

Methods for the Preservation of Periodontal Ligament Cells using Different Storage Media

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Abstract— This paper presents a review of different storage media and their efficiency in retaining periodontal ligament (PDL) cell vitality before replantation. After avulsion of a tooth, immediate replantation is recommended, however this is often not possible. Therefore, storage media that are affordable and readily available are required to avoid resorption and tooth death by preservation of the PDL cells. Numerous studies have been undergone and use of media such as Hank's Balanced Salt Solution (HBSS), milk, aloevera, coconut water, and et cetera have been trialed.

In order to find the storage media that best meets the price/quality ratio, this study compares the length of time that the aforementioned solutions for retaining PDL vitality, their pH and osmolality, and discusses their efficiency in terms of other in vitro experiments. The media that are compared in this study are Hank's Balanced Salt Solution (HBSS), milk, aloevera, coconut water, egg albumin, and contact lens solution. Results provide that HBSS and coconut water have the longest retention time, however milk, aloevera and egg albumin are also effective for shorter periods of time.

Keywords— *Periodontal ligament cells; avulsion; resorption; storage media; vitality*

I. INTRODUCTION

The development of biomedical engineering enables improvement of quality of service provided in healthcare [1-4]. Innovative techniques have been used in various aspects of healthcare [5-11], and therefore it is interesting to investigate the impacts of new methods in stomatology, especially ones used for treatment of avulsions.

The avulsion of a tooth is a severe form of dental trauma that is characterized by the rupture of the neurovascular bundle and periodontal ligament (PDL) exposing the tooth to the environment [12-15]. Ideally, avulsed teeth should be replanted immediately after removal to preserve the vitality of PDL cells. The vitality of PDL cells ensures the optimization of healing and minimization of root resorption when returned to the mouth. However, immediate

replantation rarely occurs [12]. Therefore, storage conditions, and also the amount of extraoral time, are the most crucial factors in determining the vitality of the remaining PDL cells and the avulsed tooth.

Sardana et al [13] describes a 3 year follow up case report of a 12 year old female with delayed replantation (of 15 hours) of maxillary central incisors without interim storage media. Results revealed ankylosis and inflammatory resorption, which demonstrates replantation failure. This example provides evidence that effective storage media should be used to retain PDL cell vitality and avoid ankylosis and inflammatory resorption.

Cases that use interim storage media before replantation have more positive outcomes. Ines and Nabiha [14] deliver a case report about a 9 year old male who, due to a car accident, required treatment for an avulsed maxillary right central incisor. The tooth was left in milk for 24 hours before replantation, and after a 1 year follow up, the tooth revealed a lack of ankylosis and inflammatory resorption. Furthermore, another positive case study performed by Rutar [15] describes a 9 year old boy who required treatment for an avulsed 11th tooth. It was left in milk for 30 minutes before successful replantation.

The following 3 case studies confirm that suitable storage media are advantageous for successful tooth replantation as they successfully retain the vitality of the PDL cells due to their added vitamins and minerals, pH levels and mitogenicity ratios.

The various media employed for storage of avulsed teeth are HBSS (Hank's Balanced Salt Solution), milk, egg albumin, and ect. HBSS is extensively used as a storage medium in dentistry, however it is not widely available and is costly [9, 10]. Therefore, a storage media that can be readily purchased, readily available and affordable, such as milk, should be identified [12-16]. In terms of duration,

storage media should be able to sufficiently sustain PDL cells until replantation of the avulsed tooth can occur. Ideally, an avulsed tooth should be replanted 1 to 3 hours after extraction [6,8,17].

Additionally, an ideal storage medium should be capable of preserving the viability, mitogenicity, and clonogenic capacity of the damaged PDL in order to prevent further root resorption. Essentially, it should have properties similar to physiological osmolality and pH, 300 mosm/kg and 7.4 respectively [8], and contain minerals and vitamins for effective preservation of cells. The storage media with parameters closest to those mentioned above will allow optimal PDL cell growth or survival, and successful replantation of the avulsed tooth [7,8,17,18].

II. STORAGE MEDIA

This review employs the results from other papers inspecting the effectiveness of different storage media, and compares their ability to retain cell vitality, pH and osmolality. Results, in terms of the aforementioned criteria, were taken from 18 review and experimental papers [12–29]. The comparison is presented in Table 1 with an in detail explanation of the storage media's properties and advantages in cell retention.

TABLE 1. CHARACTERISTICS OF STORAGE MEDIA

Storage Media	Characteristics
Milk	Physiological pH of 6.3–6.5, osmolality of 300–400 mosm/kg, ability to preserve the viability of PDL cells, low bacterial count and commonly available.
HBSS	Sterile, non-toxic media, pH of 6.7-7.8, osmolality of 270-305 mosm/kg, and physiologic-isotonic salt solution
Egg Albumin	High protein content, vitamins, and water, pH of 7.6-8.5, osmolality of 251-298 mosm/kg lack of microbial contamination and easy accessibility.
Aloe vera	Antiviral and antimicrobial, pH of 4.5-5.5, osmolality of 100-500 mosm/kg ability to inhibit the synthesis of thromboxane.
Contact Lens Solution	Readily available, pH of 6.5-7.6, osmolality of 310 mosm/kg and short term storage.
Coconut Water	Isotonic fluid, pH of 9.04, osmolality of 288 mosm/kg biologically pure and sterile, rich in amino acids, proteins, vitamins and minerals.

MILK

Milk can be used as a storage solution for avulsed teeth as it meets the following criteria for storage media: (1) it has physiological pH, (2) ability to preserve the viability of PDL cells, (3) low bacterial count and (4) commonly available [17,18]. Monica M. Chamorro et al [16] reported that milk has proven to be superior to saliva in maintaining vitality, both *in vitro* and *in vivo*. This is attributed to the physiologic osmolality of milk. Also, they found that milk can maintain viability, mitogenicity and clonogenic capacity of PDL cells for as long as 24 hours.

After 24 hours of storage at 4°C in milk, there is a 2% reduction in the number of viable PDL cells, and 38% reduction in clonogenicity. N. Malhotra correlates that due to milk's physiologic osmolarity and nutritive value, it is considered an acceptable interim transport medium for 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 six hours) [17].

Chilled milk has shown better results in maintaining the viability of PDL and for a longer time period [7, 19]. Elif B. Tuna et al [18] discovered that milk can maintain vitality of PDL cells for two hours. Consequently, chilled milk is recommended by the International Association of Dental Trauma. Milk with a lower fat content is more suitable for retaining the vitality of PDL cells than milk with a higher fat content [20]. Additionally, Monica M. Chamorro et al [16] noted that in the presence of ice in low fat milk, apoptotic cell death of PDL is further inhibited. Additionally, if chilled, low fat milk is a suitable alternative to HBSS [7, 21].

HANK'S BALANCED SALT SOLUTION (HBSS)

Hank's balanced salt solution (HBSS) is a sterile, non-toxic, pH balanced, isotonic salt solution commonly used for disinfection of wounds and as a cell culture medium [7]. HBSS is considered as the gold standard and is often used as a comparison reference medium to deduce the clinical efficacy of other media. It is a sterile, physiologically balanced isotonic salt solution. 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). It is superior to other media in preserving the vitality and viability of PDL cells, and has shown the highest mitogenicity for PDL cells after 8 hours and 24 hours [8].

In an experiment conducted by Ashkenazi et al [19], it was found that culture medium and ViaSpan, followed by HBSS, were the most effective solutions for preserving PDL cells after 24 hours of storage. After 24 hours of storage at 4°C in HBSS, there is a 5% reduction in the number of viable PDL cells, and a 38% reduction in clonogenicity [7]. In its 1994 guidelines for the Treatment of the Avulsed Permanent Tooth, the American Association of Endodontists recommends Hank's balanced salt solution (HBSS) as the most suitable transport medium for the avulsed tooth that cannot be replanted immediately. If HBSS is not available, milk is the second best, followed by saline, saliva (buccal vestibule), and water [17, 21].

EGG ALBUMIN

Egg Albumin is considered as a suitable storage medium due to its high protein content, vitamins, water, lack of microbial contamination and easy accessibility [8, 19]. Results from the experiment conducted by Khademi et al [20] revealed that egg albumin and HBSS media have no difference in cell viability, but there was a higher incident of PDL healing when compared to milk as a storage media. It is observed to be an excellent medium for up to 10 hours with the principle advantage being its availability [8, 20, 21].

ALOEVERA

Aloe vera is a very popular plant used in alternative medicine. It is commonly available and widely used in alternative medicine. Hence, the chances of availability of aloe vera near the site of injury could be relatively high [8]. The parenchymatous cells of the plant contain a transparent jelly that is referred to as aloe vera gel. It has been reported to have anti-viral, anti-diabetic, wound healing, anti-cancer, antioxidant, antigenotoxic, anti-inflammatory, angiogenic, antimicrobial and hepato-protective properties [8, 22]. In dentistry, aloe vera has shown to enhance defense mechanisms and accelerate healing process in periodontal diseases by slowing or inhibiting the synthesis of thromboxane [23].

An experiment conducted by Fulzele et al [22] found that aloe vera presented a maximum mean percentage of viable PDL cells (81%). At 30, 60 and 90 mins, aloe vera demonstrated 71%, 59%, 57% viable cells respectively. In the study done by Badakhsh et al [24], aloe vera at concentrations of 10%, 30% and 50% concentrations performed similarly as supplemented culture media for up to 9 hours. Aloe vera at these concentrations maintained cell viability over 90% and was superior to 100%.

CONTACT LENS SOLUTION

Contact lens use is rather popular in America, and solutions for storing and rinsing are sterile and saline with some preservatives. A study conducted by Shiang-Chi Huang et al [25] revealed that contact lens solutions gave poor results in terms of preserving the vitality of PDL cells. Within the aforementioned experiment, most of the cells were rounded and shriveled, and eventually detached from the culture dishes.

Chamorro et al [16] also found that the level of apoptosis of PDL cells at room temperature in contact lens solutions were higher when compared with HBSS and milk groups. It was suggested that the preservatives of contact lens solutions (edetate disodium, polyquaternium, or sorbic acid) may be detrimental to PDL cells causing them to become detached from the culture wells or the induction of necrosis [25]. Sigalas et al [26] concluded that contact lens solutions can be used as short term storage medium when other solutions cannot be reached.

COCONUT WATER

Coconut water is a naturally isotonic fluid that is biologically pure and sterile. It is rich in amino acids, proteins, vitamins and minerals [8]. Recent investigation by Omar et al [27] has proposed coconut water as a promising medium for avulsed teeth and has revealed its superiority to HBSS and milk in maintaining the viability of PDL cells. A further experiment also proposed coconut water as a promising medium, suggesting that it might be due to the nutrients within, such as proteins, minerals, amino acids and vitamins, which help nourish the cells [27 - 29].

According to Nwangwa et al [29], coconut water can store avulsed teeth for a longer period of time such as 45 minutes. Another study states that if the pH of coconut water is adjusted to 7.0, it can be used as a storage media for up to 24 hours [28]. Due to its superior osmolality, easy availability and cost effectiveness, coconut water can be advocated as a viable storage media [27, 28].

III. COMPARISON OF THE EFFECTIVENESS OF STORAGE MEDIA

There are many different natural substances that can be used as storage media for avulsed teeth, however this paper explores the ability of HBSS, milk, coconut water, aloe vera, contact lens solution and egg albumin to retain the vitality of PDL cells before replantation. In terms of analyzing the effectiveness of the aforementioned storage media, number of hours, pH value and osmolality of the media were compared. Table 2 compares the different media in terms of number of hours, pH value and osmolality.

TABLE 2. COMPARISON OF STORAGE MEDIA

Storage Media	Number of Hours	pH Value	Osmolality
HBSS	48	7.2	290
Milk	24	6.4	350
Coconut Water	24	9.04	288
Aloe vera	9	5	350
Contact Lens Solution	2	7	310
Egg Albumin	10	8	275

Figure 1 depicts the numbers of PDL vitality in storage media. HBSS, for a longer period of time, can preserve PDL cell when compared to the other solutions. Next is coconut water, egg albumin and aloe vera, and then milk and contact lens solution which less effectively preserves PDL cells.

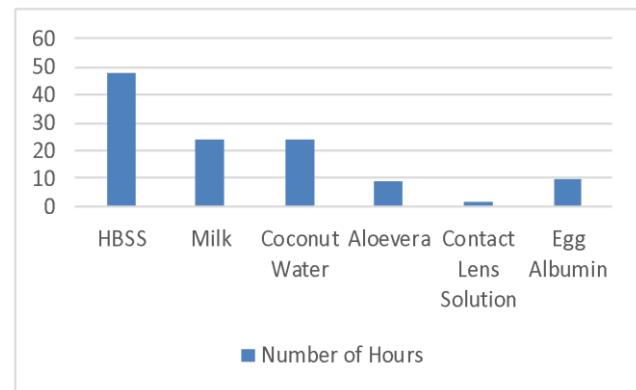


Fig 1. Number of hours of PDL vitality in storage media

In terms of pH value, storage media should have a pH close to 7.4 (physiological pH). All solutions trialed in this review have pH values similar to the physiological pH of the body, however HBSS, milk and egg albumin have the closest pH values. This is evident in Figure 2.

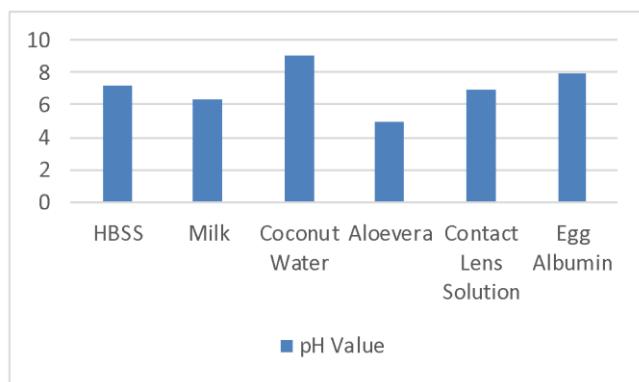


Fig 2. pH value of different storage media

Figure 3 describes the osmolality of the different storage solutions. HBSS, coconut water and contact lens solution have osmolality values closest to physiological osmolality.

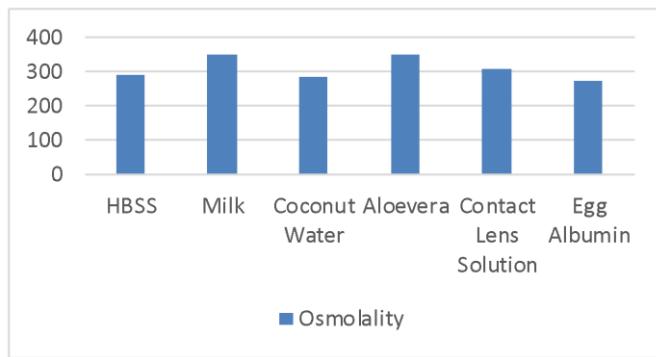


Fig 3. Osmolality of different storage media

IV. DISCUSSION

Factually, it is not possible to immediately replant an avulsed tooth. Therefore, the need for a storage media arises to retain the vitality of PDL cells for replantation. Results from previous studies have demonstrated natural or convenient substances that can be used as storage media, such as coconut water, egg albumin, contact lens solution, aloevera, HBSS and milk.

Mentioned previously, the avulsed tooth should be replanted 1 to 3 hours after extraction to ensure regrowth. However, if medical care cannot be attained during this period, the storage media should be able to effectively retain PDL cells until it is possible. In Figure 1, all storage media are able to retain PDL vitality for 1 to 3 hours. However, for a longer period of time, HBSS, milk and coconut water can effectively retain PDL cells for up to 24 hours (and longer in the case of HBSS). Egg albumin is also suitable as it can maintain vitality for up to 10 hours according to previous experiments.

In terms of pH and osmolality, storage media should have a pH and osmolality similar to physiological parameters (7.4 and 300 mosm/kg, respectively). Figure 2 depicts that the tested storage media each have pH values similar to 7.4, although HBSS, milk and egg albumin have the closest values to the physiological pH. Although this might not dramatically affect the storage media's ability to maintain PDL cells, it can give the storage media an advantage in terms of retention.

Osmolality of the storage media, as shown in Figure 3, should be similar to 300 mosm/kg. According to the graph, HBSS, coconut water and contact lens solution have an osmolality closest to this value. When observing all 3 graphs and the parameters of the different solution media, it is evident that HBSS has the closest specifications to physiological parameters and is therefore the ideal solution for PDL maintenance. However, HBSS is not readily available. In terms of readily available solutions, milk and coconut water, which have suitable parameters, are more suited selections. Egg albumin also contains adequate parameters for PDL cells.

The results displayed in the following figures are in vitro, and very few studies focus on in vivo experimentation. In vivo experiments are crucial as they conclude whether a solution can inhibit resorption and loss of a tooth when returned to the mouth.

Availability is another major factor in deciding on the storage media of PDL cells. Referring to the examples above, aloevera is the only storage media that must be processed before use and HBSS must be purchased from manufacturers. The other substances are readily available for use. Additionally, after a certain amount of time, the effectiveness of different storage media to preserve vitality decreases. At colder temperatures, specific solutions retain their vitality more efficiently. This is evident especially in milk solution, where amount of apoptosis is considerably less.

V. CONCLUSIONS

Appropriate storage media can help maintain the viability of PDL cells, and also lead to successful replantation of avulsed teeth. Avulsed teeth, on most occasions, cannot be immediately replanted back into the mouth. Therefore, a storage media is required to sustain the tooth before its replantation. Natural products such as coconut water, milk and egg albumin can act as appropriate storage media due to their availability, pH and osmolality values, and potential to maintain viability of PDL cells for longer durations.

VI. REFERENCES

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