How to Cite:
Tank, U., Jawdekar, A., & Tiku, A. (2022). Comparative evaluation of the efficacy of aloe vera juice, amla juice, and milk as storage media in maintaining the periodontal ligament cell viability: An in-vitro study. *International Journal of Health Sciences*, *6*(S5), 4514–4528.
https://doi.org/10.53730/ijhs.v6nS5.10068

Comparative evaluation of the efficacy of aloe vera juice, amla juice, and milk as storage media in maintaining the periodontal ligament cell viability: An in-vitro study

Dr. Urvashi Tank
III-year PG Student, Department of Pediatric and Preventive Dentistry

Dr. Ashwin Jawdekar
Professor and Head, Department of Pediatric and Preventive Dentistry

Dr. Amita Tiku
Former Professor and Head, Department of Pediatric and Preventive Dentistry

***Abstract*---**Background: Tooth avulsion implies total displacement of the tooth out of its socket. Many storage media such as milk, saliva, Hank's Balanced Salt Solution (HBSS), propolis, Viaspan, have been studied as storage medias for preserving PDL cell viability. Amongst these, HBSS has been proposed as the storage medium of choice for treatment of avulsed teeth by the American Associations of Endodontists. However, the major disadvantage is that it is not easily available at places where these traumatic injuries occur. Aim: To evaluate and compare the efficacy of aloe vera juice, amla juice, milk and water in maintaining the periodontal ligament cell viability of avulsed teeth. Study Design: Forty premolars extracted for orthodontic therapeutic purposes were randomly and equally divided into four groups based on storage media used [Group I: aloe vera juice; Group II: amla juice; Group III: milk; Group IV: water. Following extractions, the teeth were dried for 30 mins and were placed in one of the four different storage media for 45 minutes, following which the scrapings of the PDL from these teeth were collected in Falcon tubes containing collagenase enzyme in 2.5 mL of phosphate buffered saline. The tubes were subsequently incubated for 30 minutes and centrifuged for five minutes at 800 rpm. The obtained PDL cells were stained with Trypan Blue and were observed under optical microscope. The percentage of viable cells was calculated. Statistical analysis: Normality of data was checked by Kolmogorov Smirnov test. Inferential statistics was performed using one way analysis of variance (ANOVA) for overall

Manuscript submitted: 27 Feb 2022, Manuscript revised: 9 April 2022, Accepted for publication: 18 June 2022

comparison within four groups. Post hoc pairwise comparison was done using Tukey test. Results: Aloe vera juice showed the highest percentage of viable cells (77.62 ± 3.59), followed by milk (70.01 ± 4.13), amla juice (55.85 ± 4.88) and water (46.20 ± 4.06). The differences across the groups and in the pairwise comparisons were statistically significant. Conclusion: The preferred order of storage medium for maintaining the PDL cell viability was aloe vera juice followed by milk. Amla juice and water are least preferred.

***Keywords*---**evaluation, HBSS, cell viability.

Introduction

Tooth avulsion implies total displacement of the tooth out of its socket and is seen commonly where root formation is incomplete in young age group. [1,2] In permanent dentition, the incidence of traumatic dental injuries seen is 1-16%.[2,3,4,5] Avulsion causes tearing of the periodontal ligament. Periodontal ligament cells comprise of fibroblasts, these fibroblasts are responsible for reattachment after reimplantation of the avulsed tooth. Immediate replantation is an appropriate treatment for avulsed teeth as viable periodontal ligament cells are seen on root surface. However, immediate replantation cannot be carried out always. After avulsion, periodontal ligament cells begin to dehydrate,[2,4] to avoid dehydration, appropriate storage media is required. External root resorption and viability of periodontal ligament cells can be retained with the use of tooth storage media.[2] Various studies have been conducted in maintaining the viability of periodontal ligament cells using storage media such as HBSS, propolis, milk, saliva, Viaspan, etc. International Association of Dental Traumatology and American Association of Endodontists (AAE) recommends Hank's balanced salt solution (HBSS) as the storage medium of choice for avulsed tooth.[2, 6] Hank's balanced salt solution (HBSS) has a major disadvantage that it is not readily available at the time of trauma.[7]

Practice of Herbal medicine continues to be used for various reasons.[2] Adverse effects of various available synthetic drugs and their increased cost has led to a need of studying plants as source for medicines. According to the information provided by National Health Portal, World Health Organization (WHO) estimated that 80 % of people worldwide for some aspect of their primary health care needs depend on herbal medicines. Herbal products such as aloe vera gel, neem, green tea, turmeric have been studied as storage media. Various studies have been done on aloe vera gel and aloe vera pure extract to test its efficacy as storage media for avulsed tooth.[2]

It has been reported that aloe vera not only has ability to show an increase in the production of fibroblast cells faster than normal cell production but also improves fibroblast cell structure and accelerates the collagen production process.[8] Aloe vera also contains allantoin, which helps to stimulate the fibroblast activity. Addition of ascorbic acid has been said to stimulate type 1 collagen production. Increased alkaline phosphatases activity on periodontal ligament cells were seen by the effect of ascorbic acid, which is essential for binding of periodontal

ligament cells to type 1 collagen, this binding is seen via beta 1 integrin, whose effect is again increased by ascorbic acid.[9] One such herbal product, amla is said to be the richest source of ascorbic acid. Veena (2015) suggested ascorbic acid could be a potential storage medium for maintaining the viability of PDL cells.[10]

According to literature no research is available on evaluating the efficacy of amla juice in maintaining periodontal ligament cell viability. Also, in our literature search we did not come across any study in which comparison of aloe vera juice and amla juice had been done. However, Navit et al11 (2017) studied the efficacy of pure aloe vera extract (100% concentration). As pure aloe vera extract is highly viscous and it entirely covers the surface of the experiment cell, it is said to prevent the accessibility of oxygen, thereby leading to cell death thereby its effectiveness in gel form is questionable. [11, 12] Therefore, there is a need for research on testing the efficacy of aloe vera juice as storage medium.

Aloe vera and amla juice are economical and readily available in India as herbal products. Their efficacy in maintaining the viability of periodontal ligament cells warrants research. Therefore, this study aims at evaluating the efficacy of these commonly available and cost-effective storage media, such as: aloe vera, amla juice, milk and water in maintaining viability of periodontal ligament cells.

Materials and Methods

Forty extracted intact premolars were collected from Dental College and Hospital, as well as from Private Dental Clinics in Mumbai, Navi Mumbai and Thane areas. The research protocol was initially submitted to the Institutional Ethical Committee and Review Board and ethical approval was taken prior to commencement of the study. Forty freshly extracted intact premolars, extracted for orthodontic reason were included; carious teeth, cracked teeth, teeth having any other defects such as fractures, hypoplastic enamel, were excluded.

a) b)

Figure 1- a) Aloe vera juice, b) Amla juice

Sample Preparation

Forty premolars were randomly and equally assigned into four groups. Group I: extracted teeth stored in aloe vera juice, Group II: extracted teeth stored in amla juice; Group III: extracted teeth stored in milk. Group IV: extracted teeth stored in water. Samples were prepared using the standard protocol.[14] After extractions, Teeth were dried in normal daylight in sterile petri dish for 30 mins following which it was placed in one of the four different storage media for 45 minutes, and then the teeth were transferred to storage media by holding only crown portion, without disturbing the viable cells present on root surface. The scrapings of the PDL from the root surface of the teeth were collected in Falcon tubes containing 0.5 mg of type I collagenase enzyme in 2.5 mL of phosphate buffered saline.[14] The tubes were incubated for 30 minutes following which centrifugation was done for five minutes at 800 rpm.[14] Staining of obtained periodontal ligament cells was done with 0.4% Trypan Blue stain (100 µL of solution with 100 µL of dye) and the cells were observed under optical microscope. The percentage of viable cells were calculated.[22]

Figure 2- Rinsing of extracted teeth.

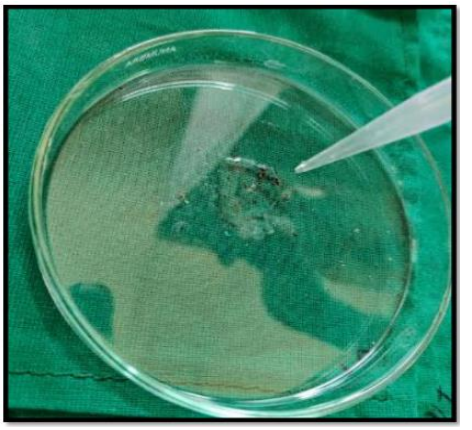

Figure 3- Scraping of PDL cells from extracted teeth

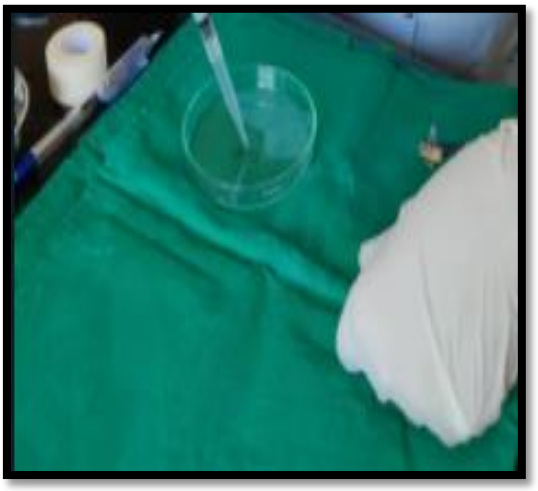

Figure 4 - Addition of collagenase

Figure 5- Centrifugation and balancing of tubes.

Staining and Counting of Cells with Assessment of color change

Staining was done in 1:1 ratio using 0.4% (w/v) Trypan blue stain (HiMedia, Mumbai, India) (100 µL of solution with 100 µL of dye). After 10 minutes, 10µL of the staining solution was taken and the number of viable cells and non-viable were counted with a hemocytometer under a light microscope at 10x magnification. Viable cells appear visible as clear cells as they do not take up the stain and nonviable cells absorb the stain.

Figure 6- Charging of Hemocytometer (Neubauer's counting chamber)

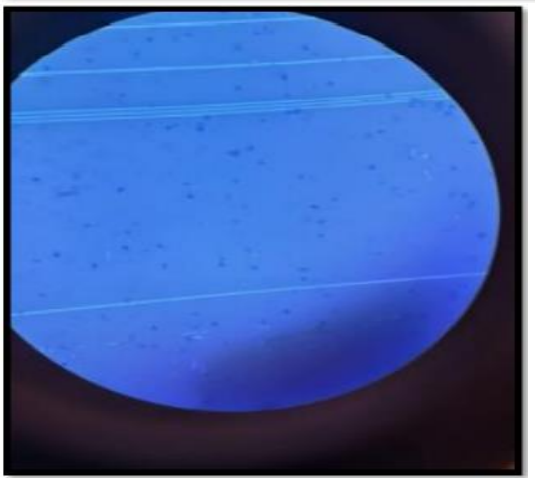

Figure 7- Cells observed under microscope (Viable and non-viable ones)

Percentage of viable cells = (No of viable cells / No of total cells) X 100

Data Analysis

Data were organized and tested for normality using the Kolmogorov-Smirnov test for normal distribution. As the data were normal, the comparison of the four groups was done using one-way analysis of variance (ANOVA) followed by post-hoc pairwise comparisons using the Tukey-Kramer test. All testings were done using two-sided tests at alpha 0.05. The p value <.05 was considered significant.

Results

Table 1 - Data chart for viable cells in aloe vera juice

ALOE VERA			
Samples	**Total cells**	**Viable cells**	**% Viable cell**

			count
1	103	77	74.75
2	115	89	77.39
3	107	85	79.43
4	112	91	81.25
5	119	90	75.63
6	111	88	79.27
7	120	98	81.66
8	116	83	71.55
9	109	89	81.65
10	114	84	73.68
MEAN ± SD	112.6	87.4	77.62 ± 3.59

In the aloe vera group, total cells and viable cells were counted and the mean for % viable cells is 77.62 ± 3.59.(Table 1)

Table 2 - Data chart for viable cells in amla juice

AMLA			
Samples	**Total cells**	**Viable cells**	**% Viable cell count**
1	102	62	60.78
2	109	60	55.04
3	111	64	57.65
4	132	67	50.75
5	124	66	53.22
6	119	63	52.94

7	116	69	59.48
8	128	64	50.00
9	122	65	53.27
10	104	68	65.38
MEAN ± SD	116.7	64.8	55.85 ± 4.88

In the amla group, total cells and viable cells were counted and the mean for % viable cells is 55.8 5± 4.88.(Table 2)

Table 3 - Data chart for viable cells in milk

MILK			
Samples	**Total cells**	**Viable cells**	**% Viable cell count**
1	109	78	71.55
2	111	77	69.36
3	132	87	65.90
4	128	85	66.40
5	126	89	70.63
6	119	83	69.74
7	118	93	78.81
8	114	74	64.91
9	116	81	69.82
10	116	86	74.13
MEAN ± SD	118.9	83.3	70.01 ± 4.13

In the milk group, total cells and viable cells were counted and the mean for % viable cells is 70.01±4.13. (Table 3)

Table 4 - Data chart for viable cells in water

WATER			
Samples	**Total cells**	**Viable cells**	**% Viable cell count**
1	104	51	49.03
2	102	49	48.03
3	101	54	53.46
4	99	47	47.47
5	101	49	48.51
6	98	46	46.93
7	107	48	44.85
8	106	45	42.45
9	101	41	40.59
10	103	42	40.77
MEAN ± SD	102.2	47.2	46.20 ± 4.06

In the water group, total cells and viable cells were counted and the mean for % viable cells is 46.20±4.06. (Table 4)

Graph of all group means is placed with % viable cells on Y axis and groups on X axis. (Figure-8)
Viable cells and % cell count mean was 77.62 ± 3.59, 55.85 ± 4.88, 70.01 ± 4.13, 46.20 ± 4.06 for aloe vera, amla, milk and water, respectively.(Table1- 4) [Differences in mean and SD were statistically significant. ($p<0.05$)]

GRAPH

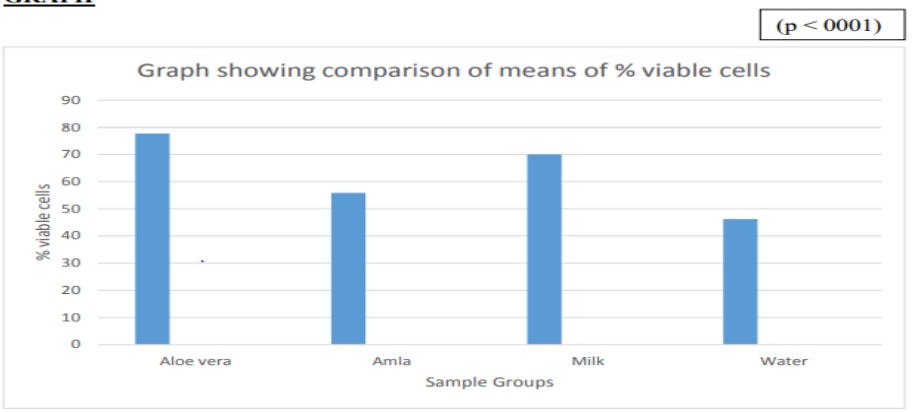

Figure 8- Comparison of means of percentage viable cells

Discussion

The importance of PDL cell viability prior to replantation was first addressed by Hammer in 1955.[23] The number of viable PDL cells available determine the survival of a replanted tooth. There has been continuous search in literature to find an optimum set of conditions that will keep the highest quantity of PDL cells alive post replantation. According to Andreasen and Hjorting- Hansen (1966), better success rate with replantation was seen with teeth those were replanted within 30 mins when compared to teeth those were stored for a longer duration of extra oral dry time[24]. Numerous studies have evaluated the optimum requirements of storage media for cell viability and preservation.

An ideal storage medium should[25]

- Inhibit microbial invasion or growth.
- Preserve the feasibility of PDL cells.
- Be able to preserve the proliferative capacity of cells
- Be non-allergic.
- Not promote failures such as ankylosis and post-reimplantation root resorption.
- Be stable when stored
- Be effective in different climatic conditions.
- Be able to wash off toxic waste products.
- Aid in the reconstitution of reduced cellular metabolites.

The basic principle behind the use of different storage media is to transport tooth to an environment similar to that of oral cavity. HBSS is considered as gold standard among all storage media, HBSS is a pH-balanced (7.2) salt solution, has physiological osmolarity (320 mOsm/kg) and has said to have no bacterial content. It has essential nutrients such as glucose, calcium and magnesium ions which help in reconstituting the deleted cellular components of the PDL cells. According to Hiltz and Trop (1991), cells after storage in HBSS appeared normal and about 70% viable fibroblasts were maintained after a span of 96 hours in HBSS.[26]

Various other medias like viaspan, Eagles's medium have been found suitable for maintaining cell viability of avulsed teeth and for transportation of avulsed teeth,

However, high cost of the medium and lack of availability in rural areas are the major disadvantages of these media. Therefore, there is a need to find storage medium that is easily available and cheap. Hence the present study was carried out to evaluate and compare the efficacy of commonly available and cost-effective storage media aloe vera juice, amla juice and milk in maintaining PDL cell viability.

Age, trauma and inflammation affects fibroblast function. Therefore, mature human premolars, non-carious indicated for orthodontic purposes were selected. Teeth without periodontal disease from healthy young individuals were included in the study. [27]

In the literature, various techniques have been used to evaluate the number of PDL cells. Reinholdt et al [28] used 3 stepwise trypsinization procedure, Soder et al used chromogenic stain to count viable PDL cells. As suggested by Pileggi et al, [29] to minimize the exposure of cells to active trypsin and to preserve maximum cell viability, Collagenase Type I was used in the current study to treat the root surfaces; it maintained maximum cellular integrity and allowed rapid cell retrieval. Trypan blue exclusion assay helps in quickly and easily differentiating between non-viable cells from viable ones. Chromophore is present on cell membranes and is negatively charged, trypan blue stain is not taken unless the membrane is damaged, all the viable cells are excluded as they do not pick up the dye.[30,31]

Trypan blue stain used in this study assessed viability of the cells, the actual physiologic health and metabolic capabilities was not assessed. [32]
In the present study, aloe vera juice was chosen as storage media as "it contains over 75 nutrients, it also contains 20 minerals, 19 amino acids and 12 vitamins. For maintaining better health human body needs 22 amino acids, 8 of which are essential and our body cannot synthesize these essential amino acids. All of these eight essential amino acids and 11 of 14 secondary amino acids are present in aloe vera. It promotes healing and has antibacterial, anti-inflammatory, antioxidant, immune boosting and hypoglycemic properties.[13] In the field of dentistry, aloe vera has been used to enhance defense mechanisms and fasten the healing process of periodontal diseases by slowing or inhibiting the synthesis of thromboxane.[33]

Pure aloe vera gel was not used in this study, the reason which may reduce its effectiveness is that the pure aloe vera (at 100% concentration) is highly viscous and at the time of experiment it may entirely cover the surface of the experimental cells which may possibly prevent the accessibility of oxygen to cells. As a result, more the deficiency in supply of oxygen, more the cell death will occur. [34] One of the plant's unique ability is increasing the production of human fibroblast cells faster than normal cell production by six to eight times. Aloe vera accelerated collagen production process and also improved fibroblast cell structure.

Amla juice has not been studied as storage media, amla is a rich source of ascorbic acid. Type I collagen production was stimulated by addition of ascorbic acid to osteoblastic cell lines which was followed by specific markers expression associated with osteoblastic phenotypes such as alkaline phosphatises (ALP) and

osteocalcin. Ishikawa et al observed that ascorbic acid increased the ALP activity, which is required for binding of PDL cells to type I collagen via 2 beta integrin, whose efficacy is again increased by ascorbic acid. As type I collagen production is supposed to be an initial process in differentiation of PDL cells, it may serve as a potential storage medium.[9]

Our analysis reveals the order of preferred storage material for PDL cell viability as: aloe vera, milk, amla and water. Better results were expected with amla but the possible reasons for not achieving favorable results could be due to its high pH, and also in the procedure incubation was carried out, amla has vitamin C which is heat labile, so the properties of amla and vitamin C could have been destructed during incubation.

Limitations of this study

Limitation of this in vitro study is that it does not mimic a real life clinical scenario. Most avulsion injuries are likely to occur due to sports, playground or road traffic injuries and accidents; in such cases the avulsed tooth might come in contact with surfaces such as the floor, ground, soil or road surfaces, which may result in bacterial contamination, thereby adversely affecting the viability of PDL cells. Furthermore, the extraoral dry time taken in this study was 30 minutes, whereas, in clinical scenario, there may be variations in the extraoral dry time leading to adverse effects on PDL cells. Moreover, cell viability does not always confirm reattachment always. Despite such limitations, this research lays a foundation for the easily available storage media like aloe vera juice, amla juice especially in India. Further research regarding their efficiency in maintaining the viability of PDL cells is needed.

Conclusion and Recommendations

Based on the observations of our study, following are the specific conclusions drawn with respect to each of the study objectives:
Cell viability of PDL cells was maximum in aloe vera juice (77.62%) followed by milk (70.12%), amla juice (55.85%) and water (46.20%). Within the limitation of this study it can be concluded that among all the four experimental groups aloe vera juice is the most effective storage media for an avulsed tooth. The order of preference for storage medium for maintaining the PDL cell viability can be: aloe vera juice > milk > amla juice > water. Aloe vera and milk can be recommended as an alternative storage media for an avulsed tooth in areas where HBSS might be inaccessible and expensive. Based on our results, amla juice and water are the least recommended.

Conflict of interest- Authors declare no conflict of interest.

Funding- No external source of funding.
Acknowledgment-Department of microbiology, Department of physiology, Department of dental anatomy and histology. (Bharati Vidyapeeth dental college and hospital, Navi Mumbai.)

LIST OF ABBREVIATIONS

1	HBSS	Hank's balanced salt solution
2	AAE	American Association of Endodontists
3	WHO	World Health Organization
4	PDL	Periodontal ligament
5	SEM	Standard Error of Mean
6	ANOVA	Analysis of variance
7	DMEM	Dulbecco's Modified Eagle Medium.
8	MTT	Thiazolyl Blue Tetrazolium Bromide
9	SD	Standard Deviation

References

1. J.O Andreasen, F.M. Andreasen, Textbook of traumatic injuries of the teeth, Page no-486,4th Edition.
2. Jain D, Dasar PL, Nagarajappa S, Natural products as storage media for avulsed tooth, Saudi Endodontic journal, 2015, volume 5(2), 107-113.
3. 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, Journal of conservative dentistry, 2013 Jan;16(1),71-74.
4. Balto HA, Halawany HS, Jacob V , Abraham NB, The efficacy of Salvadora persica extracts in preserving the viability of human foreskin fibroblasts, Saudi dental Journal.,2015, Jul;27(3): 137-40
5. 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 Journal of Dental Research-September 2013, 2(2): 42-47
6. Chamorro MM, Regan JD, Opperman LA, Kramer PR, Effect of storage media on human periodontal ligament cell apoptosis. Dental Traumatology, 2008 Feb; 24(1):11- 6.
7. Khademi AA, Saei, Mohajeri MR, A new storage medium for avulsed tooth. Journal of Contemporary Dental Practice. 2008 Sep 1; 9(6):25-32.
8. Arbaz Sajjad and Samia Subhani Sajjad, Aloe vera: An Ancient Herb for Modern Dentistry—A Literature Review, Journal of Dental Surgery, Volume 2014, ID 210463, 6 pages

9. Ishika S, Iwasaki K, Komaki, Role of ascorbic acid in periodontal ligament cell differentiation. Journal of periodontology, 2004 May; 75(5):709-16.
10. Navin HK, Veena A, Rakesh CB, Advances in storage media for avulsed tooth review. International journal of preventive and clinical dental research, 2015, 2(3):41-47.
11. Saumya Navit, Niharika Shahi, Suleman Abbas khan, Nature's benefaction as a life saver for an avulsed Tooth: an in vitro study, Journal of Clinical and Diagnostic Research, 2017 Jun; 11(6): ZC01–ZC04
12. Samaneh Badaksh, Tahereh Eskandarian, Tahereh Esmaeilpour, The Use of Aloe Vera Extract as a Novel Storage Media for the Avulsed Tooth, Iranian Journal of Medical Sciences, 2014 Jul; 39(4): 327–332.
13. Babaji P, Melkundi M, Devanna R In vitro comparative evaluation of different storage media (hank's balanced salt solution, propolis, Aloe vera, and pomegranate juice) for preservation of avulsed tooth. Journal of Dentistry, 2017 Jan-Mar; 11(1): 71–75.
14. Sharma M, Sharma S, Reddy Y, Mittal R, Agarwal V, Singh C , Singh A, Evaluation of Periodontal Ligament Cell Viability in Three Different Storage Media: An in Vitro Study. Journal of Dentistry, 2015 Jul; 12(7): 524–531.
15. Resende K, Faria G, Longo D, Martins L, Costa C, In vitro evaluation of plants as storage media for avulsed teeth: A systematic review, Dental Traumatology / Volume 36, Issue 1 / p. 3-18, 2019.
16. Priyenka Patil, Viddyasagar Mopagar, Saurabh Joshi, Comparative assessment of periodontal ligament cell viability using three different storage media for avulsed teeth. International Journal of Scientific Research, volume 7(6) 2018
17. Souza BD, Lückemeyer DD, Reyes-Carmona JF, Felippe WT, Simões CM, Felippe MC, Viability of human periodontal ligament fibroblasts in milk, Hank's balance salt solution and coconut water, as storage media. International Endodontic Journal. 2011 Feb;44(2):111-5
18. Adnan S, Lone S, Khan F, Husain S, Nagi S, Which is the most recommended medium for the storage and transport of avulsed teeth? A systematic review, Dental Traumatology / Volume 34, Issue 2 / p. 59-70, 2018.
19. Longo DL, Fumes AC, Kuchler EC, Paula- Silva WG, Nelson-Filho P LAB, Efficiency of different storage media for avulsed teeth in animal models: a systematic review, Dental Traumatology / Volume 36, Issue 1 / p. 3-18, 2018.
20. Osmanovic A, Halilovic S, Kozaric A, Hadziabdic N, Evaluation of periodontal ligament cell viability in different storage media based on human PDL cell culture experiments—A systematic review, Dental Traumatology / Volume 34, Issue 6 / p. 384- 393, 2018.
21. De Brier N, O D, Borra V, Singletary EM, Zideman DA, De Buck E, International Liaison Committee on Resuscitation First Aid Task Force, Bendall JC, Berry DC, Carlson JN, Cassan P. Storage of an avulsed tooth prior to replantation: A systematic review and meta-analysis, Dental Traumatology / Volume 36, Issue 5 / p. 453-476.
22. Pranjali Dhimole, Deepak P Bhayya, Shilpi Gupta, Prabhat Kumar, Saurabh Tiwari, Swarnam Pandey, Evaluation of efficacy of neem and turmeric as storage media in maintaining periodontal ligament cell viability: an invitro study. Journal of Indian society of Pedodontics and Preventive Dentistry: 2019, Volume: 37(2), 140-145.

23. Hammer H. Replantation and implantation of teeth. Int Dent J 1955, 439-57.
24. Andreasen JO, Hjorting- Hansen E. Replantation of teeth part 1. Radiographic and clinical study of 22 replanted anterior teeth in humans. Acta Odontol Scand 1966 24:287- 306.
25. Vineet IS Khinda, Clinical and Practical Implications of Storage Media used for Tooth Avulsion Int J Clin Pediatr Dent 2017; 10(2):158-165.
26. Hiltz J, Trope M. Vitality of human lip fibroblast in milk, hank's balanced salt solution and viaspan storage media. Endod Dent Traumatol. 1991; 7:69-72
27. Pohl Y, Filippi A, Kirschner H. Results after replantation of avulsed permanent teeth. II. Periodontal healing and the role of physiologic storage and antiresorptive-regenerative therapy. Dent Traumatol. 2005. April; 21 (2): 93– 101.
28. Reinholdt J, Andreasen JO, Soder PQ, Otteskog P, Dybdahl R, Riis I, Cultivation of periodontal ligament fibroblasts on extracted monkey incisors. A histological study of three culturing methods. International journal of oral surgery 1977, 6 215-25
29. Pileggi R, Dumsha TC, Nor JE, Assessment of post traumatic PDL cell viability by a novel collagenase assay, Dental Traumatology 2002, 18:186-9
30. Pohl Y, Filippi A, Kirschner H. Results after replantation of avulsed permanent teeth. II. Periodontal healing and the role of physiologic storage and antiresorptive-regenerative therapy. Dent Traumatol. 2005. April; 21 (2): 93– 101.
31. Thaib, P. K. P., & Rahaju, A. S. (2022). Clinicopathological profile of clear cell renal cell carcinoma. International Journal of Health & Medical Sciences, 5(1), 91-100. https://doi.org/10.21744/ijhms.v5n1.1846
32. Davis RH, Leitner MG, Russo JM, Byrne ME. Anti-inflammatory activity of aloe vera against a spectrum of irritants. Journal of Med Assoc. 1989. June; 79 (6): 263– 76
33. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. International Journal of Health Sciences, 6(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
34. Martin MP, Pileggi R. A quantitative analysis of propolis: a promising new storage media following avulsion. Dental Traumatology. 2004. April; 20 (2): 85– 9
35. Fulzele P, Baliga S, Thosar N, Pradhan D. Evaluation of aloe vera gel as a storage medium in maintaining the viability of periodontal ligament cells- an in vitro study. Int J Clin Pediatr Dent. 2016; 40(1):49-52.
36. Badakhsh S, Eskandarian T, Esmaeilpour T. The use of aloe vera extracts as a novel storage media for the avulsed tooth. Iran J Med Sci. 2014; 39(4):327-32