Original Article

Comparative evaluation of four transport media for maintaining cell viability in transportation of an avulsed tooth – An *in vitro* study

Makonahalli Jaganath Bharath, Chickmagravalli Krishnegowda Sahadev, Praveen Kumar Makonahalli Ramachandra, Sandeep Rudranaik, Jijo George, Ashna Thomas[1]

Department of Conservative Dentistry and Endodontics, Sri Hasanamba Dental College and Hospital, Hassan, Karnataka, [1]Orthodontist, Private Practitioner, Jijos Dental Clinic, 15/F, T.D.K. Road, Coonoor, Niligiris, Tamil Nadu, India

Corresponding author (email: <jijogeorge.15483@gmail.com>)
Dr. Jijo George, Department of Conservative Dentistry and Endodontics, Sri Hasanamba Dental College and Hospital, Hassan, Karnataka, India.

Abstract

Objectives: The study was performed to compare and evaluate the efficacy of four experimental storage media (Hank's balanced salt solution, Ringer's lactate solution, tender coconut water, and green tea extract) for maintaining cell viability of human periodontal cells at different time intervals of 15 min 30 min, 60 min, and 90 min. Materials and Methods: Human periodontal cells were cultured and stored in the four media. After 15 min 30 min, 60 min, and 90 min, the different media were examined under optical microscope and viabilities analyzed using an optical calorimeter. Mean and standard deviation were estimated from the results that were statistically analyzed using one-way analysis of variance (ANOVA) to identify the significant groups. Results: The results indicated that there was no difference in cell viability between the four media up to a period of 60 min, whereas green tea extract showed a lower cell viability after 90 min. Conclusion: Within the limitations of the present study, it appears that due to superior osmolality, cost effectiveness, and easier availability, Ringer's lactate, tender coconut water, and green tea extract can be used as alternate storage media for avulsed tooth.

Key words: *Cell viability, optical calorimeter, storage media*

INTRODUCTION

Traumatic dental injuries in children and adolescents are frequently associated with the avulsion of tooth. Clinical surveys have shown an increase in prevalence of such injuries.[1] Immediate tooth reimplantation of the avulsed tooth is considered as the ideal clinical treatment.[2]

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	DOI: 10.4103/2231-0762.151981

For successful reimplantation of an avulsed tooth, it is most important to preserve the vitality of periodontal ligament (PDL) cells attached to the root surface to re-establish nutrient supply to the cells.[3] When an avulsed tooth is reimplanted after 15 min, damaged PDL cells cause partial root resorption. Moreover, a 30 min delayed reimplantation can cause fatal damage to cells and a 60 min delayed reimplantation in dry condition can cause PDL necrosis, leading to extensive root resorption. However, the lack of specific dental knowledge may lead a patient to fail to reimplant an avulsed tooth in a timely manner.[4]

The ability of transport medium to support cell viability can be more important than the extra oral time for preventing ankylosis and resorption. Various storage media such as tap water, saliva, milk, and culture media have been investigated for the ability to maintain cell viability.[5]

The purpose of this study was to compare and evaluate the efficacy of Ringer's lactate, Hank's balanced salt solution (HBSS), tender coconut water, and green tea extract in maintaining the cell viability for transportation of an avulsed tooth within time intervals of 15 min, 30 min, 60 min, and 90 min, by using the 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT) assay.

MATERIALS AND METHODS

Ten grams of green tea leaves was soaked in 100 ml of boiling distilled water for 5 min and filter sterilized by using a Whatman filter paper [Figure 1].

Preparation of green tea extract

In this *in vitro* experimental study, cell culture of human gingival fibroblast cells was obtained from clinically healthy premolars extracted for orthodontic purposes from the Department of Oral and Maxillofacial surgery, Sri Hasanamba Dental College, Hassan. The protocol was reviewed and approved by the Ethical Review Committee of Sri Hasanamba Dental College. Extraction was performed as atraumatically as possible, and the tooth was washed with sterile solution to wash out residual blood. The tooth was held with forceps at the coronal region and the PDL cells were obtained by scraping with a No. 2 scalpel blade from the lower third of the root surfaces. The teeth were stored in air-tight vials containing antibiotic solutions under aseptic conditions. These vials were transported to the International Stem Cells Private Limited, Sri Raghavendra Biotechnologies, Bangalore to harvest and culture the human gingival fibroblasts.

The tissues were split into small pieces and cultivated in Dulbecco's modified Eagle Medium supplemented with 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37°C. After incubation, the fibroblasts migrated out of the tissue pieces and started proliferating.

The 96-well culture plates were incubated at 37°C for 24 h with a layer of fibroblasts attached to the well culture plates and they were seeded at a density of 40,000 cells/well onto the 96-well culture plates using a microlittre pipette [Figures 2 and 3].

The storage media tested in the study were divided into four major groups to be inoculated into the 96-well culture plates as follows [Figure 4]:

- Group I: HBSS
- Group II: Ringer's lactate solution
- Group III: Tender coconut water
- Group IV: Green tea extract

Figure 1: Filter sterilized – Whatman filter paper

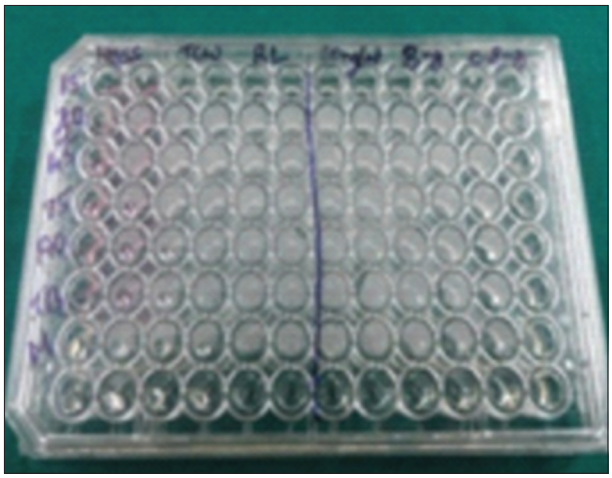


Figure 2: 96 well culture plates

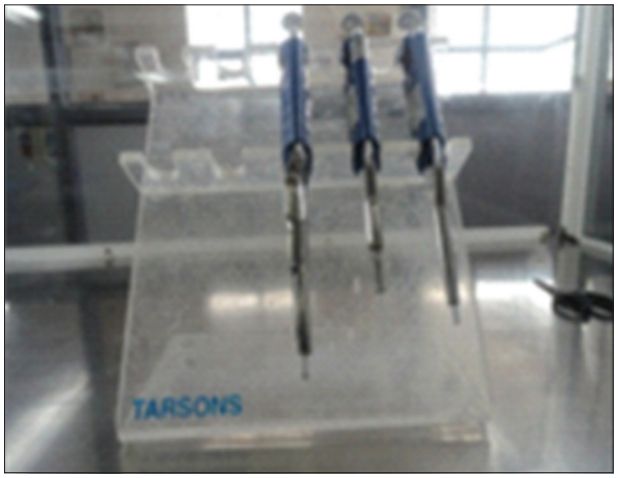


Figure 3: Microlittre pipette

Figure 4: The four storage medias used in the present study

The four groups were then divided to denote the storage time periods of 15 min, 30 min, 60 min, and 90 min, respectively. These time periods are considered because one of the sequelae following replantation of avulsed tooth includes inflammatory or replacement resorption. The development of replacement resorption depends on both the degree of damage to the periodontium at the time of avulsion and the extent to which the viability of periodontal ligament cells remaining on the tooth surface is maintained. Hence, the prognosis of an avulsed tooth is largely dependent on the status of the periodontal ligament cells at replantation. When an avulsed tooth is reimplanted after 15 min, the damaged PDL cells cause partial root resorption. Moreover, a 30 min delayed reimplantation can cause fatal damage to the cells and a 60 min delayed reimplantation in dry condition can cause PDL necrosis, leading to extensive root resorption. The best prognosis of tooth reimplantation is obtained when the extra-alveolar time does not exceed 5 min.

To standardize the number of cells, a cell suspension was made to get a cell count of 40,000 cells/ml. The cells were counted with a Neubauer counting chamber under light microscope. One milliliter of each of the storage media was inoculated into the 96-well culture plates containing the fibroblasts cells and placed in an incubator of 5% CO_2 at 37°C for 24 h. Then 50 µl of MTT dye was added to each well and the contents were incubated for 4 h at 37°C. After incubation, the MTT dye was removed and dimethyl sulfoxide (DMSO) solvent was added to solubilize the colored products. The viable fibroblast cells absorbed the dye and a purplish residue containing formazan crystals was formed in most of the wells. The absorbance was measured using an optical microplate reader or called as optical calorimeter [Figure 5] at a wavelength of 540 nm and the percentage of viable cells was calculated for 15 min, 30 min, 60 min, and 90 min. A graph of concentration versus inhibition was plotted.

RESULTS

Table 1 shows the cell viability of human periodontal cells at different time intervals with four experimental transport media. The mean and standard deviation were estimated from the results that were statistically analyzed using one-way analysis of variance (ANOVA) to identify the significant groups at $P \leq 0.5$. Graph 1 shows the percentage of cell viability found using MTT assay.

Figure 6 shows the scanning electron microscopic analysis.

Figure 5: Optical calorimeter

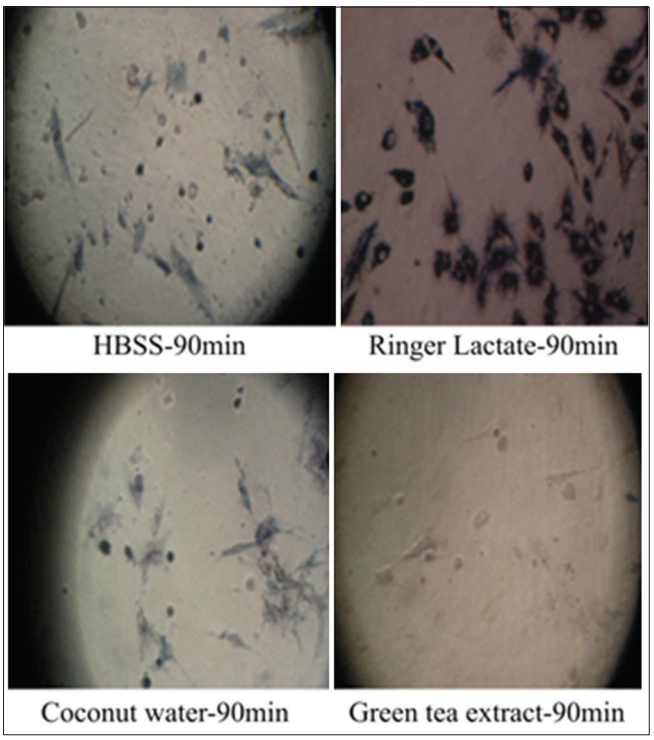
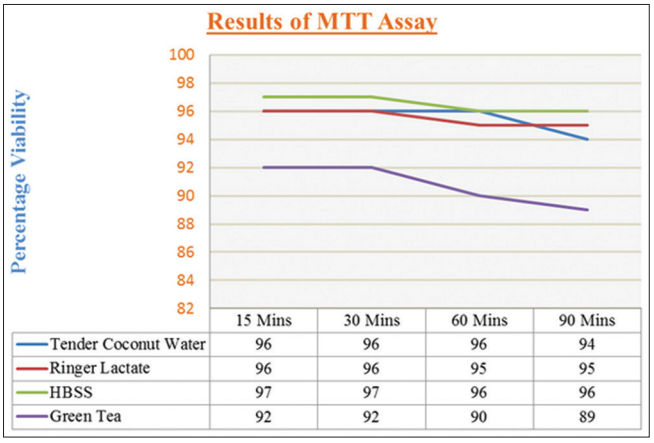


Figure 6: Scanning electron microscopic analysis



	15 Mins	30 Mins	60 Mins	90 Mins
Tender Coconut Water	96	96	96	94
Ringer Lactate	96	96	95	95
HBSS	97	97	96	96
Green Tea	92	92	90	89

Graph 1: Percentage of cell viability found using MTT assay

Table 1: Cell viability of human periodontal cells at different time intervals with four experimental transport media

Time	Group	Mean±SD	*P* value
15 min	Ringer's lactate	71.6±19.3	0.002
	HBSS	76.4±18.6	
	Coconut water	56.3±11.3	
	Green tea extract	56.3±12.9	
30 min	Ringer's lactate	61.3±18.2	0.003
	HBSS	71.4±12.6	
	Coconut water	46.6±11.2	
	Green tea extract	56.3±10.2	
60 min	Ringer's lactate	60.6±11.6	0.004
	HBSS	69.1±19.2	
	Coconut water	44.2±16.2	
	Green tea extract	56.2±10.2	
90 min	Ringer's lactate	59.4±18.6	0.02
	HBSS	60.1±10.6	
	Coconut water	41.3±16.0	
	Green tea extract	42.3±10.2	

SD=Standard deviation, HBSS=Hank's balanced salt solution

Results indicated that there was no statistically difference in the cell viability of Ringer's lactate, HBBS, and tender coconut water. Green tea extract showed lower viability after 90 min, when compared to the other three media.

DISCUSSION

Studies confirm that root resorption is a frequent complication in reimplanted teeth. Two of the most crucial factors affecting the prognosis of an avulsed tooth are extraoral dry time and the storage medium in which tooth is placed before reimplantation. Experimental studies have indicated that storage medium is a more critical prognostic factor than the extra-alveolar period.[6] Physiological storage media such as saliva, milk, HBSS, and viapan have been used for preserving the vitality of periodontal ligament cells.[7]

Tap water was found to be unsuitable due to its hypotonicity leading to rapid cell lysis. Tap water showed a decrease in periodontal ligament cell viability and increased external root resorption.[8]

Saliva was found to be more effective than tap water, but also has the potential for bacterial contamination. The osmolarity of saliva (60–80 Osm/l) was found to be much lower than the normal range (230–400 Osm/l) required for cell growth. Saliva is a hypotonic solution, causing periodontal cells to swell and burst.[9] Practically speaking, HBSS is not commonly available to a majority of the people at the time of an accident, hence milk has been advocated as an appropriate storage medium.[10]

Apart from maintaining the cell viability, a medium should be easily available, inexpensive, and simple to use. Tender coconut water has been proved to be a blood plasma substitute because of its sterility and biocompatibility.[11]

Green tea was chosen as a test solution because people in Asia generally drink green tea and it is easily available in the event of an accident. Substance in green tea used as allograft material and for cell study. In the dental field, green tea extract was known to protect alveolar bone resorption from periodontal disease because it inhibited the expression of matrix metalloproteinase-9 (MMP) in osteoblasts and the formation of osteoclasts. The commercial green tea used in this study showed very low osmolality, which might lead to cell death.[12]

The human gingival fibroblasts are the predominant cell type in the soft connective tissues of the periodontium and consequently play a central role in normal function and in pathologic alterations.[13] Fibroblasts used in the present study were cultured from atraumatically extracted premolar tooth with clinically healthy periodontium.

Browne *et al*. postulated that established cell lines such as BHK-21/C13 (baby hamster kidney fibroblasts), L929 fibroblasts, and human cervical carcinoma epithelial cells were used commonly for endodontic research. In the present study, human gingival fibroblasts were used at a density of 40,000 cells/ml, as they are easily cultured and are consistent in quality.[14]

Barnhart *et al*. conducted an *in vitro* study to determine the cytotoxicity of various irrigating solutions using the Cy-QUANT assay. This non-radioactive assay provides the potential to evaluate cytotoxicity in terms of cell proliferation.[15] In the present study, the cell viability was evaluated using the MTT tetrazolium method. The MTT assay is a quantitative colorimetric assay for *in vitro* cytotoxicity tests. Viable cells with active mitochondria cause cleavage of MTT dye into water-insoluble dark blue formazan crystals, whereas dead cells remain uncolored.[16] The main advantages of MTT assay are its rapidity, accuracy, and not requiring the use of radioisotopes. Therefore, it is a useful tool for evaluating the toxicity of newly developed drugs.

In the present study, the results obtained from the scanning electron microscopic analysis were similar to the cytotoxicity results. Numerous vacuoles occurred

in the cell cytoplasm with changes in cell shape and attachment, indicating cell death.

CONCLUSION

With the limitations of this *in vitro* study, the following conclusions are drawn:

- Within 15 min, 30 min, and 60 min, HBSS, Ringer's lactate, tender coconut water, and green tea extract were the most effective storage media
- Viability of cells in green tea extract after 90 min was slightly statistically inferior to the other three media
- Due to superior osmolarity, easier availability, and cost effectiveness, green tea extract, Ringer's lactate solution, and coconut water can be advocated as alternate and viable storage media.

REFERENCES

1. Hwang Young J, Choi Chu S, Park Hong J, Kang Wook S. The use of green tea extract as a storage medium for the avulsed tooth. J Endod 2011;37:962-7.
2. Andreasoe JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 4. Factors related to periodontal ligament healing. Endod Dent Traumatol 1995;11:76-89.
3. Söder PO, Otteskog P, Andreasen JO, Modéer T. Effect of drying on viability of periodontal membrane. Scand J Dent Res 1977;85:164-8.
4. Lindskog S, Blomlöf L, Hammarström L. Mitoses and microorganisms in the periodontal membrane after storage in milk or saliva. Scand J Dent Res 1983;91:465-72.
5. Layug ML, Barrett EJ, Kenny DJ. Interim storage of avulsed permanent teeth. J Can Dent Assoc 1998;64:357-63, 365-9.
6. Khademi AA, Saei S, Mohajeri MR, Mirkheshti N, Ghassami F, Torabi nia N, *et al*. A new storage medium of an avulsed tooth. J Contemp Dent Pract 2008;9:25-32.
7. Malhotra N, Cyriac R, Acharya S. Clinical implications of storage media in dentistry: A review. Endo 2010;4:179-88.
8. Blomlöf L, Otteskog P, Hammarström L. Effect of storage in media with different ion strengths and osmalalities on human peridontal ligament cells. Scand J Dent Res 1981;89:180-7.
9. Weine FS. Endodontic emergency treatment. In: Weine FS, editor. Endodontic Therapy. 6th ed. St. Louis: Mosby; 2004. p. 72-103.
10. Hiltz J, Trope M. Vitality of human lip fibroblasts in milk, Hanks balanced salt solution and Visapan storage media. Endod Dent Traumatol 1991;7:69-72.
11. Ediriwera ND. King coconut. Jakarta, Indonesia: Cord Asian Pacific Coconut Community (APCC); 1996. p. 43-7.
12. Shin BC, Ryu HH, Chung JH, Lee BR, Kim HL. The protective effects of green tea extract against L-arginine toxicity to cultured human mesangial cells. J Korean Med Sci 2009;24(Suppl):S204-9.
13. Ghabanchi J, Moattari A, Darafshi R, Andisheh Tadbir A, Khorshidi H, Shakib M. Effects of three commercial mouth rinses on the cultured fibroblasts: An *in vitro* study. J Dent (Shiraz) 2013;14:64-7.
14. Brown RM. The *in vitro* assessment of the cytotoxicity of dental materials--does it have a role? Int Endod J 1988;21:50-8.
15. Prado M, Santos Júnior HM, Rezende CM, Pinto AC, Faria RB, Simão RA, *et al*. Interactions between irrigants commonly used in endodontic practice: A chemical analysis. J Endod 2013;39:505-10.
16. Chowdhary KY, George JP, Gowda P, Rao JA. Human peroidontal ligament fibroblast response to rhPDGF-BB application on periodontally diseased root surfaces--*in vitro*. Growth Factors 2013;31:130-8.

How to cite this article: Bharath MJ, Sahadev CK, Ramachandra P, Rudranaik S, George J, Thomas A. Comparative evaluation of four transport media for maintaining cell viability in transportation of an avulsed tooth - An in vitro study. J Int Soc Prevent Communit Dent 2015;5:69-73.

Source of Support: Nil, **Conflict of Interest:** None declared.