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READILY AVAILABLE STORAGE MEDIA FOR AVULSED TEETH IN SCHOOL AND COMMUNITY: A REVIEW.

Dental Science

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ABSTRACT

Dental avulsion is the most severe type of traumatic tooth injuries because it causes damage to several structures and results in the complete displacement of the tooth from its socket in the alveolar bone. Replantation is widely accepted as an effective treatment option for an avulsed tooth. However, the long-term fate of replanted teeth is unpredictable; it is dependent on various factors such as the time interval between avulsion and replantation, the storage method of teeth during the extra-alveolar period (dry storage or storage media), the vitality status of pulp or periodontal tissues, and the type and period of splinting. The appropriate use of storage media is an important clinical factor affecting the postoperative prognosis of avulsed teeth following replantation. However, the major disadvantage of conventionally proven effective material is that they are not easily available at places where these traumatic injuries occur. Hence, there is a need to distinguish storage media that will be readily available, yet effective. This material should produce conditions that closely resemble the original socket environment, with adequate osmolality (cell pressure), pH, nutritional metabolites and glucose, and thus create the best possible conditions for storage. The present review discusses the readily available storage media for avulsed teeth and their potential maintenance of the vitality of periodontal ligament cells.

KEYWORDS

Tooth avulsion, periodontal ligament, Aloe Vera, Milk, Egg White, Coconut water, Green tea, Neem, Pomegranate Juice, Ricetral, Propolis, and Turmeric.

INTRODUCTION

Absolute treatment for an avulsed tooth is its immediate replantation into the socket, which significantly improves the prognosis. Andreasen reported in his retrospective study that 90% of avulsed teeth could be successfully retained when they were replanted within the first thirty minutes of the accident. [1] When immediate replantation of an exarticulated tooth is not possible, the PDLF (Periodontal Ligament Fibers) should be incubated in a physiological storage medium to maintain its viability during transportation to the dental office because dry storage of avulsed teeth leads to the death of PDL cells of the root. Partial or total loss of PDL leads to ankylosis since the activity of cells derived from the PDL plays a crucial role in the prevention of ankylosis. Both the storage media and extra-alveolar duration are significant critical factors in determining the final prognosis.

Until now, various types of wet storage media for avulsed teeth have been investigated, which may vary from cell and tissue culture solutions like Hank's balanced salt solution (HBSS); medical/hospital products developed specifically for organ storage purposes, like ViaSpan, Euro-Collins culture media, Minimum Essential Medium (MEM), Saline. However, the major disadvantage of HBSS and many of the other aforementioned media is that they are not easily available at places where these traumatic injuries occur. Other natural products like water, saliva, Bovine milk and its variations, Propolis, Green tea, Morus rubra (red mulberry), Egg white, and Coconut Water can be used as a storage media which are easily available.

An ideal storage medium should be capable of maintaining PDL and pulp cell viability while presenting clonogenic capacity, compatible physiological pH and osmolality, antioxidant property, no or minimal microbial contamination, maintained at an appropriate temperature, high availability, and low cost. [2] It also should be readily accessible, especially to families with children. The present review summarizes the role of readily available storage media in periodontal healing and its ongoing developments in this field.

STORAGE MEDIA

Hank's Balanced Salt Solution (HBSS) has been specially developed for cell maintenance and thus, theoretically, it allows better conservation of tissues for long time periods. It has been widely employed as a reference solution in studies on dental avulsion as it has the ideal osmolality and pH for preserving the vitality of cells. [3-6] Hwang *et al* [7] reported 94% cell viability after storage of cultured human PDL cells for 24 h in this medium, which is considered an excellent result and Pillegi *et al* [8] observed approximately 90% cell viability. However, its use is restricted to laboratory environments and is not available at an accident site, which makes it impracticable as a storage medium. Viaspan® and MEM cell culture medium also have demonstrated their excellent efficacy but their lack of availability and high cost make their routine use unviable, and thus these solutions are used in very special cases, such as laboratory studies.[9-11]

This present review further discusses easily accessible storage media for an avulsed tooth. They have further benefits: cost-effective, longer shelf life, and easy usage. Thus they can be easily introduced to normal community people and society and they can use it while any case of avulsion injury to promote good reimplantation.

Saliva

Previous studies suggested that saliva can be used as an interim storage medium for avulsed teeth to prevent desiccation. According to Weine [12], patients' 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. However, more recent studies have indicated that saliva may not be the most suitable medium for extended (greater than 1 h) storage of avulsed teeth. [13] Non-physiological osmolarity, less favorable composition and the presence of microorganisms make saliva a less desirable storage medium. Storage of avulsed teeth in saliva for 2 to 3 h causes swelling and membrane damage of PDL cells. [14] In 1 h, it can cause approximately twice as much damage as HBSS or milk. [15] However, saliva storage produces one-third less cell damage than dry storage or storage in tap water. A study by Lekic *et al* [13] investigated the clonogenic capacity of human PDL cells stored for 15 min in autologous saliva (at 23°C), followed by storage in saliva, milk and HBSS (at 4°C) for an additional 15 and 45 min. A clonogenic capacity above 3% is considered a

requirement for wound healing. The PDL cells of the avulsed teeth stored in saliva for 30 min had a clonogenic capacity of 7.6% and for 60 min the clonogenic capacity was 1.5%. 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.

Water

Tap water is an unacceptable storage media for avulsed teeth. [16] Blomlof *et al* [15] found that storing 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. They attributed the increased cell damage to the cell lysis caused by the very low osmolarity of tap water. Thus, tap water is not a suitable interim storage medium for retaining the viability of PDL cells. However, as it is readily available, even in athletic fields, it can be used as a media of last resort, as opposed to allowing the tooth to dry out. This will decrease the speed with which PDL cells will die. [12]

Normal saline

Cvek *et al* [17] suggested that wet (saline or saliva) storage for 25 to 60 min can lower the occurrence of ankylosis before replantation (20%) when compared with dry conditions (60%). They proposed that if an avulsed tooth has been kept dry for more than 15 min, it should be stored in an isotonic saline solution for about 30 min before replantation, presuming that PDL cells might be reconstituted or reconditioned by this procedure. Based on his findings, Andreasen [16] concluded that the storage of avulsed teeth in saline offered good protection against root resorption for the extra-alveolar duration of up to 2 h. In contrast, other studies have shown negligible effects of saline storage if the extra-alveolar duration exceeds 30 min. Andreasen and Schwartz [18] observed that following a 30-min dry period, saline storage under experimental conditions does not affect the development of root resorption and pulpal repair, presumably because within 30 min maximal damage to the PDL has been inflicted. Patil *et al* [19] observed no significant difference between the PDL cells recovered from the saline storage group and the cells recovered immediately after extraction. However, as discussed earlier, it is of clinical importance that in cases of delayed replantation the tooth should be kept wet (saline or saliva), rather than keeping it dry. Thus it can be concluded that storing avulsed teeth in saline is only acceptable when other storage media (HBSS) are not immediately available and when required for a short period of time.

Milk

Milk has been studied extensively and has gained acceptance as a medium capable of maintaining PDL viability. [20-22] Because of its physiological osmolarity, composition, and markedly fewer bacteria, milk is a superior storage medium. [14] The nutritive value of milk and the presence of growth factors in milk are considered to be the contributing factors. Cultivated human PDL cells, stored in milk for 1 to 3 h, displayed approximately the same (minimal) cellular leakage as occurred with storage in HBSS.[9] Lekic *et al* [13] demonstrated that milk was as effective as HBSS for storing avulsed teeth for up to 1 h and superior to saline, saliva or water. Trope and Friedman [11] concluded that milk is an excellent storage medium for up to 6 h, after which it loses its effectiveness. However, milk is not always available and may contain many antigens that could act negatively from an immunological standpoint on the reattachment process. Also, not all types of milk are equally as effective as storage media. Some evidence supports the use of chilled milk as an interim storage medium for avulsed teeth. Avulsed teeth stored in chilled milk for up to 1 h can maintain sufficient numbers of viable PDL cells to support the replantation of the tooth and the possibility of PDL healing. [13,20]

Harkacz *et al* [23] showed that milk with lower fat content may be more appropriate at maintaining cell viability than milk with higher fat content. Regular pasteurized milk has a short shelf life and requires refrigeration, which makes it less readily available at the trauma site. Thus long shelf-life milk having an identical composition, pH, and osmolarity to regular milk with a storage capability of 6 hours without the need for refrigeration has gained more acceptance. [24] Therefore milk, chilled or otherwise, can be used as a storage medium of choice for extended extra-alveolar storage (1 to 6 h).

Egg white

Egg white from a single egg contains 4.7 grams of 40 different proteins, 0.3 grams of carbohydrate, 62 milligrams of sodium and the remainder being water. [25] It is considered a good choice as a storage media for teeth undergoing delayed replantation because apart from proteins it also contains vitamins and water, the absence of microbial contamination and easy access. [2] It has shown better cell viability and significantly higher incidence of PDL healing as compared to milk and equivalent cell viability as HBSS. [26] Khademi *et al* [27] compared milk and egg white as solutions for storing avulsed teeth, and found that teeth stored in egg white for 6 to 10 h had a better incidence of repair and lower surface resorption than those stored in milk for the same amount of time and index than the controls. It is observed to be an excellent medium for up to ten hours with the principle advantage being its availability. Mahal NK et al [28] showed no significant difference in a number of viable PDL cells between HBSS and egg white in his study. A study performed by Badakhsh S *et al* [29] showed that approximately 80% of cells survived during first-hour exposure to egg white storage media. The great number of proteins, vitamins, and water may lead to positive or negative results in relation to the efficacy of egg white and its protein as a storage medium for avulsed teeth. Some experiments indicate that this is a very good medium to maintain cell viability, but others show a small loss of efficacy over time, possibly due to egg's high pH (9.89) and also because the PDL cells could target the several egg proteins as strange bodies. Further studies are required to confirm theses adverse effects, as there are wide variations in egg composition and quality.

Aloe vera

Aloe Vera gel is a good choice of transport medium because it contains 99% water and over 75 nutrients, which include 20 minerals, 19 amino acids, and 12 vitamins. The human body requires 22 amino acids to maintain good health, eight of which are essential, as the body cannot synthesize them. All of these eight essential amino acids and 11 of 14 secondary amino acids are found in Aloe Vera. [30] Many studies have demonstrated that Aloe Vera has superb properties, such as anti-inflammatory, antibacterial, antifungal, anticancer and even antioxidant activities. [31,32] Aloe Vera also contains allantoin which has been found to stimulate fibroblast activity and collagen proliferation. [33] The number of viable cells in Aloe Vera group might also be because of the presence of catalase enzyme, an antioxidant enzyme that converts hydrogen peroxide (H_2O_2) to water and oxygen and suppression of the generation of these free radicals may improve the effectiveness of cell preservation and prevent lipid peroxidation. Hence, the presence of antioxidants in storage media is necessary for inhibiting the generation of free radicals thereby minimizing cell damage. Buttke *et al* [34] suggested that reimplantation success may be increased by storing avulsed teeth in medium containing one or more antioxidants. The osmolality of Aloe Vera was found to range from 280-300 mOsm/l which is similar to the osmolality of a normal cell. [35] Fulzele P *et al* [35] investigated the effectiveness of aloe vera gel in maintaining the viability of periodontal ligament cells. Results showed that at 15 min HBSS presented the maximum mean percentage of viable PDL cells (89%) followed by aloe vera at 81% and packaged drinking water at 10%. Sharma M *et al* [30] evaluated the viability of periodontal ligament (PDL) cells of avulsed teeth in three different storage media and results demonstrated that the aloe vera group had the highest percentage of viable cells followed by egg white and milk.

Green tea

Some studies have proven that the green tea extract has strong antioxidant, anti-inflammatory, and antibacterial properties, good anticarcinogenic effect, good ability to extend the survival of grafts and the capacity to protect the periodontal tissues against the resorption of alveolar bone as a result of infectious processes caused by pathogenic microorganisms.[36] Additionally, Hwang *et al* [7] and Jung *et al* [36] in the search for a medium capable of minimizing the infections after tooth replantation, maintaining PDL cell viability and reducing root resorption and ankylosis, reported enthusiastic results with green tea, with the maintenance of 90% of cell viability for up to 24 h, similar to the HBSS control. Jung *et al* [36] 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 an alternative for the conservation of avulsed teeth and its beneficial effect is enhanced by higher extract concentrations. The antibacterial and anti-inflammatory actions of green tea demonstrate their capacity to inhibit prostaglandin synthesis, aiding the immune system in the phagocytic activity and promoting healing effects in the epithelial tissue. [7, 38] Additionally, one or more antioxidant composites in these substances may increase the success rate of tooth replantation because they prevent the harmful effects of the free radicals, modulating the osteoblast and osteoclast activity.

Coconut water

Coconut water is a natural, biologically pure, sterile product rich in amino acids, proteins, vitamins, and minerals. This is a medium with easy access and good biological characteristics that Storage media for avulsed teeth could be promising for its indication. Several studies have been performed to use this substance as a storage medium for avulsed teeth, but the results are contradictory. Gopikrishna *et al* [3] and Gopikrishna *et al* [4] found greater efficacy of coconut water over HBSS and milk for the viability of PDL. Thomas *et al* [9] found that 15 to 120 min storage in coconut water is as efficient as storage in HBBS. On the other hand, Pearson *et al* [37] and Thomas *et al* [9] observed that inflammatory resorption was more frequent when the tooth was maintained in coconut water compared with milk. Moreira-Neto *et al* [38] and Souza *et al* [39] also reported that milk presented a better performance than coconut water in relation to the cell viability. These results can be attributed due to an acidic pH of coconut water (4.1) which is deleterious to cell metabolism. However, overall, the use of coconut water as a storage medium for avulsed teeth is not feasible under clinical conditions because of the difficulty of neutralizing the coconut water to obtain a pH of 7.0

Propolis

Propolis is a natural substance produced by honeybees from the buds or bark of trees, chiefly conifers. It consists of the following components: resin (rich in flavonoids) (45 to 55%), waxes and fatty acids (23 to 35%), essential oils (10%), pollen proteins (5%), and other organic compounds and minerals. [40] Flavonoids are thought to account for much of the biological activity of Propolis. Propolis has antiseptic, antibiotic, antibacterial, antifungal, antiviral, antioxidant, anticarcinogenic, antithrombotic and immune-modulatory properties. Propolis also contains iron and zinc, important for collagen synthesis, and bioflavonoids that help in the contention of hemorrhages of the PDL tissue and stimulate the stimulate enzymes that fortify the walls of the blood vessels in the periodontium. [5] Martin and Pillegi [41] reported that teeth stored in Propolis demonstrated the highest viability for PDL cells when compared with HBSS, milk, and saline. Shaher *et al* [42] observed that with Propolis, the viability of PDL fibroblasts can be maintained for as long as 20 h. Therefore Propolis can act as a good alternative natural storage medium for avulsed teeth. Mori *et al* [43] investigated the ideal period for maintaining the tooth in Propolis and concluded that the efficacy of the medium increases if maintained for 6 h because the contact with the product is beneficial for cell maintenance.

Red mulberry

Morus rubra (red mulberry) is a natural product available in different climates, which contains flavonoids, alkaloids, and polysaccharides, all of them very important for cell preservation. Ozan *et al* [44] reported that when teeth were stored in red mulberry for up to 12 h, its capacity to maintain the viability of PDL cells was better than that of HBSS; however, if a longer storage time is required, it is advisable to employ higher concentrations of the fruit juice. There are very few studies evaluating the use of red mulberry juice as a transport medium for avulsed teeth and its biological proprieties have not been yet established. Further research is necessary before its use can be recommended. [44, 45] This fruit seems to contain a great amount of flavonoids, alkaloids, and polysaccharides in addition to antioxidant substances. [44,45] Even though these natural products present promising results as media for avulsed teeth, their lack of availability in all region limit their indication.

Ricetral (Oral Rehydrate Solution)

Dehydration, as in diarrhea cases, is treated with oral rehydration solutions like Ricetral, which contain essential cells and nutrients like glucose and vital salts in concentrations considered adequate for cell metabolism. These solutions are available in drugstores at low cost and their components can maintain the body hydrated by reposition of liquids lost in the intestine. Rajendran *et al* [46] evaluated the PDL cell viability of extracted human teeth by the Trypan blue exclusion method and the results showed that Ricetral was similar to the HBSS control and both were superior to milk. Ricetral may also retain some cell viability and provide conditions for the maintenance of cell metabolism.

Contact lens solution

The number of people using contact lenses is growing and consequently, there is also great availability of solutions for cleaning contact lenses in homes, schools, and centers of physical activities. [10,20] These solutions are fatty acid monoester composites with an antimicrobial cationic component. Sigalas et al [20] studied the efficacy of different contact lens solutions in maintaining the viability of cultured PDL cells by the Trypan blue exclusion method and the results showed that the preservatives in the formula damaged the cells. Nonetheless, in the absence of another storage medium, they may be used instead of water or saline for short periods of time. Contact lens solutions do not present quite positive results, probably because their characteristics are not very favorable to the cells; however, they may replace water and saliva if required. [10,20]

Honey milk

`It is 8% non – fat solid milk, 3 gm protein, 11 gm carbohydrate, 0.1 gm calcium, 0.6 gm minerals & 0.12gm phosphorous & natural honey (5%). The storage capacity of honey milk is of at least 6 months without the need for the refrigerator so it is appropriate for school use. After 9 hours long shelf life of honey milk showed a better result than fresh milk. [11]

Castor oil

Vegetable oil with antimicrobial and antioxidant properties, low toxicity, and glutathione preservation capability, low cost, and high availability. It also has the capacity to repair bone defects. Mohammadreza Nabavizadeh evaluates and compares the capacity of castor oil with HBSS and milk. The percentage of viable cells treated with castor oil, HBSS, and milk counted immediately after removal from these media was 46.93%, 51.02 %and 55.10 % respectively. Castor oil was not able to preserve the viability of PDL cells efficiently comparable to HBSS and milk. [47]

Pomegranate juice

Pomegranate effects fibroblast cell proliferation. This proliferative effect is found for 1 hour 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 the 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. [48]

Neem (Azadirachta indica)

It is an extremely healthy plant that has attracted worldwide attention. Neem leaf and its constituents have been verified to exhibit immunomodulatory, anti-inflammatory, antihyperglycemic, antifungal, antiviral, antioxidant, antimutagenic, and anticarcinogenic properties. It has several active constituents such as nimbidin, nimbin, nimbolide, azadirachtin, mahmoodin, and cyclic trisulfide which are responsible for its antibacterial action. [49,50] It is biocompatible with pH balanced at 7–7.5 and has an osmolality of 270 mOsmol/kg. Dhimole P. *et al* [51] showed around 88% of cells were viable in the case of the neem extract storage medium in his study. This herbal product is readily accessible at the trauma site might prove as a boon for effective storage capacity and maintenance of cell viability.

Turmeric (Curcuma longa)

Turmeric (Curcuma longa) is a common antiseptic belonging to the family Zingiberaceae. The proven properties of curcumin include anti-inflammatory, antioxidant, antimicrobial, antiseptic, and antimutagenic. The antimicrobial effect of curcumin inhibits the growth of various microorganisms. It is a natural medicament with a wide spectrum of biological actions. [50,52] It has a pH balance of 7.1 with an osmolality of 260 mosmol/kg. Dhimole P. *et al* [51] had evaluated the efficacy of neem and turmeric as storage media in maintaining periodontal ligament (PDL) cell viability in his study and showed 81.63% of viable cells in turmeric extract. The results can be attributed due to turmeric's Chemical constituents of turmeric include volatile oil (6%), curcumin, and α- and β-turmerone. [53] Mandrol et al [52] investigated the in vitro cytotoxicity of curcumin against primary dental pulp fibroblasts by MTT assay. No cytotoxicity was detected for curcumin at any of the concentrations used (25%, 50%, and 100%). The results revealed that the viability of primary dental pulp fibroblasts increased with an increasing concentration of curcumin. Curcumin promotes cell viability and induces the proliferation of dental pulp fibroblasts and thus can be used as a suitable natural storage medium.

CONCLUSION

An appropriate storage media is recommended for the protection of

PDL cells following trauma as they maintain the viability of PDL cells and can lead to successful replantation of avulsed teeth. HBSS, Tooth rescue box, ViaSpan, and EM are effective media but are not feasible due to factors, such as cost and lack of availability. The natural products such as coconut water, milk, Egg white, Aloe vera, Neem, Green tea, 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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