

Storage Media for Avulsed Tooth: A Review

¹Dr. N Surya Vamshi, Postgraduate Student, Department of Oral Medicine and Radiology, Meghna Institute of Dental Sciences, Mallaram Village, Varni Road, Nizamabad, Telangana

²Dr. Anusuya Mishra, Senior Resident, Department of Pediatrics and Preventive Dentistry, S.C.B. Dental College and Hospital, Manglabag, Cuttack, Odisha

³Dr. Ch. Aparanjitha, Postgraduate Student, Department of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, Hyderabad

⁴Dr. Revathi Rajeshwarkar, Postgraduate Student, Department of Oral Medicine and Radiology, Kamineni Institute of Dental Sciences, Hyderabad

⁵Dr. T. Aravindh Raja, Senior Lecturer, Department of Oral Medicine and Radiology, Vivekanandha Dental College for Women, Salem

⁶Dr. Bikkina Akhila, Private Practitioner, Vishnu Dental College, Hyderabad

Corresponding Author: Dr. N Surya Vamshi, Postgraduate Student, Department of Oral Medicine and Radiology, Meghna Institute of Dental Sciences, Mallaram Village, Varni Road, Nizamabad, Telangana

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Abstract

After avulsion, immediate re-plantation is the treatment of choice to prevent further damage to the PDL cells from desiccation. If the tooth cannot be replanted for any reason, the extraoral dry time must be minimized by placing the tooth in a suitable storage medium. The optimum storage medium should have the ability to preserve the viability, mitogenicity and clonogenic capacity of the injured PDL cells and their progenitors. While HBSS, Viaspan[®], milk, normal saline, saliva and tap water are some suggested storage media for an

avulsed tooth, recent studies have verified the application of some other storage media with natural sources such as propolis, coconut water and green tea extract. Previous studies have indicated that inflammatory resorption and ankylosis are frequent sequelae after tooth replantation. For increasing the replantation success, using a suitable medium with anti-inflammatory, antioxidant and antibacterial properties would be of great advantage. The purpose of this study was to review the literature regarding all available storage media and to highlight their characteristics.

Keywords: Avulsion, Traumatic injury, Storage media

Introduction

Avulsion is one of the most complicated types of trauma to the teeth, frequently seen in children aged 7–9 years, where a total displacement of the tooth from its socket occurs. It represents 0.5%–3% of the traumatic injuries to the permanent dentition.¹ Avulsion causes the severing of the periodontal ligament (PDL) and disruption of the vascular nerve bundle, with a consequent loss of blood supply to the tooth, which can lead to pulp necrosis.²

Avulsion is characterized by complete displacement of the tooth out of the socket, resulting in severely compromised neurovascular supply of tooth, which may lead to the loss of vitality. After the avulsion, the PDL tissues begin to dehydrate.³ If the PDL attached to the root surface does not dry out, the consequences of tooth avulsion will be minimal. As immediate replantation is not always practically possible at the trauma site, an “interim transport” media often required to maintain the vitality (clonogenic and mitogenic capacity) of PDL cell during the extra-alveolar time period.⁴ The type of storage medium used following avulsion affects the prognosis of tooth replantation. As a result of the critical role of these storage media, an informed choice of a suitable medium is essential for successful replantation. The purpose of this study was to review the literature regarding all available storage media and to highlight their characteristics.

Table 1: Ideal Properties of Storage Media^{4,5,6}

- Should have antimicrobial characteristics
- Should be able to maintain the viability of periodontal fibers for an acceptable period of time
- Should not react with body fluids
- Be capable of preserving the feasibility of cellular

PDL

- Favour proliferative capacity of cells and should have the same osmolality as that of body fluids
- Not produce any antigen antibody reactions
- Reduce the risk of post-reimplantation root resorption or ankylosis.
- Have a good shelf life
- Effective in different climate and under different conditions.
- Wash off extraneous materials and toxic waste products
- Aid in the reconstitution of depleted cellular metabolites
- Readily accessible and having a low cost

Storage Medium

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.⁷

There are many solutions that have been proposed and/or tested as storage media for avulsed teeth. The following were identified and reviewed as a result of the literature search: Hank’s balanced salt solution (HBSS), Eagle’s medium (EM), milk, ViaSpan, Gatorade, propolis, tooth rescue box (Dentosafe), conditioned medium, contact lens solution, tap water, egg white, saliva, normal saline, ORS, and coconut water.⁸

Saliva: Human saliva is used as a storage medium due to its easy availability. It has a pH of 7.4–7.79 and osmolality of 30 mOsmol Kg. This hypertonic osmolality leads to cell lysis and higher rates of replacement resorption. Although it is readily available, avulsed teeth should not be stored for longer than 30 min in saliva.⁹

Saliva contains potentially harmful substances, such as enzymes, bacteria and their by-products. Patient's own saliva is the best immediate transport medium for an avulsed tooth. It is also an immediately available storage medium at all the accident sites. After trauma, several ml of saliva can easily be collected in a cup and the tooth dropped into this, or the tooth can be placed in the patient's mouth under the tongue. In an animal study, Andreasen showed that saline and saliva were suitable storage medium for protection against root resorption for short extra-alveolar periods.¹⁰ Thus saliva can be considered to be an acceptable short-term storage medium (less than 30 min) and its use should be limited to cases where the extra-alveolar duration is less and other superior storage media are not available.¹¹

Tap Water: Use of tap water to store avulsed teeth is not recommended as it is not compatible with PDL cells because of its hypotonic osmolality which causes cell lysis, and is reported to causes replacement resorption in avulsed teeth when they are place in it. It is considered the least desirable storage medium.

Blomolf et al. (1981) found that cultured human PDL cells in tap water for 1 h caused more PDL cell damage than the other physiological and non-physiological storage media tested. Hence, its use should be limited to the case where the extra-alveolar duration is less.

Normal Saline: Normal saline consist of solution of 0.90% w/v of NaCl with osmolality of 280 mOsm/kg. Despite being compatible to the cells of PDL, it lacks essential nutrients such as magnesium, calcium and glucose which are required for normal metabolic activity of PDL cells. Thus, it is not a widely accepted storage media but can be used for a short period of time if no other option is available.¹⁴ **(Figure 1: Normal Saline)**

Cvek found that avulsed teeth soaked in an isotonic saline for 30 minutes before replantation showed less resorption than those stored dry between 15 and 40 minutes.¹⁵ Martin and Pileggi found that saline had a worse behavior compared with HBSS and milk but it may be employed for short periods of time.¹⁶



Figure 1: Normal Saline

Milk: Milk can be employed as a storage media for avulsed teeth. It meets the following criteria for an interim storage media: (1) it has physiologic pH (2) ability to preserve the viability of PDL cells (3) has low bacterial count and (4) commonly available.¹⁶

Due to its physiologic osmolality and nutritive value milk is considered an acceptable interim transport medium for avulsed teeth. Its clinical efficacy is considered equivalent to HBSS for maintaining the vitality of the PDL cells of an avulsed tooth for an extended period of time (up to six hours). Low fat content milk and chilled milk has shown better results in maintaining the viability of PDL and for a longer time period. The main drawback is the presence of antigens that may interfere with the reattachment process.^{17,18}

HBSS: Hank's balanced salt solution, which is considered the gold standard for storage media. (**Figure 2: HBSS**) It comprises of sodium chloride, D-glucose, potassium chloride, sodium bicarbonate, monobasic potassium phosphate, calcium chloride, and magnesium sulfate anhydrous. It has a pH of 7.4 and osmolality of 280 mOsm/kg. Hank's balanced salt solution possesses optimal properties for the maintenance of PDL cell viability.^{19,20}

A study by Hiltz and Trope concluded that after 96 hours' storage in HBSS, 70% fibroblasts retained their vitality.²¹

The American Academy of Endodontics has accepted HBSS as an acceptable medium for the avulsed teeth because of its capability to maintain vitality and proliferative capacity of PDL for an extended period of time. HBSS is marketed as Save-A-Tooth (Save-A-Tooth; Phoenix-Lazerus Inc., Pottstown, PA, USA), to maintain PDL cell viability. Unfortunately, HBSS is not widely used in India because it is not readily available.^{22,23}

Blomlof et al. showed that the important factor in maintaining the viability is the osmolarity of the transport media. It has been reported that cell growth can occur at a range of 230-400mOsm/l. When measured in an osmometer, the osmolarity of the HBSS, milk and coconut water was found to be 295mOsm/l, 232mOsm/l, and 372mOsm/l respectively. The osmolarity of HBSS was found to be well within the range, which has enabled it to keep up the viability of the cells for a period of two hours. Blomlof found that HBSS was slightly better than milk, saliva or saline in maintaining the cell integrity. This was mainly due to its physiologic osmolarity.^{13,16}



Figure 2: HBSS

Coconut Water: Coconut water is biologically pure and sterile. It is rich in amino acids, minerals and vitamins. It is known to possess regenerative and antioxidant properties. Storage media having antioxidant properties can be more effective in maintaining the viability of PDL.²⁴

Gopikrishna et al. (2008) compared the efficacy of coconut water with propolis, HBSS and milk in maintaining viable periodontal ligament cells on simulated avulsed teeth using Collagenase-Dispase assay. They found that coconut water had significantly more PDL cells viable compared with propolis, HBSS, or milk.

Coconut water can be used to store avulsed tooth for a relatively longer period (45 minutes).²⁴ Moura et al., claimed that if the pH of coconut water is adjusted to 7.0, it can be used as storage media for upto 24 hours. This finding holds high relevance in clinical practice, where presence of life-threatening conditions like complex fractures of jaw can delay the replantation of tooth.²⁶ Due to its superior osmolarity, easy availability

and cost effectiveness, coconut water can be advocated as a viable storage media.²⁴

Eagle's Medium: Eagle's medium is a synthetic cell culture medium developed by Harry Eagle first published in 1959 in Science that can be used to maintain cells in tissue culture. It is based on 6 salts and glucose described in Earle's salts in 1934: (calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, sodium phosphate and sodium bicarbonate), supplemented with 13 essential amino acids, and 8 vitamins: thiamine (vitamin B₁), riboflavin (vitamin B₂), nicotinamide (vitamin B₃), pantothenic acid (vitamin B₅), pyridoxine (vitamin B₆), folic acid (vitamin B₉), choline, and myo-inositol (originally known as vitamin B₈).²⁷

Eagle's Minimal Essential Medium contains 4 ml of L-Glutamine; 105 IU/L of Penicillin; 100µg/mL of Streptomycin, 10µg/mL of Nystatin and calf serum (10% v/v). Ashkenazi et al. stated that Eagle's medium had relatively high viability, mitogenic and clonogenic capacity up to 8 hours of storage at 4°C. When the storage time was up to 24 hours, Eagle's medium was less effective than milk or HBSS.⁴⁷

Contact Lens Solution: Contact lenses are growing and consequently there is also great availability of solutions for cleaning contact lenses in homes, schools and centres of physical activities. These solutions are fatty acid mono ester composites with an antimicrobial cationic component. It was found to be better than tap water and gatroade but not as superior like HBSS and milk.²⁸

Oral Rehydration Solutions: Ricetral is a commercially available oral rehydration formulation, consisting of essential nutrients like glucose and vital salts which help in maintaining cell metabolism. They are marketed in sealed sterile pouches and easily

available over the counter in addition to being economical. It does not promote cell mitosis and regenerative capacity of the PDL fibroblasts. Its ability to maintain PDL cell viability was demonstrated to be equal to HBSS in a study, both retaining PDL vitality better than milk.²⁹ Mousavi et al. observed that by using ORS, the viability of the PDL cells was maintained for at least 12 hours and was similar to HBSS.³⁰

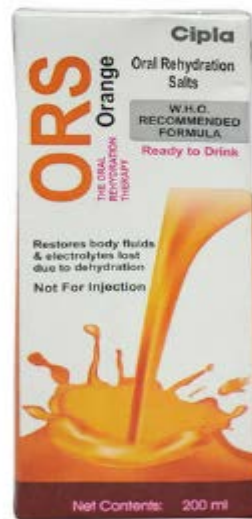


Figure 3: Oral Rehydration Solutions

ViaSpan: The ViaSpan is a medium used for the transportation of organs which are going to be transplanted and it has been very effective for storing avulsed teeth. It has an osmolality of 320 mOsm/kg and its pH is around 7.4 at room temperature, which is ideal for the cellular growth.

Hiltz and Trope have compared the vitality of lip fibroblasts, at room temperature which were stored in milk, Hank's balanced salt solution and ViaSpan. The ViaSpan was the best storage medium observed at all times, and after 168 hours, there was still 37.6% of living cells.³¹

Aloe Vera: Aloe Vera belongs to family Liliaceae with the inner gel of Aloe containing more than 75 active ingredients. The gelatinous substance contains 96% water and active properties such as vitamins, enzymes, minerals, sugars, salicylic acids, and amino acids. It has been reported that Aloe Vera has significant anti-inflammatory, antioxidant, antibacterial, antifungal and anticarcinogenic activities. A great wound healing effect has been also reported.³²

Buttke and Trope suggested that if the storage media has antioxidant ingredients, the efficacy of the media will be improved. Since Aloe Vera has enough antioxidant properties, it is thought to be useful in preserving the PDL cell viability.³³

Propolis: It is a sticky resin that sweeps from the buds of trees and mainly consists of resin, waxes fatty acids, essential oils, pollen proteins and other organic compounds and minerals. Due to its antiseptic, antibiotic, antifungal, antiviral, antioxidant, anticarcinogenic, antithrombotic and immunomodulatory properties considered as a promising medium for the maintenance of avulsed teeth.³⁴

One of the major components of propolis is flavonoids, the most important pharmacologically active constituent and powerful antioxidant, which would explain its ability to maintain cell viability. Propolis also has an antibacterial property, which assists with successful replantation and decreases the chance of inflammatory resorption-a common sequel in delayed replantation. Iron and zinc which are used in collagen synthesis are also found in propolis.

Ozan et al. (2007) compared the efficacy of propolis 10%, propolis 20%, milk and HBSS. He found that propolis was significantly more effective than HBSS and

milk at 3, 6, 24, 72 hours. However at one hour no significant difference existed among the groups.³⁵

Pomegranate Juice: Pomegranate is the fruit of *Punica granatum* (Punicaceae) that has been extensively used in the folk medicine of many cultures. In traditional medicine, pomegranate fruit has been used to treat acidosis, dysentery, microbial infections, diarrhoea, haemorrhage, respiratory pathologies, and hypertension. Besides, pomegranate is a rich source of polyphenolic flavonoids; which possess direct antioxidant properties, such as radical scavenging ability, and indirect antioxidant properties such as induction of endogenous antioxidant enzymes. Pomegranate flavonoids also have anti-inflammatory properties such as restriction of low stimuli activation of inflammation.³⁶

Tavassoli et al. (2014) in their study found that pomegranate affects the fibroblast cell proliferation. Pomegranate juice and HBSS can preserve the spindle-like morphology of periodontal fiber for 24 h after storage. Hence, it can be a good storage media. Further research is required to assess the efficacy of pomegranate juice.³⁷

Green tea: Green tea is the second most consumed drink in the world, losing only to water. Due to this fact, the effectiveness of the green tea extract (GTE) to maintain PDL cell viability was assessed and showed similar results to HBSS and better than milk. The presence of catechin in GTE, an antioxidant agent highly effective in the maintenance of cell viability, antibacterial, and anti-inflammatory properties, might explain the better result than HBSS, but this has to be confirmed by future studies, as its efficacy to prevent root resorption.^{4,6}

Hwang and Park investigated the efficacy of GTE as a storage medium for avulsed teeth and found that there

was no difference in the PDL cell viability between GTE and HBSS medium, whereas GTE showed higher viability than milk, water, and commercial green tea.⁵

Tooth Rescue Box: Dentosafe (Miradent, Germany) is the commercial name of a tooth rescue box containing special cell culture medium which is a combination of amino acid, vitamins, and glucose. In the USA, it is marketed as EMT tooth saver (Phoenix, USA). It has demonstrated the maintenance of vitality of PDL cells for 48 h at room temperature. If unopened, this medium has a shelf life of 3 years. The use of this system is self-explanatory and simple to understand for layperson. Avulsed teeth can be stored in the tooth rescue box for a longer duration, and its early availability can result in an excellent healing prognosis after replantation.^{4,38}

Conclusion

Avulsion is the worst traumatic injury to teeth; which leads to detachment of periodontal ligament from the alveolar socket. The treatment of choice for avulsion is immediate replantation of the tooth. However, due to the lack of knowledge, stress and different conditions at the time of accident, immediate replantation rarely occurs.

In this situation, maintenance of PDL vitality until provision of dental treatment is of high importance for a good prognosis. The prognosis depends on two factors: the interim transport medium and the extra-alveolar time. The capacity of a storage medium to preserve cell vitality is considered more critical than the extra-alveolar time in prevention of inflammation and replacement root resorption. To date, several studies have suggested various substances as storage media.

Because of its acceptable performance, ease of availability and lesser cost, milk remains the storage medium of choice in cases where avulsed teeth cannot be immediately reimplanted.

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