Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/6427
DOI URL: http://dx.doi.org/10.21474/IJAR01/6427

*RESEARCH ARTICLE*

# VARIOUS TRANSPORT MEDIA FOR AVULSED TOOTH: A REVIEW.

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## *Manuscript Info*

***Manuscript History***

Received: 03 December 2017
Final Accepted: 05 January 2018
Published: February 2018

***Key words:-***
Dentaltrauma,avulsion, periodontal ligament fibre viability,transport medium.

## *Abstract*

Dental avulsion is a consequence of injury that results in the complete displacement of a tooth from its alveolar socket.When dental avulsion occurs; immediate replantation at the trauma site is the ideal procedure for maintaining the viability of PDL cells. However, immediate replantation is rarely achieved.The type of storage medium used for 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.



## Introduction:-

Dental avulsion is a consequence of injury that results in the complete displacement of a tooth from its alveolar socket, and may affect multiple tissues, such as the periodontal ligament (PDL), alveolar bone, cementum, dental pulp, and gingival mucosa[1].It corresponds to 1% to 16% of all types of tooth injuries involving the permanent dentition.[2-5]This wide variation can be explained by the differences in the evaluated populations, including the levels of interpersonal violence, involvement in motorcycle and bicycle road and traffic accidents, especially without use of helmets, and practice of contact sports, especially without use of mouth guards.[2] When dental avulsion occurs, immediate replantation at the trauma site is the ideal procedure for maintaining the viability of PDL cells. However, immediate replantation is rarely achieved.[6] Immediate tooth replantation leads to a better PDL repair and reduces significantly the occurrence of root resorption. Therefore, shortening as much as possible the time elapsed between trauma and tooth replantation and maintaining the avulsed tooth in a suitable transport medium may attenuate the deleterious effects of the extrabuccal period on root surface and increase the prognosis considerably.[2,7,8] Inflammatory resorption and replacement resorption subsequent to dental alveolar ankylosis are the most significant and common complications after replantation of avulsed teeth.[9]

Successful tooth replantation with a favourable long-term prognosis particularly depends on the maintenance of PDL cell viability. Several storage media have been proposed for the purpose of transporting teeth following avulsion. The ideal storage medium should preserve cell vitality, adherence and clonogenic capacity and it should be readily available or easily accessible at the site of an accident.[10] Both pH and osmolality are more important than chemical composition of the medium in preserving the viability of PDL cells. Cellular growth occurs at an osmolality of 230-400 mosmol/kg and a pH of 6.6-7.8, but its optimal growth happens at an osmolality of 290-300 mosmol/kg and pH of 7.2-7.4.[11]

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.

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The purpose of this study was to review the literature regarding all available storage media and to highlight their characteristics.

**Types of storage media:-**

**Tap water:**

Tap water has been used as a storage medium for the avulsed tooth. It is not effective in maintaining PDL cell viability and is as good as air-drying. Water osmolality and its pH are 3 mosmol/kg and 7.40-7.79, respectively. Since water is hypotonic, its use results in rapid cellular lysis [11, 12].

**Normal saline:**

Isotonic saline has been used successfully as a storage medium by researchers in both animal and human studies. It has a comparable osmolality to that of PDL cells. However, unlike HBSS, milk and egg white, normal saline contains no nutrients and Lauer et al. showed that physiologic salt solution was unable to maintain the metabolism of the fibroblasts. Alacam et al. showed that normal saline was a poor medium, lacking metabolically essential ions and glucose required by PDL cells. Only a few cells remained viable after about 3h of storage.Cvek et al found that a tooth stored in normal saline for 30 min showed less resorption than a tooth stored dry for between 15 and 40 min. This contrasts with a human study where Trope reported little difference in the development of ankylosis between teeth stored in normal saline and teeth kept dry. Hence, normal saline appears to be suitable for short-term storage of avulsed teeth as it will maintain the vitality of the cells for about 2 h but it is potentially damaging if the cells are stored for longer than this.The saline solution provides osmolality of 280 mosm/kg and despite being compatible to the cells of the periodontal ligament, it lacks essential nutrients such as magnesium, calcium and glucose; necessary to the normal metabolic needs of the cells of the periodontal ligament [10, 13-17].

**Milk:**

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[17].Milk, which contains amino acids and vitamins, is capable of inactivating enzymes harmful to the PDL cells.[18]Blomlof et al. [19] reported that milk is a compatible short-term storage medium for teeth if they were placed in it within 15 to 20 min of being avulsed. Milk has a pH of 6.5 to 7.2 and osmolality of 270 mosmol/kg, which is similar to extracellular fluid. Milk can potentially maintain PDL cell viability for up to 2 h.[20] Huang et al found that milk at 40°C provided short-term viability to cells, but they did not remain attached after 48 h. When milk was used at 20°C, 24.4% retention of cells after 72 hrs was observed. HBSS was the best medium with 47% of the cells remaining attached after 72 h of exposure. [21]

**Saliva:**

Human saliva (buccal vestibule) is used as a storage medium due to its availability, but it has unfavourable characteristics, such as non-physiological pH and osmolality,high microbial contamination and hypotonicity.Though very readily available, avulsed teeth should not be stored for longer than 30 min in saliva.Saliva contains potentially harmful substances, such as enzymes, bacteria and their by-product.[10,11]Its osmolality is much lower than the physiologic(60-70 mosm/kg), thus it boosts the harming effects of bacterium contamination. Its only advantage is its availability. [17]

**Gatorade:**

Gatorade is a sports drink used for rehydration. This substance has pH 3 and osmolality between 280 and 360 mosm, which may cause damage to the cells due to the low pH and hypertonicity.Gatorade contains 20 mEq/L sodium, 3 mEq/L potassium,11.5 mEq/L chlorine, 22 mEq/L phosphorous, and 333 mmol/L carbohydrate sucrose/glucose.Because of the favourable osmolality and carbohydrate content, which provides an energy source for the PDL, it appears to be a suitable medium for the avulsed tooth.Studies have shown Gatorade to be a potential storage medium, although it was inferior to tap water in maintaining cell viability. The pH of Gatorade was thought to be the principal cause for maintaining decreased cell viability, as opposed to electrolyte composition and osmolality.[23,24]

**Hank's balanced salt solution:**

HBSS is essentially a pH-balanced salt solution containing all of the essential metabolites and glucose necessary for the maintenance of cells. HBSS was originally developed for scientific research purposes. It contains the following ingredients: 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 osmolality (280 mosmol kg-1) are ideal.HBSS has been useful in supporting the growth of many cell types. It can maintain the viability of PDL cells for several hours with a success rate of 90% reported when degenerated PDL cells were stored in HBSS for less than 30 min. The vitality, clonogenic and mitogenic capacity of PDL cells using this medium are excellent. HBSS is the only medium that can replenish metabolites in depleted PDL cells. [10,20, 25]

**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 fattyacid mono ester composites with an antimicrobial cationic component. Sigalas et al studied the efficacy of different contact lens solutions in maintaining the viability of cultured PDL cells by the Tripan 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.[22,26]

**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.ViaSpan is a clear to light yellow sterile, non-pyrogenic solution that has an approximate calculated osmolality of 320 mosmol $kg^{-1}$, a sodium concentration of 29 mEq $L^{-1}$, a potassium concentration of 125 mEq $L^{-1}$ and 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.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 as well as not being available in smaller containers.[10,27]

**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).Eagle's medium, alpha-Minimum Essential Media (MEM) and αMEM-S (supplemented with foetal calf serum and antibiotic) have been shown to maintain the viability and proliferative activity of PDL cells for an extended period of time (48-53 hours) with a reduced rate of inflammatory resorption. This can be attributed to the availability in the culture medium of all the required essential nutrients for the growth and proliferation of PDL cells. Supplementation and the addition of growth factors (platelet derived growth factor, insulin-like growth factor, epidermal growth factor, recombinant human platelet-derived factor-AB, natural human platelet-derived growth factor, transforming growth factor, synthetic human insulin-like growth factor-I, polypeptide growth factors etc) in a culture medium has also been shown to increase the mitogenic and clonogenic capacity of PDL cells for as long as 24 hours. As potent biologic mediators they are proposed to aid in regeneration of PDL.Similarly the addition of antioxidant-like, catalase supplementation to a medium have shown beneficial effects on PDLcells and a reduced rate of surface resorption.[17,28,29,30]

**Dubelco's storage medium:**
There is a variation of the Eagle's modified essential medium (EMEM), called Dubelco's modified Eagle'smedium (DMEM), which contains approximately fourtimes 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. However, it is not available to the public and therefore of little value as a storage medium for avulsed teeth.[10,31]

**Egg white:**
Egg white and ovalbumin, the major protein in egg 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.It has a pH of 8.6 to 9.3 and its osmolality is 258mosmol/kg.It has shown better cell viability and significantly higher incident of PDL healing as compared to milk and equivalent cell viability as HBSS.[2,11]

**Tooth rescue box (dentosafe):**
A tooth rescue box (Dentosafe_; DentosafeGmbH, Iserlohn, Germany; EMT Tooth Saver,SmartPractice.com, Phoenix, AZ, USA) was introduced and distributed in schools in parts of Germany and Switzerland and in all schools in Austria. The tooth rescue box contains a tissue culture medium similar to a medium used during islet cell transplantation. Besides different salts, the medium also contains amino acids, vitamins, and glucose. The medium was shown to maintain vitality and proliferative capacity of PDL cells for up to 48 h at room temperature in vitro. Due to an added protect medium and a preservative the unopened box has a shelf life of 3 years at room temperature (below $37^{o}$ C).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.[32,33]

**Green tea:**
Green tea (GT), extracted from Camellia sinensis, is a widely consumed beverage throughout the world that is second only to water. Green tea extracts (GTEs) contain catechin, which is one of the polyphenols from GT. Catechins in GT are catechin,epicatechin, epicatechingallate, epigallocatechin, epigallocatechin-3-gallate (EGCG), and so forth. It has been reported that GTEs have remarkable anti-inflammatory, antioxidant, and anticarcinogenic effects in a number of animal tumors,cell culture systems, and epidemiological studies.GTE has enough catechin, which is an antioxidant-rich ingredient and thus is thought to be effective in maintaining PDL cell viability.[34]

**Propolis:**
Propolis, a substance made by the honeybee, is a potent antimicrobial, antioxidant and anti-inflammatory agent. Recently, it has attracted much attention as a useful substance applied in medicine and cosmetics because of its antibacterial and antifungal activities.In general, propolis is composed of 50% resin and vegetable balsam, 30%wax, 10% essential and aromatic oils, 5% pollen, and 5% various other substances including organic debris depending on the place and time of collection.Mori et al 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 product is beneficial for cell maintenance. Another variable that could produce contradicting results is the propolis dilution vehicle. The most frequently used vehicle is ethanol, but the Dulbecco's modified Eagle's medium (DMEM) culture medium has also been used. Depending on the methodology, ethanol could have had any interference on the results, but propolis has the capacity to neutralize the toxic effects of ethanol, at least in part. This makes propolis a promising medium for the maintenance of avulsed teeth. [2,35,36]

**Morus rubra:**
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 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 properties have not been yet established yet. Further research is necessary before its use can be recommended. [37]

**Coconut water:**
Coconut water has a high osmolarity(372 mosm/L) because of the sugars present, which are primarily glucose and fructose.It is also rich in many essential amino acids including lysine, cystine, phenylalanine, histidine, and tryptophan.This natural isotonic fluid having pH of 4.1 is available in its natural form directly from the coconut. The predominant cations are potassium, calcium, and magnesium.Sodium, chloride, and phosphate are found inmuch lower concentrations.Superior maintenance of viability of the PDL cells in the coconut water group may be due to the nutrients that are present in coconut water such as proteins, amino acids, vitamins, and minerals, which help in nourishing the cells and maintaining their viability.[38]

**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.ORS is a glucose-electrolyte solution whose compositions keep the optimal osmolality as well as pH and can even provide nutrients. The osmolality and pH of ORS is 270 mosm/L and 7.8, respectively which makes it suitable for cellular growth. In addition, ORS is easily available and inexpensive.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. [39,40]

**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 at5% 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 h after storage. Hence, it can be a good storage media.[41]

**Ascorbic acid:**
The 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 phosphatases (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 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.[42]

**Emdogain:**
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. The diminishing can happen due to the lack of an adherent surface or the increase on the difference of fibroblasts, which grow in its presence.Emdogain increased the differentiation of cells mineralized tissue forming cells. 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, 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.[43]

**Salvia extract:**
Salvia extract is the largest genus of Lamiaceae and has about 900 species. Salvia officinalis extracts are useful spasmolytics,antiseptics and astringents.They also have antimicrobial and scavenging activities. Oxygen radicals and oxygen tension have been reported to modulate osteoblastic and osteoclastic activities. It is thought that oxidative damage may promote root surface resorption via toxic effects by enhancing resorptive activity of clastic cells and that media containing anti-oxidants might increase replantation success. The anti-oxidant effects of Salvia officinalis are due to its phenolic constituents – namely, rosmarinic acid, camosic acid, salvianolic acid and its derivatives.Ozan et al reported that Salvia officinalis not only keeps PDL cells alive, but also has antimicrobial, anti inflammatory and anti-oxidant properties.[44,45]

**Aloe Vera:**
Aloe Vera is a member of liliaceae family. This medicinal plant is cactus like with green, tapered leaves that are filled with a transparent viscous gel.[13] This gelatinous substance contains 96% water and 75 active properties such as vitamins, enzymes, minerals, sugars, salicylic acids, and amino acids. It has been reported that Aloe Verahas significant anti-inflammatory, antioxidant, antibacterial, antifungal and anticarcinogenic activities.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.[46]

## Conclusion:-

The prognosis of replanted tooth depends on the existence of viable periodontal ligament cells(PDL).If immediate replantation is not possible, storing in a proper storage media might be an alternative fact which may lead to better prognosis and prolong the tooth survival. Both the storage media and extra-alveolar duration are major critical factors in determining the final prognosis.However, the capacity of the storage media in maintaining PDL cells viability has been shown to be a more important factor.

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