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Save the Avulsed Tooth- Review of the storage media

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ABSTRACT

Dental avulsion is a common type of trauma that results in the complete displacement of tooth from the alveolar socket. Although the ideal treatment would be the immediate re-implantation of the tooth at the site where the trauma took place, this may not be practically possible in every case. Hence, the avulsed tooth may have to be placed in an appropriate storage or transport medium until it is re-implanted. The biological properties of the storage medium have significant impact on the success of re-implantation, as it must be capable of preserving the vitality, clonogenic and mitogenic ability of the PDL cells for successful re-implantation.

Keywords: Dental trauma, Avulsed teeth, storage media, PDL cell viability.

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INTRODUCTION

Tooth avulsion is seen in 0.5-16% of all the traumatic injuries to the anterior permanent teeth.[1]Other injuries can result in crown or root fractures or luxations. Avulsion is the complete exarticulation of tooth from the socket. It is a complex type of injury which affects pulp, periodontal ligament and alveolar bone. The prevalence of avulsed teeth is expected to surpass the prevalence of dental caries.[2]A good prognosis is anticipated if the avulsed teeth are stored in a suitable storage media and replanted in 20 minutes. This minimizes risk of post-replantation inflammatory or replacement resorption.[3]

Although it is known that minimum extra-alveolar period is recommended for a good prognosis, but under practical situations it is not possible as at the site of the trauma the patient might be unconscious, there is lack of consent for the desired treatment and also unawareness and lack of first aid knowledge. So usually the extra-alveolar time extends beyond 20 minutes. This extra-alveolar time leads to desiccation of the root surface, increasing the risk of loss of vitality of the periodontal ligament (PDL) cell.[4]

The clinical success of replantation depends on various factors:[5]

- Age of the individual,
- Stage of root development, mechanical damage
- During trauma and replantation, treatment of the
- Socket, type of splinting, mastication, endodontic
- Treatment, antibiotics, time of replantation, extra
- Oral dry time, macroscopic contamination,
- Storage media and storage period

Properties of an ideal storage media:

- Be capable of preserving the feasibility of cellular periodontal ligament
- Should be mitogenic
- Should be clonogenic
- Should preserve the functional capacity of cells of periodontal ligament
- Thus should be capable of forming clones of the damaged fibroblasts of periodontal cells and its generating cells

Following trauma, the periodontal ligament cells shed off the root surface, an ideal storage media should be capable enough to repopulate the root surface with fibroblasts. This also avoids the adherence of osteoclasts on the root surface.[6]

The mode of transport of the avulsed tooth is also important for the prognosis of the treatment. If the dental root is repeatedly touched, periodontal ligament cells get damaged and may become non-vital. Thus, the container should have some desirable properties:[6]

- Unbreakable
- Non-toxic
- Leak proof
- Ease of handling
- Internal walls made of soft material
- Sterile
- Protect the tooth during transport
- Makes the removal ofthe tooth easy without any traumas.

Through this review we want to bring forward various storage media used.

TAP WATER

It is not a widely recommended storage media. It is known that if an avulsed tooth is stored in tap water the death of PDL starts in 1 hour. So it can be said that it is the most harmful storage media when compared to any other physiological or non-physiological media.[7]The reason suggested is low osmolality of tap water which causes cell lysis. The only advantage of using tap water as a storage media is that it keeps the tooth hydrated but according to Gopikrishan et al 2008 it causes cellular death rapidly similar to dry storage.[8]

NORMAL SALINE

It has osmolality 280mOsm/kg which shows its compatibility with living cells but it lacks the essential nutrients such as magnesium, calcium and glucose. Thus, the metabolic needs of the periodontal ligament cells are not met. Cvek et al 1974 compared dry storage for 15 to 40 minutes to storage in normal saline 30 minutes before replantation and it was concluded that dry storage showed more resorption. Hence it was said that if a tooth has been in dry storage, it should be kept in normal saline for 30 minutes before replantation, this will facilitate reconstitution and reconditioning of PDL cells.[4]On the other hand according to Andreason et al 1986 storage in saline after prolonged dry storage has no effect on prevention of root resorption and pulpal repair as the maximum damage happens within 30 minutes.[9] Similarly, according to other authors storage in normal saline for more than 2 hours can be more harmful than useful to the cells. 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.[10]

SALIVA

For storage of avulsed tooth for a time period of 1 hour saliva is an advised storage media as if used for more than 1 hour it can cause damage to periodontal ligament cells.[12]As it is hypotonic storage of any viable cells for more than 2-3 hours can cause swelling of cells and damage of cell membrane. Another disadvantage of using saliva as a storage media is the presence of micro-organisms. On comparing saliva with milk in an animal study it was concluded that milk was a more reliable storage media. It has also been shown that dry storage produces three times more damage when compared to saliva.[13] For evaluating clonogenic potential of saliva a study was conducted by Lekik et al 1998 PDL cells were kept in autologous saliva at room temperature followed by storage in saliva, milk andHBSS (at 4°C) for an additional 15 and 45 min. For uneventful wound healing clonogenic capacity of 3% is deemed necessary. In this experiment, it was proven that saliva at room temperature has clonogenic capacity of 7.6% for first 30 minutes, this capacity comes down to 1.5% in next 30 minutes.[14] In a case report by Sonada et al 2007, avulsed teeth was kept in oral environment for 90 minutes and at 3 years follow up, no root resorption, mobility or ankylosis was seen.[15] Thus, it was concluded that saliva is a potential storage media and has a positive effect on prevention of PDL dessication.

MILK

Milk has several advantages over above mentioned storage medias:

- Phsiological compatibility with the cells
- Easy availability
- Absence of bacteria as it is pasteurized
- Presence of suitable nutrients

The only problem is that it can be used only for 20 minutes. Also the milk that is available is pasteurized which although kills bacterias but also activates enzymes responsible for damaging the fibroblasts of periodontal ligament cells. Various authors have also suggested that although for 6 hours milk can be used as a storage media for avulsed teeth but beyond that as damage to PDL cells start, milk lacks the potential of regeneration of damaged cells.[9]According to Lekik et al 1998, milk when used for 1 hour had the potential equivalent to HBSS but was better than saliva, saline or tap water. Another point to be considered is that different types of milk have different potential as a storage media. Lekik et al 1998 compared chilled milk to warm milk and concluded that chilled milk for 1 hour had a good potential to maintain the viability of cells.[14] Also milk with a lower fat content was proven better than the normal milk.[12]

GATORADE

Composition of Gatorade is:

- Water
- Sodium
- Sugar
- Potassium
- Phosphate
- Lemon juice

It is a sports drink manufactured by PepsiCo. The major disadvantage of using it as a storage media is its physiological incompatibility with the cells. The cell membrane can get damaged due to low pH of the medium. Also it is hypertonic which can cause swelling of the cell and hence cause loss of water.[12] According to Sigalas et al 2004 Gatorade is better than tap water at all the temperature.[16]

COCONUT WATER

Since ages coconut water is being used for replacing fluids and electrolytes in case of dehydration. The advantages are that it is:[17]

- Biologically pure
- Sterile
- Rich in amino acids, proteins, vitamins andminerals
- Isotonic
- Physiologically acceptable equilibrium
- Long shelf life
- Easily available world wide

Gopikrishan et al 2008 conducted a study where the potential of coconut water to maintain the vitality of periodontal ligament cells was compared to HBSS, propolis and milk. Coconut water was found to be superior to all other storage media.[18]

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; SoftWear®, Ciba Vision Opti Care, 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.[19]

ASCORBIC ACID

Ascorbic acid stimulates production of collagen type I which stimulates osteocalcin and alkaline phosphatase. Thus, when used as a storage media it increases the activity of ALP which facilitates binding of PDL cells to type I collagen. Collagen is deemed essential for differentiation of PDL cells.[20]

HANK'S BALANCED SALT SOLUTION (HBSS)

It is the most widely and successfully used medium not only as a storage medium but also used in biomedical research. It has various advantages:

- Non toxic
- Biocompatible
- pH 7.2

- 320mOsm/kg osmolality

Its composition is 8 g/L sodium chloride; 0.4 g/L ofD-glucose; 0.4 g/L potassium chloride; 0.35 g/L sodium bicarbonate; 0.09 g/L sodium phosphate; 0,14 g/L potassium phosphate; 0.14 g/L calcium chloride; 0.1 g/L magnesium chloride and 0.1 g/L magnesium sulphate.

All the components are found to be important in maintaining and replenishing periodontal ligament cells.

Ashkenazi et al 2001 compared HBSS to Eagle's media, milk, ViaSpan and conditioned media for a period of 8hours and 24 hours, HBSS was found to be most effective in terms of its clonogenecity, mitogenecity and ability to maintain the viability.[21]

Save-A-Tooth an avulsed tooth storage system was developed by Krasner et al 1992. This system contains HBSS, a net for holding the tooth atraumatically and a container for taking the submerged tooth to a clinician.[22] According to Hiltz1991, it is the most recommended storage media even if the extra-alveolar storage time is 72-96 hours.[23]

PROPOLIS

Propolis is a bee hive product which is both antibacterial and anti-inflammatory in nature. Its composition is:

- Vegetable balsams (50%)
- Waxes (30%)
- Essentialoils (10%)
- Pollen (5%)
- Other constituents: amino acids, minerals, vitamins a, bcomplex, e and the highly active bio-chemicalsubstance known as bioflavenoid (vitamin p),phenols and aromatic compounds.

Martin and Pileggi et al 2004 conducted a study wherein comparison of Propolis was done with HBSS, milk and saline in their potential to maintain the viability of PDL cells. This was a quantitative analysis. Following the study it was concluded that Propolis was better than the other tested media. Also in this study the various concentrations of Propolis were compared to evaluate the toxicity of these to the PDL cells. The result for this comparison was that Propolis 100% was not significantly different from Propolis 50%, thus concluding that no difference in toxicity was seen with the concentrations.[24]

Ozan et al 2007 compared Propolis with, HBSS and low fat milk for their potential as a storage media and 10% Propolis was found to be the best amongst the three.[25]

Hence, propolis is suggested as a suitable storage medium.

ViaSpan

Originally it is a medium used for organ transport for transplantation. So it was suggested as a very effective storage media. It has good physiological properties required for cellular growth; osmolality 320mOm/kg, pH 7.4. Hiltz 1991 et al compared the vitality of lip fibroblasts stored in milk and ViaSpan at room temperature. ViaSpan was found to be an effective storage medium.[23]

Ashkenazi et al 2000 compared 6 different storage media Eagle's medium, milk, Hank's balanced salt solution, ViaSpan and conditioned medium. Clonogenic capacity was evaluated for 24 hours and it was concluded that at 8 hours ViaSpan had the capacity equivalent to HBSS and the potential reduced down to 65% which was less than that of milk and HBSS. The vitality and the mitogenic capacity of the cells stored in ViaSpan was lower than that of the other two tested media.[21]

Although a very effective storage media, it has various disadvantages:

- Short shelf life
- High Cost
- Difficult availibilty

EAGLE's MEDIUM

Up to at 8 hours at 4ºC it serves as an ideal storage media, but at 24 hours it is less reliable than vitality, mitogenecity and clonogenecity. Its composition is:[26]

- 4 ml of L-Glutamine
- 105 IU/L of Penicillin
- 100µg/mL of Streptomycin
- 10µg/mL of Nystatin
- Calf serum (10% v/v)

According to Hiltz 1991 at 37^0 C it was found to be beneficial to be used as storage media for extended period of time before replantation. It can be used as a storage media for as long as 1 year before replantation where the cells were trypsinised and cultured before preserving them in culture media.[27]

Andreason 2004 used MEM containingheat-inactivated calf serum further supplemented with L-glutamine, penicillin, streptomycin and mycostatin as storage medium, after 5-7 days minimal inflammatory root resorption was seen.[28]Sigalas et al 2004 had a high clonogenicity, mitogenecity and viability of the PDL cells at 0^0C and at 37^0C.[16]

Thus culture media possess a superior capacity to maintain the health of the PDL cells.

Dubelco's modified Eagle's medium(DMEM) is its variation which contains vitamins and amino acids four times of regular EMEM and glucose 2-4 times of the normal. Also it contains iron and phenol red. Chandha MH reported that it is equivalent to HBSS but is not easily accessible and hence is not a popular storage medium.[29]

GROWTH FACTORS

It is effective in regulating wound healing and has a potential for periodontal ligament cell regeneration. Lynch et al 1991 used periodontal growth factors and insulin like growth factors for early phase of wound healing to increase the formation of periodontal attachment apparatus.[30] Matsuda et al 1992 evaluated the effects of individual growth factors and the effect of various combinations of growth factors on the proliferation of PDL cells. Evaluation was done for a combination of Epidermal Growth Factors, Platelet Derived Growth Factors, Transforming Growth Factors, human—Insulin like Growth Factors. On using these combinations, an increase in the mitogenic capacity was seen as the cells maintained their spindle shape with a high degree of polarisation and well developed golgi bodies.[31]

Ashkenazi et al 2000 evaluated the effect of various storage media supplemented with growth factors on the clonogenic and mitogenic capacity of PDL cells and it was found that at 24 hours 20-37% increase in both clonogenic and mitogenic capacity was seen but no difference was seen at 2-8 hours. Thus, it was suggested that if storage is required for less time then supplementation with growth factors is not recommended while it is considered better if storage for 24 hours or more is required.[26]

EMDOGAIN

According to Shaked et al 2006Emdogain was used as storage media based on the basis of its use in post periodontal surgeries but the mechanism of it being used as a storage media for replantation of teeth post traumatic is different. With the use of Emdogain the capacity of fibroblasts to repopulate the cells diminishes due to the lack of an adherent surface or the increase on the difference of fibroblasts, which grow

in its presence. Although the use of Emdogain as a storage media can delay replacement resorption but cannot prevent it. Thus, Emdogain is not considered an ideal storage media for avulsed teeth.[32]

EGG WHITE

On comparing egg white with milk to be used a storage media for 6 -10 hours Khademi et al 2008 found out that egg white was better than milk when avulsed teeth were stored. Also they showed that pH and osmolarity of egg white was equivalent to physiologic system.[33] Sousa et al 2008 compared the viability of human PDL cells of avulsed teeth with dry extra-alveolar period of 1 hour and those stored in milk, egg white and artificial saliva. Milk and egg white showed good results with artificial saliva showing poor results, hence it was proven that egg white could be considered as an ideal storage medium.[34]

L-DOPA

L-DOPA is a mitogenic drug. It promotes healing by stimulating secretion of growth hormone from anterior portion of pituitary gland. According to Mandana et al 2002 levodopa has a dual function, it promotes the local growth of cells and also promotes healing when used as a storage medium.[35]

CRYOPROTECTIVE AGENT

Replantation of mature teeth has been in question, use of cryoprotective agents can be considered as a blessing in this regard as it facilitates healing without any PDL damage. Andreason 1983 conducted an animal study and studied the effects of 5% and 10% dimethyl sulphoxide(DSMO) and 10% glycerol, on PDL. The study concluded that a combination of various cryopreservatives at a temperature of -196^{o}C helped in preservation of PDL cells.[36]

CATALASE SUPPLEMENTATION

Buttke et al 2003 said that catalase helps significantly in reduction of surface resorption as catalase is an antioxidant. Also it is useful in cases if the tooth is stored in storage media containinh hydrogen peroxide which is toxic to PDL cells. Catalase reduces the toxicity of PDL cells.[37]

MORUS RUBRA

Recent studies have recommended juice of red mulberry to be used as storage media.[37] It belongs to Moraceae family which are popular for their therapeutic effects as they are rich in flavonoids (rutin, quercetin, isoquercitrin,quercetin) , alkaloids and polysaccharides. Ozan et al 2008 compared 4% concentration M. rubra with HBSS for 12 hours wherein M rubra was found to be more effective than HBSS.[38]

GREEN TEA

Green tea is a very popular and healthy beverage. It consists of epicatechin,epigallatocatechin, epicatechingallate,epigallate and catechin which combine and contribute to its anti-inflammatory and anti-bacterial action. Another advantage is that it ia easily available at the site of accident. But the commercially available green teaand green tea extract have low osmolality which can cause death of PDL cells and hence are not considered ideal storage media. But on comparing green tea extract and commercially available green tea with HBSS, tap water and milk, Green tea extract was found to give best results. Hwang et al showed the method of preparation of Green Tea to be used as storage media: Boil 10gm of green tea leaves in 100ml of boiling distilled water for 5 minutes and then filter the sterilized extract.[39] Jung IH conducted a series of experiments on the constituents of Green Tea and their various concentrations and it was concluded that treatment with (-)-epigallocatechin-3-gallate or green tea can be a great therapeutic strategy for tooth transplantation , as storage for avulsed teeth in a medium containing EGCG will allow sufficient time for the desired dental treatment.[40]

POMEGRANATE JUICE

It is popularly known as "Pharmacy unto itself" as all the parts of the fruit its peel. juice and seeds are used for medicinal purpose because of its potential anti-oxidant, anti- carcinogenic and anti-inflammatory purpose. It is recommended to be used as storage media as it facilitates cell attachment and fibroblast proliferation but this proliferative effect is seen for 1 hour at the concentrations of 1% and 2.5%. As the concentration is increased to 5% and 7.5% the proliferative ability increases. Peak proliferative ability is seen at 6 hours.[41]According to Tavassoli et al 2014 its ability is equivalent to HBSS and it can preserve the morphology of PDL cells for 24 hours. But they have also suggested that there is sparse literature available and more research is needed.[42]

ALOE VERA

It is a medicinal plant which belongs to the ciliacea family. It is made of 98-99% water and remaining 1-2% contain about 75 active ingredients in gel form-aloesin, aloin, aloeemodin, aloemannan,acemannan, aloeride, naftoquinones, methylchromones,flavonoids,saponin, sterols, amino acids, vitamins. Fani M 2012 suggested that it is a natural remedy because of its properties like anti-inflammatory action, anti-bacterial, anti-oxidant, immune-boosting and hypoglycemic properties.[43]

Badakhsh et al 2014 compared 10%,30% and 50% of aloe vera to culture media for 9 hours, at these concentrations cell viability could be maintained upto 90% and these concentrations were considered superior to 100% A vera and egg white.[44]

Pattamapun et al 2014 conducted a study to compare aloe vera to milk and HBSS where the PDL cell fibres were evaluated using Scanning Electron Microscope and they concluded that periodontal fibres of the teeth stored in aloe vera were more intact when compared to milk and HBSS.[45]

SALVIA OFFICINALIS

It is a newer type of storage media which is increasingly gaining popularity these days. It is a medicinal plant with antimicrobial and antioxidant properties. It belongs to family Labiatea. Its major constituents are α and β thujone and camphor. Other minor components are manool, ledene, viridiflorol, 1-8 cineole, limonene and trans-carryophyllene. Ozan et al observed that PDL cells viability at 1-3 hours is similar for 2.5% S. officianalis and HBSS whereas at 24 hours, the efficacy of 2.5% S officianalis is significantly better than HBSS. Thus, S officianalis can be recommended as a suitable transport medium for avulsed teeth.[46]

SOY MILK

It is the watery extract of soya bean which has minimal saturated fatty acids and lacks cholesterol. It was used as a storage medium by Mozami et al 2012on the basis that it serves as an excellent culture medium and has good biochemical activities. When they compared it with HBSS and milk it showed comparable results.[47]

ORAL REHYDARTION SOLUTION

Commercially it is available by the name of Ricetral which is composed of glucose and vital salts which help maintain cell metabolism. Its advantage is that it is available OTC in sealed sterile pouches. Rajendra P et al compared it with HBSS and concluded that although it does not promote cell mitosis and regenerative capacity but it maintains the viability of PDL cells equivalent to HBSS.[48]

CONDITIONED MEDIUM'

Human gingival fibroblasts are used to derive this medium, these fibroblasts release stimulatory growth factors which facilitate proliferation of PDL cells. But it is not a popular agent as it is not easily available.[49]

CUSTODIAL

It is a registered medium by the trademark Dr Franz. Its chief ingredient is histidine-tryptophan ketoglutarate with low potassium content. Originally it is used as a organ transport medium which served as a basis for it being used as a storage media for avulsed teeth. On comparing it with HBSS Alacam et al 1996 concluded that it is equivalent to HBSS in its ability to maintain the cell viability. But similar to other organ transport media, this also has the disadvantage of difficult availability.[50]

CONCLUSION

An ideal storage media should immediately prevent dessication of PDL cells stored in it. Various storage media have been suggested and many have been considered as more acceptable like culture media, HBSS and milk. Some other storage media like tap water, saline, saliva have been studied but not recommended widely because of their non-physiologic conditions. With time all storage media loose their effectiveness but a new technique of cryopreservation helps prolong the effectiveness. ViaSpan, Growth Factors, coconut water and propolis are other newer agents which have shown good results under in-vitro conditions but their clinical effectiveness has to be proven still. In conclusion, HBSS and chilled milk are the most appropriate, clinically recommended storage media for avulsed teeth.

REFERENCES

[1] Andreasen JO. Etiology and pathogenesis of traumaticdental injuries. A clinical study of 1298 cases. Scand JDent Res 1970; 78:329–42.

[2] Andreasen J. A time-related study of periodontalhealing and root resorption activity after replantationof mature permanent incisors in monkey. Swed Dent J1980;4: 101-110.

[3] Hamilton FA, Hill FJ, Mackie IC. Investigation oflay knowledge of management of avulsed permanentincisors. Endod Dent Traumatol 1997;13:19-23.

[4] Cvek M, Granath L, Holender L. Treatment of nonvitalpermanent incisors with calcium hydroxide. III.Variation of occurrence of ankylosis of reimplantedteeth with duration of extra-alveolar period and storageenvironment. Odontol Revy 1974;25:43-56.

[5] Andreasen JO, Borum MK, Jacobsen HL, AndreasenFM. Replantation of 400 avulsed permanent incisors.2. Factors related to pulpal healing. Endod DentTraumatol 1995;11:59-68.

[6] Hammarstrom L, Pierce A, Blomlof L, Feiglin B,Lindskog S. Tooth avulsion and replantation: a review.Endod Dent Traumatol 1986;2:1-6.

[7] Mackie IC, Worthington HV. An investigation ofreplantation of traumatically avulsed permanentincisor teeth. Br Dent J 1992;172:17-20.

[8] Gopikrishna V, Baweja PS, Venkateshbabu N, ThomasT, Kandaswamy D. Comparison of coconut water,Propolis, HBSS, and milk on PDL cell survival. JEndod 2008;34:587-589

[9] Blomlof L. 1981. Milk and saliva as possible storagemedia for traumatically exarticuled teeth prior toreplantation. Swed Dent J, 8: 1–26

[10] Courts FJ, Mueller WA &Tabeling HJ. 1983. Milk asan interim storage media for avulsed teeth. PediatricDentistry, 5: 183–6.

[11] Krasner P. 1992. Tooth avulsion in the school setting.J SchNurs, 8: 20–6.

[12] Harkacz O, Carnes D &WalkerW. 1997. Determination of periodontal ligament cell viability in the oral rehydration fluid Gatorader and milk of varying fat content. J Endod, 23: 687–90.

[13] Lindskog S, Bloml¨of L &Hammarstr¨om L. 1983.Mitoses and microorganisms in the periodontalmembrane after storage in milk or saliva. Scand J DentRes, 91: 465–472.

[14] Lekic PC, Kenny DJ & Barrett EJ. 1998. The influenceof storage conditions on the clonogenic capacity ofperiodontal ligament cells: implications for toothreplantation. IntEndod J, 31: 137–40.

[15] Sonoda CK, Poi WR, Panzarini SR, Sottovia AD &Okamoto T. 2008. Tooth replantation after keepingthe avulsed tooth in oral environment: case report of a3-year follow-up. Dent Traumatol, 24: 373–6.

[16] Sigalas E, Regan JD, Kramer PR, Witherspoon DE,Opperman LA. Survival of human periodontal ligamentcells in media proposed for transport of avulsed teeth.Dent Traumatol 2004;20:21-28

[17] Gopikrishna V, Thomas T, Kandaswamy D. Aquantitative analysis of coconut water: a new storagemedia for avulsed teeth. Oral Surg Oral Med OralPathol Oral RadiolEndod 2008;105:61-65.

[18] Gopikrishna V, Baweja PS, Venkateshbabu N, ThomasT, Kandaswamy D. Comparison of coconut water,Propolis, HBSS, and milk on PDL cell survival. JEndod 2008;34:587-589.

[19] Pettiette M, Hupp J, Mesarso S, Trope M. Periodontalhealing of extracted dog's teeth air-dried for extendedperiods and soaked in various media. Endod DentTraumatol 1997;13:113-118.
[20] Ishikawa S, Iwasaki K, Komaki M, Ishikawa I.Role of ascorbic acid in periodontal ligament celldifferentiation. J Periodontol 2004;75:709-716.
[21] Ashkenazi M, Marouni M, Sarnat H. In vitro viability,mitogenic and clonogenic capacities of periodontalligament fibroblasts after storage in four mediasupplemented with growth factors. Dent Traumatol2001;17:27-35.
[22] Krasner P, Person P. Preserving avulsed teeth forreplantation. J Am Dent Assoc 1992;123:80-88.
[23] Hiltz J, Trope M. Vitality of human lip fibroblast inmilk, Hank's balanced Salt Solution and ViaSpanstorage media. Endod Dent Traumatol 1991;7:69-72.
[24] Margaret PM, Pileggi R. A quantitative analysis ofPropolis: a promising new storage media followingavulsion. Dent Traumatol 2004;20:85-89.
[25] Ozan F, Polat ZA, Er K, ¨ Ozan ¨U &Deger O. 2007. Effectof propolis on survival of periodontal ligament cells: newstorage media for avulsed teeth. J Endod, 33: 570–3.
[26] Ashkenazi M, Marouni M, Sarnat H. In vitro viability,mitogenic and clonogenic capacities of periodontalligament cells after storage in four media at roomtemperature. Endod Dent Traumatol 2000;16:63-70
[27] Hiltz J, Trope M. Vitality of human lip fibroblast inmilk, Hank's balanced Salt Solution and ViaSpanstorage media. Endod Dent Traumatol 1991;7:69-72.
[28] Andreasen JO, Reinholdt J, Dybdahl R, Soder PO,Otteskog P. Periodontal and pulpal healing of monkeyincisors preserved in tissue culture before replantation.Int J Oral Surg 1978;7:104-112.ligament. J Endod2004;30:359-361.
[29] Chandha MH. Extraalveolar storage media of tooth. Autotransplants and replants. J Med Nurs 2006;27:64-7
[30] Lynch SE, de Castilla GR, Williams RC, Kiristy CP,Howell TH, Reddy MS. The effect of short termapplication of a combination of platelet-derived andinsulin-like growth factors on periodontal woundhealing. J Periodontol 1991;62:458-467.
[31] Matsuda N, Lin WL, Kumar NM, Cho MI, Genco RJ.Mitogenic, chemotactic and synthetic responses of ratperiodontal ligament fibroblastic cells to polypeptidegrowth factors in vitro. J Periodontol 1992;63:515-525.
[32] Ashkenazi M &Shaked I. 2006. In vitro clonogeniccapacity of periodontal ligament fibroblasts culturedwith Emdogainr. Dent Traumatol, 22: 25–9.
[33] Khademi AA, Atbaee A, Razavi S-M &ShabanianM. 2008. Periodontal healing of replanted dog teethstored in milk and egg albumen. Dent Traumatol, 24:510–4
[34] Sousa HA, Alencar AHG, Bruno KF, Batista AC &Carvalho ACP. 2008. Microscopic evaluations of theeffect of different storage media on the periodontalligament of surgically extracted human teeth. DentTraumatol, 24: 628–32.
[35] Partovi M, Sadeghein A, Azizi E, Ostad SN. Mitogeniceffect of L-dopa on human periodontal ligamentfibroblast cells. J Endod 2002;28:193-196.
[36] Schwartz O, Andreasen JO. Cryopreservation ofmature teeth before replantation in monkeys. Int J OralSurg 1983;12:425-436.
[37] Buttke TM, Trope M. Effect of catalase supplementationin storage media for avulsed teeth. Dent Traumatol2003;19:103-108
[38] Ozan F, Tepe B, Polat Z A, Er K. Evaluation of in-vitro effect of Morusrubra(red mulberry) on survival of periodontal ligament cells. Oral Surg Oral Med Oral Pathol P Oral RadiolEndod 2008;105:e66-69
[39] Hwang J, Choi SC, Park JH, Kang SW. The use of green tea extract as a storage media for avulsed teeth. J Endod 2011;37:962-7
[40] Jung IH, Yun JH, Cho AR, Kim CS, Chung WG, Choi SH. Effect of (-)- epigallocatechin-3-gallate on maintaining the periodontal ligament cell viability of avulsed teeth. preliminary study. J Periodontal Implant Sci 2011;41:10-6
[41] Subramaniam P, Dwivedi S, Uma E, Girish KL. Effect of pomegranate and aloe vera extract on Streptococcus mutans: an in-vitro study. Dent Hypotheses 2012;3:99-105
[42] Tavassoli- Hojjati S, Aliasghar E, Babaki FA, Emadi F, Parsa M, Tavajohi S et al. Pomegranate (PunicaGranatum): A new storage medium for avulsed tooth. J Dent (Tehran)2014;11:225-32
[43] Fani M, Kohanteb J. Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. J Oral Sci 2012;54:15-21

[44] Badakhsh S, Eksandarian T, Esmaeilpour T. The use of aloe vera extract as a novel storage media for avulsed teeth. Iran J Med Sci 2014;39:327-32
[45] Pattamapun K, Ruchikeeratikul P, Handagoon S, Chaiyasuk K. Effect of aloe vera gel on periodontal tissue after avulsion. IADR 2014
[46] Ozan F, Polat Z A, Er K. Influence of storage media containing Salvia officianalis on survival of periodontal ligament cells. J Contemp Dent Prac 2008;9:17-24
[47] Mozami F, Mirhadi H, Geramizadeh B, Sahebi S. Comparison of soymilk, powdered milk, HBSS and tap water on periodontal ligament cell survival. Dent Traumatol 2012;28;132-135
[48] Rajendran P, Varghese NO, Varughese JM, Murugaian E. Evaluation using extracted human teeth, of Ricetral as a storage medium for avulsion: an in-vitro study. Dent Trauamatol 2012;27:212-230
[49] UdoyeCl, Jafarzadeh H, Abott PV. Transport media for avulsed teeth: A Review. AustEndod J 2012;38:129-136
[50] Alacam T, Gorgul G, Omurulu H, Can M. Lactate dehydrogenase activity in periodontal ligament cells stored in different transport media. Oral Surg Oral Med Oral Pathol Oral Radiol Oral Endod 1996;82:321-323