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ORIGINAL RESEARCH

Comparative Evaluation of Different Storage Media on the Survival of pdl Cells: An In-Vitro Study

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ABSTRACT

Introduction: The extraoral dry time and the storage media used to store teeth before reimplantation have the greatest impact on the prognosis of avulsed teeth.

Aim: The purpose of this study was to evaluate and compare the efficacy of different storage media on viability of periodontal ligament cells at different time periods.

Materials and Methods: Fifty freshly extracted sound teeth with healthy PDL were selected for the present study. Teeth were divided into four groups with 10 teeth each depending on the storage media (HBSS, COCONUT WATER, ALOE VERA, MILK) used for storing freshly extracted teeth. Remaining 10 samples were divided into 2 groups with 5 teeth each to serve as control. Cell viability in each group was checked at 1hr, 2hrs, 4 hrs, 8 hrs and 24 hrs time period. The data were tabulated and subjected to statistical analysis using One way Analysis of Variance (ANOVA) with post hoc analysis (Tukey HSD) for comparison of means. The statistical software namely SPSS 21.0 was used to analysis of the data.

Results: The results indicated PDL cell viability was maximum at 1 hour time interval in all the groups and decreased over a period of time. Also, HBSS showed maximum percentage of viable PDL cells followed by Coconut water, Aloe Vera and milk at all time intervals tested.

Conclusion: It can be concluded that HBSS is the gold standard for the preservation of viable cells for a longer period followed by Coconut water and Aloe Vera.

Keywords: Periodontal Ligament Cells, HBSS, Coconut Water, Aloe Vera, Milk

INTRODUCTION

Tooth avulsion is defined as the complete loss of a tooth out of the alveolar bone socket as a result of an accident and represents severe traumatic dental injury. In children and adolescents, tooth avulsion typically affects the incisors, and it is frequently accompanied with unpredictability in the course of therapy and a financial burden. The most common age range is between 7 and 14 years.[1] Prognosis after reimplanting an avulsed tooth is largely dependent on extra-alveolar time and the storage media used to store the avulsed tooth. Therapeutic effectiveness of storage media depends on its osmolarity, pH, nutrient content,

and temperature of the media in order to sustain the viability of periodontal cells. The periodontal ligament cells should be able to undergo mitosis to create clones of damaged PDL fibroblasts that will cover the damaged surfaces of the root. Storage media should also be sterile, inexpensive, and readily available.[2] Various storage mediums are available like Hanks Balanced Salt Solution (HBSS), Aloe Vera, Coconut Water, Milk, tap water, saliva, ViaSpan, propolis, culture media, egg albumin, salvia officinalis, morusrubra, Emdogain, Eagle's medium, cryoprotective agents are available.[3] HBSS is a sterile, physiologically balanced isotonic standard salt solution that is commonly used in biomedical research to support the growth of various cell types. This solution is nontoxic; biocompatible with PDL cells; and the ingredients in HBSS can sustain and reconstitute the PDL cells depleted cellular components. HBSS is essential for cell survival because it is non-toxic, highly nutritive, and has an appropriate pH balance and osmolality. However, HBSS is not readily available to be used by the patient at the accident site.[4,5] Aloevera is a cactus-like plant in the Liliaceae family. It contains anti-inflammatory, antioxidant, antibacterial, antifungal, and anticarcinogenic properties as well as vitamins, enzymes, minerals, sugars, salicylic acids, and amino acids. For up to 9 hours, aloe vera at 10%, 30%, and 50% concentrations performed similarly to supplemented culture media.[4,6] The coconut, also known as the "tree of life," is a natural drink that is produced biologically and hermetically inside the coconut. Coconut water is the liquid endosperm of the coconut, and it is high in amino acids, proteins, vitamins, and minerals. It is a hypotonic solution that is more acidic than plasma and has a specific gravity of about 1.020, which is comparable to blood plasma.[5,7] Milk is significantly better than other solutions because its physiological properties, such as pH and osmolality, compatibility with PDL cells; however, because it is easy to obtain and free of bacteria, it is critical that it be used within the first 20 minutes after avulsion. Milk, as a gland secretion, contains epithelial growth factor, which stimulates the proliferation and regeneration of Malassez's epithelial cell rests and activates alveolar bone resorption. This eventually helps to isolate the bone tissue from the tooth and reduces the likelihood of ankylosis. Milk was as effective as HBSS for storing avulsed teeth for up to 6 hours, but it could not revive the degenerated cells.[5,8] In addition to storage media, extraoral time has been shown to influence maintaining cell viability. The purpose of this study is to evaluate and compare the efficacy of different storage media on viability of periodontal ligament cells at different time periods. Till date, no study has been done to comparatively evaluate different storage media (HBSS, Aloe Vera gel, Coconut water, Milk) at different timings on the survival of PDL cells. So, the aim of the study was to compare & evaluate the different storage media on the survival of periodontal ligament cells.The null hypothesis was that there is no difference in the number of viable cells in different storage media at different time intervals.

MATERIALS AND METHODS

The current study was conducted in the Dept. of Conservative Dentistry and Endodontics at IDST, Modinagar, UP after ethical approval from the Institutional Ethical Committee. Fifty freshly extracted teeth with intact crowns, closed apexes, and healthy PDL were obtained from the Dept of Oral & Maxillofacial Surgery in, IDST College, Modinagar.UP. Teeth with intact crown, closed apex and healthy PDLwere selected for the study. Teeth with cracks, fractures and bone losswere excluded from the study. Immediately after extraction, all the teeth were stored in respective storage media for the study purpose in order to maintain equal baseline standardity for viable cells. Fifty teeth were divided into four groups with 10 teeth each depending on the storage media used for storing freshly extracted teeth. Remaining 10 samples were divided into 2 groups to serve as control:

Group 1 (N = 5) Positive Control, Group 2 (N = 5) Negative Control (Tap Water), Group 3 (N = 10) Hbss, Group 4 (N = 10) Aloe Vera Gel, Group 5 (N = 10) Natural Coconut, Group 6 (N = 10) Milk.

Coronal 3mm of periodontal ligament was removed with sterile curette to remove the cells that might have been damaged during extraction and the teeth were transferred to storage media by holding the crown portion with extraction forceps and not disturbing the viable cells on root surface. The PDL tissue was scrapped and collected from the root portions of the teeth with the help of sterile curette from different storage media. These were incubated for 30 minutes in 15ml Falcons tubes with a 2.5ml solution of 0.2mg/ml-1 of collagenase and 2.4mg/ml-1 dispase grade II in phosphate buffered saline. After incubation, 50 µl of foetal bovine serum was added to each test tube. All the tubes were centrifuged for 4 minutes at 1000rpm.After centrifugation, the supernatant was removed with sterile micropipettes, and the cells were labelled with trypan blue staining. After the trypan blue exclusion test, the cells are viewed under 40X magnification with the help of hemocytometer to count the viable and non-viable cells.[4] The cells that take up the stain are non-viable cells and the cells that do not take the stain are viable cells. [(Total cells - Stained cells)/ Total Cells] X100 was used to calculate the viable cell percentage. Cell viability in each group were checked at 1 hr, 2 hrs, 4 hrs, 8 hrs and 24 hrs time period. Between each time period, the samples were stored in their respective storage media. The results were tabulated and subjected to statistical analysis using One way Analysis of Variance (ANOVA) with post hoc analysis (Tukey HSD) for comparison of means. The statistical software namely SPSS 21.0 was used to analysis of the data. P value <0.05 was considered statistically significant.

RESULTS

Positive control (collagenase and dispase II) showed maximum number of viable PDL cells at different time periods (1,2,4,8 and 24 hrs) while the negative control (Tap water) showed least number of viable cells. The results indicated that HBSS showed maximum percentage of viable PDL cells followed by Coconut water, Aloe vera and milk. (Table 1) Viability of cells in decreasing order was Group 1 (Positive Control)>Group 3 (HBSS)>Group 5 (Coconut water)>Group 4 (Aloe Vera)>Group 6 (Milk)>Group 2 (Tap Water) at all the time periods tested. Same superscript in each row depicts, statistically non-significant difference at different time intervals. Same superscript across a column depicts, statistically non-significant difference between the groups.

Table 1: Inter and intra group comparison for percentage of viable cells at different time intervals among different groups

Groups/ Time intervals (hrs)	1 hr	2 hr	4 hr	8 hr	24 hr
Group 1 (positive control)	67.0±2.75	-	-	-	-
Group II (Tap water)	9.2±0.83^{a}	2.2±2.48^{abcd}	0.2±0.4^{be}	0.0±0.0^{ce}	0.0±0.0^{d}
Group III (HBSS)	54.0±2.0^{a}	52.5±1.0^{ab}	51.6±0.84^{b}	49.9±0.99	47.0±0.84
Group IV (Alovera Gel)	37.2±0.91^{1}	30.9±0.99^{a}	29.4±1.42^{a}	26.2±3.22	11.8±1.54
Group V (Coconut water)	42.8±1.22^{1}	42.0±1.15^{ab}	40.2±1.61^{b}	38.0±1.41^{c}	36.8±0.78^{c}
Group VI (Milk)	26.0±0.94	21.2±0.99^{a}	19.2±0.99^{a}	11.7±1.25	3.50±1.08

DISCUSSION

The aim of this study was to compare & evaluate different storage media on the survival of periodontal ligament cells. Results of our study showed that maximum percentage of viable cells was seen in Group 3 (HBSS) followed by Group 5 (Coconut water), Group 4 (Aloe Vera gel), Group 6 (Milk) and Group 2 (Tap Water) at all time intervals tested. Group 3 (HBSS) showed maximum number of viable cells at all time periods when compared to other groups. This can be attributed to its optimal pH (7.4), osmolality (280 mosmol kg-1) and its constituents. It contains sodium chloride, D-glucose, potassium chloride, sodium bicarbonate, potassium phosphate, calcium chloride, and magnesium sulphate (monobasic) anhydrous.[2] These key metabolites helpreconstitute the depleted cellular components of the PDL cells, thus maintaining their viability for longer duration. Studies have reported that root resorption is delayed when avulsed tooth is soaked in HBSS for 30 minutes after extra oral dry time of 15-60 minutes.[9] The results of the study are in agreement with those of Hwang et al.who reported that HBSS maintains 90% of cell viability for 24 hours.[10]Adeli et al also reported maximum cell viability with HBSS as storage media when compared with tap water, whole milk, green tea extract and sucrose.[11] However, the results of this study are contradictory to study by Souza et al who reported that HBSS is inferior to milk. This difference could be attributed to lower temperature of HBSS and milk in their study. Lower temperature decreases the efficacy of HBSS due to low nutrient availability and formation of tetrazolium salts in formazan crystals. Group V (Coconut water) showed less percentage of viable cells than HBSS but more than other groups at all time intervals. Natural coconut water is sterile and has 93% water and 5% sugar, which gives it a high osmolalityIt contains a lot of proteins, vitamins, and minerals like potassium, calcium, and magnesium. Also, it shows mitotic, clonogenic activity and growth promoting characteristics that helps maintain the viability of PDL cells. Quimol et al stated that coconut water helps the cells form a monolayer by adhering to the culture wells and helps in maintaining viability similar to HBSS.[5] The findings of the study disagree with Moreira-Neto etal who stated that coconut water at 37^0C was less effective than milk in maintaining the cell viability.[13] Aloe vera gel (Group 4) showed less cell viability than HBSS and Coconut water but more than milk and tap water. This could be attributed to its optimal pH and its constituent parenchymal tissue (inner pulp) which contains proteins, lipids, amino acids, and other vital nutrients. Also, it contains catalase enzyme, an antioxidant that converts hydrogen peroxide to water and oxygen and suppression of the generation of these free radicals may improve the effectiveness of cell preservation and prevent lipid peroxidation.[14] Martin etal stated that the high success rate of alovera extract in protecting the cell viability might be due to its antibacterial and antifungal properties.[15] The results of our study are in accordance to the study conducted by Fulzele et al who demonstrated that aloe vera maintained PDL cells viability over a period of 120 mins.[14]Buttke et al. also proposed that storing avulsed teeth in medium containing one or more antioxidants found in alovera extract could improve reimplantation success.[16] Moazzami F. et al stated that aloe vera may be useful in the replantation of avulsed teeth because of its fibroblast stimulating properties.[17] Group 6 (Milk) showed less percentage of viable cells than HBSS, coconut water and aloevera but more than tap water at all time intervals tested. Milk has osmolality of 270 mOsm/kg and pH of 6.5 to 7.2, which is similar to extracellular fluid. The current study's findings contradict those of Olson et al, who reported that milk had a significant advantage over HBSS at 8 and 12 hours. Lekic et al stated that milk was effective for a short period of time and lost its effectiveness after 2-6 hours in vitro, and only cold milk was suitable for the preservation of the proliferation capacity of PDL cells.[18] Since milk is readily available in almost all situations, so it is widely accepted as a storage medium for short-term storage of avulsed teeth. Group 2 (Tap Water) showed least number of viable cells initially and gradually become zero at 24 hrs time interval. In this

study, tap water was used as a negative control. The pH of tap water ranges from 7.4 to 7.79, with an osmolality of 30 mOsm/kg. It is unsuitable for use as a storage medium for avulsed teeth due to bacterial contamination, hypotonicity, non-physiological pH, and osmolality, which promotes PDL cell lysis.[10,19] The results of our study are in agreement with several studies who found that cells stored in water do not retain their morphology, resulting in visible destruction and rapid cell death.[20] The findings were consistent with Blomlof's study, which found that water is damaging to PDL cells and is not a good storage medium at any time.Some studies have suggested that it could be used as a storage medium for very short periods of time where there are no other options. However, results of our study indicated that tap water was the least desirable storage medium. In view of this, tap water should be used only to avoid tooth dehydration, but it is inadequate for conservation of avulsed teeth.[21,22] Results of the present study showed that PDL cell viability was maximum at 1 hour time interval in all the groups and decreased over a period of time. Since there is no study done till date to evaluate the efficacy of HBSS, Coconut water, Aloe vera, Milk and tap water on the survival of the periodontal ligament cells at different time intervals, the results of this study cannot be contradicted or corroborated. Since there is a difference in the number of viable cells in different storage media at different time intervals, the null hypothesis was rejected. There is no ideal storage media till date, hence according to the results obtained HBSS and Coconut water can be used as long-term storage media and Aloe vera and Milk can be used as a short-term storage media.

CONCLUSION

Within the limitations of the study, it can be concluded that HBSS is the gold standard for the preservation of viable cells for a longer period followed by Coconut water and Aloe vera. In absence of HBSS, Coconut water is the best choice for the storage of avulsed teeth as it is easily available. Milk can also be used as short-term storage media of avulsed teeth. Further studies should be directed with different storage medias and also with different time periods, to evaluate which storage media best suits for the preserving the avulsed teeth for a longer period of time.

REFERENCES

1. Hegde, Mithra. Tooth Avulsion- A Dental Emergency. Indian J. Appl. Res.2013;3(11):370-372.
2. Lekic PC, Kenny DJ, Barrett EJ. The influence of storage conditions on the clonogenic capacity of periodontal ligament cells: implications for tooth replantation. Int. Endod. J. 1998;31(2):137-140.
3. Jain D, Dasar PL, Nagarajappa S. Natural products as storage media for avulsed tooth. Saudi Endod J.2022 Oct 23;5(2):107-13.
4. Babaji P, Melkundi M, Devanna R, S SB, Chaurasia VR, V GP. 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 Jan-Mar;11(1):71-75.
5. 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 May;34(5):587-9.
6. 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 Jul;39(4):327-32.
7. Protyusha GB, Sabarinath B. Coconut water. Br Dent J. 2021 Sep;231(5):268.
8. Ulusoy AT, Kalyoncuoglu E, Kaya S, Cehreli ZC et al evaluation of the effectiveness of goat milk in maintaining PDL cell viability in comparison with bovine milk and

commonly used and/or investigated storage media. Dent Traumatol. 2015 March;28(1):130-5.
9. Matsson L, Andreasen JO, Cvek M, Granath LE. Ankylosis of experiementally reimplanted teeth related to extraveolar period and storage environment. Pediatr Dent 1982; 4:327–30.
10. Hwang J Y, Choi SC, Park JH, Kang SW. The use of green tea extract as a storage medium for the avulsed tooth. J Endod. 2011 Jul;37(7):962-7.
11. Adeli F, Zabihi E, Abedian Z, Gharekhani S, Pouramir M, Khafri S, Ghasempour M. Comparative in vitro study of the effectiveness of green tea extract and common storage media on periodontal ligament fibroblast viability. European Journal of Dentistry. 2016 Jup Sep; 10(3):408-412.
12. Souza BD, Lückemeyer DD, Reyes-Carmona JF, Felippe WT, Simões CM, Felippe MC. Viability of human periodontal ligament fibroblasts in milk, Hank's balanced salt solution and coconut water as storage media. Int Endod J. 2011 Feb;44(2):111-5.
13. Moreira-Neto JJ, Gondim JO, Raddi MS, Pansani CA. Viability of human fibroblasts in coconut water as a storage medium. Int Endod J. 2009 Sep;42(9):827-30.
14. 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 Winter;40(1):49-52.
15. Poi WR, Sonoda CK, Martins CM, Melo ME, Pellizzer EP, de Mendonça MR, Panzarini SR. Storage media for avulsed teeth: a literature review. Braz Dent J. 2013 Sep-Oct;24(5):437-45.
16. Buttke TM, Trope M Effect of catalase supplementation in storage media for avulsed teeth. Dent Traumatol 2003:19;103-8.
17. Moazzami F, Asheghi B, Sahebi S. Effect of Four Different Media on Periodontal Ligament Cells Viability of Dry- Stored Dog Teeth. J Dent (Shiraz). 2017 Mar;18(1):24-29.
18. Chen F, Qi S, Lu L, Yuanzhi, Xu. Effect of storage temperature on the viability of human periodontal ligament fibroblasts. Dental Traumatology. 2014 Sep;31(1)24-28.
19. Hammarstrom L, Pierce A, Blomlöf L, Feiglin B, Lindskog S. Tooth avulsion and replantation – a review. Endod Dent Traumatol 1986;2:1–8.
20. Ozan F, Polat ZA, Tepe B, Er K et al. HBSS and milk with natural product propolis on enhancement of PDL cell viability. J Contemp Dent Pract. 2007 Sep1;9(6):17-24.
21. Blomlof L. Milk and saliva as possible storage media for traumatically exarticulated teeth prior to replantation. Swed Dent J Suppl. 1981;8:1-26.
22. Trope M. Clinical management of the avulsed tooth. Dent Clin North Am 1995;39:93–112.