Published online: 2019-09-25

Original Article

In vitro comparative evaluation of different storage media (hank's balanced salt solution, propolis, *Aloe vera*, and pomegranate juice) for preservation of avulsed tooth

Prashant Babaji[1], Mahesh Melkundi[2], Raghu Devanna[3], Suresh B.S.[4], Vishwajit Rampratap Chaurasia[5], Gopinath P.V.[6]

[1]Department of Pedodontics and Preventive Dentistry, Sharavthi Dental College & Hospital, Shimoga, Karnataka, India, [2]Department of Oral and Maxillofacial Pathology, College of Dental Sciences, Rau, Indore, Madhya Pradesh, India, [3]Department of Orthodontics, Faculty of Dentistry, Taif University, Kingdom of Saudi Arabiya, [4]Department of Pedodontics, Subbaiah Institute of Dental Sciences and Hospital, Shimoga, Karnataka, India, [5]Department of Conservative Dentistry and Endodontics, Daswani Dental College, Kota, Rajasthan, India, [6]Department of Periodontics, Malbar DentalCollege and Research Centre, Edappal, Kerala, India

Correspondence: Dr. Prashant Babaji
Email: babajipedo@gmail.com

ABSTRACT

Objectives: Prognosis of the avulsed teeth is mostly affected by extraoral dry period and storage medium used to store teeth before reimplantation. However, ability of storage media can affect cell viability and success of treatment. Various storage media were tried with some success. The present study was undertaken to comparatively evaluate the efficacy of hank's balanced salt solution (HBSS), propolis, *Aloe vera*, and pomegranate juice (PJ) in preserving the vitality of periodontal ligament (PDL) cells of avulsed teeth. **Materials and Methods:** Fifty orthodontically extracted sound teeth with healthy PDL were selected for the present study. Selected teeth were randomly divided into study groups (10 in each) and 5 each as positive and negative control groups. All the teeth were immersed immediately after extraction into respective storage media. Data were statistically analyzed using IBM SPSS software for Windows, Version 19.0., IBM Corp., Armonk, NY, USA. Analysis of variance and multiple range were done using Tukey's honestly significant difference with level of significance at 5% ($P > 0.05$). **Results:** Propolis (285,000 viable cells with standard deviation 4.11028 and standard error of 1.38097) showed more viable PDL cells followed by HBSS, *A. vera*, and PJ. **Conclusion:** Propolis, *A. vera*, and PJ can be used as an alternative tooth storage media.

Key words: *Aloe vera*, avulsion, periodontal ligament, pomegranate, propolis, storage media, tooth

INTRODUCTION

Tooth avulsion is more common in young age group where root formation is incomplete.[1] The incidence

Access this article online

Quick Response Code:

Website: www.eurjdent.com

How to cite this article: Babaji P, Melkundi M, Devanna R, Suresh BS, Chaurasia VR, Gopinath PV. *In vitro* comparative evaluation of different storage media (hank's balanced salt solution, propolis, *Aloe vera*, and pomegranate juice) for preservation of avulsed tooth. Eur J Dent 2017;11:71-5.

DOI: 10.4103/ejd.ejd_101_16

of tooth avulsions ranges from 1% to 16% of traumatic injuries in permanent dentition.[1-4] Tooth avulsion is defined as the complete displacement of the tooth from its alveolar socket which causes damage to the periodontal ligament (PDL) structure, cementum, and alveolar bone, gingival tissue, and dental pulp.[4] Immediate replantation is an appropriate treatment for avulsed tooth. After avulsion, PDL tissues begin to dehydrate.[1,3] A vital periodontal membrane (PDL) is important for the successful healing of replanted teeth.[1,5] During avulsion, complete separation of tooth from the socket results in tearing of the PDL, leaving viable PDL cells on the root surface. A quarter of PDL cells comprise PDL fibroblasts, which are responsible for the reattachment of the avulsed tooth after its reimplantation into its socket.[6] However, immediate replantation cannot be possible always. In cases of delayed replantation of avulsed tooth, it can be stored in appropriate storage media to avoid dehydration and to preserve the tooth vitality. External root resorption and pulp necrosis can result commonly as failure of replanted tooth.[1] The most critical factor in success of replanted avulsed teeth is extraoral dry time and tooth storage media. Use of tooth storage media can prevent external root resorption and retain viability of PDL cells.[1,6]

Ideal requirements of storage media should have low bacterial content, physiological osmomolarity, neutral pH, and essential nutrients; should maintain viability of periodontal fibers, no antigen-antibody reaction, antimicrobial, sterile; and should be inexpensive.[1,2,6] Various storage media suggested are hanks balanced salt solution (HBSS), milk, soya milk, honey milk, pomegranate juice (PJ), oral rehydration solution (ORS), normal saline, propolis, viaspan, Gatorade, saliva of patient, coconut water, etc.[2,4,6-9] The HBSS is suggested as storage media by the International association of dental traumatology.[1] HBSS has been found in many studies as best storage media, but it is expensive and not easily available. There is not yet a single solution that fulfills all requirements to be considered as the ideal medium for temporary storage of avulsed teeth. Hence, there is search for accessible and economical storage media.[1] *Aloe vera* and PJ are easily available and economical and hence can be considered as tooth storage media. Propolis is naturally occurring substance. Several studies have shown the efficacy of *A. vera* and propolis in retaining cell viability.[10,11]

The present study was undertaken to comparatively evaluate efficacy of different storage media (HBSS, propolis, *A. vera*, and pomegranate).

MATERIALS AND METHODS

Fifty orthodontically extracted premolar teeth with intact crown, closed apex, and with healthy PDL were selected for the present study. The teeth were randomly divided into six groups – test (10 samples in each group) and control (positive and negative control with five samples in each) groups. Group-1: positive control, collagenase dispase Grade II, Group-2: HBSS, Group-3: porpolis, Group-4: *A. vera*, Group-5: pomegranate, and Group-6: bench drying (negative control). Immediately after the extraction, all the teeth were immersed in respective storage media for the study purpose to maintain equal baseline standardity for viable cells. Teeth were transferred to storage media by holding only crown portion with extraction forceps and without disturbing the viable cells on root surface.

HBSS is commercially available. *A. vera* gel was obtained from fresh leaves. In fresh *A. vera* leaves (*Barbadensis*), lower one inch of the leaf base and sharp spines along leaf margins were removed with bard parker blade no. 15 with same blade inner gelatinous part between the top and bottom rind was removed and the inner gel was collected into test tube.[10]

Solid propolis was grounded into fine particles with help of mortar and pestle. Propolis (50%) was prepared by adding 50 mg ground propolis/250 ml to 0.4% ethanol solution. This solution was shaked for 15 min before transforming the study teeth.[2,11]

The grains of fresh fruits of pomegranate (*Punica granatum*) were separated carefully to extract the juice. PJ was prepared by squeezing the grains and filtering the juice. Pure juice was placed in Rotary Flash evaporator until the optimal concentration was obtained. It was then filtered using filter paper to be suitable for passing through the 0.2 μm filter. The prepared PJ was kept in dark at +4°C until tested.

Teeth in positive control group were treated immediately with collagenase dispase Type-II. Moreover, those for negative control group were bench-dried for 8 h without use of any storage media and then placed in dispase and collagenase and incubated at 37°C for 30 min. After incubation, fetal bovine serum (50 ml) was added to each tube. Other teeth were kept for 45 min in respective test group media.

All tubes were subjected to centrifugation at 1000 rpm for 5 min and supernatant was removed with sterile micropipettes. 0.4% trypan blue was used

to label to determine viability of these PDL cells at 40X magnification under light microscope with hemocytometer.[2]

Data were statistically analyzed using IBM SPSS software for Windows, Version 19.0., IBM Corp., Armonk, NY, USA. Analysis of variance and multiple range were done using Tukey's honestly significant difference with level of significance at 5% ($P > 0.05$).

RESULTS

Positive control media, collagenase dispase Type-II showed 360,000 viable PDL cells whereas negative control with bench drying showed only 2000 viable cells. Results indicated that propolis (285,000 viable cells with standard deviation (SD) 4.11028 and standard error (SE) of 1.38097) showed more viable PDL cells followed by HBSS, *A. vera*, and pomegranate [Table 1]. Viability of cells in decreasing order is positive control > propolis > HBSS > *A. vera* > PJ > negative control. Least was seen in negative control. Table 2 shows pH and osmolality of various storage media.

DISCUSSION

The prognosis of replanted tooth depends on minimal damage to PDL cells since it helps in regeneration, attachment, and to prevent resorption. Several studies have shown that an avulsed tooth can be replanted after 1–3 h without complications if stored in suitable storage media.[2] Blomlöf *et al*. suggested ideal requirement for storage media as 290–330 μ OSM/L and 6.6–7.8 pH.[12] We tested storage media such as *A. vera* and PJ since they are easily available and less expensive. *A. vera* is widely cultivated thorough out the world. Propolis showed more viable PDL cells followed by HBSS, *A. vera*, and pomegranate [Table 1]. Table 2 shows osmolality and pH of various storage media. All the tested media had pH and osmolality in expectable range suggested by Blomlöf. Here, propolis had suitable pH (7.4) and osmolality (350 osml/L) and hence can maintain more viable cells compared to other test groups.

HBSS is considered as gold slandered as storage media. It has balanced pH (7.2) and contains essential metabolites necessary for PDL cells viability.[6,10] It has been found that it can maintain viability of PDL cells up to 48 h.[13] However, storage of avulsed tooth maximum for 30 min in HBSS has been recommended. It is nontoxic and commercially available as Save-A-Tooth, PA, USA. It is expensive and not easily available.[6] Nozari *et al*. observed HBSS was better than honey milk and milk to maintain PDL cell viability.[4] Gopikrishna *et al*. found coconut as superior to HBSS, propolis, and milk.[11] Sunil *et al*. and Thomas *et al*. found coconut water as better storage media than HBSS and milk.[14,15]

Propolis is a natural wax-like substance used by bees in construction of their hives. It is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various other organic substances. It has antibacterial, antifungal, anti-inflammatory, immune-modulatory, anti-ulcer, and antitumor properties.[1,2,6,7,11] It is nontoxic and inhibits osteoclastic resorption of teeth.[1] It is not easily available. Ozan *et al*. from their study found that propolis was superior to HBSS or milk in maintaining PDL cell viability after storage of avulsed tooth.[16] Similarly, efficacy of propolis in maintaining PDL cell was observed in many studies.[17,18] We found propolis (28,5000 viable cells with SD 4.11028 and SE of 1.38097) as superior to HBSS, *A. vera*, and PJ in maintaining cell viability [Table 1]. It has been found that 10% propolis can be used to store avulsed tooth for as long as 24 h.[1] Sanghavi *et al*. found viable PDL cells with coconut, 50% propolis and ORS solution.[2]

A. vera (*Barbadensis*) gel contains more than 75 ingredients. It is commonly available plant. This gel is made up of water (98%), active compounds

Table 1: Mean and standard deviation of groups

Groups	Number of samples	Mean number of viable cells	SD	SE
Positive control - collagenase dispase	5	360,000	6.45726	1.76899
HBSS	10	262,000	3.12743	1.28678
Propolis	10	285,000	4.11028	1.38097
A. vera	10	226,000	3.42785	0.98565
Pomegranate juice	10	214,000	3.41709	1.08654
Negative control - bench drying	5	2000	0.35578	0.01568

Analysis of variance by Tukey test ($P<0.05$). Viability of cells in decreasing order is - positive control > propolis > HBSS > *A. vera* > pomegranate juice > negative control. Least was seen in negative control. HBSS: Hank's balanced salt solution, SD: Standard deviation, SE: Standard error, *A. vera*: *Aloe vera*

Table 2: Osmolality and pH of transporting media

Medium	Osmolality (mosm/L)	pH
HBSS	320	7.2
Propolis	350	7.4
A. vera	280-300	6.8
Pomegranate juice	280	6.7
Ideal	230-400	6.6-7.8

HBSS: Hank's balanced salt solution, *A. vera*: *Aloe vera*

such as amino acids, vitamins, aloin, aloesin, aloe emodin, aloemannan, aloeride, naphthoquinones, methylchromones, flavonoids, saponin, and sterols. It has antibacterial, anti-inflammatory, antioxidant, immune boosting, and hypoglycemic properties.[1,10] Badakhsh *et al*. found that *A. vera* at concentration of 10%, 30%, 50% maintained cell viability and recommended it as suitable storage media for avulsed tooth.[19] Fulzele *et al*. from their study concluded that highest viable cells found in HBSS followed by *A. vera* over a period of 120 min.[10] Similar results were found in our study relevant to *A. vera* gel as storage media. Sharma *et al*. found highest viable cells with *A. vera* when compared to egg white and milk.[20]

Pomegranate (*Punica Granatum*) juice is considered in ayurvedic medicine as "pharmacy unto itself" since it has complete medicinal power in its seeds and juice. It has antioxidant, anti-inflammatory, anti-carcinogenic properties, and helps in fibroblast cell proliferation and cell attachment.[1] We found viable PDL cells in PJ. It can be used as a storage media for avulsed tooth because of its physical properties and maintains cell viability. Tavassoli-Hojjati *et al*. from their study found that PJ is equally effective as HBSS. They further suggested that 7.5% concentration of PJ is more effective and cell viability increases with increased concentration.[21]

We used trypan blue staining technique since it is easy and quick to perform and differentiates viable cells from nonviable one. We checked whether cells take up stains or not. Viable cells will have a clear cytoplasm but a nonviable cell will have a blue cytoplasm. In our study, PDL cells were treated with collagenase and dispase Grade II to preserve maximum viability and to minimize exposure of cells to active trypan. It has been observed that collagenase and dispase help in maintaining maximum cellular integrity.[2] Aydin *et al*. form their study concluded that 12 months storage of the avulsed teeth resulted in a statistically significant decrease in microhardness when compared to microhardness of teeth stored for 2 months. Hence, teeth kept up to 2 months in deionized water, glutaraldehyde, HBSS, NaOCl, or in thymol solutions can be used for mechanical *in vitro* tests.[22]

Further studies are required to evaluate the efficacy of propolis, *A. vera*, and PJ as storage media at time interval from 30 min to 24 h.

CONCLUSION

A. vera gel and PJ are easily available and less expensive. Propolis, *A vera*, and PJ can be used as an alternative tooth storage media since we observed viable PDL cells in theses storage media.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Jain D, Dasar PL, Nagarajappa S. Natural products as storage media for avulsed tooth. Saudi Endod J 2015;5:107-13.
2. Sanghavi T, Shah N, Parekh V, Singbal K. Evaluation and comparison of efficacy of three different storage media, coconut water, propolis, and oral rehydration solution, in maintaining the viability of periodontal ligament cells. J Conserv Dent 2013;16:71-4.
3. Balto HA, Halawany HS, Jacob V, Abraham NB. The efficacy of *Salvadora persica* extracts in preserving the viability of human foreskin fibroblasts. Saudi Dent J 2015;27:137-40.
4. Nozari A, Esmaeilpour T, Fijan S, Salmannejad M. Evaluation of the long-shelf life honey milk as a storage media for preservation of avulsed teeth. Caspian J Dent Res 2013;2:42-7.
5. Poi WR, Sonoda CK, Martins CM, Melo ME, Pellizzer EP, de Mendonça MR, *et al*. Storage media for avulsed teeth: A literature review. Braz Dent J 2013;24:437-45.
6. Adnan S, Khan FR. Storage media for avulsed teeth: A review. J Pak Dent Assoc 2014;23:54-60.
7. Gomes MC, Westphalen VP, Westphalen FH, da Silva Neto UX, Fariniuk LF, Carneiro E. Study of storage media for avulsed teeth. Braz J Dent Traumatol 2009;1:69-76.
8. Manoj Kumar KR, Pradeep DR. Usage of different media for restoring tooth in tooth avulsion. Int Organ Sci Res J Dent Med Sci 2015;4:53-5.
9. Siddiqui F, Karkare S. Storage media for an avulsed tooth: Nature to the rescue. Br J Med Health Res 2014;1:1-10.
10. Fulzele P, Baliga S, Thosar N, Pradhan D. Evaluation of *Aloevera* gel as a storage medium in maintaining the viability of periodontal ligament cells – An *in vitro* study. J Clin Pediatr Dent 2016;40:49-52.
11. Gopikrishna V, Baweja PS, Venkateshbabu N, Thomas T, Kandaswamy D. Comparison of coconut water, propolis, HBSS, and milk on PDL cell survival. J Endod 2008;34:587-9.
12. 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.
13. Blomlöf L, Otteskog P, Hammarström L. Effect of storage in media with different ion strengths and osmolalities on human periodontal ligament cells. Scand J Dent Res 1981;89:180-7.
14. Sunil O, Devadathan A, Jacob J, Mathew J, Rex I. A comparative evaluation of post traumatic periodontal ligament cell viability using four different storage media – An *in vitro* study. Health Sci 2013;2:1-9.

15. Thomas T, Gopikrishna V, Kandaswamy D. Comparative evaluation of maintenance of cell viability of an experimental transport media "coconut water" with Hank's balanced salt solution and milk, for transportation of an avulsed tooth: An *in vitro* cell culture study. J Conserv Dent 2008;11:22-9.
16. Ozan F, Polat ZA, Er K, Ozan U, Değer O. Effect of propolis on survival of periodontal ligament cells: New storage media for avulsed tooth. J Endod 2007;33:570-3.
17. Ahangari Z, Alborzi S, Yadegari Z, Dehghani F, Ahangari L, Naseri M. The effect of propolis as a biological storage media on periodontal ligament cell survival in an avulsed tooth: An *in vitro* study. Cell J 2013;15:244-9.
18. Mahal NK, Singh N, Thomas AM, Kakkar N. Effect of three different storage media on survival of periodontal ligament cells using collagenase-dispase assay. Int Endod J 2013;46:365-70.
19. 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: 327-332
20. Sharma M, Sharma S, Reddy YG, Mittal R, Agarwal V, Singh C, *et al.* Evaluation of periodontal ligament cell viability in three different storage media: An *in vitro* study. J Dent (Tehran) 2015;12:524-31.
21. Tavassoli-Hojjati S, Aliasghar E, Babaki FA, Emadi F, Parsa M, Tavajohi S, *et al.* Pomegranate juice (punica granatum): A new storage medium for avulsed teeth. J Dent (Tehran) 2014;11:225-32.
22. Aydin B, Pamir T, Baltaci A, Orman MN, Turk T. Effect of storage solutions on microhardness of crown enamel and dentin. Eur J Dent 2015;9:262-6.