Storage Media – A review

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Abstract—Management protocol for avulsed teeth includes the maintenance of viable periodontal ligament (PDL) cells for good prognosis and long-term survival of these teeth. The desirable treatment is immediate replacement and replantation of the avulsed tooth but it cannot always be accomplished for a number of reasons. Thus, the tooth should be transported in a suitable storage medium to maintain the cell viability. Considering the critical role of these media, an informed choice of a suitable medium is essential for a favourable outcome. This review paper focuses on the various storage media available and highlights their specific features or drawbacks.

Index Terms—Avulsion; osmolality; replantation; storage medium.

I. INTRODUCTION

Avulsion or exarticulation is a complete displacement of a tooth from its alveolar socket as a result of trauma. This injury is comparable to losing one’s organ, such as a finger in an accident and therefore has severe psychological implications [1]. Management protocols for avulsion should include management of the pulp and the periodontal ligament (PDL) cells, with the latter being more important to improve the long-term survival and prognosis of avulsed teeth [2]. According to Andreasen, the factors that play a role in healing of the periodontal ligament after reimplantation of an avulsed tooth are primarily the amount of physical damage to the root surface, type of medium in which the avulsed tooth is stored and also on the extra oral dry time [3]. The fundamental philosophy for the storage of avulsed teeth is that it should be stored in an environment that most closely replicates the oral environment. 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[4]. The ideal requirements of storage media are[5]:

1. Should have antimicrobial characteristics.
2. Should be capable of preserving the feasibility of cellular PDL.
3. Should be able to maintain the viability of periodontal fibers for an acceptable period of time.
4. Should favor proliferative capacity of cells
5. Should have the same osmolality as that of body fluids.
6. Should not react with body fluids.
7. Should not produce any antigen antibody reactions.
8. Should reduce the risk of post-reimplantation root resorption or ankylosis.
9. Should have a good shelf life.
10. Should be effective in different climate and under different conditions.
11. Should wash off extraneous materials and toxic waste products.
12. Should aid in the reconstitution of depleted cellular metabolites.

This review paper focuses on many solutions that have been proposed and/or tested as storage media for avulsed teeth. In vitro and in vivo research published during 1972-2019, allowing open access on National Center for Biotechnology Information (NCBI) database and articles on EBSCO host (EBSCO-Elton B. Stephens Company) were included. The following were identified and reviewed as a result of the literature search. Table 1 and Table 2 summarises the characteristics and properties of each of these solutions as storage media.

TAP WATER

Several studies have shown that cells stored in water did not maintain their morphology, with visible destruction and rapid cell death. Blomlof found that storing 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. They attributed the increased cell damage to the cell lysis caused by the very low osmolality of tap water[6]. In view of this, tap water should be used only to avoid tooth dehydration, but it is inadequate for conservation of avulsed teeth.

HUMAN SALIVA

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DOI: 10.24018/ejmed.2019.4.6.82
Saliva can be used as an immediate interim storage medium. Although it is readily available, avulsed teeth should not be stored for longer than 30 min in saliva. Saliva contains potentially harmful substances, such as enzymes, bacteria and their by-products. Patients’ own saliva is the best immediate transport medium for an avulsed tooth[7]. It is also an immediately available storage medium at all the accident sites. After trauma, several ml of saliva can easily be collected in a cup and the tooth dropped into this, or the tooth can be placed in the patient’s mouth under the tongue. In an animal study, Andreasen showed that saline and saliva were suitable storage medium for protection against root resorption for short extra-alveolar periods [8]. Thus saliva can be considered to be an acceptable short-term storage medium (less than 30 min) and its use should be limited to cases where the extra-alveolar duration is less and other superior storage media are not available.

NORMAL SALINE
Normal saline is a solution of 0.90% w/v of NaCl despite being compatible to the cells of the PDL, it lacks essential nutrients, such as magnesium, calcium, and glucose, which are fundamental to the normal metabolic needs of the cells of the PDL[9]. Cvek found that avulsed teeth soaked in an isotonic saline for 30 minutes before replantation showed less resorption than those stored dry between 15 and 40 minutes[10]. Thus, it is not a widely accepted storage media but can be used for a short period of time if no other option is available.

MILK
Milk has been accepted by the American Association of Endodontists as a suitable transport medium for avulsed teeth. Blomhoff[11] found that milk was capable of preserving 50% of the periodontal ligament cells from culture for up to 12 hours. Marino[12] compared milk with several other storage media and found that milk was gold standard to the others in maintaining the viability. Marino[12] showed that both regular pasteurized milk and long shelf life milk were more effective in maintaining human periodontal ligament cell viability than other storage medias. Some evidence supports the use of cold milk as an interim storage medium for avulsed teeth. Avulsed teeth stored in chilled milk for up to 1 hour can maintain sufficient number of viable periodontal ligament cells to support replantation of the tooth and the possibility of periodontal ligament healing [13]. There is evidence that milk with a lower fat content may be more appropriate at maintaining cell viability than milk with a higher fat content[14].Pearsson [7] indicated that powdered milk, Enfamil ® is an effective storage medium in maintaining PDL cells viable.

EAGLE’S MEDIA (EM)
EM is an excellent storage media although it is not practical to recommend it for general use because of limiting factors. One study has shown that human PDL cells proliferated in EM’s storage medium [25]. In another human study, Pearson [26] showed that soaking teeth in EM for up to 60 min after they had been stored dry for 5–14 days resulted in better PDL healing than if the teeth had been immediately replanted. Lekic [8] reported that teeth extracted for orthodontic reasons, stored in saliva for 15 min and then transferred to various other media had the highest number of viable cells after both 30 and 60 min when EM was used.

ORAL REHYDRATION SOLUTION (ORS)
Commercially available ORS like Ricetral, which contains essential cells and nutrients like glucose and vital salts in concentrations was found to be a suitable storage medium due to its similar composition with HBSS[15]. These solutions are available in drugstores at low cost and their components are able to maintain the body hydrated by reposition of liquids lost in the intestine[16]. ORS showed a significantly higher number of viable cells as compared to milk [17]. In countries where HBSS is unavailable, ORS could be a suitable storage medium for avulsed teeth. Due to its ease of storage, long shelf life and inexpensive packages, it can be used easily in schools, gyms and outdoor athletic fields and the places that tooth avulsions are most likely to occur.

COCONUT WATER
Coconut water is a natural, isotonic beverage that is readily accepted by the body because of its sterility, pH, and electrolytic balance. Some of the constituents are similar to that of blood plasma[18]. The exact composition has been related to the stage of maturation of the coconut and the region where the coconut palms grow. The idea that coconut water can be used as a storage medium arose from its past use as an intravenous resuscitation fluid for dehydrated patients, and also as an intravenous fluid for war patients. Following avulsion, the tooth has to be carried in the shell of the coconut, because once it is exposed to air or is removed from the shell, the liquid rapidly loses most of its nutritional characteristics and begins to ferment. Gopikrishna [19] assessed the potential of coconut water in comparison with propolis, Hank’s balanced salt solution (HBSS) and milk in maintaining viable periodontal ligament (PDL) cells on avulsed teeth. Results showed that coconut water kept significantly more PDL cells viable compared with propolis, HBSS or milk. Half mature coconut water followed by mature coconut water was found to be more effective than immature coconut water in preserving fibroblast cell viability. This could be explained by the changes in the composition with maturity[20].

CUSTIODIOL
It is used as a preservation solution for organ transplantation. Alaçam [21] 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.

GATORADE
According to Chamorro [22] when cells are exposed to Gatorade, it is possible that the delicate cellular membrane gets damaged because of the low pH, which makes it...
impossible for the cells to grow. The use of Gatorade yields better results for PDL survival Compared to tap water. Gatorade is often available at sporting events but it has incompatible, harmful osmolality that causes cell destruction, so it is not an ideal solution to use.

CONTACT LENS SOLUTION

Majority of injuries occur at home or at school where contact lens solution is easily available so it is suggested to be used as a storage media. The commercially available solution is physiologically acceptable as it is buffered and is isotonic. Various companies manufacture contact lens solutions and on comparing these solutions no significant difference was found in their potential of maintaining viability of cells. It was found to be better than tap water and Gatroade but not as superior like HBSS and milk.[23]

PROPOLIS

Propolis is a multifunctional material used by bees in the construction and maintenance of their hives. Propolis possesses several biologic activities such as anti-inflammatory, antibacterial, antioxidant, antifungal, antiviral, and tissue regenerative, among others[14]. The constituents of propolis vary widely because of climate, season, location, and year, and its chemical formula is not stable. Al-Shaher [24] studied the viability of PDL and dental pulp cells in vitro when they were maintained in a low concentration of propolis, and observed low toxicity with maintenance of cell viability greater than 75%. In vivo results showed that teeth maintained in propolis medium exhibited replacement resorption with significant reduction in tooth length, similar to teeth maintained in saliva and dried teeth. This resorption was less intense with the 3-h storage time than the 1-h storage time [25].

HANK’S BALANCED SALT SOLUTION (HBSS)

HBSS is the standard solution recommended by the International Association of Dental Traumatology. Hank’s Balanced Salt Solution (HBSS) has been especially developed for cell maintenance and thus, theoretically, it allows a better conservation of tissues for long time periods. It has been widely employed as a reference solution in studies on dental avulsion as it has the ideal osmolality and pH for preserving the vitality of cells[16]. It is a special kit available in some countries, which contains a small basket in which the avulsed tooth is suspended and submerged in HBSS. Gentle agitation can remove debris from the PDL during storage and lost nutrients can be replenished by the HBSS before replantation. However, its use is restricted to laboratory environments and is not available at an accident site, which makes it impracticable as a storage medium. In a study by Krasner [26], Hank’s balanced salt solution was found to be the best solution for storing avulsed teeth. It has shelf life of 2 years and does not require refrigeration. This solution is effective in preserving PDL cells, renew the degenerated PDL cells and maintain a superior success rate if an avulsed tooth is soaked in them for 30 minutes. Ashkenazi [27] conducted a study to compare the effectiveness of different storage media for preserving viability, mitogenicity and clonogenic capacities of periodontal ligament cells. Results showed that Hank’s balanced salt solution was the most effective medium for up to 24 hours at 4°C, when compared with EM, milk, ViaSpan. HBSS is commercially available as Save-A Tooth TM (Save-A-Tooth TM, Inc., Pottstown, PA) with an inner net to receive the avulsed tooth and to minimize cells trauma during transport. Blomlof [28] showed that storage of the tooth in HBSS can extend the extra-oral time up to 4 hours.

VIASPAN [18]

The ViaSpan is a medium used for the transportation of organs which are going to be transplanted and it has been very effective for storing avulsed teeth. Hiltz and Trope [29] have compared the vitality of lip fibroblasts, at room temperature which were stored in milk, Hank’s balanced salt solution and ViaSpan. The ViaSpan was the best storage medium observed at all times, and after 168 hours, there was still 37.6% of living cells. Ashkenazi[27] compared the effectiveness of the four storage media (Hank’s balanced salt solution, culture medium, Eagle’s medium and ViaSpan) concerning the preservation of fibroblasts of the periodontal ligament at room temperature (22°C). ViaSpan and culture medium, followed by Hank’s balanced salt solution were the most effective in keeping the clonogenic capacity of the fibroblasts of the periodontal ligament after 24 hours, at room temperature (22°C).

EMDOGAIN

Emdogain is a commercial Enamel Matrix Derivative (EMD) extracted from developing embryonic enamel of porcine origin and contains several matrix proteins. Emdogain has been shown to increase the incidence of healed PDL when this gel was applied to root surface of the avulsed tooth and/or inserted directly into alveolar socket before implantation. According to Ashkenazi and Shaked [27] Emdogain diminishes the percentage of fibroblasts of the periodontal ligament with capability of forming colonies and that lowers the capability for the fibroblasts to repopulate the dental radicular surface after dental avulsion. Emdogain can delay, but not stop the development of replacement resorption, one of the worst complications of dental trauma. Emdogain on its own is not efficient in the regeneration of injured periodontal tissues of the avulsed tooth.

EGG WHITE

Egg white is considered a good choice as a storage media for teeth undergoing delayed replantation due to its high content of proteins, vitamins and water, absence of microbial contamination and easy access[16]. Khademi [30] have compared milk and egg white as solutions for storing avulsed teeth, and the results have shown that teeth stored in egg white for 6 to 10 hours had a better incidence of repair than those stored in milk for the same amount of time. Sousa[31]has microscopically analyzed the human periodontal ligament attached to the extracted tooth after one hour of extra-alveolar time, compared to milk, egg
white and artificial saliva. The results of teeth stored in milk and egg white were similar concerning the organization of collagen fibers and the number of cells.

**OCIMUM SANCTUM**[32]

Ocimum sanctum may be attributed to its high content of phenolic compounds and phytochemicals like flavonoids, tannins, terpenoids, and saponins in the leaves and the stem which act as health promoting compound as a results of their anion radicals and consequently have a high anti-oxidant, anti-inflammatory, anti-fungal, and anti-bacterial properties.

**GREEN TEA**[33]

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. 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.[34] A 100 µM(−)-epigallocatechin-3-gallate can provide a preservation period of 14 days. Chen and Huang [35]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 low concentration tooth transplantation, as storage of avulsed teeth in a medium containing EGCG will allow sufficient time for the needed dental treatment.

**ALOE VER**

Aloe vera belongs to family Liliaceae with the inner gel of Aloe containing more than 75 active ingredients. The active compounds include aloesin, aloin, alo-emodin, aloemannan, acemannan, aloeride, naftoquinones, methylichromones, flavonoids, saponin, sterols, amino acids and vitamins.[33]

**MORUS RUBRA (RED MULBERRY)**[36]

Ozan reported that when teeth were stored in red mulberry for up to 12 h, its capacity to maintain the viability of PDL cells was better than that of HBSS. 4% juice of the M. rubra could be recommended as a suitable transport medium however, there are very few studies evaluating the use of red mulberry juice as a transport medium for avulsed teeth and its biological properties have not been yet established yet.

**SALVIA OFFICINALIS**[37]

Salvia officinalis (sage) extracts have been credited with a long list of medicinal uses such as spasmyotics, antiseptics, and astringents. Some phenolic compounds of plants belonging to this genus have also demonstrated excellent antimicrobial activity as well as scavenging activity of their active oxygen forms such as superoxide anion radicals, hydroxyl radicals, and singlet oxygen. These inhibit lipid peroxidation, and the corresponding extracts have been widely used to stabilize fat and fat containing foods. Antioxidant activity of *S. officinalis* is due to its phenolic contents as rosmarinic acid, carnosic acid, carnosic acid, salvianolic acid, and its derivatives carnosol, rosmanol, epirosmanol, rosmadial, and methyl carnosate. Two point five percent *S. officinalis* showed better results than HBSS, PBS, and tap water at all times except at 1 hour, where there was no significant difference between *S. officinalis* and HBSS or PBS.

**TOOTH RESCUE BOX**[38][39]

A tooth rescue box contains Special Cell Culture Medium (SCCM) including amino acids, vitamins and glucose has been developed. In Europe it is marketed as Dentosafe (Dentosafe GmBH, Iserlohn, Germany) and in the USA as EMT Tooth Saver (SmartPractice.com, Phoenix, AZ, USA). The medium was shown to maintain vitality and proliferative capacity of PDL cells for up to 48 h at room temperature in vitro and to be successful for storage of avulsed teeth in clinical use. In a long-term clinical study, all teeth that were rescued and stored in the tooth rescue box within 15 min after avulsion exhibited favourable healing after replantation, irrespective of the root development stage and irrespective of the storage duration that was up to 53 h [40] Avulsed teeth stored in a rescue box for 15 minutes have exhibited functional healing irrespective of storage duration. This medium has shown to maintain the vitality and viability of PDL cells at room temperature for at least 48 hours. The usage of the tooth rescue box is self-explanatory and plausible to lay people (teachers, pupils). 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. Since their introduction and distribution in Germany, the rate of functional healing after replantation of avulsed teeth has increased to 50%. Thus it is advised to distribute tooth rescue boxes at locations prone to tooth traumas (schools, kindergartens, sporting facilities, public pools) and at emergency units (hospitals, ambulances) to enhance the prognosis of avulsed teeth.

**ASCORBIC ACID**

Ascorbic acid stimulates osteoblasts to lay down Type I collagen and also helps in expression of specific markers associated with osteoblastic phenotypes such as alkaline phosphates (ALP) and osteocalcin. Ishikawa[41] studied the effect of ascorbic acid on PDL cells and stated that ascorbic acid increased the ALP activity, which is required for binding of PDL cells to Type I collagen via α2β1 integrin whose expression is again increased by ascorbic acid, so it may serve as potential storage media.

**L-DOPA**

L-Dopa (Levodopa; Sigma chemical, Perth, WA, Australia) is a drug with possible mitogenic effect. It stimulates the
secretion of growth hormone from the pituitary gland which aids in the healing process. Partovi[42] observed the effect of levodopa on human PDL fibroblasts and stated that it can also have a local effect on growth of cells and can act as preserving medium for avulsed teeth.

**CRYOPROTECTIVE AGENTS**
The effect of low-temperature storage on clinical success of reimplantation has been studied. Schwartz and Andreasen [43] studied the effects of the cryopreserving agents, 5% and 10% dimethyl sulfoxide and 10% glycerol on PDL. They observed that use of different cryoprotectives, combined with controlled freezing rates can preserve the PDL of reimplanted teeth.

**MIMUSOPS ELENGI (BAKUL)**
Mimusops elengi (bakul) have been found to be having antimicrobial, antiinflammatory, analgesic, and antipyretic properties and used to treat conditions like bleeding gums, arthritis, bronchitis, skin diseases and diabetes. However, Mimusops elengi showed a dismal performance due to the fact that pH and osmolality of the prepared solution was not favourable for the growth of the PDL cells.[44]

**POMEGRANATE JUICE (Punica Granatum)[45]**
In traditional medicine, pomegranate fruit has been used to treat acidosis, dysentery, microbial infections, diarrhea, hemorrhage, respiratory pathologies, and hypertension. Pomegranate affects 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 warranted.

Table 1: Classification of solutions that have been proposed and/or tested as storage media for avulsed teeth.

| Laboratory prepared | Natural source           |
|---------------------|--------------------------|
| Normal saline       | Tap water                |
| Contact lens solution | Human Saliva            |
| Hank’s Balanced Solution | Milk                   |
| Eagle’s medium      | Propolis                 |
| ViaSpan             | Ocimum sanctum           |
| Ascorbic acid       | Coconut water            |
| Custodiol           | Egg white                |
| Dubelco’s storage   | Endogain                 |
| Tooth rescue box    | Morusrubra               |
| Conditioned medium  | Salvia officinalis extract |
| Gatorade            | Green tea                |
| Oral Rehydration Solution | Aloe Vera               |
| Cryoprotective agents | Catalase supplementation |

Table 2: Characteristics and properties of solutions that have been proposed and/or tested as storage media for avulsed teeth
| Storage Media                        | Ph  | Osmolarity (mosm/kg) | Availability at site of trauma | Refrigeration Requirement | Viability of pdl | Nutrient content | Cost Effective |
|-------------------------------------|-----|----------------------|--------------------------------|---------------------------|------------------|------------------|----------------|
| Tap Water[30]                       | 7.5 | 3                    | Readily available              | Not required              | Cell lysis due to hypotonicity [6] | None             | Cost Effective  |
| Normal Saline[11]                   | 7.0 | 280                  | Not always readily available   | Not essential             | Does not facilitate | No essential metabolites | Cost effective  |
| Hank’s Balanced Salt Solution [46]  | 7.2 | 270-320[13]          | Limited access                 | Not essential             | Excellent        | Glucose, calcium and magnesium ions | Prohibitive     |
| Human Saliva[47]                    | 6.3 | 110-120              | Readily available              | Not essential             | Good             | -                | Cost Effective  |
| Oral Rehydration solution[17]       | 7.2 | 320                  | Readily available              | Not essential             | Good             | Has essential nutrients         | Cost effective  |
| ViaSpan [16]                        | 7.4 | 320                  | Limited access                 | Not essential             | Excellent        | Hydrogen buffer and lactobionate, raffinose which prevent cellular swelling and maintain vitality of cells. | Prohibitive     |
| Egg White[48]                       | 8.6-9.38 | 258               | Available but not always readily available | Not essential             | Not different from Ca2+/Mg free HBSS | Rich             | Cost-effective  |
| Eagle’s medium                      | 7.2±0.2 | 335±30           | Not readily available except in research laboratories. | Essential                 | Good             | Amino acids, vitamins and bicarbonates | Prohibitive     |
| Custodiol® [49]                     | 7.0 | 310                  | Not readily available          | Recommended by the manufacturer to be used at 4 °C | Mineral composition similar to that of the intracellular fluid. | Histidine-tryptophan ketoglutarate solution and low potassium content. | Prohibitive     |
| Tooth rescue box[38]                | -   | -                   | -                              | Not required              | Vitality an proliferative capacity of PDL cells for up to 48 h at room temperature in vitro and to be successful for storage of avulsed teeth in clinical use. | Contains aminoacids, vitamins and glucosis and preservative | -              |
| Gatorade [22]                       | 2.91 | 280-360             | Limited access                 | -                         | -                | Sodium, sugar, potassium, phosphate and lemon juice. | Cost-effective  |
| Contact lens solution               | 7-7.36 | 275-310            | Limited access                 | Not essential             | Presence of preservative is harmful to the cells of PDL cells. | -                | -              |
| Milk[17]                             | 6.5 - 7.2 | 230 – 270 | Readily available              | Essential                 | Potentially maintain PDL cell viability for up to 2 h | Amino acids, carbohydrates and vitamins. | Cost effective  |
| Propolis [14]                       | -   | -                   | Not always readily available   | -                         | -                | In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various other substances | -              |
| Coconut water[50]                   | 6.2 | 288                  | Readily available              | -                         | Composition similar to that of the intracellular fluid. | Potassium, calcium, and magnesium, Sodium, chloride, and phosphate ions | Cost effective  |
| Emdogain [27]                       | -   | -                   | Not readily available          | Delay the development of replacement resorption. [51] | -                | -                | -              |
| Green tea                           | 5.9 | 87                   | Not essential                 | -                         | -                | Contains calcium         | -              |
REFERENCES

[1] R McDonald, D Avery, J Dean, J Jones, "Management of Trauma to the teeth and supporting tissues" in Dentistry for the child and adolescent, R McDonald, D Avery, J Dean, New Delhi, Moby Elsevier, 2011, pp. 403–44.

[2] JI Ingle, LK Bakland, JC Baumgartner, Ingle’s endodontics. 6th ed. Hamilton, ON: B.C. Decker Inc; 2008.

[3] JO Andreasen, FM Andreasen, L Andreasen, Traumatic injuries to the teeth, 4th edn. Copenhagen: Blackwell Munksgaard; 2007.

[4] R Pileggi, TC Damsha, JE Nor, "Assessment of post-traumatic PDL cells viability by a novel collagenase assay", Dent Traumatol, vol 4, pp 186–189,2002.

[5] N Malhotra, R Cyriac, S Acharya, "Clinical implications of storage media in dentistry: a review" Endodontic Practice Today, vol 4, 2010.

[6] I Mackie, H Worthington, "An investigation of re plantation of traumatically avulsed permanent incisor teeth" Br Dent J, vol 172, pp 17-20,1992.

[7] RM Pearson, FR Liewehr, LA West, WR Patton, JC McPherson, RR Runner, "Human periodontal ligament cell viability in milk and milk substitutes", J Endod, vol 29, pp 184–6,2003.

[8] Weine F. Endodontic emergency treatment. In: Endodontic therapy. Mosby,Inc; 1996, pp. 74–103.

[9] VI Khanda, G Kaur, GS Brar; S Kallar, H Khurana, "Clinical and Practical Implications of Storage Media used for Tooth Avulsion" Int J Clin Pediatr Dent, vol 10, pp 158–165,2017.

[10] M Cvek, L Granath, L Holender, "Treatment of non-vital permanent incisors with calcium hydroxide. Variation of occurrence of reimplanted teeth with duration of extra-alveolar period and storage environment" Odontol Revy, vol 2 pp 43–56,1974.

[11] L Blomlof, P Otteskog, "Viability of human periodontal ligament cells after storage in milk or saliva", Scand J Dent Res, vol 88, pp 436–440,1980.

[12] TG Marino, LA West, FR Liewehr, JM Mailhot, TB Buxton, "Determination of periodontal ligament cell viability in long shelf-life milk", J Endodon, vol 26, pp 699–702,2000.

[13] Sigalas E, Regan JD, Kramer PR, Witherspoon DE, Opperman LA, "Survival of human periodontal ligament cells in media proposed for transport of avulsed teeth," Dent Traumatol, vol 20, pp 21–28, 2004.

[14] Özan F, Polat ZA, Er K, Özcan Ü, Değer O, "Effect of Propolis on Survival of Periodontal Ligament Cells: New Storage Media for Avulsed Teeth", J Endod, vol 33, pp 570–573,2007.

[15] Rajendra P, Vaishnav B, Varghese NO, Varghese JM, Maruagaan E, "Evaluation, using extracted human teeth, of Ricetral as a storage medium for avulsed teeth", J Endod, vol 27, pp 217–220, 2011.

[16] Poi WR, Sonoda CK, Martins CM, Melo E, Pellizzer EP, Mendonça MR De, "Storage Media For Avulsed Teeth: A Literature Review", Scand J Dent Res, vol 24, 437–445, 2013.

[17] Subramaniam P, Girija P, Essara U, Girish Babu KL, "Oral rehydration salt-liquid as a storage medium for avulsed teeth", Dent Traumatol, vol 31, pp 62–66,2015.

[18] Dharmani CKK, Singh N, Dharmi UK, "Storage Media for Avulsed Teeth: An Overview" IOSR J Dent Sci, vol 16, 138–142, 2017.

[19] Gopikrishna V, Bawpe J, Venkateshbabu N, Thomas T, Kandaswamy D, "Comparison of coconut water, propolis, HBSS, and milk on PDL cell survival", J Endod, vol 34, pp 587–593,2008.

[20] Awaddeh L, Haimoum RN, Al-Jundi SH, Al-Quod K, "Human periodontal fibroblasts viability stored in Custodiol®, coconut water, and propolis. An ex vivo study", Dent Traumatol, vol 34, 264–270, 2018.

[21] T Alacağ, G Gökülg, H Ömürülü M Can, "Lactate dehydrogenase activity in periodontal ligament cells stored in different transport media", Oral Surg Oral Med Oral Pathol Oral Radiol Endod, vol 82, pp 321–323,1996.

[22] MM Chamorro, JD Regan, LA Opperman, PR Kramer, "Effect of storage media on human periodontal ligament cell apoptosis", Dent Traumatol, vol 24, pp 11–16,2008.

[23] V Taneja, K Gupta, N Sridhar,S Kumar, K Kaur, M April, "Save the Avulsed Tooth- Review of the storage media" pp 2593–603.

[24] A Al-Shafer, M Alagwar, S Bretz, H Baugh, D, "Effect of propolis on human fibroblasts from the pulp and periodontal ligament", J Endod, vol 30, pp 359–361,2004.

[25] AR Casarotto, MM Hidalgo, AM Sell AM, Franco SL, Cuman RNK, Moreschi E, "Study of the effectiveness of propolis extract as a storage medium for avulsed teeth", Dent Traumatol, vol 6, pp 323–331,2010.

[26] P Krasner, "Management of tooth avulsion in the school setting", J Sch Nurs, vol 8,20–26,1992.

[27] M Ashkenazi, M Marouni, H Sarna, "In vitro viability, mitogenicity and clonogenic capacity of periodontal ligament cells after storage in four media at room temperature", Dent Traumatol, vol 16, pp 63–70,2000.

[28] L Blomlof, P Otteskog, L Hammarstrom, "Effect of storage in media with different ion strengths and osmolalities on human periodontal ligament cells", Scand J Dent Res, vol 89, pp 180–187,1981.

[29] J Hilz , M Trope M, "Vitality of human lip fibroblasts in milk, Hanks balanced salt solution and ViaSPAN storage media" Endod Dent Traumatol, vol 7, 69–72, 1991.

[30] AA Khedmi, S Sai, MR Mohajeri, N Mirkhehti, F Ghassami, N ordibi nia, S Alavi, "A new storage medium for an avulsed tooth", J Contemp Dent Pract, vol 9, pp 25–32,2008.

[31] Souss HA De, "Microscopic evaluation of the effect of different storage media on the periodontal ligament of surgically extracted human teeth" 2008:628–32.

[32] T Vyas, P Sood, M Kaur, "The use of herbal Extracts of Ocimum sanctum and Minusus elengi as a Novel Storage Media for the Avulsed Tooth", J Adv Med Dent Sci Res, vol 6, pp 53–62, 2018.

[33] D Jain, S Nagarajappa, P Daras, "Natural products as storage media for avulsed teeth", Saudi Endod J 2015;9(2),107.

[34] JY Hwang, SC Choi, JH Park, SW Kang, "The use of green tea extract as a storage medium for the avulsed tooth", J Endod, vol 37, 962–967,2011.

[35] Chen H, Huang B, (-)Epigallocatechin-3-gallate: A novel storage medium for avulsed teeth. Dent Traumatol 2012;28:158–60.

[36] F Özan, B Tepe, ZA Polat, Er K, "Evaluation of in vitro effect of Morus rubra (red mulberry) on survival of periodontal ligament cells", Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontol, vol 105,66–69,2008.

[37] F Özan, ZA Polat, B Tepe, Er K, "Influence of storage media containing Salvia officinalis on survival of periodontal ligament cells", J Contemp Dent Pract, vol 9, pp17–24,2008.

[38] C Filippi, H Kirscher, A Filippi, Y Pohl, "Practicability of a tooth rescue concept - The use of a tooth rescue box", Dent Traumatol, vol 24, pp 422–429,2008.

[39] N Malhotra, "Current developments in interim transport (storage) media in dentistry: An update", Br Dent J, vol 211, pp 29-33, 2011.

[40] Y Pohl, LA Filippi, H Kirscher, "Results after re plantation of avulsed permanent teeth. II. Periodontal healing and the role of physiologic storage and antiserosptive-regenerative therapy (ART)." Dent Traumatol 2005;21:93–101.

[41] S Ishikawa, K Iwasaki, M Komaki, I Ishikawa, "Role of ascorbic acid in periodontal ligament cell differentiation" J Periodontol, vol 75,709-716,2004

[42] M Partovi, A Sadeghein,E. Azizi,SN Ostad, "Mitogenic effect of L-dopa on human periodontal ligament fi broblast cells", J Endod, vol 28,193-196,2002.

[43] O Schwart, JO Andreasen,"Cryopreservation of mature teeth before re plantation in monkeys", Int J Oral Surg,vol12,425-436,1983

[44] T Vyas, P Sood, M Kaur, "Diseases and Future Prospects and their Application in Dentistry", J Adv Med Dent Sci Res, vol 6, pp 53–62, 2018.

[45] S Tavassoli-Hojiati S, Aliasghar E, Babaki FA, Emadi F, Parsa M, Tavajiho S, et al. Pomegranate juice (punica granatum): a new storage medium for avulsed teeth. J Endod Traumatol, vol 6, 2015.

[46] Momes C, Westphalen F, Carneiro E. Study of storage media for avulsed teeth1. Momes C, Westphalen F, Carneiro E. Study of storage media for avulsed teeth. Brazilian J
[47] Blomlöf L, Lindskog S, Andersson L, Hedström KG, Hammarström L. Storage of experimentally avulsed teeth in milk prior to replantation. J Dent Res 1983;62:912-6.

[48] Badakhsh S, Eskandarian T, Esmaeilpour T. The use of Aloe Vera extract as a novel storage media for the avulsed tooth. Iran J Med Sci. 2014;39(4):327–32.

[49] Awawdeh L, Haimour RN, Al-Jundi SH, Al-Qaoud K. Human periodontal fibroblasts viability stored in Custodiol ® , coconut water, and propolis. An ex vivo study. Dent Traumatol [Internet]. 2018 Aug 1 [cited 2019 Aug 8];34(4):264–70. Available from: http://doi.wiley.com/10.1111/edt.12403

[50] Gopikrishna V, Thomas T, Kandaswamy D. A quantitative analysis of coconut water: a new storage media for avulsed teeth. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2008;105(2):61-5.

[51] Ashkenazi M & Shaked I. In vitro clonogenic capacity of periodontal ligament fibroblasts cultured with Emdogain®. Dent Traumatol. 2006; 22: 25–9.

[52] Adeli F, Zabihi E, Abedian Z, Gharekhani S, Pouramir M, Khafri S, et al. Comparative in vitro study of the effectiveness of Green tea extract and common storage media on periodontal ligament fibroblast viability. Eur J Dent. 2016;10(3):408–12.