Review Article

Natural products as storage media for avulsed tooth

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ABSTRACT

Avulsion of tooth is complete displacement of tooth out of its socket that results in mutilation of periodontal ligaments. The desirable treatment option is replantation of the tooth. However, unsuccessful replantation is a matter of great discontentment. Unsuccessful replantation is due to inappropriate management of the avulsed tooth. Protection of teeth from desiccation due to drying of the periodontal ligament tissue, by keeping it in storage media can improve the outcome of the treatment. This review paper focuses on the use of natural products as storage media for avulsed teeth. In vitro and in vivo research published during 1995-2014, allowing open access on National Center for Biotechnology Information (NCBI) database and articles on EBSCO host (EBSCO-Elton B. Stephens Company) were included. It was found that natural products such as milk, coconut water, propolis, green tea, red mulberry, Aloe vera, egg-white and pomegranate have shown ability to maintain viability of periodontal ligament cells of avulsed teeth. Few natural products such as coconut water and milk can be used in raw form, while other products such as green tea and red mulberry need processing. Ability to maintain periodontal cell viability for a longer time is warranted in cases of major accidents, where teeth can be replanted only after other major surgeries. Natural products have easy availability, greater efficacy and longer storage time as compared to Hank's balanced salt solution which has been recommended by the International Association of Dental Traumatology as standard solution for storage of avulsed teeth. Natural products have shown good qualities in *in vitro* experiments; further *in vivo* studies are needed to evaluate their efficacy as storage media.

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INTRODUCTION

Avulsion of teeth is one of the most acute forms of dental trauma[1] and it is quite common in the young, where root formation is still incomplete.[2] Avulsion is characterized by the complete displacement of the tooth out of its socket resulting in severely compromised neurovascular supply of tooth, which may lead to loss of pulp vitality. The appropriate treatment for an avulsed tooth is its immediate replantation.[2]

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For successful replantation of an avulsed tooth, preservation of viability, mitogenicity and clonogenic capacity of the injured periodontal ligament (PDL) fibroblasts and their progenitors is necessary. These periodontal fibroblasts quickly repopulate the denuded root surface and prevent osteoclasts from attaching to this area.[3] After tooth avulsion, the PDL tissues begin to dehydrate. To prevent the damage due to dehydration, prompt replantation of the tooth is required. However, immediate replantation is not always feasible. The replantation of teeth beyond five minutes of avulsion has been defined by Andreasen as delayed replantation.[2] In cases of delayed replantation, the avulsed tooth should be stored in appropriate media, which can prevent desiccation and subsequent loss of vitality of PDL.[1]

Common failures associated with replanted teeth are external root resorption and pulp necrosis. *Andreasen et al.*, reported a rate of 30% loss of replanted teeth due to external root resorption.[4] The pre-dentin and pre-cementum layer present in the root are essential elements which prevent the resorption of root even in the presence of inflammatory conditions. However, if an injury removes or alters the protective pre-dentin or pre-cementum layer, inflammation of the pulp induces root resorption with multinucleated clastic cells.[5] So, a storage media is desired which can maintain the viability of PDL cells as well as can prevent the development of external root resorption in future to prevent failure of the replanted tooth.

The storage media for an avulsed tooth should have low bacterial content, physiological osmolarity, a neutral pH, and essential nutrients.[6] The various media employed for storage of avulsed teeth are Hank's balanced salt solution (HBSS), normal saline, saliva of the patient, milk, propolis, Viaspan, coconut water and green tea. Milk is a medium traditionally indicated for storage of avulsed teeth.[7] The HBSS is a widely used standard solution recommended by the International Association of Dental Traumatology as a storage medium for avulsed teeth.[8] The HBSS is costly and not available readily. So, there is a need to identify other accessible and affordable storage media. This review focuses on use of natural products as storage media for avulsed teeth. *In vitro* and *in vivo* research published during 1995-2014, allowing open access on National Center for Biotechnology Information (NCBI) database and articles on EBSCO host (EBSCO-Elton B. Stephens Company) were included.

STORAGE POTENTIAL OF DIFFERENT NATURAL PRODUCTS

Milk

Milk can be employed as a storage media for avulsed teeth.[9] It meets the following criteria for an interim storage media: (1) it has physiologic pH, (2) ability to preserve the viability of PDL cells, (3) has low bacterial count and (4) commonly available.[10,11] Milk cannot revitalize the dead cells but helps in preserving vitality of PDL cells. Thus, an ex-articulated tooth that has been dried and then been kept in milk prior to replantation, has probably the same poor prognosis as any other dried and replanted tooth.[10] 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 and HBSS, 2% and 5% reduction in the number of viable PDL cells, respectively, and 38% reduction in clonogenicity, was observed.[3] Milk was found to affect the apoptosis of PDL cells. *Chamorro et al.*, studied the apoptotic cell death after storage of the avulsed teeth in milk, HBSS and SoftWear saline solution for contact lens. The number of apoptotic cells was least in PDL cells of tooth stored in milk and HBSS.[12]

Blomlof et al., reported successful healing of the PDL membrane after as long as six hours of storage in milk but not in saliva. The healing of the PDL was as good as that of immediately replanted teeth.[10]

In a study, long-shelf life whole milk and long-shelf life skimmed milk were compared with pH adjusted coconut water and soy milk; it was observed that after an initial period, long-shelf life whole milk and long-shelf life skimmed milk can maintain viability of a significantly less number of PDL cells as compared to soy milk and coconut water. However, after 24 hours the results were the same for coconut water and long-shelf life whole milk and long-shelf life skimmed milk.[6] On the other hand, regular pasteurized milk and long-shelf life whole milk were found to have no significant difference in their storage efficacy.[13]

The fat content of milk has an effect on its ability to maintain the PDL cell viability. The milk with low fat content was found to be better than the milk with high fat content.[14] *Nozari et al.,* 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.[15] According to *Sigalas et al.*, storage of tooth in ice as storage media is more effective than at room temperature. If ice is available, low fat milk is a suitable alternative to HBSS.[16] In the presence of ice in milk, the apoptotic cell death of the periodontal ligament is further inhibited.[12]

De Souza et al., evaluated whether the renewal of milk every 24 hours for up to 120 hours is able to increase its ability to maintain PDL fibroblast's viability *in vitro*. They showed that the renewal of milk every 24 hour cannot yield a greater storage time.[17]

Studies showed that milk has the ability to maintain viability of PDL fibroblast and it is superior to saliva, water or air drying, but not as good as HBSS[11] and coconut water.[1,18,19] However, *Moreira-Neto et al.*, found

milk to be better than coconut water in maintaining PDL cell viability.[20] The results of the *in vivo* study conducted by *Trope et al.*, also demonstrated that HBSS or Viaspan are better than milk, as storage media. The failure of replanted teeth because of external resorption was less in teeth stored in HBSS as compared to those stored in milk.[21] *Doyle et al.*, reported that after the passage of dry period of as low as 30 minutes, presoaking of avulsed tooth in milk or HBSS will not add any benefit.[22]

Coconut water

Coconut water is biologically pure and sterile.[1] It is rich in amino acids, minerals and vitamins. It is known to possess regenerative[23] and antioxidant properties.[24] Storage media having antioxidant properties can be more effective in maintaining the viability of PDL.[25]

As stated by *Omar et al.*, coconut water is comparable to HBSS and is more satisfactory than milk and saline for maintaining viability of PDL of avulsed tooth.[18] However, *Gopikrishna et al.*, reported coconut water to be more effective than HBSS. They found that the total number of viable PDL cells was significantly high when stored in coconut water as compared to propolis (50%), HBSS and milk.[1] Results of collagenase-dispase assay, demonstrated its properties better than oral rehydration solution (ORS).[26] Contrary to the above finding, *Souza et al.*, found milk to be desirable than coconut water.[27] *Moreira-Neto et al.*, found that addition of sodium bicarbonate to coconut water can improve its storage potential as compared to saline, but still it was not as efficacious as milk.[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 that from young fruits.[27]

Coconut water can be used to store avulsed tooth for a relatively longer period (45 minutes).[28] *Moura et al.*, claimed that if the pH of coconut water is adjusted to 7.0, it can be used as storage media for upto 24 hours. This finding holds high relevance in clinical practice, where presence of life-threatening conditions like complex fractures of jaw can delay the replantation of tooth.[6]

In a report of *Thomas et al.*, within 15 minutes, HBSS is the most effective storage media, and between 15-120 minutes, it is equivalent to coconut water.[29] *Silva et al.*, compared the cytotoxic effect of coconut water with whole milk, HBSS, tap water, using multiparametric cytotoxicity analysis employing 3T3 cells. Coconut water and HBSS expressed least cytotoxicity.[8]

Due to its superior osmolarity, easy availability and cost effectiveness, coconut water can be advocated as a viable storage media.[29]

Propolis

Propolis is a natural wax-like resinous substance found in bee hives, which is utilized by the bees as a "chemical weapon" against pathogenic microorganisms. It has a great variety of pharmacological effects such as antimicrobial, anti-inflammatory, immune-modulatory, anti-ulcer and anti-tumor properties and it is relatively non-toxic. Propolis has a complex chemical composition and is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%). The application of propolis as a storage media has been studied by many authors.[30]

Propolis is one step ahead of milk,[7,31] HBSS or saline[31] in maintaining PDL cell viability. However, its potential is less as compared to coconut water.[26] *Ahangari et al.*, compared the number of viable cells at 1 hour and 3 hours after storage in 10% propolis, 50% propolis, milk, egg white and HBSS. 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.[32] However, *Mahal NK et al.*, reported similar efficacy of propolis (15%), egg albumin and HBSS.[33]

In the study done by Gjerston *et al.*, Propolis decreased the apoptotic levels of PDL fibroblasts and increased mitochondrial enzymatic activity of PDL cells when compared with HBSS. Therefore, propolis can be a more beneficial storage medium for avulsed teeth.[34]

Propolis can be used to store avulsed tooth for a longer duration of 6-24 hours.[35,36] According to *Mori et al.*, the avulsed teeth when stored in propolis for 6 hours demonstrated better results than when stored for 60 minutes. It was believed that the highest incorporation of active principles of propolis in 6 hours was responsible for the antimicrobial, anti-inflammatory and healing actions of propolis.[35] Propolis at 10% concentration can be used to store avulsed tooth for

as long as 24 hours.[36] However, *in vitro* studies showed prominent time dependent loss of percent cell viability with the extended periods of incubation, when checked at 6, 12 and 24 hours.[36]

Propolis has synergistic effect with Dulbecco's modified eagle's medium (DMEM) for the maintenance of cell viability and preservative potential. In combination, 10% propolis + DMEM was found to be better than 20% propolis + DMEM, which shows that 20% concentration of propolis might have some cytostatic/cytotoxic effect on the cell physiology.[36]

Propolis has also been proposed to inhibit the osteoclastic resorption of teeth, which is common sequela of replantation of teeth. Propolis inhibits some aspects of the pathway leading to mature, active osteoclasts.[37] However, in an animal experiment using dog, replacement resorption cannot be prevented in replanted tooth stored in propolis.[7]

Green tea

Green tea is a beverage consumed all around the world. It has numerous health benefits which can be attributed to the presence of polyphenols such as epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, epigallate and catechin. These polyphenols contribute towards the anti-inflammatory and anti-bacterial activity of green tea.[38]

The commercially available green tea is easily available at the site of accident. Therefore it is tested as a storage media. It has low osmolality which may lead to death of the PDL cells and therefore, is not suitable for storage of avulsed teeth. Similarly, the osmolality and pH of green tea extract also is not ideal, but in experiments it showed the best ability for storage of avulsed teeth amongst HBSS, tap water, milk, commercial green tea and green tea extracts. The green tea extract is prepared by boiling 10 grams of green tea leaves in 100 ml of boiling distilled water for 5 minutes and then the sterilized extract is filtered.[39] The effect of (−)-epigallocatechin-3-gallate (major polyphenols in green tea extract) is somewhat concentration dependent. A 100 μM (−)-epigallocatechin-3-gallate can provide a preservation period of 14 days. *Chen and Huang*., tested the efficacy of 1 mg/ml concentration of epigallocatechin-3-gallate (EGCG), as only green tea or green tea solution could be achieved in markets which might have a low concentration of EGCG. The EGCG was found to be useful even at such a low concentration.[40] Thus, treatment with (−)-epigallocatechin-3-gallate or green tea can be a great therapeutic strategy for tooth transplantation, as storage of avulsed teeth in a medium containing EGCG will allow sufficient time for the desired dental treatment.[41]

Red mulberry

Red mulberry (*Morus rubra*) is common from the temperate to the subtropical regions of the northern hemisphere to the tropics of the southern hemisphere; it can grow in a wide range of climatic, topographical and soil conditions. Mulberry fruits are used medicinally as a worming agent, as a remedy for dysentery, as a laxative, odontalgic, expectorant, hypoglycemic and emetic.[42]

Red mulberry has been recommended as a storage media for avulsed teeth by *Ozan et al*., They compared 4 different concentrations of M. *rubra* (4%, 2.5%, 1.5% and 0.5%) with HBSS and tap water at 1 hour, 3 hours, 6 hours, 12 hours and 24 hours, to check the effect on PDL viability. The number of viable PDL cells was significantly high when an avulsed tooth was stored in 4.0% concentrated solution of M. *rubra as* compared to other concentrations. Upto 24 hours the efficacy of 4.0% concentration of M. *rubra* was similar to the HBSS, but 2.5% concentration was significantly less efficacious than HBSS. It was concluded that M. *rubra* can be recommended as a suitable transport media for avulsed teeth.[42]

Pomegranate juice

In ayurvedic medicine, pomegranate is considered as "pharmacy unto itself". It is an extraordinary fruit with complete medicinal power contained in its juice, peel and seeds. It has potent antioxidant, anti-carcinogenic and anti-inflammatory properties.[43]

Pomegranate effects the fibroblast cell proliferation. This proliferative effect is found for 1 hour at lower concentrations of 1% and 2.5%, but at 5% and 7.5% concentration a general proliferative effect is exhibited. The peak increase in cell viability is observed at 6 hours. It also promotes strong cell attachment. Pomegranate juice and HBSS can preserve the spindle like morphology of periodontal fibers for 24 hours after storage. So, it can be a good storage media. Since research conducted to assess its efficacy is very less, further research is needed.[44]

Aloe Vera

Aloe vera is a cactus like plant that belongs to family Liliaceae. The inner gel of *Aloe vera* contains more

than 75 active ingredients. 98-99% of gel is made up of water and remaining 1-2% contains active compounds including aloesin, aloin, aloe-emodin, aloemannan, acemannan, aloeride, naftoquinones, methylchromones, flavonoids, saponin, sterols, amino acids and vitamins. The pharmacological actions of *Aloe vera* gel include anti-inflammatory, antibacterial, antioxidant, immune-boosting and hypoglycemic properties.[45] It is a natural remedy which is available in many herbal shops.

In the study done by *Badakhsh et al.*, *A. vera* at concentrations of 10%, 30% and 50% concentrations performed similarly as supplemented culture media for upto 9 hours. *Aloe vera* at these concentrations maintained the cell viability over 90% and was superior to 100% *A. vera* and egg white. *Badakhsh et al.*, recommended *A. vera* as a suitable storage media for avulsed teeth.[46] *Pattamapun et al.*, studied the structure of PDL fibers of avulsed teeth stored in *A. vera*, milk and HBSS. Under the scanning electron microscope it was observed that periodontal fibers near the cementum of the tooth stored in *A. vera* were thick and intact, however, the periodontal fibers associated with teeth stored in milk and HBSS were loose.[47]

Chantarawaratit et al., studied the effect of acemannan on periodontal regeneration in furcation defect models. They found that acemannan significantly increased PDL cell proliferation, vascular endothelial growth factor, type I collagen and alkaline phosphatase activity.[48] The potential of *A. vera* towards successful replantation can be attributed to periodontal cell proliferating potential of its active components.

Egg white

Egg white consists of proteins, vitamins, and water. In an animal study, *Khademi et al.*, reported that the teeth stored in egg white for 6 to 10 hours were restored more successfully than those kept in milk.[49] *Khademi et al.*, in another study reported no significant difference between egg white and HBSS, and also found egg white to be superior to tap water and milk.[50] Due to high nutrient value as well as availability at the trauma site, egg white may be considered as a good alternative storage media.[51]

DISCUSSION

Studies on duration of dry period and successful replantation of avulsed teeth have sharpened 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.[36] Factually, 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 accidents, emergencies and unavailability of dentists, so a storage media which can maintain the PDL cell viability for a long duration is needed. Results of the previous *in vitro* studies demonstrated that natural products can maintain the PDL cell viability for an extended duration, for example coconut water (upto 24 hours),[3] propolis (6-24 hours),[22,23] 100 μM (−)-epigallocatechin-3-gallate (upto 14 days),[27] pomegranate juice (upto 6 hours) etc., However, most of these studies are confined to the *in vitro* research. Very few studies have tried to find out the effects *in vivo*. In the study by *Casaroto et al.*, the *in vitro* results showed good potential of propolis in maintaining PDL cell viability, but when teeth were replanted, the resorption of teeth cannot be prevented and ultimate result was the failure of teeth replantation. So, it is necessary to conduct *in vivo* studies so that the features of successful replantation can be assessed.

Another requirement of the ideal storage media is easy availability. Although propolis and green tea need to be processed, other natural products such as coconut water and milk are easily and readily available in the market and they are usable in their unprocessed form. The storage potential of pasteurized milk is as same as long-shelf life milk. The long-shelf life milk can be recommended as a suitable storage media, due to its ease of storage and long shelf life; it can be made available in schools, gyms and outdoor athletic fields, where tooth avulsion is more likely to occur.[13] Similarly coconut water may prove to be a suitable storage media because of its sterile nature; no need of processing and availability at different sites. All storage media have shown to lose their effectiveness with time. Cooling has shown to enhance the preservation effects of milk. Effect of cooling on preservation potential of other media should be studied. Research conducted to date have checked the storage at fixed concentrations whereas it is required to check the storage at different concentrations. Storage potential especially at low concentrations should be assessed as in actual situation of the accident as it may not be possible to prepare a predetermined concentration.

So, it can be concluded that natural products can be used as storage media but further studies are required

to demonstrate the optimum storage time of different media, the form and manner of use. The studies on stimulated animal models are required to verify the results of various *in vitro* studies.

CONCLUSION

An appropriate storage media can help maintain the viability of PDL cells and can lead to successful replantation of avulsed teeth. The natural products such as coconut water, milk and propolis can act as appropriate storage media because of their easy availability and potential to maintain viability of PDL cells for longer durations.

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