A comparative evaluation of post traumatic periodontal ligament cell viability using four different storage media – an in vitro study

OMAR SUNIL L¹, DEVADATHAN A², JOSE JACOB², JOSEY MATHEW², IGNATIUS REX³

¹Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, 162, Poonamalle high road, Velappan chavadi, Chennai 600 077, Tamil Nadu, ²Department of Conservative Dentistry and Endodontics, Pushpagiri College of Dental Sciences, Thiruvalla 689 507, Kerala, ³Formerly at Department of Conservative Dentistry and Endodontics, Rajas Dental College, Vadakkangulum, Tirunelveli, Tamil Nadu, India.

Correspondence to: drdevaendo@gmail.com

Abstract

Maintaining the viability of the periodontal ligament cells is the key to success following replantation. The purpose of this study was to evaluate Tender Coconut Water, a potential new storage medium in maintaining viable periodontal ligament cells after simulated avulsion injury. Forty eight freshly extracted human mandibular premolars were divided into four experimental and two control groups. The positive and negative controls corresponded to 0-min and an 8-h dry time, respectively. The experimental teeth were stored dry for 30 minutes and then immersed in one of the four storage media, Hank's Balanced Salt Solution (HBSS), Milk, Saline or Tender Coconut Water (TCW) for 45 minutes. Each experimental tooth was incubated for 30 min in Falcon tubes with Collagenase and Protease in phosphate buffered saline (PBS) and centrifuged for 5 minutes at 800rpm. The supernatant were then removed with sterile micropipette, the cells
labeled with 0.4% Trypan blue and the number of viable PDL cells were counted with a hemocytometer, under a light microscope. The results were statistically analyzed with ANOVA and Scheffe’s test. The results of the present study showed the highest percentage of viable periodontal ligament cells with teeth stored in HBSS (56%) followed by TCW (47.8%). Within the parameters of this study, it appears that Tender Coconut Water may be able to maintain the periodontal ligament cell viability comparable to that of HBSS and better than milk and saline.

**Introduction**

Traumatic injuries are a common occurrence that requires both expedient and informed management by the practitioner. Andreasen *et al* (1995) predicted that the incidence of these injuries might eventually surpass the incidence of dental caries.¹

Avulsion injury, one of the most severe form of dental trauma constitute as much as 16% of all traumatic injuries to permanent anterior teeth.² Avulsion is characterized by complete displacement of the tooth from its alveolar socket. Due to the complexity of this injury, the neurovascular supply is severely compromised and usually results in a loss of pulpal vitality. Successful treatment of an avulsed tooth by replantation is dependent upon the prevention of progressive root resorption by minimizing damage to the periodontal ligament and timely endodontic treatment.²,³ In addition, it is important to preserve the vitality of the periodontal ligament tissue remaining on the root surface.³

The greatest success of a replanted avulsed tooth occurs when it is immediately replanted, which is not always feasible. Periodontal ligament cell viability can be preserved by replantation of the tooth within 15-20 minutes after avulsion or by immersing the tooth in a suitable storage medium until it can be replanted.²,⁴ According to Andreasen *et al* the factors that play a role in healing of the periodontal ligament after avulsion injuries are primarily the amount of physical damage to the root surface and the type of medium in which the exarticulated tooth is stored.⁵

Several methods have been suggested to preserve the viability of the periodontal ligament cells. Previous studies have tested a variety of storage media for their ability to maintain periodontal ligament cells viability including water, saliva, milk, saline, Hank’s balanced salt solution (HBSS), cell culture media, ViaSpan and Propolis. The media were compared for their effectiveness in preserving the viability of periodontal ligament cells as well as prevention of replacement resorption. Although there are some differences, in general, the results of
those studies showed that milk was better than saliva or water. HBSS and ViaSpan were better than milk. ViaSpan was equal to, or better than, HBSS in preserving periodontal ligament cells. Unfortunately, despite its effectiveness in maintaining periodontal ligament cells viability, ViaSpan and HBSS are expensive and are not readily available. Therefore, it is of interest to identify an effective, readily available and economically favorable storage media that can be as good as Via Span and HBSS, to maintain the periodontal ligament cells viability.

The purpose of this study was to investigate tender coconut water, a potentially new storage media in maintaining periodontal ligament cells (PDL cells) after simulated avulsion injury.

**Materials and methods**

Forty-eight caries free human mandibular premolar teeth with apparently normal periodontium and closed apices undergoing extraction for therapeutic reasons were selected for this study. Extractions were performed as atraumatically as possible. Following extraction, the teeth were held with the forceps by the coronal region, and the coronal 3 mm of PDL was scraped with the BP blade to remove the cells that may have been damaged.

The teeth were then randomly assigned into four experimental groups and two control groups. The teeth in the experimental groups were dried for 30 min, followed by a 45 min immersion in one of the four following experimental storage media: HBSS, milk, saline and tender coconut water (Figure 1). Neither the positive control teeth after extraction were not dried, nor were they stored in any solution, but rather they were immediately treated with Collagenase and Protease. The negative control teeth were bench dried for 8 hours, with no follow-up storage time, and then placed in collagenase and protease.
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Figure 1: Storage media- Normal saline, Milk, Saliva, TCW

Each tooth, after drying and immersing was incubated for 30 min in Falcon tubes with 2.5 ml solution of 0.2mg/ml of Collagenase Type IA (Sigma Chemicals) and 2.5 ml solution of 2.4 mg/ml of Protease Type IX (Sigma Chemicals) in PBS (Figure 2). All the tubes were then centrifuged for 5 minutes at 800 rpm. The supernatant were then removed with sterile micropipette, and the cells were stained with 0.4% trypan blue (Sigma chemicals) for determination of viability. The number of viable PDL cells was counted using a hemocytometer under a light microscope at 45x magnification. The results were statistically analyzed with an ANOVA and Scheffe’s test.

Results

The ANOVA demonstrated a significant difference (Table 1) among the groups. The mean values and SD in all groups are given in table 2. The teeth stored in HBSS Had the highest number of viable PDL cells followed in rank order by tender coconut water (TCW), saline and milk. The Scheffe’s test revealed statistically significance among different groups. Percentage of viable PDL
cells obtained with the four storage media compared in the study is plotted in figure 3.

**Table 2: Mean viability of cells using different media**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>± SD</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>1047.88</td>
<td>52.43</td>
<td>961</td>
</tr>
<tr>
<td>HBSS</td>
<td>586.88</td>
<td>26.20</td>
<td>544</td>
</tr>
<tr>
<td>Milk</td>
<td>240.88</td>
<td>22.07</td>
<td>199</td>
</tr>
<tr>
<td>Saline</td>
<td>334.75</td>
<td>24.42</td>
<td>296</td>
</tr>
<tr>
<td>Tender Coconut Water</td>
<td>501.00</td>
<td>26.11</td>
<td>475</td>
</tr>
<tr>
<td>Negative Control</td>
<td>3.88</td>
<td>2.36</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 1: Analysis of Variance (One Way ANOVA) comparing different**

**Table 1: Results of analysis of variance**

<table>
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<th></th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
</tr>
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<tr>
<td>Between Groups</td>
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<td>5</td>
<td>1015671.783</td>
</tr>
<tr>
<td>Within Groups</td>
<td>80325.000</td>
<td>42</td>
<td>1912.500</td>
</tr>
<tr>
<td>Total</td>
<td>5158683.917</td>
<td>47</td>
<td></td>
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**P < 0.001**

**Table 3: Results of Scheffe's Test**

<p>| Group                            | Subset alpha = 0.05 |</p>
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<th></th>
<th>Subset I</th>
<th>Subset II</th>
<th>Subset III</th>
<th>Subset IV</th>
<th>Subset V</th>
<th>Subset VI</th>
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<td>Negative Control</td>
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<tr>
<td>Milk</td>
<td>240.875</td>
<td>334.750</td>
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<tr>
<td>Saline</td>
<td>240.875</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Tender Coconut Water</td>
<td>501.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBSS</td>
<td>586.875</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>1047.88</td>
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Discussion

Tooth avulsion is the complete displacement of the tooth from the alveolus. Due to avulsion, the tearing of the periodontal ligament occurs, which leaves viable periodontal ligament cells on most of the root surface. When the tooth is outside the socket, the cells of the periodontal ligament begin to deteriorate. The prognosis of an avulsed tooth is largely dependent on the status of the periodontal ligament cells at the time of replantation. Successful management of the avulsed tooth is therefore predominantly concerned with replanting the tooth while maintaining the viability of these cells. Cellular damage to the periodontal ligament is most often the result of an extended extra-oral period (the time elapsed from avulsion until replantation), desiccation of periodontal ligament cells or storage of the tooth in an unsuitable condition prior to replantation. Andreasen and Hjorting-Hansen demonstrated that teeth replanted within 30 minutes had a better success rate. In this study, the dry time of 30 minutes was chosen, as this seems to be a critical time at which much damage has occurred to many periodontal ligament cells, yet some viable cells remain for assessment. Also, 30 minutes represents a typical clinical scenario during which the avulsed tooth may remain dry before being placed in a storage medium.

When this is not possible, placing the tooth in a storage medium is recommended. The hydrated periodontal ligament cells will maintain their viability allowing them to reattach on replantation without causing any more than minimal damage. Previous studies have used various storage media such as tap water, saliva, milk, saline, Hank’s balanced salt solution, ViaSpan, and Propolis. Hiltz and Trope in an experimental study showed HBSS to be extremely effective and matched that of ViaSpan for first 24 hours period in maintaining cell viability. Ashkenazi and colleagues reported that HBSS was effective in preserving the viability of periodontal ligament cells for up to 24 hours at 4°C and at room temperature. Physiologic saline has been recommended for many years as storage media for exarticulated teeth. Patil et al showed that the teeth air-dried for 10 minutes followed by 2 hours storage in either milk or saline demonstrated no significant difference in the number of viable cells. Studies by Blomlof et al, Oikarinen and Seppa, Pearson et al support the use of milk as a storage medium.
Tender Coconut Water (TCW) has been tested for its potential benefits on periodontal ligament cells of an avulsed tooth.\textsuperscript{21} 22 The water of tender coconut, technically the liquid endosperm, is the most nutritious wholesome beverage that nature has provided us. TCW is one of the highest sources of electrolyte known to man. The TCW contains various ions as potassium, sodium, calcium, magnesium, phosphorus and vitamins. TCW also contain various amino acids as alanine, arginine, aspartic acid, cysteine, glutamic acid, histidine, leucine, lysine, proline, phenylalanine, serine, and tyrosine. TCW is a sterile, natural isotonic beverage, with the same level of electrolyte balance as we have in our blood. TCW is compatible with the body fluids and is sterile. There are reports of TCW being used as a bacterial and plant tissue culture media.\textsuperscript{23} Mantena et al showed TCW to possess antioxidant property attributed to the presence of ascorbic acid.\textsuperscript{24} Buttke et al showed that the media possessing antioxidant property performed better than the one without antioxidant property in maintaining viability.\textsuperscript{25} The results of the present study reveals that the highest percentage of viable periodontal ligament cells are from teeth stored in HBSS(56%) followed by TCW (47.8%). The higher percentage of viable cells seen with TCW in our study may be due to the aforementioned factors. Gopikrishna V et al compared the efficacy of coconut water with Propolis, HBSS and milk in maintaining viable periodontal ligament cells and found that coconut water had significantly more PDL cells viable compared with Propolis, HBSS or milk.\textsuperscript{21,22}

As TCW could be beneficial to avulsed and replanted teeth, further in vitro and in vivo studies need to be conducted with varying dry time and longer storage time to determine the effectiveness.

**Conclusions**

Tender coconut water is able to maintain the viability of periodontal ligament cells as comparable to HBSS and better than milk and saline.

**References**


4. Oswald RJ, Harrington GW, Van Hassel HJ. A post replantation evaluation of air-


17. Martin MP, Pileggi R. A quantitative analysis of Propolis: a promising new storage


