

Storage Media for Avulsed Teeth: An Overview

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Abstract: Avulsion or ex-articulation is a type of traumatic dental injury in which there is complete displacement of the tooth from its alveolar socket. The treatment option for this type of injury is reimplantation i.e. reinserting the tooth in its socket. The success of reimplantation depends on 2 factors: extra alveolar dry time (EODT) and vitality of periodontal ligament (PDL) cells attached to the tooth. The vitality of PDL cells further depends on the type of storage media used while transporting the avulsed tooth from the site of avulsion injury to the dentist. The ideal storage medium should be capable of preserving the viability of PDL cells so that cells can undergo mitosis and form clones of fibroblasts. The pH and osmolality of storage medium must be physiologic. The present article reviews various storage media from simple readily available ones to the ones that are commercially available along with the ongoing research in this field.

Keywords: avulsion, reimplantation, storage media.

I. Introduction

Dental avulsion is complete displacement of the tooth from its socket causing damage to periodontal ligament, cementum, alveolar bone, gingival and pulp tissues. Avulsed teeth can be reimplanted which means reinsertion of the tooth back to its socket. After avulsion, the portion of periodontal ligament (PDL) attached to alveolar wall remains alive and does not need treatment while that attached to the tooth affects the repairing process after reimplantation. When reimplantation is immediate; that is, when it is done up to one hour after avulsion, there is increased probability of reinsertion of dental fibers with the alveolar ones. Besides the promptness of reimplantation, the prognosis of tooth reimplantation also depends on the type of storage medium used. The maintenance of vitality of periodontal ligament attached to the tooth is lower in a dry environment. The tooth should, necessarily, remain in a humid place. There are solutions which are capable of preserving the life of cells from the periodontal ligament during the time they are out of their alveolar socket. These solutions must be used when immediate reimplantation is not possible.

The ideal storage medium should be capable of preserving the viability of PDL cells so that they undergo mitosis and form clones of the damaged fibroblasts. This is important so that fibroblasts cover the nude surface of root thus avoiding the adherence of osteoclasts. An ideal storage medium should preserve the majority of functional capacities of the cells of periodontal ligament. The pH and osmolality of storing environments must be physiologic, for both interfere in the surviving of PDL cells. It is reported that the 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.

II. Types Of Storing Environments

There are many types of storing environments for avulsed teeth.

2.1 Tap water

Tap water has been shown to have the least desirable results, though it protects the tooth from dehydration – for being a hypotonic medium – it causes rapid cellular lysis of periodontal ligament, similar to dry storage.

2.2 Saliva

Saliva can be used as a storing medium for a short period of time, since it can damage the cells of periodontal ligament if used for longer than an hour. Its osmolality is much lower than the physiologic (60-70 mOsm/kg), thus, it boosts the harming effects of bacterial contamination. Its only advantage is its availability [1].

2.3 Saline solution

The saline solution provides osmolality of 280 mOsm/kg and despite being compatible to the cells of periodontal ligament, it lacks essential nutrients such as magnesium, calcium and glucose; necessary for normal metabolic needs of the cells of periodontal ligament [2]. Krasner stated that saline solution was harmful to PDL cells in avulsed teeth if it is used for longer than two hours [3].

2.4 Contact lenses solutions

The solutions for keeping contact lenses are worse than saline solution, milk and Hank's Balanced Salt Solution (HBSS). The presence of preservatives in its formula are harmful to the cells of the periodontal ligament [4].

2.5 Propolis

Propolis is a resinous yellow brown to dark brown substance that is collected from tree buds, shrubs or other botanical sources by honey bees (*Apis mellifera*). It is used by honey bees to seal unwanted open spaces in the hive, protecting it from outside contaminants. Flavonoids, phenolics and other aromatic compounds are the main chemical compounds present in propolis. Flavonoids possess antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory properties. Martin and Pileggi [5] conducted a study and compared various storage media and it appeared that propolis may be a better alternative to HBSS, milk, or saline in terms of maintaining PDL cell viability after avulsion. Ozan et al [6] determined the ability of propolis to serve as a temporary storage medium for the maintenance of periodontal ligament (PDL) cell viability of avulsed teeth. PDL cells were obtained from healthy third molars and cultured in Dulbecco's Modified Eagles Medium (DMEM). Cultures were subjected to 10% propolis solution, 20% propolis solution, milk with lower fat content (milk), Hank's Balanced Salt Solution, tap water and Eagle's medium. The authors found that 10% propolis was more effective than other groups and it was concluded that propolis can be recommended as a suitable storage medium.

2.6 Coconut water

Coconut water is a natural, isotonic beverage that is readily accepted by the body because of its sterility, pH, and electrolytic balance. Some of the constituents are similar to that of blood plasma. The botanical name for coconut is *Cocos nucifera*, which is a nut-bearing plant. It is sterile because water permeates through the filtering husk. Components of coconut water: Potassium (mEq/L) 51.4, Sodium (mEq/L) 32.5, Magnesium (mEq/L) 16, Chloride (mEq/L) 515, Calcium (mg/dL) 17.5, Phosphate (mg/dL) 7.6. The idea that coconut water can be used as a storage medium arose from its past use as an intravenous resuscitation fluid for dehydrated patients, and also as an intravenous fluid for war patients. It has a specific gravity of approximately 1.020, comparable with blood plasma. Following avulsion, the tooth has to be carried in the shell of the coconut, because once it is exposed to air or is removed from the shell, the liquid rapidly loses most of its nutritional characteristics and begins to ferment.

Coconut water serves as a good storage medium for the avulsed tooth, which might be attributed to nutrients present in it, such as proteins, amino acids (lysine, cystine, phenylalanine, histidine, and tryptophan). This helps in nourishing the cells and maintains their viability. The osmolality of coconut water, which is close to that of plasma, and the presence of glucose and amino acids, which will nourish the PDL cells, enables it to maintain their viability. Gopikrishna et al [7] assessed the potential of coconut water in comparison with propolis, Hank's balanced salt solution (HBSS) and milk in maintaining viable periodontal ligament (PDL) cells on avulsed teeth. Results showed that coconut water kept significantly more PDL cells viable compared with propolis, HBSS or milk.

2.7 Emdogain

Although it has been shown that enamel matrix derivative (Emdogain) promotes periodontal regeneration in the treatment of intrabony periodontal defects, there is little information concerning its regenerative capacity in cases of delayed tooth replantation. According to Ashkenazi and Shaked [8] Emdogain diminishes the percentage of fibroblasts of the periodontal ligament with capability of forming colonies and that lowers the capability for the fibroblasts to repopulate the dental radicular surface after dental avulsion. Emdogain can delay, but not stop the development of replacement resorption, one of the worst complications of dental trauma. Emdogain on its own is not efficient in the regeneration of injured periodontal tissues of the avulsed tooth.

2.8 Egg white

Khademi et al [9] have compared milk and egg white as solutions for storing avulsed teeth, and the results have shown that teeth stored in egg white for 6 to 10 hours had a better incidence of repair than those

stored in milk for the same amount of time. The osmolality of the egg white is between 251 and 298 mOsm/kg. Sousa et al [10] has microscopically analyzed the human periodontal ligament attached to the extracted tooth after one hour of extraalveolar time, compared to milk, egg white and artificial saliva. The results of teeth stored in milk and egg white were similar concerning the organization of collagen fibers and the number of cells. Artificial saliva had an inferior result.

2.9 Milk

Milk is indicated as a storage medium for avulsed teeth by American Association of Endodontics [3]. Milk is significantly better than others solutions for its physiological properties, including pH and osmolality compatible to those of the cells from the periodontal ligament; the easy way of obtaining it and for being free of bacteria but it is important that it is used in the first 20 minutes after avulsion [11].

The following reasons might explain why milk is a good storage medium for the avulsed tooth:

- (a) 275 mosmo/kg osmolality;
- (b) pH 6.5–6.8, which supports cell viability;
- (c) pasteurized milk has markedly few bacteria;
- (d) milk provides essential nutrients such as aminoacids, carbohydrates and vitamins.

Trope and Friedman [12] recommend milk as an excellent storing solution for 6 hours, however, milk can not revive the degenerated cells. An avulsed tooth which has remained in a dry medium and later has been put into milk before reimplantation, will probably have as undesirable prognosis as that which has been into a dry medium and has undergone reimplantation. Studies have shown that the lower the fat content of the milk, the higher the tendency to maintain cell viability.

2.10 Gatorade

Gatorade, according to Harkacz et al [13] did not turn to be an adequate storing medium for avulsed teeth due to its pH around 2.91 and its osmolality of 407 mOsm/kg. According to Chamorro *et al.* when cells are exposed to Gatorade, it is possible that the delicate cellular membrane gets damaged because of the low pH, which makes it impossible for the cells to grow [4]. As for osmolality, because Gatorade is hypertonic, it can make cells lose water. Compared to tap water, the use of Gatorade yields better results for PDL survival [11].

2.11 Green Tea Extract

Epigallocatechin-3-gallate (EGCG) is a major polyphenol of green tea, having anti-oxidative, anti-carcinogenic, anti-mutagenic, anti-inflammatory, anti-microbial and anti-viral activities. A study showed that EGCG can be used adequately as a storage medium, with a higher potential than HBSS and milk to promote favourable reimplantation with less risk of root resorption and ankylosis [14]. Jung et al [15] also observed that the higher the extract concentration, the more efficient the medium. In view of this, the use of green tea extract and its compounds may be used as an alternative for the conservation of avulsed teeth.

2.12 Ascorbic acid

Addition of ascorbic acid to osteoblastic cell lines can stimulate type I collagen production followed by expression of specific markers associated with osteoblastic phenotypes such as alkaline phosphatase (ALP) and osteocalcin [16]. Ishikawa et al [17] studied the effect of ascorbic acid on PDL cells and observed that ascorbic acid increased ALP activity which is required for binding of PDL cells to type I collagen via 2 beta 1 integrin, whose expression is again increased by ascorbic acid.

2.13 Red mulberry (Morusrubra)

Very few studies have been done to see the effectiveness of red mulberry juice as a storage medium. Ozan et al [6] reported that when teeth were stored in red mulberry for up to 12 hours, its capacity to maintain the viability of PDL cells was better than HBSS. Malhotra N [18] found that at 4 % concentration M. rubra was more effective than HBSS up to 12 hours for maintaining PDL cell viability. More studies are required to be done before its use can be recommended.

2.14 Hank's Balanced Salt Solution (HBSS)

Hank's balanced salt solution is a standard saline solution that is widely used to support the growth of many cells types. This solution is non-toxic; it is biocompatible with periodontal ligament cells, pH balanced at 7.2 and has an osmolality of 320 mOsm/kg [19]. It is composed of 8 g/L sodium chloride, 0.4 g/L of D-glucose, 0.4 g/L potassium chloride, 0.35 g/L sodium bicarbonate, 0.09 g/L sodium phosphate, 0.14 g/L potassium phosphate, 0.14 g/L calcium chloride, 0.1 g/L magnesium chloride and 0.1 g/L magnesium sulfate (Biological

Industries, Beit Haemek). It contains ingredients such as glucose, calcium and magnesium ions which can sustain and reconstitute the depleted cellular components of the periodontal ligament cells [20].

In a study by Krasner [3], Hank's balanced salt solution was found to be the best solution for storing avulsed teeth. It has shelf life of 2 years and does not require refrigeration. This solution is effective in preserving PDL cells, renew the degenerated PDL cells and maintain a superior success rate if an avulsed tooth is soaked in them for 30 minutes. Ashkenazi et al [21] conducted a study to compare the effectiveness of different storage media for preserving viability, mitogenicity and clonogenic capacities of periodontal ligament cells. Results showed that Hank's balanced salt solution was the most effective medium for up to 24 hours at 4°C, when compared with Eagle's medium, milk, ViaSpan. HBSS is commercially available as **Save-A Tooth™** (Save-A-Tooth™, Inc., Pottstown, PA), with ideal osmolality and pH. It has an inner net to receive the avulsed tooth and to minimize cells trauma during transport.

Blomlof et al [11] showed that storage of the tooth in HBSS can extend the extraoral time up to 4 hours. Hiltz et al [22] in an extensive study showed that HBSS is effective for 24 hours with 71.3% vital cells remaining, at 48 hours, 38% cells were vital and at 120 hours, no cell survived.

2.15 Viaspan

The ViaSpan (Belzer VW-CSS, Du Pont Pharmaceuticals, Wilmington, DE, USA) 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. Main components are a hydrogen buffer and lactobionate, raffinose which prevent cellular swelling and maintain vitality of cells. ViaSpan has osmolality of 320 mOsm/kg, which enables excellent cellular growth. Its pH is around 7.4 at room temperature; ideal for the cellular growth [21]. Hiltz and Trope [22] 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. Ashkenazi et al [20] compared the effectiveness of the four storage media (Hank's balanced salt solution, culture medium, Eagle's medium and ViaSpan) concerning the preservation of fibroblasts of the periodontal ligament at room temperature (22°C). ViaSpan and culture medium, followed by Hank's balanced salt solution were the most effective in keeping the clonogenic capacity of the fibroblasts of the periodontal ligament after 24 hours, at room temperature (22°C). The culture medium, followed by Hank's balanced salt solution and ViaSpan were the most effective in keeping vitality and mitogenicity.

The small functional capacity of the fibroblast of the periodontal ligament kept in ViaSpan, its high cost, its short vitality expiration date (a couple of months) and the difficulty to find it make it difficult to find and use this storage medium.

2.16 Eagle's medium

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). Many studies demonstrated that the cell culture medium (Eagle's medium at 37°C) can preserve periodontal ligament fibroblasts for extended periods before dental reimplantation [23].

In a study by Ashkenazi et al [21] it was seen 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 as compared to milk or Hank's balanced salt solution. This might be because the low temperature (4°C) of storage may have induced aggregation and thus decreased the cell's functional capacity. Ashkenazi et al. concluded that the lower functional capacities were encountered on periodontal ligament fibroblasts stored in Eagle's medium when compared to Hank's balanced salt solution, culture medium and ViaSpan.

III. Conclusion

Success of transplantation of an avulsed tooth depends on the viability of cells of periodontal ligament attached to the tooth. Several storage media for avulsed teeth have been studied. Although commercial media like HBSS, ViaSpan and Eagle's medium have been shown to be very effective for preserving viability, mitogenicity and clonogenic capacities of PDL cells, but they are costly, have limited shelf life and not readily available. Storage media like propolis, coconut water and milk have been shown to be potential alternatives to HBSS. It is fundamental to have a storage medium easy and commercially available, for more avulsed teeth can be reimplanted, with better prognosis.

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