

CURRENT DEVELOPMENTS IN TRANSPORT MEDIA FOR AVULSED TEETH: AN UPDATE

SHREYA HEGDE, ROMA M*

Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences, Manipal University, Mangalore, Karnataka, India. Email: enettem20@gmail.com

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ABSTRACT

Dental avulsion is one of the common types of trauma that results in the complete displacement of tooth from the alveolar socket. Although the ideal treatment would be the immediate reimplantation of the tooth at the site where the trauma took place, this may not be practically possible in every case. Hence, the avulsed tooth may have to be placed in an appropriate storage or transport medium until it is reimplanted. The biological properties of the storage medium have a significant impact on the success of reimplantation, as it must be capable of preserving the vitality, clonogenic, and mitogenic ability of the periodontal ligament cells for successful reimplantation.

Keywords: Dental trauma, Avulsed teeth, Storage media, Periodontal ligament cell viability, Transport media.

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INTRODUCTION

Avulsion is defined as the complete displacement of the tooth out of its socket. This kind of dental trauma causes the periodontal ligament (PDL) fibers to be severed with or without fracture of the alveolus [1,2]. Reported incidence of dental avulsion is 1-11% of all dental injuries to the permanent dentition, with the maxillary central incisor being the most frequently involved tooth. The age group of 7-10 years appears to be most affected [3]. A variety of factors such as age of the individual, stage of the root development, mechanical damage during trauma and replantation, treatment of the socket, type of splinting, mastication, endodontic treatment, antibiotics, time of replantation, extraoral dry time, macroscopic contamination, and storage media and storage period are important and can influence the clinical success of replantation [4].

The ideal storage medium should have the capability of preserving the cellular PDL so that the cells could go through the process of mitosis, and form clones of the damaged fibroblasts of the PDL, and its generating cells. This is essential so that the nude surface of the root be repopulated with fibroblasts, thus, avoiding the adherence of osteoclasts on this area. An ideal storage medium should preserve the majority of the functional capacities of the cells of the PDL [5].

The way by which the tooth is transported also affects significantly the degree of success. The root of the tooth should not be touched, for each time this happens, the cells of the PDL are damaged and they die. The container used for the transport of avulsed teeth must be unbreakable, nontoxic, leak proof and of easy handling, with internal walls made of soft material, sterile and it must protect the tooth during transport, besides making the removal of the tooth easy without any trauma [6]. The present review summarizes the role of storage media in periodontal healing, the available media and the ongoing developments in this field.

VARIOUS STORAGE MEDIA**Tap water**

Tap water is an unacceptable storage media for avulsed teeth. Blomlof *et al.* found that storing cultured human PDL cells in tap water for 1 hr caused more PDL cell damage than the other physiological and nonphysiological storage media tested. They attributed that increased cell damage to the cell lysis caused by the very low osmolarity of tap

water [7]. Among the studied environments, tap water has shown to be the one with the least desirable results, though it protects the tooth from dehydration - for being a hypotonic medium - it causes rapid cellular death of the PDL, similarly to a dry storage [8].

Normal saline (NS)

NS is a solution of 0.90% w/v of sodium chloride, and osmolality of 280 mOsm/kg and despite being compatible to the cells of the PDL, it lacks essential nutrients such as magnesium, calcium, and glucose; necessary to the normal metabolic needs of the cells of the PDL [6]. Cvek *et al.* 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 [9]. They proposed that if an avulsed tooth has been kept dry for more than 15 minutes, it should be stored in an isotonic saline solution for about 30 minutes before replantation, presuming that PDL cells might be reconstituted or reconditioned by this procedure but in contrast Andreasen and Schwartz observed that following a 30 minute dry period, saline storage under experimental conditions has no effect on the development of root resorption and pulpal repair presumably because within 30 minutes maximal damage to the PDL has been inflicted [10].

Saliva

Saliva can be used as a storing medium for a short period, for it can damage the cells of the PDL if used for longer than an hour. Its osmolality (60-70 mOsm/kg) is much lower than the physiologic, thus, storage of avulsed teeth in saliva for 2-3 hrs causes swelling and membrane damage of PDL cells and also the presence of microorganisms makes saliva a less desirable storage medium [11]. Although readily and most easily available, saliva is not considered an effective interim transport medium. Recent literature indicates that saliva may not be a suitable transport medium for avulsed teeth due to its nonphysiologic osmolality and the presence of microorganisms [12].

Milk

Milk is considered as one of the suitable storage medium for avulsed teeth, as it presents physiologically compatible pH and osmolarity, essential nutrients, growth factors besides being easily accessible [13]. Milk is significantly better than other solutions for its physiological properties, including pH and osmolality. It is also considered compatible to the cells from the PDL; the easy way of obtaining it and for being

bacteria free, but it is important that it is used in the first 20 minutes after avulsion. The favorable results of milk probably occur due to the presence of nutritional substances such as amino acids, carbohydrates, and vitamins. The pasteurization of milk is responsible for diminishing the number of bacteria and bacteriostatic substances also for the inactive presence of enzymes, which could be potentially harmful to the fibroblasts of the PDL [6].

Gatorade

Gatorade is a brand of sports-themed food and beverage products manufactured by PepsiCo and distributed in over 80 countries. It consists of a mixture of water, sodium, sugar, potassium, phosphate, and lemon juice. According to Chamorro *et al.* [14] 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. As for osmolality, Gatorade is hypertonic, it can make the cells lose water. Gatorade preserves more viable cells than tap water but fewer than all other media, both at room temperature and on ice. Gatorade is often available at sporting events but it has incompatible, harmful osmolality that causes cell destruction, so it is not an ideal solution to use.

Coconut water

Coconut water is biologically pure, sterile and rich in amino acids, proteins, vitamins, and minerals. It has been widely consumed to replace fluids, electrolytes (potassium, calcium, and magnesium), and sugars lost from the body during heavy physical exercise. This natural isotonic fluid having pH of 4.1 is available in its natural form directly from the coconut or in long shelf life packages and plastic bottles mainly available in several geographical locations worldwide. This is the reason, its use as a storage medium for avulsed teeth has been investigated recently. More recently, biologically pure tender coconut water, which aids in replenishing the fluids, electrolytes, and sugars lost from the body during heavy physical work, has been suggested as a promising storage medium for avulsed teeth [15].

Contact lens solution

Contact lens solution is one of the convenient preservation medium for teeth after avulsion injuries. These solutions are available in school or athletic grounds and at home, where most injuries occur. They contain buffered, isotonic saline solutions with the addition of preservatives that may preserve the viability of PDL cells. Although various single bottle systems (SoftWear®, Ciba Vision Opti Care) have been examined as potential temporary storage media for avulsed teeth, no significant difference has been detected in the ability of different contact lens solutions to maintain the cell viability [16]. However, subsequent studies showed that these solutions were damaging to PDL cells, and therefore, they are not recommended [17].

Ascorbic acid

In 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. It is also required for *in-vitro* mineralized nodule formation of osteoblasts. Ishikawa *et al.* studied the effect of ascorbic acid on PDL cells and observed that ascorbic acid increased the ALP activity, which is required for the binding of PDL cells to Type I collagen through 2 beta 1 integrin, whose expression is again increased by ascorbic acid. As Type I collagen production is considered an initial process in the differentiation of PDL cells, it may serve as a potential storage medium [18].

Hank's balanced salt solution (HBSS)

Hank's balanced salt solution is a standard saline solution that is widely used in biomedical research to support the growth of many cells types. This solution is nontoxic; biocompatible with PDL cells, pH balanced at 7.2 and has an osmolality of 320 mOsm/kg. 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. It contains ingredients such as glucose, calcium, and magnesium ions which can sustain and reconstitute the depleted cellular components of the PDL cells [6]. Krasner and Person have developed an avulsed tooth storage system and emergency tooth preserving system (SAVE-A-TOOTH®, Pottstown, PA, USA), which contains HBSS, a net for holding the tooth atraumatically and a container for taking the submerged tooth to a clinician. It is a desirable storage medium for avulsed teeth, even when the extra-alveolar period is extensive (between 72 and 96 hrs) [19].

ViaSpan

The ViaSpan (Belzer VW-CSS, DuPont Pharmaceuticals, and Wilmington, DE, USA) is a medium used for the transportation of organs which are going to be transplanted and it has been considered effective for storing avulsed teeth. ViaSpan has osmolality of 320 mOsm/kg and pH is around 7.4 at room temperature; ideal for the cellular growth. Hiltz and Trope have compared the vitality of lip fibroblasts, at room temperature 37°C (Biological Industries) and recommended it as one of the storage medium, as it can preserve the PDL for extended periods before replantation [20].

Growth factors

The use of polypeptide growth factors, which function as potent biological mediators regulating numerous activities of wound healing, has been suggested for the promotion of PDL regeneration. Lynch *et al.* demonstrated that short-term application of a combination of platelet-derived growth factor and insulin-like growth factor can enhance the formation of the periodontal attachment apparatus 5-10 fold during the early phase of wound healing. Matsuda *et al.* studied the effects of individual and a combination of growth factors on PDL fibroblasts [21,22].

Emdogain

Emdogain diminishes the percentage of fibroblasts of the PDL with capability of forming colonies and that lowers the capability for the fibroblasts to repopulate the dental radicular surface after dental avulsion. The diminishing can happen due to the lack of an adherent surface or in the increase in the differentiation of fibroblasts, which grow in its presence. Emdogain increased the difference on the cells of mineralized tissues. In cases of periodontitis, Emdogain has improved the periodontal recovering after surgeries. In contrast, the post-traumatic periodontal healing needs contrary differentiation. Emdogain can delay, but not stop the development of replacement resorption, which is 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 [23].

Egg white

Khademi *et al.* have compared milk and egg white as solutions for storing avulsed teeth, and the results have shown that teeth stored in egg white for 6-10 hrs had a better incidence of repair than those stored in milk for the same amount of time ($p < 0.05$). The osmolality of the egg white is between 251 and 298 mOsm/kg [24]. de Sousa *et al.* have microscopically analyzed, the human PDL attached to the extracted tooth after 1 hr of extra alveolar 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. The authors suggest that egg white can be considered as the perfect medium for storing avulsed teeth [25].

L-DOPA

L-DOPA (Levodopa; Sigma Chemicals, Perth, WA, Australia) is a drug with possible mitogenic effects. Levodopa stimulates dopaminergic systems in the anterior portion of the pituitary gland to secrete growth hormone, which is considered as a promoter of the healing process. Partovi *et al.*, observed the effect of levodopa on human PDL fibroblasts and indicated that levodopa can also have a local effect on the growth of cells, including the PDL cells, and can be used as a preserving medium for avulsed teeth [26].

Cryoprotective agents

The effect of low temperature storage on the clinical success of replantation has been studied. Mature teeth can be replanted after cryopreservation and can heal *in-vivo* with a normal PDL, provided that a cryopreservative is used. Schwartz and Andreasen studied the effects of the cryopreserving agents (cryoprotectives) 5% and 10% dimethyl sulfoxide and 10% glycerol, on PDL. They observed that the use of different cryoprotectives, combined with controlled freezing rates to -196°C , can preserve the PDL of the replanted teeth to a greater or lesser extent [27].

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 [28].

Propolis

Propolis is a natural wax-like resinous substance found in bee hives, which is utilized by the bees as a "chemical weapon" against pathogenic microorganisms. Propolis is a mixture of plant resins and bee secretions. It has a great variety of pharmacological effects such as antimicrobial, anti-inflammatory, immune-modulatory, antiulcer, and antitumor properties, and it is relatively nontoxic. Propolis has a complex chemical composition and is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%). The application of propolis as a storage media has been studied by many authors [29-31]. Propolis has synergistic effect with Dulbecco's modified eagle's medium (DMEM) for the maintenance of cell viability and preservative potential. In combination, 10% propolis with DMEM was found to be better than 20% propolis with DMEM, which shows that 20% concentration of propolis might have some cytostatic/cytotoxic effect on the cell physiology [32]. Propolis has also been proposed to inhibit the osteoclastic resorption of teeth, which is common sequel of replantation of teeth. Propolis inhibits some aspects of the pathway leading to mature, active osteoclasts [33]. It is used for many dental implications such as pulp capping agent, mouthwash, intracanal irrigant, cariostatic agent, dentinal hypersensitivity, and treatment of periodontitis, treatment of dental stomatitis and as an intracanal medicament.

Catalase supplementation

Buttke and Trope observed that supplementation with catalase resulted in a significant reduction of surface resorption. It is suggested that the roots stored in antioxidant, demonstrate lower levels of surface resorption. As some storage media contain hydrogen peroxide, which may be toxic to PDL cells, addition of an antioxidant such as catalase can have beneficial effects on PDL cells [34].

Red mulberry

Red mulberry (*Morus rubra*) is common from the temperate to the subtropical regions of the northern hemisphere to the tropics of the southern hemisphere. It can grow in a wide range of climatic, topographical and soil conditions. Mulberry fruits are used medicinally as a deworming agent, as a remedy for dysentery, as a laxative, odontalgic, expectorant, hypoglycemic, and emetic. Red mulberry has been recommended as a storage media for avulsed teeth by Ozan *et al.* They compared four different concentrations of *M. rubra* (4%, 2.5%, 1.5%, and 0.5%) with HBSS and tap water at 1 hr, 3 hrs, 6 hrs, 12 hrs, and 24 hrs to check the effect on PDL viability. The number of viable PDL cells was significantly high when an avulsed tooth was stored in 4.0% concentrated solution of *M. rubra* as compared to other concentrations [35].

Pomegranate juice

Pomegranate effects the fibroblast cell proliferation. This proliferative effect is found for 1 hr at lower concentrations of 1% and 2.5%, but at

5% and 7.5% concentration a general proliferative effect is exhibited. The peak increase in cell viability is observed at 6 hrs. It also promotes strong cell attachment. Pomegranate juice and HBSS can preserve the spindle such as morphology of periodontal fibers for 24 hrs after storage. Hence, it can be a good storage media. Since research conducted to assess its efficacy is very less, further research is needed [36].

Aloe vera

A. vera is a cactus such as plant that belongs to family Liliaceae. The inner gel of *A. vera* has issue of dry period. The dry period should be as less as possible and it would be better if immediate replantation can be achieved. As a fact, it is not always possible to replant the tooth immediately, so, the need for storage media arises. The delay in replantation can vary from few minutes to hours, as in cases of accidents, emergencies and unavailability of dentists, so a storage media which can maintain the PDL cell viability for a long duration is needed [32,37].

Oral rehydration solutions

Ricetral is a commercially available oral rehydration formulation, consisting of essential nutrients such as 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 [38].

Dentosafe

Dentosafe (Miradent, Germany) is the commercial name of a tooth rescue box containing special cell culture medium which is a combination of amino acids, vitamins, and glucose [39]. In the USA, it is marketed as EMT Tooth Saver (Phoenix, USA). It has demonstrated the maintenance of vitality of PDL cells for 48 hrs at room temperature [40]. If unopened, this medium has a shelf-life of 3 years. A study by Pohl *et al.* showed that avulsed teeth placed in Dentosafe solution showed functional healing and recommended that Dentosafe should be included in all first aid kits, especially in locations prone to tooth avulsion injuries such as schools, sports ground and facilities, and public pools as well as emergency units such as hospitals and ambulances. The use of this system is self-explanatory and simple to understand for lay persons. Although effective, this medium is still not easily available in many countries [41].

CONCLUSION

An appropriate storage media can help maintain the viability of PDL cells and can lead to successful replantation of avulsed teeth. The natural products such as coconut water, milk, and propolis can act as appropriate storage media because of their easy availability and potential to maintain the viability of PDL cells for longer durations. Although research has been undertaken on a wide variety of materials to be used as storage media for the transport of avulsed teeth, lack of availability and high cost limit the use of the majority of these media.

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