Extra-alveolar storage media for avulsed teeth

ABSTRACT
Tooth avulsion is characterized by complete displacement of a tooth from its alveolar socket. During the extra-alveolar period, adherent cells on the root are subjected to contamination and dehydration and may become necrotic. Immediate repositioning of the tooth is not always possible, so the choice of a suitable storage medium for maintenance of periodontal ligament cell vitality is of extreme importance for the success of replantation. The American Association of Endodontists has recommended Hank's balanced salt solution as a storage medium. However, this solution is not readily available in many locations where tooth avulsions are likely to occur. This article reviews the advantages and disadvantages of various storage media for avulsed teeth reported in the literature, including known and new media, such as powdered milk and green tea extracts.

Key words: Tooth avulsion; Tooth replantation

Introduction
Tooth avulsion is characterized by complete displacement of a tooth from its alveolar socket. It is a fairly common and complicated injury that involves damage to the supporting tissues of the tooth, as well as the dental pulp. The reported incidence of complete avulsion ranges from 1 to 16% of all injuries of permanent dentition. Immediate tooth replantation is widely accepted as the best clinical treatment for an avulsed tooth. The healing pattern of an avulsed tooth after replantation in the alveolar socket will depend upon the healing potential of each cellular component of the tissues involved. Furthermore, it will depend on replantation management and patient-specific factors. Minimal damage to the periodontal ligament (PDL) is critical for regeneration of the attachment apparatus and to protect the root from resorption.

Extraoral time and storage conditions are the most crucial factors in determining the viability of the remaining PDL cells, and thus the prognosis of the avulsed tooth. Studies showed that an avulsed tooth can be replanted without complications after 1 to 3 hours of being placed in suitable storage conditions.

This article reviews the various storage media for avulsed teeth reported in the literature. A literature search was performed for relevant citations via the internet, using the key words “tooth avulsion”, “storage media”, and “PDL cell viability” to retrieve around 160 citations.
Ideal qualities of a storage medium

An ideal storage medium would be the one that is capable of preserving the vitality, mitogenicity, and clonogenic capacity of the damaged PDL in order to facilitate repopulation of the denuded root surface, thereby preventing further root resorption. According to Marino et al., the hydrogen ionic potential (pH) and osmolality of storage environments must be physiologic, as they both are important for the survival of PDL cells. The authors reported that cellular growth may occur between 290 and 330 mOsm/kg. The pH must be between 7.2 and 7.4, but growth may occur between 6.6 and 7.8. Therefore, the storage medium should have a physiological osmolality and pH, and should be maintained at an appropriate temperature to allow optimal cell growth or survival. Finally, the ideal storage medium should be readily available for use in emergency situations.

The advantages and disadvantages of the various storage media discussed in this review are detailed below and summarized in the Table.

**Tap water**

Tap water as a storage medium has shown the least desirable results. Tap water being a hypotonic medium causes rapid

<table>
<thead>
<tr>
<th>Table</th>
<th>A summary of advantages and disadvantages of storage media for avulsed teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage medium</td>
<td>Advantage</td>
</tr>
<tr>
<td>Tap water</td>
<td>Availability is not a concern</td>
</tr>
<tr>
<td>Isotonic saline</td>
<td>Has osmolality compatible with PDL cells</td>
</tr>
<tr>
<td>Human saliva</td>
<td>Available at every accident site</td>
</tr>
<tr>
<td>Eagle's culture medium</td>
<td>Can preserve PDL cells for extended periods</td>
</tr>
<tr>
<td>Milk</td>
<td>Widely available to the general public</td>
</tr>
<tr>
<td>Enfamil (milk substitute)</td>
<td>Similar to milk</td>
</tr>
<tr>
<td>Contact lens solutions</td>
<td>Available to the general public</td>
</tr>
<tr>
<td>Hank's balanced salt solution (HBSS)</td>
<td>Nearly ideal storage medium</td>
</tr>
<tr>
<td></td>
<td>Preserves teeth for up to 24 hours</td>
</tr>
<tr>
<td></td>
<td>Needs no refrigeration</td>
</tr>
<tr>
<td>Gatorade</td>
<td>Available at some sporting events</td>
</tr>
<tr>
<td>Propolis</td>
<td>As good as HBSS</td>
</tr>
<tr>
<td>Natural coconut water</td>
<td>Biologically and hermetically packed inside the coconut in a hygienic way</td>
</tr>
<tr>
<td>Probiotic solutions</td>
<td>Electrolyte composition resembles intracellular fluid</td>
</tr>
<tr>
<td>Green tea extracts</td>
<td>Able to maintain PDL cell vitality</td>
</tr>
<tr>
<td></td>
<td>• Have anti-inflammatory, antioxidant, anticarcinogenic effects</td>
</tr>
<tr>
<td>Salvia officinalis extracts</td>
<td>Have antioxidant and antimicrobial properties of flavonoids</td>
</tr>
<tr>
<td>Egg white</td>
<td>More likely to be available at the site of a traumatic event</td>
</tr>
<tr>
<td></td>
<td>• As good as HBSS</td>
</tr>
<tr>
<td>Ricetral</td>
<td>• New media and more studies are required</td>
</tr>
</tbody>
</table>
PDL cell destruction, similar to dry storage.  

**Isotonic saline (normal saline)**

Burley and Grabb used isotonic saline as a storage medium for transplants. Andreasen also used the same for mature permanent incisor replacements in monkeys during his analysis of the topography of surface and inflammatory root resorption on these replacements. They believed that isotonic saline could maintain the viability of the periodontal membrane. Isotonic saline has osmolality compatible with PDL cells. However, it does not contain metabolites necessary for PDL cell metabolism.

**Human saliva**

Andreasen compared tap water, normal saline, and human saliva as potential storage media before replantation, which showed that ankylosis (replacement resorption) was rarely found among teeth stored in saline or human saliva, whereas it was significantly increased among teeth stored in tap water. The author concluded that normal saline and saliva were suitable for storage during the extra-alveolar period. The disadvantage is that it contains potentially harmful substances, such as enzymes, bacteria and their products, which can cause harm to PDL cells.

**Eagle’s culture medium**

Various culture media have been used for storing teeth for later replantation. Eagle’s culture medium has been used for storing teeth in many studies showing that this method allowed the proliferation of the vital parts of the periodontium to cover areas of the root surface denuded of periodontal membrane or areas covered by necrotic periodontal membrane. The Eagle’s culture medium also contains a number of amino acids and vitamins, and bicarbonate that acts as a buffer. The disadvantages of Eagle’s culture medium are that it must be refrigerated and is only available in research laboratories.

**Milk**

Blomlof and Otteskog compared the survival of human PDL fibroblasts in milk and saliva. The cells were incubated in the media for varying periods of time, at different temperatures, and analyzed for viability. Milk was found to be superior to saliva as a storage medium with respect to the number of viable cells, cell size, and ability to recover. The reasons for this, according to the authors, may be that milk contains important nutritional substances such as amino acids, carbohydrates, and vitamins. Also, commercially available milk is pasteurized, which may inactivate enzymes that are potentially harmful to the PDL. Saliva on the other hand contains potentially harmful substances, such as enzymes, as well as bacteria and their products.

Huang et al. exposed cultured PDL cells from healthy extracted human teeth to milk, Alcon Optic-Free contact lens solution, K-Mart contact lens solution, saline, and Hank’s balanced salt solution (HBSS). The appearance and rate of loss of cells from the culture dishes were recorded over time at both room temperature (20°C) and 4°C. The results indicated that saline was superior to either of the contact lens solutions in its ability to maintain the viability of PDL cells. Milk at 4°C provided good short-term viability. The study supported milk as a good short-term storage medium for maintaining the viability of PDL cells in vitro.

Layug et al. proposed that milk packed in ice was the best alternative to modified Eagle’s culture medium, HBSS, saline, and saliva for temporary storage of avulsed teeth, due to its wide availability and the minimal detrimental effect it has on PDL cells. Harkacz et al. reported that milk with a lower fat content might be more appropriate in maintaining PDL cell viability than that with a higher fat content.

Ashkenazi et al. compared six different media and concluded that HBSS and milk were the most effective media for preserving the viability, mitogenicity, and clonogenic capacity of PDL fibroblasts, after storage for up to 24 hours at 4°C.

Milk has physiologic osmolality of 230 to 270 mOsm/kg and pH of 6.5 to 6.8, and provides nutrients and growth factors to cells. In addition, it is widely available to the general public, thus making it a suitable medium for storing avulsed teeth.

**Milk substitute**

Pearson et al. compared the efficacy of several milk...
substitutes, including reconstituted powdered milk, evaporated milk, or baby formula (e.g. Enfamil from Mead Johnson) with whole milk in maintaining the viability of human PDL cells on avulsed teeth. They reported that Enfamil (which is supplied in a powdered form that does not require special storage and has a shelf-life of 18 months) is a more effective storage medium for avulsed teeth than pasteurized or whole milk, for at least 4 hours. The advantage of this milk substitute is that it is readily available to the general public. However, it must be kept cold during transport and is only viable for short-term storage.

**Contact lens solution**

Contact lens solutions were found to be worse for storage than saline solution, milk, and HBSS. Sigalas _et al._ reported that the presence of preservatives in contact lens formulas was harmful to PDL cells. However, they recommended that contact lens solutions or Gatorade on ice could serve as a short-term (1 hour) storage medium, if other solutions were not readily available.

**Hank’s balanced salt solution**

Hank’s balanced salt solution is a sterile, non-toxic, pH-balanced and isotonic salt solution commonly used for irrigation of wounds and as a standard cell culture medium. Being commercially available in the Save-A-Tooth system (Phoenix-Lazerus Inc., Pottstown [PA], USA), HBSS has been recommended by the American Association of Endodontists as the storage medium to maintain PDL cell viability. Several experiments showed that it is an effective medium for the storage of avulsed teeth. A study by Ashkenazi _et al._ showed that the addition of growth factors, such as insulin-like growth factor 1 and platelet-derived growth factor–BB to HBSS made the medium more effective for preserving the viability, mitogenicity, and clonogenic capacity of PDL cells stored for 24 hours at room temperature. However, for short periods of storage (2-8 hours), the study favoured HBSS without growth factors.

The advantage of HBSS is that it is the most tested storage medium. It preserves teeth for at least 24 hours if fortified with growth factors. It also needs no refrigeration. However, the main drawback of HBSS is that it may not be readily available in many locations in which tooth avulsions are likely to occur.

**Gatorade**

Gatorade is an oral rehydrating fluid commonly sold as sports drinks and available at some sporting events. Harkacz _et al._ found Gatorade to be hypertonic (407 mOsm/kg). According to a study, apoptosis plays a major role in cell death in cells treated with Gatorade in comparison to other storage solutions. Cell death by apoptosis differs from necrosis in that the former does not elicit any inflammatory reaction in the surrounding tissues. Apoptosis may be preferable in cases of tooth avulsion because the establishment of an inflammatory reaction by necrosis in the surrounding tissues can lead to resorption. Subtle cell death by apoptosis lacks the inflammatory response, and less inflammatory reaction following replantation may result in less resorption and a better prognosis.

The advantage of Gatorade is its availability at some sporting events, and it was shown that Gatorade on ice could serve as a short-term storage medium for avulsed teeth. The disadvantage is that its osmolality is not compatible with PDL cells and thus can result in their death. However, the cell death is generally by apoptosis and less by necrosis.

**Propolis**

Propolis is a resinous yellow brown to dark brown substance that honey bees (_Apis mellifera_) collect from tree buds, sap flows, shrubs, or other botanical sources to seal unwanted open spaces in the hive, protecting it from outside contaminants. The main chemical classes present in propolis are flavonoids, phenolics, and other various aromatic compounds. Flavonoids are well-known plant compounds that have antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties.

Martin and Pileggi conducted a study indicating that propolis may be a better alternative to HBSS, milk, or saline in terms of maintaining PDL cell viability after avulsion and storage. Ozan _et al._ also determined the ability of propolis to serve as a temporary storage medium for the maintenance of PDL cell viability of avulsed teeth. The PDL
cells were obtained from healthy third molars and cultured in Dulbecco's Modified Eagle's Medium (DMEM). Cultures were subjected to 10% propolis solution, 20% propolis solution, milk with low fat content, HBSS, tap water as the negative control, and DMEM as the positive control. Tissue culture plates were incubated with experimental media at 37°C for 1, 3, 6, 12 or 24 hours. Assessment of PDL cell viability was by trypan blue exclusion test. The results showed that 10% propolis was a more effective storage medium than other media tested. The authors recommended propolis as a suitable medium for avulsed teeth.

**Natural coconut water**

Natural coconut (Cocos nucifera) water is a drink produced biologically and hermetically packed inside the coconut in a hygienic way without any contamination. Natural coconut water is sterile and has 93% water and 5% sugar composition, which gives its osmolality. It is rich in proteins, vitamins, and minerals such as potassium, calcium, and magnesium. The electrolyte composition of coconut water resembles intracellular fluid more closely than extracellular plasma. It is a hypotonic solution that is more acidic than plasma and has a specific gravity of approximately 1.020, comparable with blood plasma. Coconut water is also rich in essential amino acids including lysine, cystine, phenylalanine, histidine, and tryptophan.

Gopikrishna et al. found coconut water to be better than HBSS, propolis, or milk in maintaining viable PDL cells on simulated avulsed teeth. Being hygienic with essential amino acids and compatible osmolality, natural coconut water was also recommended as a superior transport medium for avulsed teeth. However, there were studies showing that coconut water with long shelf-life (industrialized) is not a good storage medium for avulsed teeth. The lower pH (4.7) and presence of other products in its composition, such as acidulants, antioxidants and preservatives, also interfered with its performance.

**Probiotic solutions**

Caglar et al. tested the viability of fibroblasts using HBSS, saline, *Lactobacillus reuteri* solution (probiotics), and milk. They concluded that no significant difference in the number of viable PDL cells among them was noted. Within the parameters of their study, it appears that probiotics may be able to maintain PDL cell vitality as well as HBSS, milk, or saline.

**Green tea extracts**

Green tea, extracted from *Camellia sinensis*, is a widely consumed beverage throughout the world, second only to water. Green tea extracts (GTE) contain a number of catechins including epicatechin, epicatechin gallate, and epigallocatechin. It has been reported that GTE have remarkable anti-inflammatory, antioxidant, and anticarcinogenic effects in a number of animal tumors and cell culture systems.

A recent study showed that GTE had an antimicrobial effect as a root canal irrigant. Another recent study also revealed the efficacy of GTE in maintaining the viability of human PDL cells which is similar to that of HBSS and higher than that of milk. They concluded that GTE could be a suitable alternative storage medium for avulsed teeth.

**Salvia officinalis extracts**

Ozan et al. completed a study to determine the suitability of *Salvia officinalis* (sage) extracts as a storage medium for the maintenance of PDL cell viability of avulsed teeth. They compared *S. officinalis* extract with other storage media such as HBSS, phosphate-buffered saline, and tap water. They found that *S. officinalis* extract was a more effective storage medium than the other experimental solutions. They attributed this to the antioxidant and antimicrobial properties of flavonoids in the extract. The flavonoids include phenolic compounds such as rosmarinic acid, carnosic acid, salvianolic acid, and their derivatives.

**Egg white**

Khademi et al. compared egg white, milk, HBSS (as positive control), and tap water (as negative control) as media for storing avulsed teeth. Their results indicated no significant difference between HBSS and egg white, and both were more suitable than water or milk as storage media. They concluded that egg white is a suitable storage medium for avulsed teeth, that it was as good as using HBSS and may be preferable because it is more likely to be available at the site.
of a traumatic event than HBSS.

**Ricetral**

Ricetral (FDC, India) is an oral rehydrating solution of glucose and vital salts in concentrations claimed as suitable for cell metabolism 42. A study 43 comparing Ricetral with two currently recommended solutions, HBSS and milk, revealed that PDL cell vitality was high with Ricetral and HBSS, but poor with milk 43.

**Conclusion**

Immediate repositioning of avulsed teeth is not always possible. In such cases, a storage medium is used to preserve PDL cell viability. Many storage media including isotonic saline, saliva, milk, and HBSS have been reported in the literature. The newer media reported in the literature include powdered milk, Gatorade, contact lens solutions, probiotic solutions, propolis, natural coconut water, GTE, S. officinalis extracts, egg white, and Ricetral.

Trauma (avulsion) can occur in many different locations and situations, including rural or urban areas or during sporting events. It is important to be aware of different suitable storage media for an avulsed tooth, as the availability of these storage media varies from place to place. By knowing their advantages and disadvantages, one can select the best possible storage media if more than one medium is available in a particular location or situation. More future research should be focused on new storage media to know more about their properties and how best to use them.

**References**

42. CIMS-111. CMP Medica India Pvt. Ltd. Oct-Dec 2010 (update 4); 426.

Answers to CPD Programme
Hong Kong Dental Journal
June 2012 issue

Hong Kong Dent J 2012;9:33-8

Oral bisphosphonate-related osteonecrosis of the jaws: report of six Hong Kong Chinese patients and review of the literature

1. b 2. c 3. c 4. d