How to Cite:
Mahendra, P., Manali, B., Pooja, K., Kunjal, M., Vaidehi, T., & Sawan, M. (2022). Evaluation and comparison of efficacy of different storage media in maintaining the viability of periodontal ligament cells-in vitro study. *International Journal of Health Sciences*, *6*(S1), 3016–3025.
https://doi.org/10.53730/ijhs.v6nS1.5297

Evaluation and Comparison of Efficacy of Different Storage Media in Maintaining the Viability of Periodontal Ligament Cells-In Vitro Study

Patel Mahendra
Professor and Head, Department of Conservative dentistry and Endodontics, College of Dental Sciences and Research Centre

Batra Manali
M.D.S Conservative Dentistry and Endodontics

Kesharani Pooja
Reader, Conservative dentistry and Endodontics, College of Dental Sciences and Research Centre

Mistry Kunjal
Associate Professor, Conservative dentistry and Endodontics, Dharamsinh Desai University, Nadiad, Gujarat

Trivedi Vaidehi
Post graduate student, Department of Conservative dentistry and Endodontics, College of Dental Sciences and Research Centre

Makwana Sawan
Post graduate student, Department of Conservative dentistry and Endodontics, College of Dental Sciences and Research Centre

***Abstract*---**Aim: Aim of the study is evaluate the efficacy of HBSS, Green tea extract and Aloe Vera gel as storage media in maintaining the viability of periodontal ligament cell. Material and Method: Fifty-five human teeth with closed apices and with apparently normal periodontium were extracted as atraumatically as possible for orthodontic reasons were collected for study. Teeth were randomly divided into positive control group, negative control group, three experimental group. Teeth in experimental groups were dried for 30 minutes (including time taken for curetting coronal PDL cells), followed by a 45-minute immersion in one of the three storage solution groups. The teeth in positive control group after extraction

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
***Corresponding author**: Patel, M.; Email: drmahenpatel@yahoo.com*
Manuscript submitted: 18 Jan 2022, Manuscript revised: 09 Feb 2022, Accepted for publication: 27 March 2022

was immediately treated with collagenase. The teeth in negative control group were bench- dried for 8 hours, with no follow- up storage solution time, and then placed in the collagenase. Result: HBSS is the best medium for the maintaining the viability of the PDL Cells.But easier availability and cost effectiveness, GTE and Aloe Vera Gel can be advocated as a viable storage medium. Conclusion: It can be concluded that GTE and Aloe Vera Gel can be advocated as a viable storage medium.

***Keywords*---**Avulsion, Aloe Vera Gel, Green Tea Extract, HBSS, Viaspan Periodontal ligament.

Introduction

Tooth avulsion is a complete displacement of a tooth from its alveolar socket due to a traumatic injury. [1] Its management is both time and technique sensitive. It leads to damage to the cementum, periodontal ligament, alveolar bone, neurovascular supply to the pulp, and the surrounding gingiva.[2]

Tooth replantation followed by follow-up visits after a traumatic avulsion is acclaimed by the American Association of Endodontists,[3] American Academy of Pediatric Dentistry [1] and the International Association of Dental Traumatology [4]. The purpose of replantation is to have enough healing of all damaged tissues. External root resorption, principally replacement resorption and ankylosis are the most significant and prevalent complication after the replantation of the avulsed tooth.
At the time of replantation, cell viability is of paramount value for proper repair of the damaged tissues. [5,6] PDL cell viability is a key prognostic factor for replantation of avulsed teeth [7]. An avulsed tooth needs to be stored in an appropriate media to avoid desiccation and to maintain cell viability. When an avulsed tooth is exposed to air and kept in a dry condition the PDL cells will become necrotic and result in a severe inflammatory response when replanted.[8]
An ideal storage media is the one which will preserve cell viability of PDL cells and also be inexpensive and easily manageable by the patient when required for tooth storage

Hank's Balanced Salt Solution (HBSS) is currently recommended by the AAE, AAPD, and IADT for an avulsed tooth. HBSS is a sterile solution and contains essential nutrients for cell viability including: sodium chloride, D-glucose, potassium chloride, sodium bicarbonate, potassium phosphate, calcium chloride, magnesium chloride, and magnesium sulfate . [9] Hank's balanced salt solution (HBSS) and Viaspan have been recommended by many authors as an ideal storage media for avulsed teeth but they are not easily available. [10,11] Thus, the search for readily available solutions which could act as storage media for avulsed teeth is progress.

Green tea extract (GTE) as an readily available physiologic medium. It has been of interest recently because of its antibacterial, anti-oxidative, and anti-inflammatory effect.[12,13] Aloe Vera is a member of liliaceae family. This medicinal plant is

cactus with green, tapered leaves that are filled with a transparent viscous gel.[14] This gelatinous substance contains 96% water and 75 active properties such as vitamins, enzymes, minerals, sugars, salicylic acids, and amino acids. It has been reported that Aloe Vera has significant anti-inflammatory, antioxidant, antibacterial, antifungal and anticarcinogenic activities. [14,15] The aim of this study was to compare various storage media for maintaining cell viability of human periodontal ligament Cells.

Methodology
Source of data

Fifty- five human teeth with closed apices and with apparently normal periodontium were extracted as atraumatically as possible for orthodontic reasons were collected from department of oral and maxillofacial surgery, college of dental sciences and used for the study. The extractions were performed atraumatically with utmost care taken to prevent damage to periodontal ligament cells.

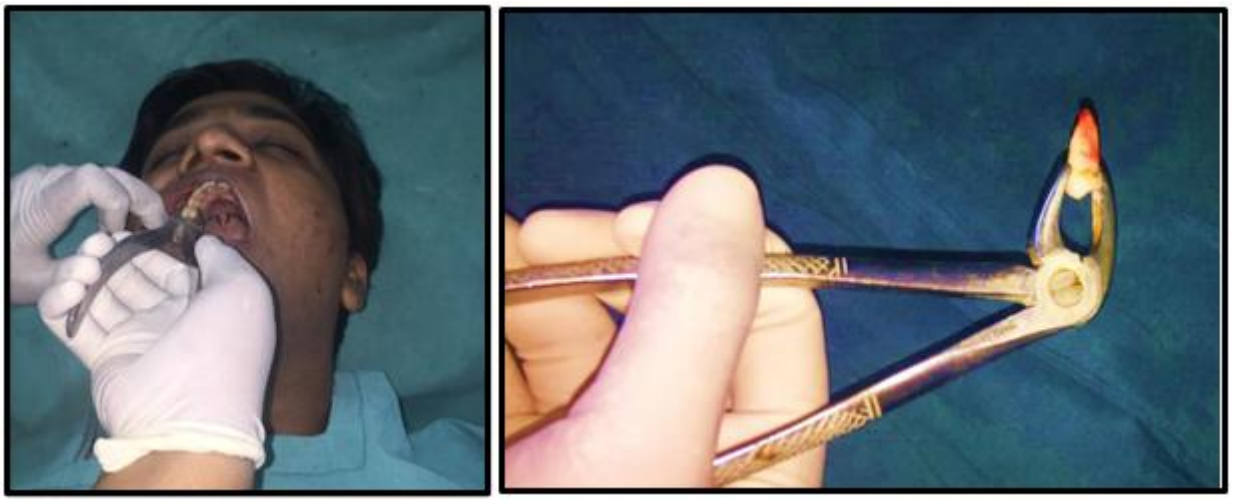

Figure 1: Atraumatic Extraction of tooth

Teeth were randomly divided into positive control group, negative control group, three experimental groups

A –Positive Control Group
B- Negative Control Group
C -Control Group (Save-A-Tooth) D –Green Tea Extracts
E- Aloe Vera gel

Figure 2: Storage medias

As Tudose A et al. described the external surface of the Aloe Vera leaf was washed and disinfected with 70% ethanol alcohol. Under a sterile hood, the insides gelatinous substance was cut from the external shield and triturated to achieve a homogenous gel.

Following extraction, the teeth were held with forceps at the coronal region and coronal 3mm of periodontal ligament cells on the root surface was scraped from the cervical margin using BP blade no.15 to remove the cells that might have been damaged during extraction.

Teeth in experimental groups were dried for 30 minutes (including time taken for curetting coronal PDL cells), followed by a 45-minute immersion in one of the three storage solution groups.

The teeth in positive control group after extraction was immediately treated with collagenase.

The teeth in negative control group were bench-dried for 8 hours, with no follow-up storage solution time, and then placed in the collagenase.

After drying and soaking of each experimental teeth, 2.5 ml of stock solution containing grade II collegenase were added to teeth and incubated for 30 min at 37°C. After incubation, 50 µL of fetal bovine serum was added to each tube with help of micropipette.

Percentage of viable cells are counted as :

$$\text{Percentage(\%)of viable cells} = \frac{\text{Total no of cells- NO of non-viable cells}}{\text{Total no of cell}}$$

All tubes were then centrifuged for 4 minutes at 1000 rpm and supernatant was removed with sterile micropipettes. The cells were labeled with 0.4% trypan blue for determination of viability. The number of viable PDL cells was counted under light microscope with haemocytometer at 40× magnification

Results

Table 1: Percentage (%) of viable cells for different groups was as follows:

SAMPLE NO.	GROUP C	GROUP D	GROUP E
1	88.57	75	70
2	87.5	78.57	76.79
3	88.09	78.37	76.31
4	85	75	78.37
5	86.84	74.35	68.57
6	86.11	71.42	65
7	88.57	78.37	76.31
8	83.33	71.79	72.22
9	86.84	70	74.28
10	87.17	73.80	72.22
11	88.89	71.05	52.63
12	86.84	72.97	74.35
13	87.5	80	72.22
14	88.09	78.94	65
15	78.94	75.67	71.42

Table 2: Pecentage Of Viable Cells (Anova Analysis)

Group	N	mean	Std. Dev.	Std. Error
A	5	95.40	1.08	0.9
B	5	24.35	5.91	.64
C	15	86.55	2.55	0.66
D	15	75.02	3.23	0.83
E	15	71.3	6.51	1.68
Total	55	74.41	18.29	2.46

Table 3: Multiple Comparisionnnnnnnn Post Hoc Tukey Test For Intergroup

(I)group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
c	**A**	-8.8457005*	2.2825	0.01
	B	62.2043049*	2.2825	0
	D	11.5300708*	1.613971	0
	E	15.2117647*	1.613971	0
D	**A**	-20.3757714*	2.2825	0
	B	50.6742341*	2.2825	0
	E	3.681694	1.613971	0.282
E	**A**	-24.0574652*	2.2825	0
	B	46.9925403*	2.2825	0

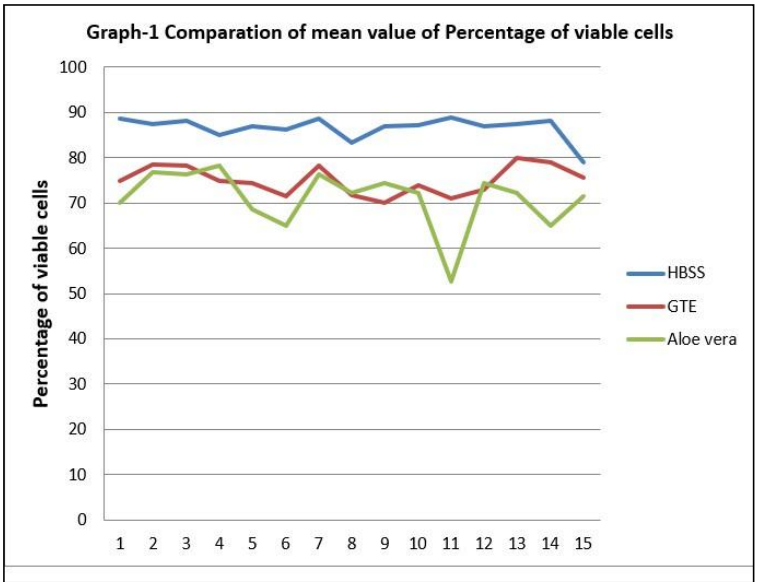

Figure 3 Graph comparing percentage of vof viable cells

Tukeys post hoc test:

Statistical analysis showed a significant difference among the groups. Tukey honestly significant difference test showed that HBSS group demonstrated a significantly higher number of viable PDL cells than GTX, and Alovera gel(table 3). There was no significant difference between alovera gel and GTE groups(Table 1).All experimental solution groups were significantly lower than positive control and higher than negative control.(Table 2,Graph 1)

Discussion

Consistent with the World Health Organization classification, for traumatized teeth, exarticulation is mentioned as the complete displacement of a tooth from its alveolar socket due to trauma.[1] This causes severe damage to the periodontal tissues. As per recent clinical studies avulsed permanent teeth should be replanted as soon as possible. Immediate replantation is the standard treatment of choice as it re-institutes the natural nutrient supply to periodontal ligament cells on the root surface, diminishing further damage, and accelerates the healing process. Unfortunately, immediate replantation is not always possible. In such conditions, the tooth should be stored in a medium which maintains periodontal ligament cell viability until conclusive dental treatment can be accomplished.[16] The healing of an avulsed tooth after replantation will depend upon the healing potential of each cellular component of the tissues involved. Prognosis of a replanted tooth will depend on minimaldamage to the PDL, which is critical for regeneration of the attachment apparatus and also to avoid root from resorption. [17,18] Extra oral time and storage conditions are the most decisive factors in determining the viability of the remaining PDL cells, and survivability of the avulsed tooth. Researches have shown that an avulsed tooth can be replanted without complications after 1-3 hours of being placed in suitable storage conditions.

An ideal storage medium is the one that is capable of preserving the viability, mitogenicity, and clonogenic capacity of the damaged PDL to facilitate repopulation of the denuded root surface thereby averting further root resorption. The storage medium should have a physiological osmolarity and pH and should be maintained at an appropriate temperature to allow optimal cell growth or survival. Finally, the ideal storage media should be readily available for use in emergency situations.[19] In an effort to find proper media that would be easily accessible for use, several solutions such as milk, egg white, coconut water, etc. have been introduced. In the present study,Viability of PDL cell is checked in different media like HBSS, GTE and Aloe Vera gel. The ability to proliferate is critical to the regeneration of oral tissue after damage.

Green tea extract medium was selected because of its special properties such as anti-inflammatory, anti-microbial, and anti-oxidative potentials. It is produced from leaves of Camellia sinensis plant which is considered a major source of polyphenols.[12] Catechin, epicatechin, epigallocatechin, epigallocatechin-3-gallate, epicatechingallate, and quercetin glycosides are the most important polyphenols of green tea.[12,20] This medium also contains critical elements for cell growth such as calcium, magnesium, selenium, zinc, iron, and fluoride as well as some carbohydrates such as glucose, fructose, sucrose, and vitamins B, C, and E.[13] Furthermore, it is an accessible popular beverage around the world and has previously been used for allografts and cell studies.[21]

Aloe Vera is a natural plant being commonly popular in herbal medicine and nowadays available in many herbal shops. This plant is well known in wound healing promotion; it has a miraculous healing potency and contains essential nutrients for cells survival.[22,23] The inner gel in Aloe Vera leave consists of 96% water and 75 other active components. Historically, many studies have demonstrated that Aloe Vera has superb activities, such as anti-inflammatory,

antibacterial, antifungal, anticancer and even antioxidant activities. Although a number of studies have reported Aloe Vera's efficacy in dentistry.[24]
In the current study to minimize the exposure of cells to active trypsin and to preserve maximum cell viability, the root surface was treated with collagenase grade II as was performed in the work by Pileggi et al.[25] This procedure allowed rapid cell retrieval and maintained maximum cellular integrity, as was demonstrated by the positive control samples. This method is more representative of the actual clinical situation because the cells are not subjected to long processing times to determine their viability status. Collagenase assay provides a combination of collagenolytic and proteolytic enzymes required for tissue disaggregation.[25]
Fetal bovine serum as growth supplement for cell culture media because of its high content of embryonic growth promoting factors. When used at appropriate concentrations, it supplies many defined and undefined components that have been shown to satisfy specific metabolic requirements for the culture of cells in vitro.[26]

The trypan blue exclusion staining technique was used because it is quick, easily performed, and distinctively differentiates nonviable cells from viable cells. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes.[27] In this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. [28,29]

Trypan blue dye exclusion test principle is, the cells with damaged membrane allow the trypan blue dye to pass through membrane into cytoplasm, whereas undamaged cells exclude dye. The test was conducted to study the cell viability by assessing the loss of membrane integrity following the method of Pant et al., with desired modifications. In brief, immediately after the completion of respective incubations, cells were aspirated and subjected to stain with trypan blue dye (0.4% solution) at a ratio of 1:5 (dye: cell suspension) and placed in hemocytometer. The counting for live (unstained transparent) and dead (blue stained) cells was made at 40× magnification in light microscope.[30]

Conclusion

The result of the study indicated that HBSS group demonstrated higher number of viable PDL cells than GTE and Aloe Vera gel. There is no significant difference between GTE and Aloe Vera gel. But ,trypan blue can only illustrate the viability of the cells not their exact healthy condition. Therefore, further in vivo investigations should be performed to validate the results of this study.
Within the limitation of this study, it can be concluded that:
HBSS is the best medium for the maintaining the viability of the PDL Cells. But easier availability and cost effectiveness, GTE and Aloe Vera Gel can be advocated as a viable storage medium.

References

1. American Academy of Pediatric Dentistry Council on Clinical Affairs. Guideline on management of acute dental trauma. Pediatr. Dent. 2008;30(7):175-183.

2. Andreasen J, Andreasen F. Textbook and color atlas of traumatic injuries to the teeth. 4th ed: Wiley-Blackwell; 2007.
3. American Association of Endodontists. The recommended guidelines of the American Association of Endodontists of the treatment of traumatic dental injuries 2013.
4. Andersson L, Andreasen JO, Day P, Heithersay G, Trope M, Diangelis AJ, et al.International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent. Traumatol.2012;28(2):88-96.
5. Cvek M, Granath LE, Hollender L. Treatment of non-vital permanent incisors with calcium hydroxide. 3. Variation of occurrence of ankylosis of reimplanted teeth with duration of extra-alveolar period and storage environment. Odontol. Revy 1974;25(1):43-56.
6. Andreasen JO. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. Int. J.Oral Surg. 1981;10(1):43-53.
7. Loe H, Waerhaug J. Experimental replantation of teeth in dogs and monkeys. Arch. Oral Biol. 1961;3:176-184.
8. Trope M. Root resorption due to dental trauma. Endodontics Topics 2002;1(1):79-100.
9. Krasner P, Person P. Preserving Avulsed Teeth for Replantation. The Journal of the American Dental Association 1992;123(11):80-88.
10. 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.
11. Kumar V, Logani A, Shah N. Intentional replantation: A viable alternative for management of palatogingival groove. Saudi Endod J 2013;3:90-4.
12. Friedman M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res
13. Cabrera C, Artacho R, Gimenez R. Beneficial effects of green tea — A review. J Am Coll Nutr. 2006;25:79–99.
14. Joseph B, Raj SJ. Pharmacognostic and phytochemical peoperties of Aloe vera linn- An overview. Int J Pharmaceutical Sciences Review and Res. 2010;4:106-10.
15. Tudose A, Celia C, Cardmone F, Vono M, Molinaro R, Paolini D. Regenerative properties of Aloe Vera juice on human keratinocyte cell culture. Farmacia. 2009;57:590-97.
16. Ayça Tuba Ulusoy ,Elif Kalyoncuoglu ,Senay Kaya ,Zafer Cavit CehreliEvaluation of goat milk as storage media to preserve viability of human periodontal ligament cells in vitro Dental traumatology August 2016
17. Hiremath G, Kidiyoor K. Avulsion and storage media. Inves and Clinical Dent 2011;1-6.
18. Gomes M, Westphalen V. Study of storagenmedia for avulsed teeth. Brazil Dent Traumatol 2009;1:69- 76
19. Mónica M. Chamorro ,John D. Regan, Lynne A. Opperman ,Phillip R. KramerEffect of storage media on human periodontal ligament cell apoptosis Dental traumatology 2008,Feb

20. Gramaza A, Korczak J, Amarowicz R. Tea polyphenols- their antioxidant properties and biological activity: A review. Pol J Food Nutr Sci 2005;14/55:219-35
21. Cavet ME, Harrington KL, Vollmer TR, Ward KW, Zhang JZ. Anti-inflammatory and anti-oxidative effects of the green tea polyphenol epigallocatechin gallate in human corneal epithelial cells. Mol Vis 2011;17:533- 42.
22. Tudose A, Celia C, Cardmone F, Vono M, Molinaro R, Paolini D. Regenerative properties of Aloe Vera juice on human keratinocyte cell culture. Farmacia. 2009;57:590-97
23. Maenthaisong R, Chaiyakunapruk N, Niruntraporn S, Kongkaew C. The efficacy of Aloe vera used for burn wound healing: A systematic review. Burns. 2007;33:713-8. doi: 10.1016/j. burns.2006.10.384. PubMed:17499928.
24. Malhotra N. Current developments in interim transport (storage) media in dentistry: an update. Br Dent J 2011;211:29-33. doi: 10.1038/ sj.bdj.2011.523. PubMed PMID: 21738189
25. Singla A, Garg S. Reimplantation: Clinical implications and outcome of dry storage of avulsed teeth. J Clin Exp Dent 2010;2:38-42
26. Giuseppe S. A. Longo-Sorbello, Guray Say&m, Debabrata Banerjee, and Joseph R. Bertino. Cytotoxicity and Cell Growth Assays, cell and tissue culture: assorted techniques;2006:315-324, Elsevier Science (USA).
27. Immunocytometry solution-Hemocytometer Counting and Cell Viability. Available from: http://www.groups.molbiosci.northwestern.edu/morimoto/research/Protocols/II.%20Eukaryotes/A.%20 Cell%20Culture/3b.%20Hemacytometer.pdf. [Last accessed on 2011 Jul 23].
28. Kenny DJ, Barrett EJ, Casas MJ. Avulsions and intrusions: The controversial displacement injuries. J Can Dent Assoc 2003;69:308-13
29. Singla A, Garg S. Reimplantation: Clinical implications and outcome of dry storage of avulsed teeth. J Clin Exp Dent 2010;2:38-42
30. Technical Reference Guide Protocol for Performing a Trypan Blue Viability Test. Available from: http://www.bio.lonza.com/uploads/tx_mwaxmarketingmaterial/Lonza_Bench Guides_Protocol_for_Perf orming_a_Trypan_Blue_Viability_Test.pdf. [Last accessed on 2011 Aug 23]