the periodontal ligament left attached to the root surface does not dry out, the consequences of tooth avulsion are usually minimal (18).

Where immediate replantation is not feasible, the extra-alveolar conditions may be modified by storing the tooth in a physiological storage medium. The use of such media has been associated with favourable healing outcomes. The rationale for soaking a tooth before replantation is that after an extended dry period, the root is covered with necrotic dying cells and inflammatory stimulators. Soaking in a suitable storage medium would wash out all the necrotic cells, and the remaining cells could be maintained and revitalized by the storage media constituents (19). Preservation of physiological and metabolic health of the PDL cells depends on several properties of the storage medium, namely compatible pH, osmolality, availability of cell metabolites and temperature of the storage media (20).

In an avulsed tooth, regeneration of the periodontal ligament is possible by retaining the viability of its fibroblasts. The cells that remain on the root surface after exarticulation are deprived of their blood supply and begin to immediately deplete their stored cell metabolites (21). To maintain optimal cell metabolism, these depleted cell metabolites must be replaced within 2 h (22).

Viability of the PDL cells depends on the extra oral dry time as well as duration of immersion in a suitable storage medium. Treatment guidelines for replantation of avulsed permanent teeth depend on open or closed apex and extra oral dry time of less than 60 min and exceeding 60 min (23). Thus, the present study evaluated the effect of 30 min and 60 min of extra oral dry time (9, 15, 19). An earlier study showed that an

<table>
<thead>
<tr>
<th>Storage media</th>
<th>Number of viable cells</th>
<th>Extra oral dry time 30 min (Mean ± SD)</th>
<th>Extra oral dry time 60 min (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n=10</td>
<td>n=10</td>
</tr>
<tr>
<td>HBSS</td>
<td></td>
<td>348 ± 19.25</td>
<td>331.10 ± 6.9</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td>297.5 ± 12.47</td>
<td>263.20 ± 10.13</td>
</tr>
<tr>
<td>ORS-L</td>
<td></td>
<td>343.5 ± 11.66</td>
<td>323.90 ± 7.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage media</th>
<th>Immersion time 45 min</th>
<th>P value</th>
<th>Immersion time 90 min</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS vs. milk</td>
<td>51</td>
<td>0.001*</td>
<td>68</td>
<td>0.001*</td>
</tr>
<tr>
<td>HBSS vs. ORS-L</td>
<td>5</td>
<td>0.676</td>
<td>7</td>
<td>0.052</td>
</tr>
<tr>
<td>Milk vs. ORS-L</td>
<td>46</td>
<td>0.001*</td>
<td>61</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Table 3. Comparison of the storage media in relation to cell viability
The avulsed tooth kept dry for 30 min did not need immersion in a storage medium. Replantation of the tooth following 30 min of extra oral dry time had a prognosis similar to that of an immediately replanted tooth. The critical period for reduced cell viability was observed at 60 min of dry time (24).

Oral rehydration salt (ORS) is available in a sachet, which is reconstituted with water to make standard ORS solution. This ORS solution is used to combat dehydration (13). Standard ORS is now available over the counter at any pharmacy in sealed sterile tetrapacks as ready to use solution and sold as ORS-L.

Hanks’ balanced salt solution can be considered as a gold standard among all storage media available. It is composed of essential cell nutrients that are needed in maintaining the viability of PDL cells. HBSS is marketed as a special kit, only in some countries, for use in the emergency management of avulsed teeth. This kit contains a small basket in which the avulsed tooth is suspended and submerged in HBSS. It has an osmolality of 320 mOsm and a pH of 7 (22). Gentle agitation can remove debris from the PDL during storage, and lost nutrients can be replenished by HBSS before replantation (22, 25, 26).

Milk is an easily available medium for short-term storage of avulsed teeth [20]. Milk has a pH of 6.5 to 7.2 and osmolality of 270 mOsmol kg⁻¹, which is similar to that of extracellular fluid (20). Amino acids and vitamins present in milk are capable of inactivating enzymes harmful to the PDL cells (27). Storage of isolated PDL cells in milk has been found to be favourable with respect to cell viability, cell recovery, cell swelling and experimental wound healing (28, 29). Different storage media are reported to be effective at different storage times (6, 9, 15, 21). Regular pasteurized milk has the tendency to become spoiled with time (30).

Retrieval of viable PDL cells from the root surface without damaging them is a critical step. Stepwise trypsinization (31), collagenase type II and dispase assay (9, 15) and collagenase type III assay (21) are methods of cell retrieval. In collagenase type II and dispase assay, both collagenase and dispase enzymes disrupt the extracellular matrix resulting in the release of cells, without excessive disruption and destruction of their own membrane. This method is more representative of an actual clinical situation involving tooth avulsion.

Quantification of the viable PDL cells is essential to evaluate the efficacy of the storage medium. Various methods have been used to assess proliferation and viability of PDL cells, following immersion in storage media. These include trypan blue dye exclusion test, neutral red uptake test (31, 32), fluorescein diacetate (FDA) staining and other assays (4, 12, 33). Many of these tests are time-consuming and subject the retrieved cells to extensive processing to determine their viability (9).

In the present study, trypan blue staining, which is quick, easy to perform and distinctively differentiates non-viable from viable cells (9), was used to determine the viability of the PDL cells. The principle of trypan blue dye exclusion assay lies in the differentiation between viable and non-viable cells. Cells with damaged membrane allow the trypan blue dye to pass through their membrane into cytoplasm, whereas undamaged cells exclude the dye (17). A limitation of this test is that it cannot assess the health of the viable cells and their ability to proliferate (17) as it only gives the measure of the number of viable cells present.

Assessment of PDL cell viability revealed that in all the storage media, the mean number of viable cells was higher when the extra oral dry time was 30 min, irrespective of the immersion time. It was also seen that irrespective of the immersion time, the highest number of viable PDL cells was found in HBSS, while milk showed the least. This was in accordance with earlier reports (22, 25, 34, 35).

The importance of immediate replantation following avulsion was further emphasized. The positive control showed maximum number of viable cells, because these teeth were not subjected to any dry time and were assessed for cell viability immediately after extraction. The negative control showed least number of viable cells as it was stored dry for 8 h extra orally, which would have lead to dehydration and complete loss of PDL cells.

A higher number of viable cells were seen with ORS-L, when the extra oral dry time was 30 min and immersion time was 45 min. This was attributed to the physiological osmolality, pH and composition of ORS-L, which provide essential nutrients to the PDL cells and aid in maintaining the viability of PDL cells (12, 21). ORS-L consists of essential cell nutrients, such as glucose and vital salts, in concentrations deemed adequate for cell metabolism to remain unhindered.

Following an extra oral dry time of 30 min, ORS-L was able to maintain cell viability at immersion time of 45 min and 90 min, which was comparable to that of HBSS. In comparison with milk, a significantly higher number of viable cells were seen with ORS-L.

The use of an inappropriate medium potentially increases the risk of cell necrosis, which leads to ankylosis and replacement resorption of the tooth root. To support the survival of PDL cells, both pH and osmolality of the storage medium are as important as its chemical composition (12, 13). Optimal cellular growth is reported to occur at an osmolality between 290 and 330 mOsm (20). The osmolality and pH of ORS-L used in the present study were 320 mOsm and 7.2, respectively, which is comparable to that of HBSS used (330 mOsm and pH 7).

Although the composition of ORS is well defined by WHO, it varies among countries (12). A commercially available oral rehydrating solution, Ricetral, was found to be a suitable storage medium due to its similar composition with HBSS (21). However, Gatorade, another oral rehydrating solution, was found to be incompatible when used as a storage medium (7). This is due to its harmful pH of three and osmolality of 280–360 mOsm that resulted in swelling of the PDL cells and their subsequent destruction.

In countries where HBSS is not available, ORS-L could be a hygienic/sterile suitable storage medium for avulsed teeth. However, clinical reports on the use of
ORS-L as a storage medium would be necessary to validate the in vitro results. As a weak relationship could exist between various in vitro measures of cell viability and clinical success, further clinical studies are warranted.

From the present study, it can be concluded that
1. ORS-L can be recommended as a suitable storage medium for avulsed teeth.
2. ORS-L is similar to HBSS in maintaining the viability of PDL cells.
3. ORS-L showed a significantly higher number of viable cells as compared to milk.
4. The mean number of viable cells in all three storage media was higher, following 30 min of extra oral dry time and immersion for 45 min.

References