



NATURAL PRODUCT AS THE STORAGE MEDIUM FOR AN AVULSED TOOTH – A SYSTEMATIC REVIEW



ABSTRACT

Dental avulsion is considered as one of the most severe types of traumatic tooth injuries because it causes damage to several structures and results in the complete displacement of the tooth from its socket in the alveolar bone. The ideal situation is to replant the tooth immediately after avulsion because the extra oral time is an important determinant for the success of the treatment and for a good prognosis. The aim of this systematic review was to identify the recommended natural storage medium to store and transport avulsed tooth based on the survival capacity of periodontal ligament cells.

This paper reviews the different storage media that have been evaluated for avulsed teeth based on full-length research papers retrieved from PubMed/Medline, Lilacs, BBO and SciELO electronic databases using the key words 'storage medium', 'avulsion', 'tooth avulsion', 'replantation', 'tooth replantation', 'milk' and 'propolis'. Based on the application of inclusion and exclusion criteria, about 14 papers have been selected and critically reviewed with respect to the characteristics, efficacy and ease of access of the storage media. The review of this study shows and includes a wide array of wet storage media that have been evaluated in laboratory-based studies on PDL cells found on adult permanent teeth.

Among the natural products other than milk, propolis, coconut water, green tea extract, egg white, green tea extract, Alovera gel, pomegranate juice, salvia officinalis followed by dragon blood sap (*Croton Lechleri*) were recommended based on the cell viability and its longevity. In an emergency, it is important for dentists to consider the circumstances of the accident, the location and suggest an appropriate transport media.

Key words: Avulsion, cell viability, periodontal ligament cell.

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INTRODUCTION

Tooth avulsion is defined as the complete loss of tooth from the alveolar socket as a result of trauma¹ and it may result in the early loss of teeth, leading to functional, psychological and aesthetic problems. Avulsion of permanent teeth is the most serious of all types of traumatic tooth injuries because the complete dislodgement of the tooth from its socket causes severe damage to the supporting tissues and vascular and nerve structures.^{2,3} It corresponds between 1% and 16% of all types of tooth injuries involving the permanent dentition.⁴⁻⁶ This wide variation can be explained by the differences in the evaluated population, including the levels of interpersonal violence, involvement in vehicular accidents and other road accidents related to traffic, which are caused especially due to the negligence of using helmets, and practice of contact sports, especially without using of mouthguards.⁷

The age group between 7-10 years is the most affected one by this injury due to their excessive involvement in sport activities because of their playfulness, the characteristic of that age stage. The prognosis of a replanted tooth and its maintenance on the dental arch for the longest possible time depend on the viability of the periodontal ligament (PDL) cells remaining on root surface, integrity of root cementum and minimal bacterial contamination⁸, which are the conditions directly related to the extra-alveolar time, type of storage after avulsion and root surface alterations. The ideal treatment for the avulsion of permanent tooth is its immediate replantation into the socket.

In most situations, important factors for the success of replantation cannot be controlled. Studies have shown that this scenario can be improved significantly with educational campaigns on dentoalveolar trauma and storage media, to create awareness among common people and non-dental health professionals, especially for those working in emergency assistance services⁹ to obtain a positive behavioural change to obtain successful treatment. Immediate tooth replantation leads to a better PDL repair and reduces significantly the occurrence of root resorption. Therefore,

shortening the time is required between trauma and tooth replantation and maintaining the avulsed tooth in a suitable transport medium, well increase the prognosis Considerably.¹⁰ As replantation of avulsed teeth occurs more frequently between 1 and 4. after avulsion, degeneration of cemental PDL fibers is a common event and the presence of necrotic PDL remnants on root surface stimulates the occurrence of inflammatory root resorption, which is the major cause for the loss of replanted teeth. In this way extra-alveolar time, taken for endodontic therapy is to administration of syc and adequate handling and maintenance of the tooth until the moment of replantation are the conditions leading to a better prognosis.¹¹

However, adverse situations may occur, such as ankylosis and different types of root resorption, depending especially on the storage time and the characteristics and temperature of the storage medium. In fact, the capacity of the storage medium to maintain the viability of PDL cells has been considered more important than the extra-alveolar time.¹² Different types of wet storage media for avulsed teeth have been investigated, which may vary from cell and tissue culture solutions like propolis, green tea, *Morus rubra* (red mulberry), egg white and coconut water.¹³ This paper reviews the different storage media that have been investigated for avulsed teeth based on full-length research papers retrieved from electronic databases considering their characteristics, efficacy and accessibility.

MATERIALS AND METHODS

Using the key words 'storage media', 'tooth replantation', 'tooth avulsion,' 'milk' and 'propolis', PubMed/Medline, Lilacs, BBO and SciELO, electronic databases were searched for research articles, reviews of literature, animal laboratory studies and laboratory studies which had been published in English between 2004 and 2017 involving cell counting in human teeth assessing the PDL cell viability after storage of avulsed teeth with different substances. Papers without an abstract and those that evaluated pulp cell Viability and root surface treatment were not included. The PubMed Advanced search was conducted using keywords. From a total of 206 papers, 14 articles were selected after application

of the inclusion criteria (table 2) and were critically reviewed for comparison of the outcomes.

Sources Used

For identification of studies included or considered for this review, detailed search strategies were carried out on the following databases.

-PubMed

-PubMed Advanced search

-MEDLINE

-Cochrane Database of Systemic Review

Language

There was no language restriction for the electronic search.

Hand Searched Journals

The following journals were hand searched

- 1) Journal of Interdisciplinary Dentistry
- 2) Journal of Khyber College of Dentistry

Types of Participants

Tooth with avulsion.

Type of Intervention

Natural storage medium.

Types of Outcome Measures

Periodontal ligament cell viability

EXCLUSION CRITERIA

The following studies were excluded,

- Case reports / case series
- Studies involving primary teeth
- Systematic reviews

INCLUSION CRITERIA

-In vitro study

-Natural storage medium

-Therapeutic Extraction

To assess the quality of the included studies, each item in the data extraction form was assigned a specific value, according to the amount of information that could be obtained from the individual study. The details of the studies included in the present systematic review are presented in table 2.

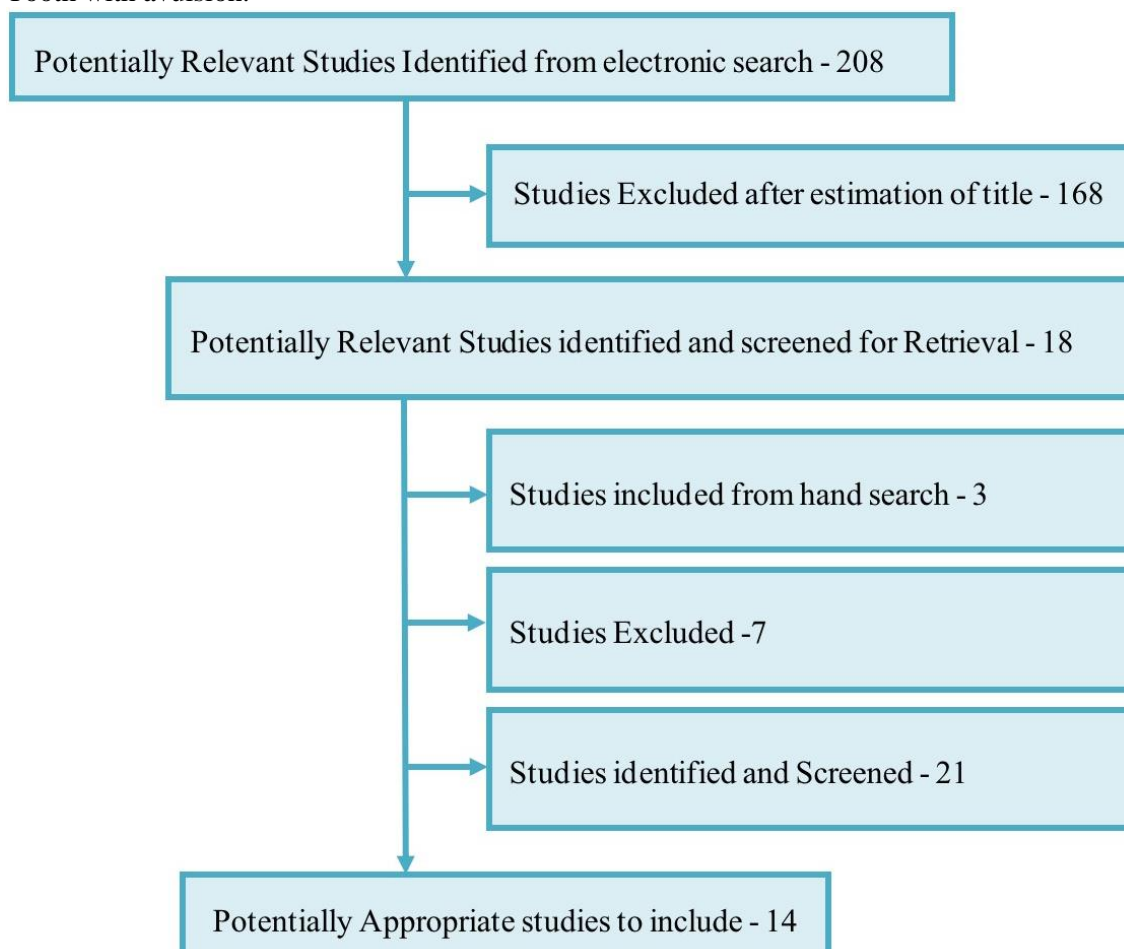


Figure 1. Search flow chart

Table 1. Included studies of Natural Storage medium

AUTHORS, YR	TYPE OF TEETH	NATURAL PRODUCTS	STORAGE TIME	ASSAY FOR EVALUATION	RESULTS	LEVEL OF EVIDENCE
D'Costa et al ⁹ (2017)	Forty sound human premolars undergoing extraction for orthodontic purpose	coconut water, milk, and saline	45 min	Trypan blue stain	Coconut water preserved significantly had more PDL cells viable (p<0.05) compared to milk and saline.	Very low
Irem BAG et al ²⁵ 2017	Orthodontic extraction	MILK	30-60min or 12hrs	MIT assay	In the HBSS groups, RUNX2 expression increased showing a direction to osgenic differentiation of PDL fibroblasts.	low
V. Kokkali et al ¹⁷ 2017	Orthodontic extraction	Milk, coconut water, butter milk	75mins	Collagenase-dispase assay	The milk group has the maximum average number of viable PDL cells compared to coconut water or Buttermilk which was the least effective.	Low
Babaji et al ⁴ 2017	Orthodontic Extraction	Propolis, Aloe vera and pomegranate juice	45 mins	Hemocytometer	Propolis showed the more viable PDL cell followed by HBSS, aloe vera and pomegranate juice	Very low
Christine Men Martins et al ²⁶ 2016	The human periodontal ligament cells were provided by the Laboratory of Applied Virology	Dragon blood sap, milk and PBS	1, 2, 3, 6, 10 and 24 hrs of incubation.	MIT assay	The dragon's blood sap showed better results than all storage media, even better than milk (p<0.05)	Moderate
Divya Saini et al ²⁷ 2016	Orthodontic extraction	coconut milk or probiotic milk) and a Hanks' Balanced Salt Solution (HBSS)	20 minutes and then immersed for 30 minutes in one of the storage media	collagenase-dispase assay	there was a significant difference (p<0.001) between Coconut milk and probiotic milk as well as HBSS.	Very low
Souza et al ²⁴ 2016		skimmed milk (S Milk), whole milk (W Milk), natural coconut water (Coconut), propolis, and egg white	3, 6, 24, 48, 72, 96, and 120 hours	MTT Assay	Coconut, Propolis and Egg, were less effective than S Milk, W Milk, and HBSS	High
Ahangari et al ² (2013)	Extracted teeth	Propolis, Hank's Balanced salt solution, milk and egg white	3 hrs	Dispase collagenase assay	Propolis significantly is found better than egg white and milk (p<0.05)	Low
Hwang et al ¹⁵ 2011	Freshly premolar	Tap water, milk, GTE, and commercial green tea.	1, 3, 6, 12, and 24 hours	Collagenase-Dispase assay	GTE showed higher cell viability than other media (p<.05).	Moderate
B. D. M. Souza et al ¹⁸ (2010)	Third extraction	skimmed milk, whole milk	3, 6, 24, 48, 72, 96 and 120	MTT assay	The greatest number of viable cells was observed for MEM. Skimmed and whole milk, followed by natural coconut water and HBSS, which were the most effective media in maintaining cell viability (p<0.05).	High
Ozan, Fatih et al ²² (2008)	healthy third molars	Salvia officinalis solutions, Hank's balanced salt solution (HBSS), phosphate buffered saline (PBS), and tap water	1, 3, 6, 12 or 24 hours.	trypan blue exclusion	The results showed 2.5% Salvia officinal is a more effective storage medium than the other experimental solutions (p<0.05).	Moderate
Khademi et al ¹⁶ 2008	Extracted teeth	egg white, and milk	1, 2, 4, 8, and 12 hours	trypan staining	There was no difference in the cell viability between egg white and HBSS (p<0.05)	Moderate
Gopikrishna et al ¹² 2008	Fifty extracted teeth	freshly human coconut water (CW) and milk	30 minutes	MIT aasay	Coconut water kept significantly had more PDL cells viable compared to either HBSS or milk.	Very low

Table 2. Natural storage media groups as reported in the included studies.

Natural Products	Total search	Inclusion criteria
MILK	103	3
COCONUT WATER	19	2
EGG WHITE	13	1
PROPOLIS	14	1
GREEN TEA EXTRACT	04	1
POMEGRANATE JUICE	03	2
RED MULBERRY	02	1
ALOVERA GEL	01	1
BLOOD DRAGON SAP	01	1

RESULTS

The search identified 208 publications, out of which many were excluded after careful review and finally it is found that only 14 publications have fulfilled all criteria for inclusion. Henceforth, we conclude that the skimmed milk has shown higher level of evidence based on the storage time, followed by Green tea Extract, coconut water and blood dragon sap.

DISCUSSION

There are numerous studies that have been reviewed and compared to find out the best storage medium for avulsed tooth.¹⁴ The systematic review compared the various natural storage media in terms of their ability to maintain PDL cell viability with particular storage time. To determine the actual physical state of the PDL cells after they have been placed in different natural storage media, only laboratory-based studies have been selected. The present systematic review demonstrates that milk is considered as the best storage medium for avulsed tooth.¹⁵ Milk has a unique combination of nutrients, capable of maintaining the PDL cell viability, and with physiological pH range of 6.5-7.2. The PDL cells have been shown to survive for 2-6 hours when immersed in milk. Sour milk should not be used as it harmful to the PDL cells.¹⁶ Recent studies have favoured other natural media for avulsed tooth.¹⁷ The low cost, presence of nutrients and easy availability make milk a more practical choice than any other medium. HBSS is a specially designed storage medium containing essential nutrients.¹⁸ Although it is not easily available in most parts of the world, it is marketed in some countries as 'Save-a-tooth'. To maintain PDL cell viability, the clonogenic as well as mitogenic capacity of the PDL fibroblast has to be analysed and demonstrated. PDL cell viability has

been studied and documented by sustaining its ability for about 48 hrs. It has been recommended that the avulsed tooth should be placed in HBSS for 30 minutes before replantation into the socket, regardless of which storage medium the tooth was placed in, prior to this procedure.¹⁹

The most common method reported that the viability of the PDL cells can be assessed by Trypan blue exclusion or staining test.²⁰ Trypan blue solution in the strength of 0.4% is routinely used as a cell stain to assess cell viability using the dye exclusion test. A disadvantage of using Trypan blue is that the dye is cytotoxic to some degree, and also stains the background which can result in inaccuracy when counting the cells.²¹ Another method that was used to determine PDL cell viability was MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) which is basically a colorimetric test determining the metabolic activity of cells. This tetrazolium-based assay is useful because of its rapid results, objectivity, and ease of manipulation as well as immediate identification of viable PDL cells.²²

Parameters indicating a good prognosis for the replanted avulsed tooth such as the lack of replacement resorption, external inflammatory resorption, external invasive resorption, pain, and swelling can only be assessed clinically over a long follow-up periods.²³ However, the clinical application of the investigated storage media can only be established after their use in clinical trials. Hence to standardize the inclusion criteria dictated by the dry time of the PDL cells would be very difficult.²⁴ Thus, identification of the most recommended storage medium for avulsed teeth in the context of clinical survival rate is difficult. This could be considered the primary shortcoming of the present review, in which no studies assessing the clinical survival of teeth were included.²⁵ This was mainly because the

parameters for the assessment of clinical success were different to those of laboratory-based studies. The inferences of the investigation would be much expressive henceforth the method to control such biases has been applied. Ideally, the most recommended storage medium should not only be able to maintain PDL cell viability but also enhance it.

However, this enhancement of PDL cell viability was observed only in few Studies.²⁶ The

actual number of cells that remained viable after being placed in the storage medium could not be compared in the selected studies because of the great variability in the methodology of studies. The best medium encompassing all the ideal characteristics may yet to be identified, but this systematic review has managed to identify the most recommended natural storage medium that can be used to store or transport the avulsed teeth.²⁷

Table 3. Criteria used for grading quality of Evidence

GRADE range	Quality of evidence	Interpretation
0- 1hours	Very low	PDL cell viability assessed (less than 1 hour)
1-12 hours	low	PDL cell viability assessed (less than 12 hours)
12-24 hours	Moderate	PDL cell viability assessed (less than 24 hours)
>24 hours	High	PDL cell viability assessed (more than 24 hours)

This systematic review has identified many storage media that have been used to preserve avulsed teeth, including coconut water, soy milk, whole milk, saline solution, saliva, propolis. However, we conclude that the best storage media for avulsed tooth is skimmed milk followed by

green tea extract, coconut water and blood dragon sap based on storage time and they were used to assesses the cell viability (Table 3). ‘High level of evidence’ studies indicate proof that the true effect lies close to the estimated effect (figure 2).



Figure 2. Showing the evidence of included studies

CONCLUSIONS

Although HBSS with its osmolality and pH similar to plasma is probably the best transport media for avulsed teeth, it may not necessarily be available at the site of accident. Any appropriate media prevents desiccation of the periodontal ligament cells following trauma and improves chances for successful replantation. Natural transport media like skimmed milk, coconut water, blood dragon sap and egg white score over HBSS based on their storage time, ease of availability and economical price. In case of emergency, it is important for dentists to consider the circumstances of the accident, the location and

suggest an appropriate transport medium for the avulsed tooth.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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