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Original Article

The Use of herbal Extracts of Ocimum sanctum and Mimusops elengi as a Novel Storage Media for the Avulsed Tooth

K. Nidesh Kumar[1], Ch Srinivas Kumar[2], Vanga V Narsimha Rao[3]

[1]Post graduate Student, [2]Professor, Department of Pedodontics and Preventive Dentistry, Gitam Dental College and Hospital, Visakhapatnam, Andhra Pradesh

***ABSTRACT*:**

Background: Maintaining the viability of PDL cells of the avulsed tooth is of utmost importance for a successful re implantation procedure. The storage medium plays a significant role in this aspect, but the immediate availability of the commercial storage medium has been a major problem. Storage media prepared with readily available herbs like Ocimum sanctum (tulasi) and Mimusops elengi (bakul) which have anti-inflammatory, anti-oxidant, anti-bacterial and anti-fungal properties would be of great help in this regard. **Aim:** To evaluate the efficacy of Ocimum sanctum and Mimusops elengi as storage media for avulsed teeth. **Materials and Methods:** The periodontal ligament cells obtained from the scrapings of orthodontically extracted teeth were stored separately in Milk, and extracts of Ocimum sanctum and Mimusops elengi for 60 minutes. They were then transferred into a falcon tube containing 2ml of growth media and incubated for 24 hours followed by suspension in phosphate buffer solution for 10 minutes. The PDL cells were then kept in 5ml of Trypsin-EDTA solution for10 minutes and centrifuged for 5 minutes at 800 rpm. The residue collected is added with 0.4% Trypan blue solution to check the viability of the PDL cells using optical microscope. **Results:** The results indicate that Milk could preserve the viability of 88% of PDL cells, where in Ocimum sanctum and Mimusops elengi could maintain viability of 70% and 30% cells respectively. **Conclusion:** In the absence of commercially available storage medium, Ocimum sanctum extracts could be recommended as potential alternatives.

Key words: Avulsed teeth, PDL cells, Ocimum sanctum, Mimusops elengi, Cell Viability.

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Corresponding author: Dr. K. Nidesh Kumar, Gitam Dental College and Hospital, Visakhapatnam, Andhra Pradesh

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INTRODUCTION:

The incidence of dental trauma has significantly increased during the last decade mainly affecting anterior teeth of children between 7 and 12 years of age. Among all traumatic injuries to permanent dentition, avulsion has 1 to 16% of incidence.[1] The tooth avulsion which is complete displacement of the tooth from the alveolar socket leads to damage of the periodontal ligament cells and disruption of the blood supply to the pulp tissue. The success of the eventual treatment depends on the viability of the PDL cells which in turn depends largely on extra-alveolar time period and storage conditions.[2] Commercial media like Hank's Balanced Salt Solution (HBSS), and Viaspan are ideally suitable for storage of the avulsed teeth. Other solutions like milk, contact lens solution, culture media have also been used with varied success rate.[3] But the cost and ready availabilityof these agents at the site of the accident have been the major problems. Commonly available plants like Ocimum sanctum (tulsi) and Mimusops elengi (bakul)have been found to be having antimicrobial, anti-inflammatory, analgesic, and antipyretic properties and used to treat conditions like bleeding gums , arthritis, bronchitis, skin diseases and diabetis.[4]

AIM: To evaluate the efficacy of the herbal extracts of commonly available plants like Ocimum sanctum and Mimusops elengi against milk as storage media for avulsed teeth.

MATERIALS AND METHODS:

Sample collection: 36single rooted teeth atraumatically extracted for orthodontic reasons and free from caries, periodontal disease, hypoplasia and restorations were collected immediately after extraction and washed with sterile isotonic saline solution to remove the residual blood.[3]They were then placed randomly in one of the three experimental groups with 12samples in each group.

Preparation of the extracts of Ocinium sanctum and Mimusops elengi:

Fresh leaves of Ocimum sanctum and Mimusops elengi were collected and dried in a hot air owen after which they were coarsely powdered and placed in 200 ml of water on a heating mantle at 100^{o}C for 1 hour. The

obtained residue was then filtered with 0.2 micron filter paper and stored at – 4°C.[5]

Collection and dissociation of periodontal ligament cells:

The extracted teeth placed in different storage media were left for 1 hour and then taken out, washed with sterile isotonic saline solution to remove the storage medium. Then periodontal ligament cells from the apical two thirds of the teeth were scraped with a scalpel and collected in a petri dish. These cells are then suspended in TC-199 culture medium in a falcon tube and incubated at 37^0C temperature with 95% air and 5% CO_2 for 24 hours. The sub confluent tissue formed was then placed in phosphate buffer solution for 5 minutes and then dissociated with 0.25% trypsin EDTA solution for 5 minutes.[1,6]

Periodontal ligament cell viability assessment:

The collected residue was centrifuged for 5 minutes at 800rpm, the supernatant was discarded and the remaining pellet obtained was stained with equal amount of 0.4%(w/v) trypan blue solution.[7]

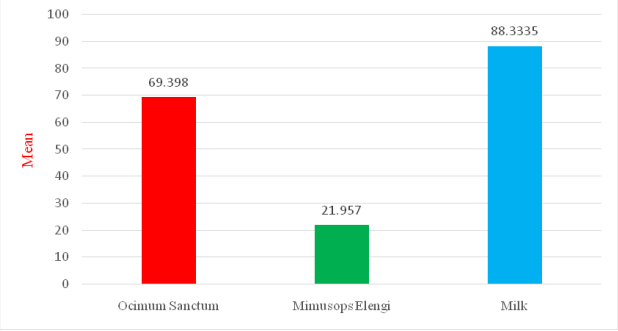
The rationale behind staining the cells with trypan blue dye was that damaged membrane of the cell allows the dye to pass through its cytoplasm and appear blue, while viable cells appear colourless or pink which can be evaluated with a light microscope at 40X magnification.[8]

STATISTICAL ANALYSIS:

Table 1: Comparison of Percentage of Viable cells among three study groups

Group	Mean no of viable PDL cells	Independent samples median test	Kruskal Willis
Ocimum Sanctum	69.3980		
Mimusops Elengi	21.9570	0.002	0.001
Milk	88.3335		

Figure 1: Bar chart of Percentage of Viable cells among three groups



The percentage of viable PDL cells was found to be 69.398 for Ocimum sanctum, 21.957 for Mimusops elengi, and 88.3335 for Milk. The ability of Ocimum sanctum in maintaining the viability of PDL cells was almost near to milk, where as Mimusops elengi was found to be least efficient of all.

DISCUSSION:

The ideal treatment option for avulsion is immediate replantation of the teeth as it re-establishes the nutrient supply, depreciates further damage, and accelerates the healing process of periodontal ligament cells on the root surface as it is stated that duration of survival for a replanted tooth is related to the number of viable periodontal ligament cells.[9]

Howevever, immediate reimplantation may not be feasible always as shown in the previous studies which have stated that approximately it takes about 10 to 15 minutes for the victim to recover from traumatic event and act properly.[10]And the study by Pool et al, has shown that periodontal ligament cells up-to 15minutes of dry time remain in a non-compromised state, and then they start deteriorating. This is the time, the role of the transport media becomes very significant. Ideally, the storage or transport media should provide an environment similar to the oral cavity with the osmolality and pH favourable for the optimal growth of the periodontal ligament cells.

HBSS is the standard solution recommended by the International Association of Dental Traumatology as the osmolality and pH of HBSS are 270 to 290mosmol/kg and 7.2 respectively providing a congenial environment for the PDL cells.[6]

Butte and Trope stated that the competence of the media will be enhanced by the presence of antioxidant ingredients. The study by Ozan et al also stated that the presence of antioxidant constituents in saliva officinal make it superior to HBSS.[3] Studies have also speculated that Aloe Vera is a better media in preserving the PDL cell viability due to the presence of antioxidant, antibacterial and antifungal activities.[1] The ability of propolis in maintaining the viability of the PDL cells was also thought to be because of its antibacterial and anti-inflammatory properties, as in the case of green tea also.[11]

Hence, the present study was intended to evaluate the efficacy of Ocimum sanctum and Mimusops elengi which have similar properties in maintaining the viability of the periodontal ligament cells. The present study has taken milk as the control group as Blomlof et al. stated that milk is a gold standard for transporting the avulsed teeth.[12]

Methods to estimate the number of viable periodontal ligament cells comprise step wise trypsinization, chromogenic stain method, step wise trypsinization along with and fluorescein diacetate etc., and the commonly used method is hemocytometer and trypan blue exclusion.[13] The trypan blue dye passes through cytoplasmic membrane of the damaged cell and the philosophy involved is that, chromosphere on the cell membranes which is negatively charged does adopt to trypan blue and appear blue, and this process does not happen for the viable cells which eventually appear colourless or pink.

In the present study, the superior results shown by Ocimum sanctum may be attributed to its high content of phenolic compounds and phytochemicals like flavonoids, tannins, terpenoids, and saponins in the

leaves and the stem which act as health promoting compound as a results of their anion radicals and consequently have a high antioxidant, anti inflammatory, antifungal, and antibacterial properties. The relative pH value of Ocimum sanctum is 7.4 which is also congenial for the health of PDL cells.[14]

The reason for the dismal performance of Mimusops elengi might be due to the fact that pH and osmolality of the prepared solution was not favourable for the growth of the PDL cells.[9]

The higher efficiency shown by milk can be attributed to the presence of preservatives in the packaged milk.[12]

CONCLUSION:

As a storage medium Ocimum Sanctum's efficiency seems to be on par with milk and could be a potential alternative to the conventional storage media with added advantage of omnipresence and easy availability.

REFERENCES:

1. 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(4):327-32.
2. Chen H, Huang B. Epigallocatechin-3-gallate: A novel storage medium for avulsed teeth. Dent Traumatol 2012;28(2):158-60.
3. Hwang JY, Choi SC, Park JH, Kang SW. The use of green tea extract as a storage medium for the avulsed. J Endod 2011;37(7):962-7.
4. Sahaa MR, Hasana SMR, Aktera R, Hossaina MM, Alamb MS, Alama MA, Mazumderc MEH. In vitro free radical scavenging activity of methanol extract of the leaves of mimusopselengilinn. Bangl. J. Vet. Med 2008;6(2):197–202.
5. Sircar B, Mandal S. Antibacterial Activity of Mimusopselengi Leaf, Seed and Bark extracts alone and in combination with antibiotics against Human pathogenic bacteria. Transl Med [internet] 2016 Nov [cited 2017 Dec 08]; 6(4): 1-6.
6. Sharma M, Sharma S, Reddy YG, Mittal R, Agarwal V, Singh C, Singh A. Evaluation of periodontal ligament cell viability in three different storage media: An in vitro study. J Dent Tehran 2015;12(7):524-31.
7. Ebenezar AV, T M, Priya J. Addition of L-dopa to HBSS in enhancing the maintenance of cell viability of Periodontal Ligament (PDL) cells: An In-Vitro Study. J Clin Diagn Res 2014;8(10):ZC79-80.
8. 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(1):71-74.
9. Khademi AA, Saei S, Mohajeri MR, Mirkheshti N, Ghassami F, Torabi NN, Alavi SA. A new storage medium for an avulsed tooth. The Journal of Contemporary Dental Practice 2008;9(6):1-7.
10. 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;21(2):93-101.
11. Martin MP, Pileggi R. A quantitative analysis of propolis: A promising new storage media following avulsion. Dent Traumatol 2004;20(2):85-89.
12. Courts FJ, Mueller WA, Henry J, Tabeling HJ. Milk as an interim storage medium for avulsed teeth.Pediatr dent 1983;5(3):183-186.
13. Pileggi R, Dumsha TC, Nor JE. Assessment of post-traumatic PDL cells viability by a novel collagenase assay. Dent Traumatol 2002;18(4):186-189.
14. Shafqatullah S, Khurram M, Asadullah A, Khaliqur rehman K, Khan FA. Comparative analyses of ocimum santum stem and leaves for phytochemicals and inorganic constituents. Middle-East Journal of Scientific Research 2013;13 (2): 236-240

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