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Advances in Storage Media for Avulsed Tooth: A Review

Abstract

Traumatic dental injuries like tooth luxation, avulsion, fracture or laceration of soft tissue often occurs in the children and young adults due to various etiological factors. Viable periodontal ligament (PL) cells are required for PL healing of avulsed teeth following replantation. If immediate replantation cannot be accomplished, the ability of PL progenitor cells to reproduce (clonogenic capacity) and recolonize the wound may be extended by prevention of desiccation and storage in physiological media. Recent research has led to the development of storage media that produce conditions that closely resemble the original socket environment, with adequate osmolality, pH, nutritional metabolites and glucose. This paper reviews the literature on the different storage media that have been investigated for avulsed teeth based on full-length papers retrieved from PubMed/ Medline, Lilacs, BBO and SciELO electronic databases using the key words 'storage medium', 'transportation medium', 'avulsion', 'tooth avulsion', 'replantation', 'tooth replantation'.

Key Words

Tooth avulsion; storage media; replantation

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INTRODUCTION

Traumatic dental injuries represent one of the most common reasons for emergency appointments in dental practice.[1] Avulsion, or exarticulation, is a complete displacement of a tooth from its alveolar socket as a result of trauma.[2] Traumatic tooth avulsions constitute 0.5%-16% of all traumatic injuries of permanent anterior teeth.[3] It corresponds to 1% to 16% of all types of tooth injuries involving the permanent dentition.[4] As immediate replantation is not always practically possible at the trauma site, an 'interim transport' media is often required to maintain the vitality of PDL structures.[5] The success of replantation depends on two factors; extra alveolar period and the viability of the PDL cells for reattachment. Viability is known to play an important role and this can be achieved by placing the tooth in a storage medium till further treatment is initiated. 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.[1] These solutions are capable of preserving the life of cells from the periodontal ligament during the time they are out of their alveolar socket.[6] The present review summarizes the role of storage media in PDL healing, the available media and the ongoing development in the field.

The ideal requirements for a storage medium are[7]

- Possess antimicrobial characteristics
- Maintain the viability of periodontal fibers for an acceptable period of time
- Favour proliferative capacity of the cells.
- Possess osmolarity as that of body fluids (290-300 mosmol/ kg) and pH balanced (7.2 – 7.4)
- Unreactive with body fluids
- Not produce any antigen-antibody reactions
- Reduce the risk of post-replantation root resorption or ankylosis
- A good shelf life
- Effective in different climates
- Capable of washing off extraneous materials and toxic waste products
- Aid in reconstitution of depleted cellular metabolites.

Authors submitting review manuscripts should include a section describing the methods used for locating, selecting, extracting, and synthesising

data. These methods should also be summarised in the abstract. This paper reviews the literature on the different storage media that have been investigated for avulsed teeth based on full-length papers retrieved from PubMed/ Medline, Lilacs, BBO and SciELO electronic databases using the key words 'storage medium', 'transportation medium', 'avulsion', 'tooth avulsion', 'replantation', 'tooth replantation'

Review of Storage Media

Tap Water

Tap water has shown the least desirable results because it has bacterial contamination, hypotonicity, and non-physiological pH of 7.4 to 7.79 and an osmolality of 30 mOsmol Kg-1.[1] Blomlof *et al*., 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.[3] Hence its use should be limited to cases where the extra alveolar duration is less.

Saline

Pileggi *et al*.,[8] evaluated the PDL cells viability when maintained in this medium for 45 min and resulted in only 20% mortality. Lauer *et al*.,[9] showed that physiologic salt solution was unable to maintain the metabolism of the fibroblasts. Hence, normal saline appears to be suitable for short-term storage of avulsed teeth for about 2 hrs.

Gatorade

Harkacz *et al*.,[10] were the first to test its effectiveness as a storage medium. Compared to tap water, the use of Gatorade as storage medium yielded better results for PDL cells survival.[11] Because of its low pH (around 2.91) and hyper tonicity (osmolality of 407 mOsmol Kg-1), it causes cell destruction and hence not recommended for long term storage media for avulsed teeth.

Saliva

Human saliva (buccal vestibule) is used as a storage medium due to its easy availability. It has a pH of 7.4 to 7.79 and an osmolality of 30 mOsmol Kg-1. This hypertonic osmaolarity leads to cell lysis and higher rates of replacement resorption. Human saliva has unfavorable characteristics, such as non-physiological pH and osmolality (60-70mosmol Kg-1), high microbial contamination and hypotonicity.[1] It also causes swelling and membrane damage of PDL cells of avulsed tooth if stored for 2-3 hours. A clonogenic capacity in excess of 3% is considered a requirement for wound healing but teeth stored in saliva for 30 min had a clonogenic capacity of 7.6% and for 60 min the clonogenic capacity was 1.5%. Andreasen showed that saline and saliva were suitable storage medium for protection against root resorption for short extra-alveolar periods.[7] Hence saliva can be considered to be acceptable for short term storage medium (less than 90 min) and its use should be limited.

Coconut Water

It resembles intracellular fluid and the predominant cations are potassium, calcium, and magnesium. 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. Compared to HBSS, propolis and milk, it was found that coconut water was the most effective in maintaining viability of PDL cells.[12] Since the pH of coconut water is 4.1, it has harmful effects on cell metabolism until sufficiently neutralized.[13] Gopikrishna *et al*.,[12] and Gopikrishna *et al*.,[14] found greater efficacy of coconut water over HBSS and milk for the viability of PDL. Thomas *et al*.,[15] found that 15 to 120 min storage in coconut water is as efficient as storage in HBBS. On the other hand, Pearson *et al*.,[16] and Thomas *et al*.,[15] observed that inflammatory resorption was more frequent when the tooth was maintained in coconut water compared with milk. Moreira-Neto *et al*.,[13] and Souza *et al*.,[17] also reported that milk presented a better performance than coconut water in relation to the cell viability. It is therefore difficult to consider coconut water as an adequate storage medium for avulsed teeth. Standardized studies with similar methods are required to avoid diverging results and eliminate doubts over its use.

Egg White

Egg white has a pH of 8.6–9.3 and its osmolarity is 258 mosmol/ kg. Egg white was found to be more suitable storage media because there was no significant difference between egg white and milk at storage times of 1, 2, 4, 8 and 12 h in cell viability.[7] White, are considered a good choice as a storage media for teeth undergoing delayed replantation due to its high content of proteins, vitamins and water, absence of microbial contamination and easy access.[4] Some studies demonstrated greater PDL healing when compared with milk. It can be used to store avulsed teeth for up to 10 hours.[18] Further studies are required to confirm theses adverse effects, as there are wide variations in egg composition and quality.

Morusrubra [red mulberry]

At 4% concentration, M. rubra is found to be more

effective than HBSS up to 12 hours, in maintaining the PDL cells' viability.[19] Ozan *et al*.,[4] 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. 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 yet. Further research is necessary before its use can be recommended.[4]

Saliva Officinalis

Salvia officinalis is a perennial, evergreen shrub which has a long history of medicinal and culinary use. This extract has been proposed as a storage medium for avulsed teeth because of the anti-oxidants effects caused due to the presence of its phenolic components like rosmarinic acid, camosic acid, salvianolic acid and derivatives.[18] Studies have shown that Salvia extract at 2.5% helps maintain PDL cells viability over longer periods of time (3, 6, 12 or 24 hours) when compared with HBSS, phosphate buffered saline and tap water.[2] Thus salvia officinalis can be recommended as suitable storage media for avulsed teeth.

Custodial

This medium is the registered trademark of Dr. Franz. It contains a histidine-tryptophan ketoglutarate solution containing high flow properties and low potassium content.[18] It has an osmolality of 310 mosmol L-1.[2] Alaçam *et al*.,[20] reported that it is comparable to HBSS for cell preservation. However, it is not available to the public and therefore of little value as a storage medium for avulsed teeth Similar to other organ storage medium, it is not available to public which limits its practicality as a storage medium for avulsed teeth.

Honey Milk

Honey milk has 8% non-fat solid milk, 3gr protein, 11gr carbohydrate, 0.1gr calcium, 0.6gr minerals and 0.12gr phosphorous and natural honey (5%). This product has extended storage capability of at least 6 months without the need for refrigeration. Long-shelf life honey milk may be considered as appropriate storage media which are comparable to HBSS and better than fresh milk medium.[7] In a study fresh milk revealed the best outcome after 1 hour. After 9 hours, long-shelf life honey milk showed better results in comparison to fresh milk. This is in agreement with the study by Marino et al. which showed that after 8 hours, the cells' viability was higher in milk compared to HBSS.[21] Long shelf life honey milk can be recommended as a suitable transport medium for avulsed teeth.

Probiotic Solution

Recently a host probiotic, L. reuteri DSM 17938 and L. reuteri ATCC PTA 5289, have been shown to preserve vitality of periodontal ligament (PDL) cells in case of dental trauma. The key event is that harmless microorganisms, such as strains of lactobacilli or bifidobacteria, can occupy a space in a biofilm that otherwise would be colonized by a pathogen.[14] Caglar *et al*., tested the viability of fibroblasts using HBSS, saline, Lactobacillus reuteri solution, and milk and concluded that there was no significant difference in the number of viable PDL cells between the materials. It appears that probiotic may be able to maintain PDL cell viability as HBSS, milk, or saline.[1] However further studies are required to confirm its beneficial effect.

Patients Own Serum

Thonner proposed the use of the patient's serum as a storage medium, He claimed that the histological picture of the periodontium of a freshly extracted tooth showed that the cementum and periodontal tissue present over the root are well vascularized. The histologic picture after the tooth had been preserved in serum for about one hour showed that the tissues still stained well, indicating that serum can maintain the vitality of the periodontal membrane during the critical extra-alveolar period during tooth transplant or replant procedure.[1]

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 phosphatises (ALP) and osteocalcin.[3] It is also required for in vitro mineralised nodule formation of osteoblasts. Ishikawa *et al*.,[22] 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 via 2 beta 1 integrin, whose expression is again increased by ascorbic acid. As type I collagen production is considered to be an initial process in differentiation of PDL cells, it may serve as a potential storage medium.

Culture Medium

Culture media can include Eagle's medium, modified Eagle's medium (MEM) and Dulbecco's storage medium. Eagle's medium contains many nutrients like amino acids, vitamins and bicarbonates considered essential for maintaining

the viability and proliferative capacity of PDL cells for longer time periods when compared with other storage media (48-53 hrs).[18] Pearson *et al*.,[2] showed that soaking teeth in EM for up to 60 min after they had been stored dry for 5–14 days resulted in better PDL healing than if the teeth had been immediately replanted. Lekic *et al*.,[23] reported that teeth extracted for orthodontic reasons, stored in saliva for 15 min and then transferred to various other media had the highest number of viable cells after both 30 and 60 min when EM was used. MEM cell culture medium contains L-glutamin, penicillin, streptomycin, Nistatin, bovine serum and nutrients for cell growth and proliferation, and several authors have reported its efficacy in preserving the viability of PDL cells and have indicated it as a storage medium before tooth replantation, but not easily available.[4] There is a variation of the Eagle's modified essential medium (EMEM), called Dubelco's modified Eagle's medium (DMEM), which contains approximately four times as much of the vitamins and amino acids present in the regular EMEM formulation and 2-4 times as much glucose. In addition, it contains iron and phenol red. DMEM is suitable for most types of cells.[2] However, it is not available to the public and therefore of little value as a storage medium for avulsed teeth.

Conditioned Medium

Conditioned medium is derived from the supernatant of human gingival fibroblasts grown in culture. It may contain stimulatory factors derived from the gingival fibroblast cells which could have a stimulatory effect on the remaining cells on the root surface. Conditioned medium is a superior medium for the extended storage of dog's teeth. Healing rates were lower for roots that had been soaked in conditioned medium compared with those that were soaked in ViaSpan and HBSS.[2] But there is lack of studies regarding storage of human teeth. Conditioned medium is not available to the public, and therefore, it is of little value as a storage medium for avulsed teeth.

HBSS

Hanks' Balanced Salt Solution (HBSS) was introduced by John H Hanks in 1975 as a solution for preservation of tissue culture. Among all the storage medium HBSS is considered as the gold standard and is often used as a comparison reference medium to deduce the clinical efficacy of other media. The American Academy of Endodontics has accepted HBSS as an acceptable medium for avulsed teeth because of its capability to maintain vitality and proliferative capacity of PDL for an extended period of time (up to 48 hours).[7] It contains the sodium chloride, D-glucose, potassium chloride, sodium bicarbonate, potassium phosphate (monobasic), calcium chloride and magnesium sulphate anhydrous. It can preserve cells and tissues for 24 h and both the pH (7.4) and the osmolarity (280 mosmol kg-1) are ideal.[24] It can maintain the viability of PDL cells for several hours with a success rate of 90%.[25] HBSS has no need of refrigeration. HBSS is marketed as Save-A-Tooth (Save-A-Tooth; Phoenix Lazerus Inc., Pottstown, PA, USA), to maintain periodontal ligament cell viability.[26] It is a special kit in which avulsed tooth can be suspended, which has been designed for the public to use for the emergency management of avulsed teeth. Gentle agitation can remove debris from the PDL during storage and lost nutrients can be replenished by the HBSS before replantation. Unfortunately, HBBS is not widely used in India, because it is not readily available.

Emdogain

Emdogain (Biora, Malmo, Sweden) is a commercial Enamel Matrix Derivative (EMD) extracted from developing embryonic enamel of porcine origin and contains several matrix proteins. It can influence the migration, attachment, proliferative capacity and biosynthetic activity of PDL cells.[5] It has also been used in anti-resorptive-regenerative therapy along with topical glucocorticoids and systemic doxycycline. Use of emdogain has been shown to increase the incidence of healed PDL when this gel was applied to root surface of the avulsed tooth and /or inserted directly into alveolar socket before implantation. It also aids in preventing or retarding root resorption and ankylosis.[27] However, no firm conclusion regarding the efficacy of EMD application on healing of replanted[28] and auto transplanted permanent teeth can be drawn because of a lack of randomised controlled trials and clinical controlled trials.[29]

Viaspan

ViaSpan is a clear to light yellow sterile, non-pyrogenic solution that has an approximate calculated osmolality of 320 mosmol kg-1and a pH of about 7.4 at room temperature. This composition is thus consistent with that of an intracellular solution. It is a very effective storage medium. PDL cell morphology remains unchanged in the medium, providing optimal pressure for cell growth. ViaSpan shows long-term superiority over HBSS and

conditioned media. The shortcomings of ViaSpan are that it must be refrigerated, it has a high cost and it is not readily available to the general public.[30,31] Viaspan clearl is the most effective medium with 37.6% vital fibroblasts after 168 hours of storage.[32] Generally, Viaspan is considered as a medium close to ideal, but it must be refrigerated, it has a high cost and it is not readily available to the general public making it difficult to use.[2]

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 favorable reimplantation, with less risk of root resorption and ankylosis.[18] Hwang *et al*., and Jung *et al*., reported enthusiastic results with green tea, with the maintenance of 90% of cell viability for upto 24 h, similar to the HBSS control. Jung et al. also observed that the higher the extract concentration the more efficient the medium.[18] 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.

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. The growth factors evaluated included mouse epidermal growth factor (EGF), recombinant human platelet-derived factor-AB (rhPDGF-AB), recombinant human PDGF-BB (rhPDGF-BB), natural human PDGF (nPDGF), transforming growth factor (TGF), and synthetic human insulin-like growth factor-I (IGF-I). Ashkenazi *et al*.,[33] observed, after 24 h of storage in different storage media supplemented with growth factors, that there was an increase in the mitogenicity of PDL fibroblasts by 20 to 37%. For short periods of storage (2 to 8 h), the use of media without growth factors is preferable.

Milk

Milk has been seen to be a compatible short-term storage medium when the teeth are placed in it within 15 to 20 minutes.[34] Milk has a pH of 6.5 to 7.2 and osmolarity of 270 mosmol kg-1, which is similar to extracellular fluid.[7] Milk only prevents cell death rather than restoring normal morphology and ability to differentiate and mitose. Blomlof *et al*.,[34] found that milk was a compatible storage medium for periodontal ligament cells, only when it was cold and fresh. However, Milk as a storage medium is the most practical transport medium for the storage of avulsed teeth because of its ready availability in almost all situations. Milk, which contains amino acids and vitamins, is capable of inactivating enzymes harmful to the PDL cells. Milk can potentially maintain PDL cell viability for up to 2 hours.[7] The disadvantages are that milk needs to be fresh and kept refrigerated, it does not replace depleted cell metabolites, and it does not facilitate cell mitosis. At a cellular level, milk is ranked equal to HBSS as a storage medium although it loses its effectiveness after 2 h. Powdered milk is one of the presentation forms of bovine milk and is considered as a feasible medium in cases of delayed tooth replantation. It has shown similar results to long shelf-life whole milk in relation to the healing process after delayed replantation of avulsed teeth. However, the powdered form is more effective than pasteurised milk as a medium only up to four hours, following which these substitutes perform worse than whole milk.[7]

Propolis

Propolis is an antibacterial and anti-inflammatory bee hive product. Propolis has antiseptic, antibiotic, antibacterial, antifungal, antiviral, antioxidant, anticarcinogenic, antithrombotic and immunomodulatory properties. Mori *et al*.,[4] in his study concluded that the efficacy of the medium increases if maintained for 6 h because the contact with product is beneficial for cell maintenance. Ozan *et al*.,[3] showed that 10% propolis was a more effective storage medium than milk with lower fat content (milk) and Hank's Balanced Salt Solution. Shaher *et al*.,[3] 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.

CONCLUSION

To date, several acceptable storage media, such as culture medium, HBSS and milk, have been proposed for avulsed teeth, with HBSS considered optimal. Apart from solutions designed specifically for storage and culture purposes, regular pasteurized whole milk is the most frequently recommended and with the best prognosis among other solutions that are likely to be available at the scene of an

accident, such as water, saline or saliva. Although HBSS, ViaSpan and Eagle's medium have great potential to maintain the PDL cells in a viable state after avulsion, the practicalities of using these solutions and the lack of ready availability to the general public make them less than ideal. However, a variety of new media like propolis, tender coconut water, honey milk, powdered milk and egg albumin have also been proposed as potential alternatives to HBSS. However, further clinical and/or in vitro studies are required before considering their usefulness and clinical effectiveness in the case of an avulsed tooth.

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