# REVIEW ARTICLE

# IMPORTANCE OF STORAGE MEDIA FOR REPLANTATION OF AVULSED TEETH: A REVIEW

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# **Abstract**

Avulsion of tooth is the total displacement of a tooth from a socket. Has a prevalence of 0.5-3% among all dental injuries1. Hank's balanced salt solution can preserve periodontal ligament cells in vitro for 120 hours (5 days) and in vivo for 96 hours (4 days). This is one of the best solutions for the storage of avulsed teeth, because it does not need refrigeration, it has two years of shelf-life and it has been recommended and used with success for clinicians and researchers. Due to minimal side effect and maximum health benefit, many natural products such as Tender coconut water, Green tea extract, Aloevera, Long Shelf Life Honey Milk, MorusRubra, Salvia officinalis and recently Pomegranate Juice, Soymilk can also be used.

Key Words Storage media, Avulsed tooth, Hank's Balanced Salt Solution, ViaSpan, Minimum Essential Medium (MEM), Propolis

#### INTRODUCTION

Tooth avulsion (exarticulation, total luxation) implies total displacement of the tooth out of its socket. Avulsion of permanent teeth is seen in 0.5-3% of all dental injuries, most commonly involving single maxillary young permanent incisor tooth. 1,2,3

The ideal treatment of choice at the time of avulsion is "Immediate Replantation", thus reestablishing the natural nutrient supply to the periodontal ligament cells, thereby minimizing further damage and enhancing the healing process. 4.5

However, in spite of its recognized therapeutic value, clinical experience has shown that immediate replantation rarely occurs due to lack of knowledge or confidence of the general population and even professionals about replantation procedures.

Where such situations exist, the tooth should be stored in a medium that maintains periodontal ligament cell viability until definitive dental treatment can be accomplished.<sup>6</sup>

"A storage medium may be defined as a physiological solution that closely replicates the oral environment and helps to preserve the viability of PDL cells following avulsion."

Different types of wet storage media for avulsed teeth have been investigated, which may vary from naturally available products to artificial agents. The present article is a review of the availability, property and clinical application of different storage media, their role in maintaining viability of periodontal ligament cells. Special focus

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has been given to present the ongoing developments in this field.

#### **METHODOLOGY**

A broad search of Medline, Research Gate, Ebscohost, Pub Med and Google Scholar databases was conducted for the years 1980 until 2016, using as index terms 'management of avulsed tooth', 'tooth avulsion', 'storage media', 'storage media in management of avulsion', 'extra alveolar storage media'. Papers other than in English were excluded.

#### IDEAL PROPERTIES OF STORAGE MEDIA 8,9

- Maintenance of the viability and proliferative capacity of periodontal ligament cell for an acceptable period of time.
- The storage medium should have a physiological osmolality and pH. The cellular growth occurs between 290 and 330 mOsm/kg and at pH 7.2 to 7.4.
- It should be maintained at an appropriate temperature to allow optimal cell growth or survival.
- Should have antimicrobial characteristics
- Does not produce any antigen-antibody reactions
- Reduces the risk of post-reimplantation root resorption or ankylosis
- Un reactive with body fluids
- Good shelf life
- Effective in different climates and under different conditions
- Should wash off extraneous materials and toxic waste products
- Should aid in reconstitution of depleted cellular metabolites
- Should be cost effective and readily available for use in emergency situations.

# FACTORS DETERMINING THE EFFICACY OF STORAGE MEDIA 10,11

#### 1. Extra-Alveolar Duration

According to Andreasen et al, among the various clinical factors, the length of the dry extra-alveolar period seems to be most crucial.

Avulsed teeth usually experience an extraalveolar duration of as long as 5 hours before replantation and longer duration is associated with a decreased incidence of PDL healing.

# 2. Osmolality and pH

Cellular growth may occur between 290 and 330mOsm/kg. The pH must be between 7.2 and 7.4, but growth may occur between 6.6 and 7.8.

#### 3. Temperature

The temperature of the storage medium appears to have little influence on periodontal healing and vitality of the cells of the periodontal membrane, as long as it is kept below 37°C.

#### **CLASSIFICATION OF STORAGE MEDIA**

Storage media may be classified as:

- 1. Artificial Hank's Balanced Salt Solution, ViaSpan, Saline, Oral Rehydration Solution (Ricetral), Contact Lens Solution, Culture Media, Euro-Collins, Custodiol, Gatorade, Emdogain, Save A Tooth, Tooth Rescue Box, Probiotic
- 2. Natural Milk, Saliva, Tap water, Propolis, Coconut water, Green Tea Extract, Aloevera, Chick Egg White, Long Shelf Life Honey Milk, MorusRubra, Salvia officinalis, Pomegranate Juice, Soymilk.

# DESCRIPTION OF INDIVIDUAL STORAGE MEDIA

#### 1. Hank's Balanced Salt Solution (HBSS)

HBSS is considered as the gold standard.

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 and the only medium that can replenish metabolites in depleted PDL cells.

This solution is non-toxic; it is biocompatible with periodontal ligament cells, pH balanced at 7.2 and has an osmolality of 320mOsm/kg.It contains ingredients such as glucose, calcium and magnesium ions which can sustain and reconstitute the depleted cellular components of the periodontal ligament cells.

Ashkenazi et al (1999)<sup>12</sup> showed that Hank's balanced salt solution was the most effective medium for preserving viability, mitogenicity and clonogenic capacities of periodontal ligament cells for up to 24 hours at 4°C

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).

However, ready access to this solution for first aid purposes is a major drawback in most places where avulsion occurs such as at sports fields or schools, where it might be most needed. Cost is also a major prohibitive factor.

# 2. ViaSpan

Hiltz and Trope (1991)<sup>13</sup> observed ViaSpan to be

an effective storage medium, with 33% vital cells at 144 hrs

ViaSpan is considered as a medium close to ideal, but the limited access to it, especially at the moment of the accident, high cost (USD 300 per liter), makes it difficult to use as regular storage medium.

#### 3. Normal Saline

Cveket al.(1974)<sup>14</sup> suggested that wet (saline or saliva) storage for 25 to 60 min can lower the occurrence of ankylosis prior to replantation (20%), when compared with dry conditions (60%). In contrast, other studies have shown negligible effects of saline storage if the extra-alveolar duration exceeds 30 min.

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.

#### 4. Milk

It is the second or third best transportation media for avulsed teeth, according to the International Association of Dental Traumatology, and the AAPD due to its beneficial effects and characteristics, and its ease of access at the moment of trauma.

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 hrs. Milk has several favorable characteristics as a storage medium for avulsed teeth, as it is an isotonic liquid with an approximately neutral pH and physiological osmolality, has low or no bacterial content, contains growth factors and essential nutrients for cells, in addition to having a high availability mostly everywhere and low cost.

Blomlof L, Otteskog P (1980)<sup>15</sup> 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. Lekicet al. (1998)<sup>16</sup> demonstrated that milk was as effective as HBSS for storing avulsed teeth for up to 1 h, and superior to saline, saliva or water.

Low fat content milk and chilled milk has shown better results in maintaining the viability of PDL and for a longer time period.<sup>17</sup>

The drawbacks are that milk needs to be fresh and kept refrigerated, it does not replace depleted cell metabolites, and it does not facilitate cell mitosis.

#### 5. Saliva

A healthy person's mean daily saliva production ranges from 1 to 1.5L. According to Weine, 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.

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. Its osmolality (60-70 mOsm/kg) is much lower than the physiologic, thus Storage of avulsed teeth in saliva for 2 to 3 h causes swelling and membrane damage of PDL cells and also presence of microorganisms makes saliva a less desirable storage medium.<sup>18</sup>

Its use should be limited to cases where the extraalveolar duration is less and other superior storage media are not available.

#### 6. Tap water

Tap water has inadequate characteristics to be used as storage medium for avulsed teeth because it has bacterial contamination, hypotonicity, and non-physiological pH (7.4 to 7.79) and osmolality (30 mosmol kg-1), which favors the PDL cells lysis.

Blomlofet al. (1983)<sup>19</sup> found that storing cultured human PDL cells in tap water for 1 hr caused more PDL cell damage than the other physiological and non-physiological storage media tested. However as it is readily available, even at athletic fields, it can be used as a media of last resort, as opposed to allowing the tooth to dry out.

# 7. Oral Rehydration Solution (Ricetral)

Oral rehydration solutions (ORS) are readily available and consist of essential cell nutrients, such as glucose and vital salts, in concentrations deemed adequate for the cell metabolism to remain unhindered. Another advantage regarding these formulations is that they are marketed in sealed sterile pouches.

Rajendranet al. (2011)<sup>20</sup> evaluated the PDL cell viability of extracted human teeth by the Tripan blue exclusion method and the results showed that Ricetral was similar to the HBSS control and both were superior to milk.

However, these solutions address only cell metabolite depletion, other major issues such as contamination and the regenerative capacity of the surviving fibroblasts also need to be considered.

#### 8. Contact Lens Solutions

They contain buffered, isotonic saline solutions with the addition of preservatives that may preserve the viability of PDL cells. The solutions preserve significantly more viable cells than tap water and Gatorade but are not as effective as HBSS and milk.

Sigalaset al. (2004)11 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.

However, in the absence of other storage medium, they may be used instead of water or saline for short periods of time.

#### 9. Culture Media

Culture media such as Eagle's medium, alpha-Minimum Essential Media (MEM) and  $\alpha$  MEM-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 of all the required essential nutrients for the growth and proliferation of PDL cells in culture media.

Ashkenazi et al. (1999)<sup>12</sup> showed high viability of PDL fibers after 24 hours of storage in alphaminimum essential media-S (MEM-S) supplemented with foetal calf serum and antibiotic. Pohl et al.(1999)<sup>21</sup> showed that the proliferative activity of periodontal ligament (PDL) cells of teeth stored in cell culture medium for up to 48 hours increased with storage time. Sigalaset al. (2004)<sup>11</sup> found that using culture media (minimum essential media) produced the highest mean number of viable cells (67%) at both room temperature and 0°C.

Although culture media have shown promising results as storage media, non-availability at the trauma site is a major disadvantage.

#### 10. Euro-Collins

Sottovia et al.(2010) <sup>22</sup> in a histological analysis of avulsed dog's teeth stored in the Euro-Collins solution, observed similar results to those observed after immediate replantation, with good repair of supporting tissues, repair and reorganization of vessels and PDL collagen fibers and neoformation of cementum.

The literature has 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.

#### 11. Custodiol

Custodiol is the registered trademark of Dr. Franz. This is a histidine-tryptophan ketoglutarate solution with high flow properties and low potassium content. It is used as a preservation solution for organ transplantation. Its composition is similar to that of extracellular fluid. It has an osmolality of 310 mosmol/ L Alaçamet al.(1996)<sup>23</sup>

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.

#### 12. Gatorade R

Gatorade was originally formulated as a drink for sportspeopleto replenish electrolytes during training and sport events.

Harkaczet al. (1997)<sup>24</sup> were the first to test its effectiveness as a storage medium& concluded that Gatorade R did not turn to be an adequate storing medium for avulsed teeth due to its pH around 2.91 and its osmolality of 407 mOsm/kg.

Therefore, Gatorade R can only serve as a storage medium if other more acceptable media are not available, rather than allowing the avulsed tooth to dry out.

# 13. Emdogain (Enamel Matrix Derivative, EMD)

Emdogain (Biora, Malmo, Sweden) is a commercial Enamel Matrix Derivative (EMD) extracted from developing embryonic enamel of porcine origin and contains several matrix proteins.

Studies have shown that it can influence the migration, attachment, proliferative capacity and biosynthetic activity of PDL cells.<sup>25</sup>

It has also been used in antiresorptive-regenerative therapy along with topical Glucocorticoids and systemic Doxycycline. Thus, it is a recommended therapeutic agent for the management of avulsed permanent teeth.

M.Schjott, J.O.Andreasen (2005)<sup>26</sup> performed a study to evaluate the benefit of Emdogain on tooth avulsion & found that Emdogain was not successful to prevent or cure replacement resorption (ankylosis). This study, however, leaves the question whether Emdogain delays the progress of replacement resorption (ankylosis). Such a study is now in progress.

#### 14. Save-A Tooth

Save-A-Tooth® is the only low FDA approved emergency tooth preserving system that can protect and preserve knocked-out teeth for 24 hours.

It is an Emergency Tooth Preserving System is a six-part device that protects from the two primary causes of replanted tooth loss: tooth root cell crushing and tooth cell nutrient depletion. It uses a scientifically engineered removable basket and net to hold the tooth, and a special pH balanced preserving fluid (HBSS) that preserves and reconstitutes tooth cells.

There is little difference in clinical resorption rates of teeth reimplanted within 15 minutes and those reimplanted after many hours of storage, with less than 9% of the teeth stored in the system demonstrating moderate or severe root resorption.<sup>27</sup> Recommended for schools, ambulances etc

#### 15. Tooth Rescue Box (Dentosafe)

A tooth rescue box containing Special Cell Culture Medium (SCCM) including amino acids, vitamins and glucose has been developed. In Europe it is marketed as Dentosafe (Dentosafe, Germany) and in the USA as EMT Tooth Saver .This medium has shown to maintain the vitality and viability of PDL cells at room temperature for at least 48 hours.<sup>28</sup>

However, currently, this solution and device is not readily available in all countries.

#### 16. Probiotic

Caglaret al. (2010)<sup>29</sup> 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 HBSS, milk, L. reuteri solution, and saline.

Thus, Probiotics can be used as a storage media but further clinical study is required.

#### 17. Propolis

Propolis is applied in the different dental specialties: oral hygiene; periodontology and oral mucosa pathologies; oral surgery; orthodontics; restorative dentistry; endodontics and prosthetic dentistry.

Margaret and Pileggi (2004)<sup>30</sup> reported that teeth stored in propolis demonstrated the highest viability for PDL cells, when compared with HBSS, milk and saline. It can inhibit the late stages of osteoclast maturation so it may be useful as an intracanal medicament to reduce resorption of traumatized teeth.

Ozanet al. (2007)<sup>31</sup> have analyzed the properties of propolis as a storing medium for the maintenance of cellular viability of the periodontal ligament of avulsed teeth. The major disadvantage of propolis is that it is not readily available to the public.

#### 18. Coconut Water

This is a natural isotonic fluid having pH of 4.1 and the osmolality is 372 mOsm/L.

Gopikrishnaet al. (2008)<sup>32</sup> concluded that Coconut Water may be better alternative to HBSS or milk in terms of maintaining PDL cell viability after avulsion and storage.

Thomas et al. (2008)<sup>33</sup> found that 15 to 120 min storage in coconut water is as efficient as storage in HBBS. The pH of 4.1 is deleterious to cell metabolism is a cause of concern for its efficacy to maintaining viability of living cells.

Standardized studies with similar methods are required to avoid diverging results and eliminate doubts over its use, as this is a medium with easy access and good biological characteristics that could be promising for its indication.

#### 19. Green Tea Extract (GTE)

Tea Extract (GTE) capitalizes on its own natural antibacterial and antioxidant properties and has been shown to specifically improve oral health. Studies show that two of the catechins found in green tea Epigallocatechin Gallate (EGCG) and EpicatechinGallate (ECG) combat oral plaque and bacteria, thus fighting bad breath. These anti-inflammatory properties of green tea protect against gingivitis, gum disease, and bleeding gums

Ji Young Hwang et al.(2011)<sup>34</sup> concluded that the efficacy of GTE in maintaining the viability of human PDL cells is similar to that of HBSS and higher than that of milk & therefore GTE could be a suitable, alternative storage medium for avulsed teeth.

#### 20. Aloe-Vera

Aloe Vera has significant anti-inflammatory, antioxidant, antibacterial, antifungal activities and great wound healing effect.

K. Pattamapunet al. (2006)<sup>35</sup> concluded that Aloe vera may be useful for maintenance of the integrity and viability of periodontal ligament cells after an extended extra-oral dry time.

Badakhshet al. (2014)<sup>36</sup> recommended that 10%, 30% and 50% concentrations of Aloe Vera may be recommended as a suitable storage media for avulsed teeth.

#### 21. Egg White

Egg white contains high protein, vitamins and water. Khademiet al.(2008)<sup>37</sup> reported no significant difference between egg white and HBSS, and also found egg white to be superior to tap water and milk.

Due to high nutrient value as well as availability at the trauma site, egg white may be considered as a good alternative to conventional storage media

#### 22. Long-Shelf Life Honey Milk

Milk remains the most convenient, cheapest and readily available solution in most situations while also being capable of keeping PDL cells alive.

Nozariet al. (2013)<sup>38</sup> demonstrated that adding honey to Long shelf life pasteurized milk (140°C for 3 hours) can enhance its storage potential by up to 9 hours.

Appropriate osmolality and pH for optimal growth of cells accompanied by the presence of nutritional substances in milk may be responsible for its acceptable results as storage media

#### 23. MorusRubra(Red Mulberry)

Recently the juice of the fruit of Morusrubra(red mulberry) has been recommended as a suitable transport medium for avulsed teeth.

OzanFatihet al. (2008)<sup>39</sup> performed a study to determine the ability of the juice of Morusrubra fruit for the maintenance of periodontal ligament (PDL) cell viability of avulsed teeth and concluded that M. rubra can be recommended as a suitable transport media for avulsed teeth

#### 24. Salvia Officinalis

Use of Salvia officinalis has been proposed as an alternate option for clinicians to transport (store) avulsed teeth until reimplantation.

Ozanet al.  $(2008)^{40}$  observed that PDL cells' viability at 1-3 hours is similar for 2.5% S. officinalis and HBSS, whereas at 24 hours, the efficacy of 2.5%

S. officinalis is significantly better than HBSS. Thus, S. officinalis can be recommended as a suitable transport medium for avulsed teeth.

# 25. Pomegranate Juice (PunicaGranatum)

Pomegranate is the fruit of PunicaGranatum (Punicaceae) that has been extensively used in the folk medicine of many years. TavassoliHojjatiet al. (2014)<sup>41</sup> found that 7.5% was the most effective solution for maintaining PDL cell viability.

Since research conducted to assess its efficacy is very less, further research is needed.

#### 26. Soymilk

Soymilk is a beverage made from soybeans. It is long shelf life milk that has many nutrients for maintaining the viability of PDL cells and for nourishing them.

FariborzMoazamiet al. (2012)<sup>42</sup> showed that HBSS, powdered milk, and soymilk preserved cell vitality as good as the positive control Dulbecco's modified Eagles medium (DMEM) at all experimental time periods.

Besides these, Growth factors, Ascorbic acids, L-DOPA, Cryoprotective agents, Catalase supplementation have also been tried as storage media.

#### COMPARATIVE EVALUATION OF DIFFERENT MEDIA

STORAGE MEDIUM	CHARACTERISTICS	EFFICACY	ACCESSIBILITY
Hank's Balanced Salt Solution (HBSS)	Physiological pH, osmolality and nutrients	Excellent	
Milk	Small bacterial contents, isotonic, physiological pH, osmolality, growth factors and nutrients	Excellent	+
Viaspan®	Physiological pH, osmolality and favorable to cell growth	Excellent	
Euro-Collins®	Physio logical pH and hypothermal capacity	Excellent	
Minimum Essential Medium (MEM)	Nutrients, antimicrobial property and growth factors	Excellent	
Propolis	Anti-inflammatory, antibacterial and antioxidant properties	Excellent	-
Green tea	Anti-inflammatory, antibacterial and antioxidant properties	Excellent	-
Coconut water	Sterile, natural product and contains nutrients	Good	+
Egg white	Low microbial contamination, contains nutrients and water	Good	+
Red mulberry	Not established	Good	-
ORS(Ricetral)	Essential cells and nutrients	Good	+
Saline	Physiological pH and osmolality	Poor	+
Gatorade	Low pH and hypertonic	Poor	+
Contact lens solution	Antimicrobial property, preservatives	Poor	+
Saliva	Microbial contamination, hypotonic, nonphysiological pH and osmolality	Very poor	++
Water	Microbial contamination, hypotonic, nonphysiological pH and osmolality	Very poor	++

# **CONCLUSION**

Till date, there is no single product or solution that possesses all the characteristics of an ideal storage medium for avulsed teeth.

Hank's balanced salt solution can preserve periodontal ligament cells in vitro for 120 hours (5 days) and in vivo for 96 hours (4 days) and is one of the best solutions for storage of avulsed teeth, it does not need refrigeration, it has two years of shelf-life and it has been recommended and used successfully by clinicians and researchers.

Even though Hank's balanced salt solution is superior to milk, milk remains the most convenient, cheapest and readily available solution in most situations while also being capable of keeping PDL cells viable.

Based on the literature, it could be stated that Tap water, saliva and salt solution must be avoided for the storage of avulsed teeth except in situation when none other media is available.

Due to minimal side effect and maximum health benefit, many natural products such as Tender coconut water, Green tea extract, Aloevera, Long Shelf Life Honey Milk, MorusRubra, Salvia officinalis and recently Pomegranate Juice, Soymilk have been tried as storage media for avulsed tooth.

Further researches need to be conducted to find the most suitable media.

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