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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/13340
DOI URL: http://dx.doi.org/10.21474/IJAR01/13340

RESEARCH ARTICLE

STORAGE MEDIUM FOR AVULSED TEETH: A LITERATURE REVIEW

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Manuscript Info

Manuscript History
Received: 29 June 2021
Final Accepted: 30 July 2021
Published: August 2021

Abstract

Avulsion injury is one of the most severe types of traumatic dental injuries. Following avulsion, periodontal ligament tissues are injured and the vessels and nerves of the pulp rupture at the apical foramen which causes pulp necrosis. In studies it was reported that the key to retention of the knocked-out teeth was to maintain the viability of the periodontal ligament. Storage media plays an important role in preserving the viability of PDL cells during extra alveolar time. This article highlights the different storage medias available for avulsed teeth, along with their merits and demerits.

Introduction:-

Dental trauma refers to injury to the teeth and periodontium (gums, periodontal ligament,alveolar bone), and nearby soft tissues such as the lips, tongue, etc .Dental trauma is accounting for a major part of dental problems in children and adolescents.Falling, fight, sports, accidents, and hittingobjects or people are among common etiologic factors. Traumatic dental injuries are neglected oral condition despite its relatively high prevalence. Among various types of dental injuries-Dental avulsion is an emergency in which prompt management (within 20–40 minutes of injury) results in favourable prognosis of the tooth.[1]

Tooth avulsion (exarticulation) implies total displacement of the tooth out of its socket. This kind of dental trauma causes the periodontal ligaments to be severed with or without fracture of the alveolus. Reported incidence of dental avulsion is 0.5-3%of all dental injuries. The age group of 7-14 years appears to be most affected.[2]At this age, the loosely structured periodontal ligament and low mineralized bone surrounding erupting teeth provide only minimal resistance to an extrusive force. When a tooth is avulsed, attachment damage and pulp necrosis occurs.[2]

Since most avulsions occur before the patient's facial growth is complete it is critical to maintain the integrity of the surrounding bone.The ideal treatment of avulsion injury is immediate replantation, but this can rarely be achieved.[3]In such conditions the avulsed tooth should be stored in appropriate storage media. Storage medium keeps the attached periodontal ligament cells in hydrated state and maintain their viability, allows healing with regenerated periodontal ligament cells when replanted. However, if excessive drying occurs before replantation , the damaged periodontal ligament cells will elicit an inflammatory response over a diffuse area on the root surface which will result in replacement resorption (ankylosis).[2]

This literature review attempts to discuss various storage media in depth, including their advantages and disadvantages for maintaining viability of attached PDL cells and allows favourable prognosis following Replantation.

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Storage Medium

The first line of treatment for an avulsed tooth is immediate replantation, however, most of the times it is not possible,therefore in situations when immediate replantation cannot be performed storage of avulsed teeth in an appropriate storage media is recommended.

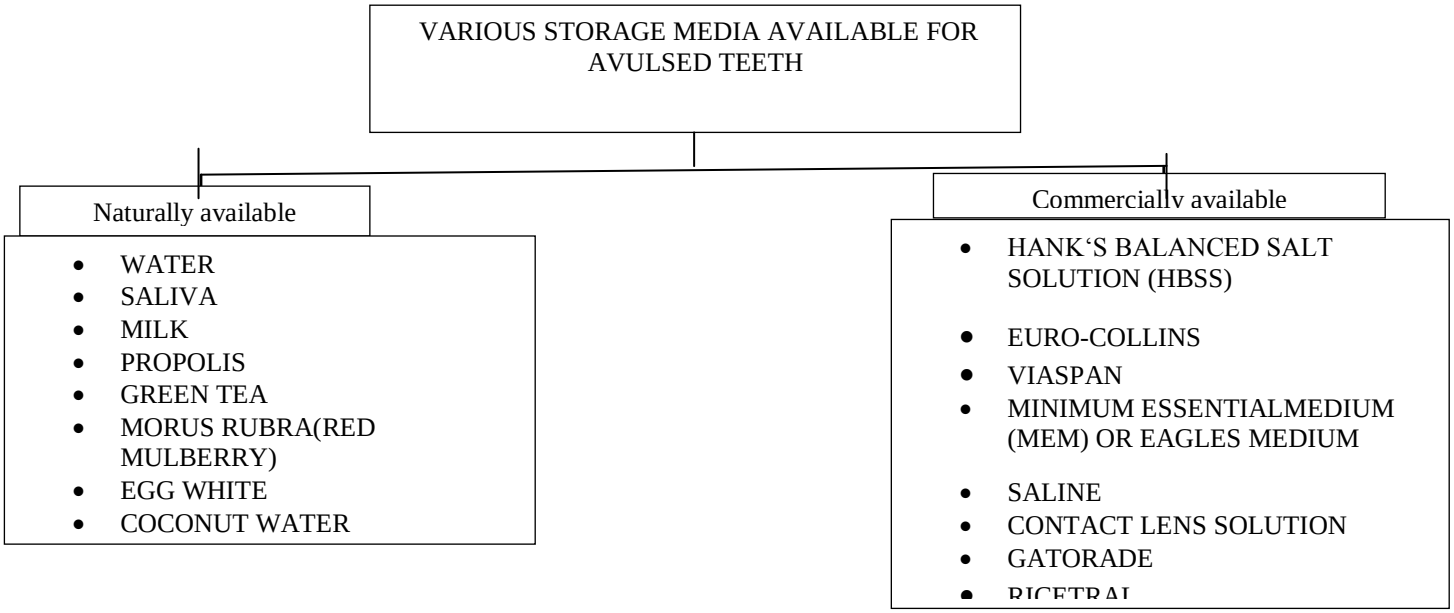
Definition

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.

Ideal requirements

1. It should have antimicrobial characteristics
2. It should maintain the viability of periodontal fibres for an acceptable period of time i.e. for at least 24 hours.
3. It should favour proliferative capacity of the cells (clonogenic and mitogenic capacity)
4. It should have the same osmolarity as that of body fluids (290-300 mOsm/ kg) and pHbalanced (7.2 – 7.4)
5. It should be unreactive with body fluids
6. It should not produce any antigen-antibody reaction
7. It should reduce the risk of post-replantation root resorption or ankylosis
8. It should have a good shelf life
9. It should be effective in different climates and under different conditions.
10. It should wash off extraneous materials and toxic waste products.
11. It should aid in reconstitution of depleted cellular metabolites.[3]

Different types of wet storage media for avulsed teeth have been investigated can be broadly classified under two broad categories.(flow chart 8)[4]



Flow Chart 1:-Classification For Available Storage Media

Most of the time the available adult or parents at the accident site place avulsed teeth in tap water because it is most readily available .But it is not an ideal storage media because several studies have shown that cells stored in water did not maintain their morphology, with visible destruction and rapid cell death.[5]Thus tap water should only be used to avoid tooth dehydration only if there is no other appropriate media available ,as water is inadequate for preservation of PDL cells attached to the avulsed teeth.(figure:1)

Advantages

It is the most readily available medium at the site of accident

Disadvantages
1. It has variable bacterial contamination
2. Hypotonic in nature
3. Non-physiologic ph(6.5-8.5) and osmolality (7-17mOsm/kg) which favours PDL cell lysis.[4]

Figure 1:- Tap Water.

Human saliva (buccal vestibule)
Saliva can be collected in a cup and tooth can be dropped into it or the tooth can be either placed in the patient mouth under the tongue or in the vestibule. But it is used as a storing medium for a short period of time as it can damage the cells of the periodontal ligament if used for longer than 1 hour.[6] (figure:2)

Advantage
It is the most immediately available storage medium at all accident sites.

Disadvantages
1. Osmolality is much lower than the physiologic (60-70 mOsm/kg).[7]
2. Has lesser amount of nutrients like glucose and essential growth factors to maintain PDL cell viability.
3. Presence of microrganisms make saliva less desirable.
4.

Figure 2:- Tooth Placed In Buccal Vestibule.

Milk
The American association of endodontics indicates milk as a storage medium for avulsed teeth for maintaining the viability of PDL cells. Milk is an excellent storage medium for upto 6 hours after which it loses its effectiveness.[8]

Not all types of milk are equally effective as storage medium. Avulsed teeth stored in chilled milk upto 6 hour can maintain sufficient number of viable PDL cells to support replantation of tooth and possibility of PDL healing.[9] Milk with lower fat content is considered to be more appropriate at maintaining cell viability than milk with higher fat content.[10] (figure:3)

Advantages

1. Isotonic liquidwith physiologic osmolality(275 mOsm /kg)
2. Neutral ph (6.5-6.8)[11]
3. Low or no bacterial content because of pasteurization
4. Contains growth factors and essential nutrients for cells
5. Milk contains epithelial growth factor which stimulates the proliferation and regeneration of epithelial cell rests of Malassez

Disadvantage

As regular pasteurized milk has a short shelf life and requires refrigeration which makes it less readily available at the trauma site.

Figure 3:- Milk.

Egg white and ovalbumin

Sousa et al. (2008) evaluating human PDL adhered to extracted tooth roots and maintained in this storage medium observed that the egg white provided cell viability and histologicalcharacteristics similar to those of milk.[12] *Khademi et al (2008)* 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.(figure:4)

Advantages

1. It has physiological osmolality in between 251-298 mOsm/kg.[7]
2. Good choice as a storage media due to high content of proteins, vitamins and water.
3. Absence of microbial contamination
4. Easy access

Disadvantages

1. Loss of efficacy if used for longer period as storage media, possibly due to egg's high ph (7.6-8.5)
2. PDL cells might recognise the egg proteins as foreign bodies and could develop antibodies against them leading to more destruction than benefit.[11]

Figure 4:- Egg White.

Coconut water

Several studies have been performed to use this substance as a storage medium for avulsed teeth, but the results are contradictory. *Gopikrishna et al. (2008)* found greater efficacy of coconut water over HBSS and milk for maintaining the viability of PDL cells.[13] *Thomas et al. (2008)* found that 15 to 120 min storage in coconut water is as efficient as storage in HBBS. On the other hand, *Pearson et al. (2003)* and *Thomas et al. (2008)* observed that inflammatory resorption was more frequent when the tooth was maintained in coconut water compared with milk. (figure:5)

Advantages

1. Biologically pure,sterile product
2. Rich in amino acids,proteins,vitamins and minerals.
3. Aids in replenishing fluids, electrolytes and sugars lost from the body

Disadvantages

The acidic ph (4.13) is a concern because of deleterious effect on cell metabolism.[14]

Figure 5:- Coconut Water.

Green tea

Hwang et al. (2011) and *Jung et al. (2011)*reported enthusiastic results with green tea, with the maintenance of 90% of cell viability for up to 24 h, similar to the HBSS control.[5] *Jung et al. (2011)* 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 preservation of avulsed teeth and its beneficial effect is enhanced by higher extract concentrations.(figure:6)

Advantages

1. Antioxidant,anti inflammatory and antimicrobial properties.
2. Have capacity to protect the alveolar bone against inflammatoryresorption (caused by pathogenic microorganisms).

Disadvantages

Very difficult to procure at the accident site.

Figure 6:- Green Tea Extract.

Propolis

Propolis is a sticky resin obtained chiefly from the buds of some conifer trees. It consists of flavonoids (45-55%), waxes and fatty acids (23-35%), essential oils (10%), pollen-proteins (>1%), vitamins and sugars (5%), other organics (ketones, lactones, quinones, steroids) and trace minerals (iron and zinc).*Martin and Pileggi (2004)* considered propolis as the most efficient medium whereas *Gopikrishna et al. (2008)* found that propolis had 50% efficacy in maintaining cell viability.[15] *Casaroto et al. (2010)* reported good results of propolis for maintenance of cell viability, but the root resorptions were visible, which compromises its efficacy as a storage media.(figure:7)

Advantages

1. Remarkable antioxidant property
2. Anti inflammatory in action
3. Antimicrobial efficacy

Disadvantages

1. It is not readily available at the site of accident.
2. Variable composition of propolisis found depending on the location of plants, climate and seasons.[16]

Figure 7:- Propolis.

Morusrubra(red mulberry)

It is a natural product usually availablein Southern Europe, Middle East,Northern Africa and Indian subcontinent which contains flavonoids, alkaloidsand polysaccharides. *Ozan et al. (2008)* 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.[17] 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 established yet. Further research is necessary before its use can be recommended.(figure:8)

Advantages
Contain essential nutrients important for cell preservation.

Disadvantage
Poor availability at the site of accident.

Figure 8:- Morus Rubura (Red Mulberry).

Commercially available storage media
Saline
Moreira-Neto et al. evaluated the viability of cultured PDL cells and found 55% of living cells after 4 h storage in saline and whereas*Pileggi et al*. evaluated the PDL cells viability when stored in saline for 45 min and found 20% mortality in PDL cells.[14,15] Using a similar methodology, *Martin and Pileggi* in an another study found that saline had much less effectiveness compared with HBSS and milk. Consequently, saline is not an adequate medium, but it may be employed when travel time is less than 1 hour..(figure:9)

Advantages
1. It has physiological osmolality 308 mOsm/kgand ph 7.[7]
2. It is readily available at local pharmacies as compared to other commercially available medium
3. Low cost

Disadvantage
It does not contain essential ions and glucose, which are fundamental for maintaining the cell metabolism.

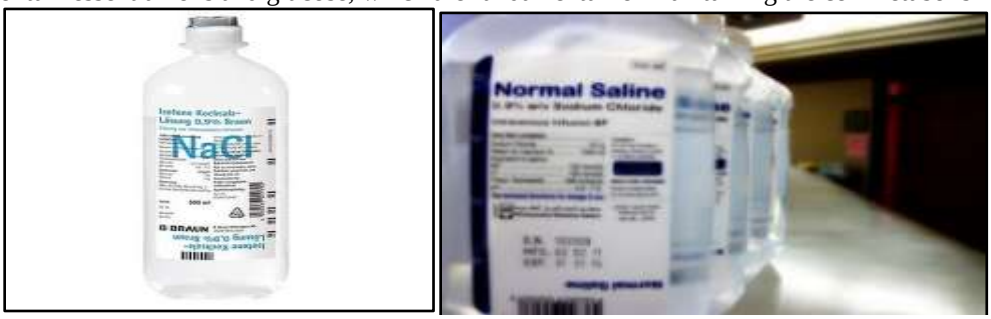


Figure 9:- Normal Saline

Ricetral:
Dehydration, as in diarrhea cases, is treated with oral rehydration solutions like Ricetral. These solutions are available in drugstores at low cost and their components are able to maintain the body hydrated by replenishment of liquids lost in the intestine. *Rajendran etal. (2011)* evaluated the PDL cell viability of extracted human teeth by the Tripan blue exclusion method and the results of the study showed that the effectiveness of Ricetral was similar to the HBSS control and both were superior to milk as storage medium. In an another study by *Subramanian P et al(2014)*the viability of PDL cells in Ricetral was maintained for 45 minutes, when it was used as storage media whereas in HBSS the viability of cells was maintained for 24 hours.[18] Thus HBSS was found to be more effectiveas compared to Ricetral in terms of storage time when both were used as storage medium for avulsed teeth.(figure:10)

Advantages
1. It contains essential nutrients like glucose and vital salts in concentrations considered adequate for the cell metabolism.
2. Low cost
3. Readily available at drug stores.

Disadvantage
Efficacy is not good as that of HBSS as it can be used as storage medium for only 45 minutes.[19]

Figure 10:- Ricteral

Contact lens solution
Contact lens solutions are fatty acid monoester composites with an antimicrobial cationic component .

These are accessible at accident site as they are readily available at pharmacies and the number of people using contact lenses is growing nowadays.

Thus in order to evaluate the efficacy of different contact lens solutions in maintaining the viability of cultured PDL cells *Sigalas et al.(2004)*did a study using different available contact lens solutions like Soft Wear, Opti Free, and Solo Careand the results of the study showed that Contact lens solutions could serve as short-term (1h) storage media if the other solutions are not readily available.[20] (figure:11)

Advantages
1. Contact lens solutions are readily available at homes, schools and centres of physical activities *(Sigalas et al. 2004, Goswami M et al.2011)*
2. It has antimicrobial activity

Disadvantages
Preservatives in the contact lens solution might harm the PDL cells if storage of avulsed teeth in contact lens solution is more than 1 hour.

Figure 11:-Contact Lenses Solution

Gatorade

It is a sports drinks used for rehydration and is available at sport's equipment stores. *Sigalas et al.* (2004) evaluated Gatorade efficacy in maintaining the viability of PDL cell culture by the Tripan blue exclusion method and the results showed that at 37^0C it was toxic to the cells; but this may be an alternate medium to HBSS and milk when used as ice cold and for a short time.(figure:12).

Advantages

It is readily available at the centre of sports activities.

Disadvantages

1. It has osmolality of 360 mOsm/kg, which may cause damage to the cells due to hypertonicity*(Goswami M et al.2011).*[7]
2. Has low ph of 3.

Figure 12:- Gatorade

Hank's Balanced Salt Solution (HBSS)

Hank's balanced salt solution has been commercially prepared storage medium for avulsed teeth.It contains active ingredients like 8 g/L sodium chloride, 0.4 g/L D-glucose, 0.4 g/L potassium chloride, 0.14 g/L calcium chloride, 0.35 g/L sodium bicarbonate, 0.09 g/L monobasic sodium phosphate, 0.1 g/L anhydrous magnesium sulphate and water as a vehicle and has the ideal osmolality of 270-290 mOsm/kgand pH of 7.2.[7] HBSS is available at Sigma Aldrich Pharmaceuticals, Bangalore, India .

Hwang et al. reported 94% cell viability after storage of cultured human PDL cells for 24 h in this medium, which is considered as an excellent result, and S*ouza et al*. had also reported similar results with HBSS. Moreover it has also beenshown to replenish metabolites which have been depleted from PDL cells. Hence, it has been recommended to place avulsed teeth in HBSS for 30 minutes before replantation in order to replenish the PDL cells, even if the avulsed teeth had been stored in an appropriate storage medium(figure:13).[11]In some countries ,HBSS is available in emergency kits [Save- A-Tooth, PA, USA]. This kit comprises a small basket to hold the avulsed tooth while it is submerged in HBSS. Each tooth that goes into Save-A-Tooth gets into its own protective slot that prevents any serious damage while transporting the avulsed tooth and the design of the slots allows the dentist to remove the tooth without accidentally damaging the root.(figure:14)

Advantages

1. It has physiologic ph of 7.2 and osmolality of 270-290 mOsm/kg.[7]
2. The presence of magnesium, glucose,calcium and other essential nutrients can maintain the PDL cell viability.
3. Replenishes the metabolites that had been depleted from the PDL cells
4. It has a long shelf life of 2 years and does not require refrigeration.

Disadvantages

1. It is not readily available at an accident site, which makes it difficult to be used as a storage medium.
2. High cost
3. It has been recommended to use HBSS at 37^0 C in a controlled incubator.[21]

Figure 13:-Hbss

Figure 14:- Save A Tooth

Culture media

Culture media such as 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 as reported by *(Andreasen JO et al.1978 ,Pohl Y et al. 1999,Ashkenazi M et al.2000,Sigalas E 2004.*[22](figure:15). In India it can be bought from Sigma Aldrich Pharmaceuticals, Banglore.

Advantages

1. The culture medium contains all the required essential nutrients for the growth and proliferation of PDL cells.

2. Supplementation and the addition of growth factors (platelet derived growth factor, insulin-like growth factor, epidermal growth factor, 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*(Ashkenazi M et al.2000)*.[22]
3. Act as a potent biologic mediator and proposed to aid in regeneration of PDL.
4. The addition of antioxidant, catalase supplementation to a medium have shown to produce reduced rate of surface resorption.

Disadvantages
Poor availabilty at the accident site.

Figure 15:- Alpha-Minimum Essential Media

Viaspan
It is a widely used solution for the storage and transportation of organs to be transplanted. In Dentistry it can be used as a storage medium for avulsed teeth.(figure:16). It can be bought online at a site www.medindia.net.

Advantages
It has 320mOsm/kg osmolality and 7.4 pH that helps to maintain the PDL cells viability.[7]

Disadvantages
The limited access to it, especially at the site of the accident, makes itdifficult to use .


Figure 16:-Viaspan

Euro-Collins solutions

It is used as a storage medium for avulsed teeth is due tothe fact that it is a hypothermal medium developed for preserving organs to be transplanted. Its characteristics include a pH of 7.4 and osmolality of 340 mOsm/kg (figure:17).[19]It is available online at www.medicineindia.org.

Advantages

1. It has physiologic ph and osmolality to maintain PDL cell viability
2. Electrolytes and phosphate present in Eurocollinshas buffering capacitythat prevents PDL cell acidosis.
3. High concentration of potassium in eurocollins decrease the intracellular cation loss.
4. Presence of essential nutrients for growth and proliferation of PDL cells.

Disadvantage

1. Poor availability at the site of accident
2. High cost

Figure 17:- Euro Collin Solution

Conclusion:-

Up to now, there is not a single product or solution that possesses all the characteristics required to be indicated as the ideal storage medium for avulsed teeth, that is, be capable of preserving the vitality of the PDL cells, while presenting compatible physiological pH and osmolality, clonogenic capacity, antioxidant property, no or minimal microbial contamination, high availability, ready accessibility at accident sites, homes, schools, hospitals and dental offices, and low cost. Based on the literature, it could be stated that, so far, apart from solutions designed specifically for storage and culture purposes, regular pasteurized low fat milk is the most frequentlyrecommended 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. Taking together the characteristics, efficacy and availability and accessibility, milk appears as the best indication of a temporary storage medium for avulsed teeth before replantation, and its use is recommended by the International Association of Dental Traumatology and the American Academy of Pediatric Dentistry.

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