

REVIEW ARTICLE

Recent Advances in Storage Media for Avulsed Tooth - A Review

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Abstract

Avulsion is complete displacement of tooth out of its socket that result mutilation of periodontal ligament. After the avulsion, the PDL tissues begin to dehydrate. If the PDL attached to the root surface does not dry out, the consequences of tooth avulsion will be minimal. As immediate replantation is not always practically possible at the trauma site, an "interim transport" media often required to maintain the vitality of PDL cell during the extra-alveolar time period. The ideal storage medium should have the capability of preserving the cellular PDL so that the cell could go through the process of mitosis and form clones of the damaged fibroblasts of the PDL and its generating cells. This review summarizes the recent advances and in brief about the newly proposed interim transport media.

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INTRODUCTION

Avulsion of teeth is one of the most common acute forms of dental trauma in young children, where root formation is still incomplete.^[1] Tooth is "separated" from the socket, mainly due to the tearing of the periodontal ligament (PDL) that leaves viable PDL cells on most of the root surface. Avulsion is characterized by complete displacement of the tooth out of the socket, resulting in severely compromised neurovascular supply of tooth, which may lead to the loss of vitality. After the avulsion, the PDL tissues begin to dehydrate.^[2] If the PDL attached to the root surface does not dry out, the consequences of tooth avulsion will be minimal.^[3] As immediate replantation

is not always practically possible at the trauma site, an "interim transport" media often required to maintain the vitality (clonogenic and mitogenic capacity) of PDL cell during the extra-alveolar time period.^[4] The ideal storage medium should have the capability of preserving the cellular PDL so that the cell could go through the process of mitosis and form clones of the damaged fibroblasts of the PDL and its generating cells.^[3] Management protocols for avulsed teeth should include management of the pulp and PDL cells to improve the long-term prognosis and survival of these teeth. The appropriate treatment for an avulsed tooth is its immediate replantation.^[5]

The type of transport media used and the storage period play a vital role in a successful clinical outcome of the replanted avulsed teeth.^[6,7] A variety of media like saliva, milk, and Hank's Balanced Salt Solution (HBSS)^[8] have been advocated as interim storage media for the avulsed teeth. However, the current research and studies have proposed few newer media in this context (e.g., propolis,^[9] culture media and growth factors,^[10,11] egg albumin,^[12] and milk powder^[13]). This review summarizes the recent advances and in brief about the newly proposed interim transport media.

Storage Medium

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.^[14]

Ideal Requirement for a Storage Medium

An ideal storage media should have osmolarity and pH closer to physiological condition to maintain the viability of PDL cells. It should not produce any antigen-antibody reaction. It should be readily available at the site of accident so that the tooth can be immediately placed into it. It should have a longer shelf life. It should have anti-inflammatory and antimicrobial properties, which reduces inflammation and replacement resorption. It should have an antioxidant property which will protect the cells from oxygen radical-mediated damage.^[15]

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The Storage Media can be classified as Laboratory Prepared and Natural Source^[16]

Laboratory prepared	Natural sources
HBSS	Milk
Normal saline	Saliva
ViaSpan	Propolis
Eagle's medium	Coconut water
Custodiol	Egg white
Dubelco's storage	Emdogain
Tooth rescue box	<i>Morus rubra</i>
Conditioned medium	Saliva officinalis extract
Contact lens solution	Tap water
Growth factors	
Ascorbic acid	
L-DOPA	

NS: Normal saline, HBSS: Hank's Balanced Salt Solution

Conventional Media

A variety of media have been used (Tap water, saline, saliva) and recommended conventionally (milk, HBSS) to be used as interim transport media for an avulsed tooth.

Tap water

Tap water is an unacceptable storage media for avulsed teeth due to the least desirable results as it has bacterial contamination, hypotonicity, and non-physiological pH of 7.4–7.79 and osmolality of 30 mOsmol Kg.^[17] Blomolf *et al.*^[7] found that 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. Hence, its use should be limited to the case where the extra-alveolar duration is less.

NS

NS is a solution of 0.09% w/v of sodium chloride and an osmolality of 280 mOsm/kg, and despite being compatible to the cells of PDL, it lacks essential nutrients such as magnesium, calcium, and glucose necessary to the normal metabolic needs of the cells of the PDL.^[18] Pileggi *et al.*^[19] conducted a study to evaluate posttraumatic PDL cell viability in NS and found that 55% of living cells after 4 h storage and 20% of mortality of cells after 45 min storage.

Saliva

Human saliva is used as a storage medium due to its easy availability. It has a pH of 7.4–7.79 and osmolality of 30 mOsmol Kg. This hypertonic osmolality leads to cell lysis and higher rates of replacement resorption.^[20] It also causes swelling and membrane damage of PDL

cells of the avulsed tooth if stored for 2–3 h.^[16] Saliva can be used as a storing medium for a short period, as there can be damage to the cell of the PDL if used for longer than an hour.^[21] Although readily and most easily available, saliva is not considered as an effective interim transport medium. Recent literature indicates that saliva may not be suitable transport medium for the avulsed teeth due to its non-physiologic osmolality and the presence of microorganism.^[4,7]

Milk

Due to its physiologic osmolality and nutritive value, milk is considered an acceptable interim transport medium for the avulsed teeth. Its clinical efficacy is considered equivalent to HBSS for maintaining the vitality of the PDL cells of an avulsed tooth for an extended period of time (up to 6 h).^[8,22–26] Marino *et al.*^[27] conducted a study to determine the ability of long shelf life milk to serve as a temporary storage medium for the maintenance of PDL cells viability on avulsed teeth, which shows that low-fat content in milk and chilled milk had shown better results in maintaining the viability of PDL for a longer time of period. However, the main drawback is the presence of antigens that may interfere with the reattachment process.

HBSS

HBSS was introduced by Hanks in 1975 as a solution for the preservation of tissue culture. Among all the storage media, HBSS is considered as the gold standard and is often used as a reference medium to deduce the clinical efficacy of other media. The American Academy of Endodontics has accepted HBSS as an acceptable medium for the avulsed teeth because of its capability to maintain vitality and proliferative capacity of PDL for an extended period of time (up to 48 h).^[15,28] It contains the sodium chloride, D-glucose, potassium, calcium chloride, and magnesium sulfate anhydrous. Its both pH (7.4) and osmolality (280 mOsmol/kg) are ideal. It can preserve the cells and tissues for 24 h.^[29] It can maintain the viability of PDL cells for several hours with a success rate of 90%. HBSS has no need of refrigeration. HBSS is marketed as Save-A-Tooth (Save-A-Tooth; Phoenix-Lazerus Inc., Pottstown, PA, USA), to maintain PDL cell viability. Unfortunately, HBSS is not widely used in India because it is not readily available.^[15]

CURRENT DEVELOPMENTS

In the past, various studies have been conducted on different avulsion media, but none of them fulfill the ideal requirement. A wide range of research has been conducted to study the efficacy of available and newer

storage media such as propolis, coconut water, aloe vera, and pomegranate juice.

Propolis

Propolis is a sticky resin obtained chiefly from the buds of some conifer trees.^[28] It is an antibacterial and anti-inflammatory beehive product. Propolis has antiseptic, antibiotic, antibacterial, antifungal, antiviral, antioxidant, anticarcinogenic, antithrombotic, and immunomodulatory properties.^[15] Mori *et al.*^[30,31] conducted a study to evaluate propolis as storage media for the avulsed teeth and concluded that efficacy of the medium was increased if maintained for 6 h because the contact with product is beneficial for cell maintenance.

Propolis is one step ahead of milk,^[9,32] HBSS, or saline^[11] in maintaining PDL cell viability. Ahangari *et al.*^[33] conducted a study to compare the number of viable cells at 1 h and 3 h after storage in 10% propolis, 50% propolis, milk, egg white, and HBSS. They observed that a significantly more number of viable PDL cells were found in propolis as compared to the other experimental groups. No significant difference was found between the performance of 10% and 50% concentration of propolis.

Gjerston *et al.*^[34] conducted a study to assess the effect of propolis on proliferation and apoptosis of PDL fibroblast and found that propolis decreased the apoptotic levels of PDL fibroblasts activity of PDL cells when compared with HBSS.

Coconut Water

Coconut water is biologically pure and sterile. It is rich in amino acid, minerals, and vitamins. It is known to possess regenerative and antioxidant properties. Storage media having antioxidant properties can be more effective in maintaining the viability of PDL.^[20]

The activity of coconut water is concentration dependent. Coconut water that is 100% concentrated is more effective storage media than coconut water that is 50% diluted. Coconut water from mature fruits performs better than from young fruits.^[35]

Coconut water can be used to store avulsed tooth for a relatively longer period of time (45 minutes).^[36] In a report of Thomas *et al.*,^[37] if the tooth is replanted within 15 min, HBSS is the most effective storage media, and between 15 and 120 min, it is equivalent to coconut water. Silva *et al.*^[38] compared the cytotoxic effect of coconut water with whole milk, HBSS, and tap water, using multiparametric cytotoxicity analysis employing 3T3 cells. They concluded that coconut water and HBSS expressed least cytotoxicity.

Due to its superior properties, coconut water can be advocated as a viable storage media.^[37]

Ascorbic Acid

Addition of ascorbic acid to osteoblastic cell lines can stimulate type I collagen production, followed by expression of a specific marker associated with osteoblastic phenotypes such as alkaline phosphatase (ALP) and osteocalcin. Ascorbic acid increases the ALP activity, which was required for the binding of PDL cells to type I collagen through 2 beta 1 integrin, whose expression was again increased by ascorbic acid. As type I collagen production is considered an initial process in the differentiation of PDL cells, it may serve as a potential storage media.^[15]

Growth Factor

The use of polypeptide growth factors, which functions as a potent biological mediator regulating numerous activities of the wound healing, has been suggested for the promotion of PDL regeneration. Lynch *et al.*^[39] demonstrated that short-term application of a combination of platelet-derived growth factor and insulin-like growth factor can enhance the formation of the periodontal attachment apparatus 5–10 fold during the early phase of wound healing.

Powdered Milk

This is the recent addition in milk substitutes and products that have been demonstrated to maintain the viability of PDL cells and has been proposed as a favorable transport medium.¹³ Powdered milk is one of the presentation forms of bovine milk and is considered as a feasible medium in case of delayed tooth replantation.^[28]

Egg White

Egg albumin is considered as a good choice because of its high protein content, vitamins, water, lack of microbial contamination, and easy accessibility. It has shown better cell viability and significantly higher incident of PDL healing as compared to milk and equivalent cell viability as HBSS.^[12] Egg white has pH of 8.6–9.3, and its osmolarity is 258 mOsmol/kg. Egg white was found to be more suitable 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.^[16]

It is considered as a good choice of storage media for teeth undergoing delayed replantation due to its high content of proteins, vitamins, and water, absence of microbial contamination, and easy access.^[28] Some studies demonstrated greater PDL healing when compared with milk. It can store avulsed teeth for up to 10 h.^[40]

Green Tea Extract

Green tea is beverage consumed all around the world. It has numerous health benefits which can be attributed to the presence of polyphenols.^[41] The commercially available tea is easily available at the site of accident. Therefore, it is tested as storage media. Epigallocatechin-3-gallate (EGCG) is a major polyphenol of green tea, having antioxidant, anticarcinogenic, antimutagenic, anti-inflammatory, antimicrobial, and antiviral activities. According to Adans *et al.*,^[40] EGCG can be used adequately as a storage medium, with a higher potential than HBSS to promote favorable reimplantation. Hwang *et al.*^[42] and Jung *et al.*^[43] reported excellent results with green tea, with the maintenance of 90% of cell viability for up to 24 h, similar to the HBSS control. Jung *et al.*^[43] also reported that the higher the extract concentration, the more efficient the medium.

Aloe Vera

A. vera is a cactus plant that belongs to family Liliaceae. The inner gel of *A. vera* has the issue of dry period. The dry period should be as less as possible, and it would be better if immediate replantation can be achieved.^[21] As a fact, it is not always possible to replant the tooth immediately, so the need for storage media arises. The delay in replantation can vary from few minutes to hours, as in cases of accident, emergencies, and unavailability of dentists, so a storage media which can maintain the PDL cell viability for a long duration is needed.^[44,45]

In a study carried out by Badakhsh *et al.*,^[46] it was revealed that *A. vera* at a concentration of 10%, 30%, and 50% performed similarly as supplemented culture media for up to 9 h. *A. vera* at this concentration maintained the cell viability over 90% and was superior to 100% *A. vera* and egg white. They recommended *A. vera* as suitable storage media for avulsed teeth.

Red Mulberry

Mulberry fruits are used medicinally as a deworming agent, as a remedy for dysentery, as laxative, odontalgic, expectorant, hypoglycemic, and emetic. Ozan *et al.*^[47] compared four different concentrations of *Myrmica rubra* (4%, 2.5%, 1.5%, and 0.5%) with HBSS and tap water at 1 h, 3 h, 6 h, 12 h, and 24 h to check the effect on PDL viability. They recommended mulberry as a storage media for the avulsed teeth. They concluded that the number of viable PDL cells was significantly high when an avulsed tooth stored in 4.0% concentrated solution of *M. rubra* as compared to other concentrations.^[47]

Pomegranate Juice

In Ayurvedic medicine, pomegranate is considered as "a pharmacy unto itself." It is an extraordinary fruit with complete medicinal power contained in its juice, peel, and seeds. It has potent antioxidant, anticarcinogenic, and anti-inflammatory properties.^[48]

Tavassoli *et al.*^[49] concluded in their study that pomegranate affects the fibroblast cell proliferation. This proliferative effect is found for 1 h at lower concentration of 1% and 2.5%, but at 5% and 7.5% concentrations, a general proliferative effect is exhibited. It also promotes strong cell attachment. Pomegranate juice and HBSS can preserve the spindle-like morphology of periodontal fiber for 24 h after storage. Hence, it can be a good storage media. Further research is required to assess the efficacy of pomegranate juice.

Emdogain (Enamel Matrix Derivatives, EMD)

Emdogain (Biora, Malmo, Sweden) is a commercial EMD extracted from developing embryonic enamel of porcine origin and contains several matrix proteins. Studies have shown that it can influence the migration, attachment, proliferation capacity, and biosynthetic activity of PDL cells.^[50]

Tooth Rescue Box

Dentosafe (Miradent, Germany) is the commercial name of a tooth rescue box containing special cell culture medium which is a combination of amino acid, vitamins, and glucose. In the USA, it is marketed as EMT tooth saver (Phoenix, USA). It has demonstrated the maintenance of vitality of PDL cells for 48 h at room temperature. If unopened, this medium has a shelf life of 3 years.^[10] The use of this system is self-explanatory and simple to understand for layperson.^[51] 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.^[52]

CONCLUSION

The success of replantation of the avulsed tooth is influenced by various factors such as width and length of root canal, degree of damage during avulsion and replantation, and storage media. However, appropriate storage media can help in maintaining the viability of PDL cells and can lead to successful replantation of avulsed teeth. None of the storage media fulfill the ideal properties completely. All storage media have been shown to lose their clinical efficacy with time. Tap water, saliva, and NS must be avoided for storage of avulsed teeth because they do not offer any benefit to the healing. Although HBSS,

tooth rescue box has a great potential to maintain the PDL cells in a viable state after avulsion, the practicalities of using this solution, and lack of ready availability to general public make them less than ideal. Natural products are more effective in maintaining the PDL cell viability compared to synthetic products. The natural products such as coconut water, milk, and propolis can act as appropriate storage media because of their cost-effectiveness and potential to maintain the viability of PDL cells for longer durations. In addition, the natural products are easily available at the site of injury which can enhance the prognosis of avulsed tooth and reimplantation.

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