Novel Herbal Storage Media for Exarticulated Teeth

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ABSTRACT

Introduction: Exarticulation is one of the most severe forms of dental trauma. Ideally, the tooth should be replanted immediately after the injury for better prognosis. Unfortunately, immediate repositioning of the tooth is not always possible. Recently researchers have proven that polyphenols can help in maintaining PDL cell viability. Such polyphenols are found in many herbal products like Cameillia sinensis, Punicia granatum, Vaccinia macrocarpon, Prunus domestica and Psidium guajava leaves which are not yet explored as storage media except Camellia sinensis. These herbal products being readily available worldwide might prove as a boon for effective storage capacity and maintenance of cell viability. This study was aimed to assess and compare the viability of periodontal ligament fibroblasts in the above mentioned herbal media.

Materials and methods: A strain of periodontal ligament fibroblasts was established from healthy premolar tooth extracted for orthodontic purpose and cultured in Dulbeco's Modified Eagle's medium. The cultivated cells were exposed to the different study media at 15mins, 30 mins, 1 hour and 3 hours. Cell viability was assessed Neutral red assay. The results obtained were statistically analysed.

Results: Vaccinium macrocarpon and Punicia granatum had greater mean optical density compared to the other study media. The optical density decreased as the time intervals increased. The viability reduction at 15 mins- 3 hours time intervals was seen least with Vaccinium macrocarpon which was 24.7%. ($p<0.05$).

Conclusion: Vaccinium macrocarpon can be used as potential storage media. Punicia granatum, Prunus domestica, Psidium guajava leaves and Camellia sinensis showed good cell viability.

Keywords: exarticulation, cell viability, storage, herbal

INTRODUCTION

Traumatic injuries to the anterior teeth occur mainly in the 7 to 10 years of age group, of which 0.5%-16% result in tooth avulsion. Avulsion injury is one of the most severe form of dental trauma. Due to the complexity of this injury, the neurovascular supply is severely compromised and usually results in loss of pulp vitality. The success for a favourable prognosis of an exarticulated tooth occurs when it is immediately replanted. This is not practically possible and hence a suitable storage medium is required to preserve the PDL cell viability till the replantation is carried out. The question is which are the various media that can be used as means of storage.

In developing countries, accessibility of HBSS storage medium is dubious. Besides, the cost is a major concern. Immense studies have been done on different storage media which can aid in maintaining the viability of periodontal ligament cells.[1] However, none of the currently used media is proficient to meet all the ideal requirements which can help in maintaining cell viability. Hence, the hunt for a suitable storage medium continues. Why can't we have a look into our backyard and try for the option which are readily available, feasible and economical. Recently, researchers have proven that the polyphenols of green tea can help in maintaining such PDL cell viability. Such polyphenolic contents are present in, Cameillia sinensis[2], Punicia granatum[3], Vaccinia macrocarpon[4], Prunus domestica and Psidium guajava leaf extract[5] and research says that they help prevention of adhesion of streptocoocus strains. Unfortunately, they are not yet explored as storage media for exarticulated teeth. These herbal products being readily accessible at the trauma site might prove as a boon for effective storage capacity and maintenance of cell viability.

Keeping in mind the benefits of these herbal media and their accessibility, this study was aimed to evaluate their potential in maintaining PDL cell viability in cases of exarticulated teeth.

MATERIALS AND METHOD

The research study was undertaken at the Department of Microbiology. The study was approved by the Institutional review board. The Human PDL fibroblasts used in the study were obtained from healthy premolar indicated for extraction for orthodontic purpose.

Following extraction, the tooth was washed with sterile solution to remove the blood and the tissue harvesting procedure was carried out.[6] The tissue culture and experiment was carried out under laminar flowhood. PDL tissue was scraped from the root surface with a No 11 sterile scalpel blade and the scrapings were further transferred to a 24 well culture plate. The explants were checked for viability after incubation at 37° with Dulbeco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 u/ml penicillin, streptomycin 100u/ml and amphotericin 2.5 mg/ml. The cells were allowed to reach confluence and passages 3-4 were used for the study.

Study media: The cells were assessed in the following study

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How to cite this article: Rajesh T. Anegundi, Shukla Nisha Paritosh, Anand Tavargeri, Shruthi Patil, Vijay Trasad, Prashant Battepati. Novel herbal storage media for exarticulated teeth. International Journal of Contemporary Medical Research 2016;3(3):729-732.

media for 15 mins, 30 mins, 1 hour and 3 hours time intervals.
Group 1: Vaccinium macracarpon (Cranberry)
Group 2 : Punicia granatum(Pomegranate)
Group 3: Prunus domestica(Plum)
Group 4: Psidium guajava leaves (Guava)
Group 5: Camellia sinensis(Green Tea)

Preparation of study media: Pure juices of Vaccinium macracarpon, Punicia granatum[7] and Prunus domestica were prepared. The psidium guajava leaf extract was prepared by soaking 400 g of leaves in 200 ml of distilled water.[8] Camellia sinensis extract was prepared by soaking 10 g of leaves in 100 ml of boiling distilled water for 5 minutes.[9]
All the prepared study media were filter sterilized with Whatman filter paper.

Assessment of PDL Cell viability in study media: The experimental PDL cells obtained from cell passages 3-4 were washed with PBS suspended with DMEM and placed in 96 well culture plate. 100 µl study media was added ontoeach well and the well plate was kept at room temperature for 15 minutes, 30 minutes, 1 hour and 3 hours time intervals. The cell viability was assessed with neutral red assay. 200 µl of neutral red solution was added into each well. The well plates were incubated for 9 mins at 37°C, 95% O2/5% CO2 conditions. To remove the neutral red dye, the well were subsequently washed with PBS, followed by addition of desorb solution. (100 µl Glacial acetic acid + 5 ml ethanol + 4.9 ml of water.) Further the well plates were incubated for 20 mins. The absorbance was recorded with spectrophotometer at 540 nm.

STATISTICAL ANALYSIS

The obtained data was subjected for statistical analysis. The statistical calculations were executed using the SPSS v21.0 Statistical software. Differences in cell viability among the various test media were statistically analysed by Kruskal Wallis ANOVA test. Intergroup comparision was carried out with Wilcoxon matched pairs test. Intergroup comparision was done with Mann Whitney U Test.

RESULTS

The mean optical density obtained at different time intervals was tabulated (Table 1, Figure 1). The optical density of Vaccinium macrocarpon was significantly more compared to the other four study media. It was noted that Vaccinium macrocarpon relatively maintained a constant cell viability even when the time intervals increased. Prunus domestica showed an increase in optical density from 15 mins to 30 mins time intervals which might indicate a proliferative capacity of Prunus domestica. The same can be implied for leaves of Psidium guajava which showed an increase in the optical density from 1 hour to 3 hours.
On intragroup comparision (Table 2), least reduction in viability from 15 minutes to 3 hours was seen with Vaccinium macrocarpon. In Vaccinium macrocarpon, Punicia grantum and Camellia sinensis, the cell viability was noted to be decreased with increased time intervals.

DISCUSSION

There has been tremendous research on the various beneficial effects of the herbal means in the fields pertaining to medicine.Be it a mention of Indian mythology or be it during the Anglo Saxon period that is from early fifth century to 1066 A.D., most cures were based on herbal remedies. Documentary sources however provide only limited evidence. The Leech books of Bald, compiled in the ninth century suggests a recipe for a broken mouth inside:[11]
"Take a plum (Prunus domestica) 's leaf, boil it, let him swill his mouth with it".

The various proven benefits are listed in following table.

Herbal media	Medicinal value
Vaccinium macrocarpon	'Super fruit', for scurvy and gastrointestinal disorders, treating urinary tract infections, preventing adhesion of Helicobacter pylori to the gastric mucosa, seasonal influenza., cardiovascular diseases, inhibition of the colonization of dental surfaces by oral streptococci [13-23]
Punicia granatum	Bactericidal, stimulant, refrigerant, astringent, styptic, laxative, diuretic and antihelminthic, suppression of prostaglandin synthesis, cardiovascular diseases, atherosclerosis, denture stomatitis, obesity, malaria, prostate cancer, inflammation, cough, Alzheimer's disease, sores and ulceration, forms bonds with collagen fibers, plaque inhibition and periodontitis as well as dental caries due to its inhibitory effect on adhesion of S.mutans and S.mitis.[24-28]
Psidium guajava	Used in food and in folk medicine, recommended for use in gastroenteritis, diarrhea, dysentery, rheumatic pain, wounds, ulcers, toothache, respiratory disturbances, miscarriages, scabies, sore throats,Pontikis in 1996 suggested its use for toothache, plaque inhibition [29]
Prunus domestica	Another variant, Prunus mume used in chinese medicine, antimicrobial and anti-inflammatory properties of prunus mume against oral pathogens.[30]
Camellia sinensis	Antiinflammatory, antioxidant and anticarcinogenic effects, used for allografts survival.[31,32]

Keeping in mind the medicinal effects of these herbal means, they were chosen as the study media.
On assessing the results obtained by Neutral red assay, greater optical density was obtained with Vaccinium macrocarpon compared to the other four groups at 15 mins time interval. The cell viability showed a further steady decline with respect to Vaccinium macrocarpon as the time intervals increased.Punicia granatum also showed a steady decline in the cell viability as the time intervals increased. A similar

Groups	15 min		30 min		01 Hrs		03 Hrs	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.753	0.035	0.643	0.024	0.545	0.024	0.441	0.027
2	0.592	0.015	0.564	0.014	0.534	0.019	0.492	0.021
3	0.505	0.010	0.515	0.009	0.481	0.012	0.415	0.016
4	0.598	0.017	0.446	0.043	0.379	0.030	0.483	0.020
5	0.585	0.017	0.554	0.008	0.511	0.014	0.472	0.020

Table-1: Mean Optical Density and Standard Deviation

Groups	Time duration			
	15 mins	30 mins	1 hour	3 hours
1 vs 2	p=0.0040*	p=0.0040*	p=0.2623	p=0.0104
1 vs 3	p=0.0040*	p=0.0040*	p=0.0040*	p=0.1093
1 vs 4	p=0.0040*	p=0.0040*	p=0.0040*	p=0.0250*
1 vs 5	p=0.0040*	p=0.0040*	p=0.0250*	p=0.0656
2 vs 3	p=0.0040*	p=0.0040*	p=0.0040*	p=0.0040*
2 vs 4	p=0.5218	p=0.0040*	p=0.0040*	p=0.4712
2 vs 5	p=0.5218	p=0.1282	p=0.0656	p=0.2002
3 vs 4	p=0.0040*	p=0.0104*	p=0.0040*	p=0.0040*
3 vs 5	p=0.0040*	p=0.0040*	p=0.0065*	p=0.0040*
4 vs 5	p=0.2002	p=0.0040*	p=0.0040*	p=0.4712

Table-2: Intergroup comparision

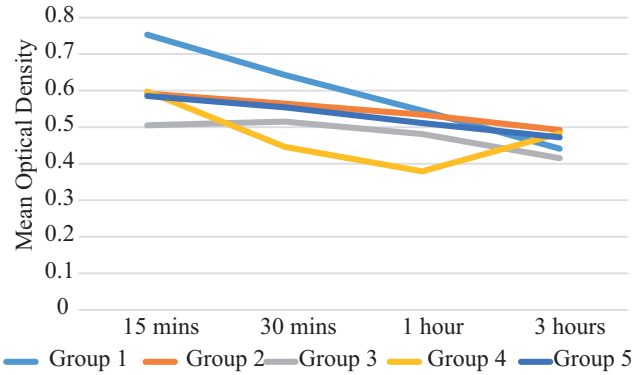

Figure-1: Intragroup comparision

study to assess the efficacy of Punicia granatum as suitable storage medium was done by Hojjati et al 2014. The results of the study indicated that 7.5% of Pomegranate juice is effective in maintaining cell viability. Thus, it can be used as a suitable transport medium.[8] In relation to Prunus domestica, the relative cell viability was noted to be increased from 15 mins to 30 mins time interval. At further time intervals it was noted to be decreased whereas in relation to leaves of Psidium guajava, the cell viability was relatively increased from 1 hours to 3 hour time intervals indicating that these two groups have a proliferative capability. Camellia sinsensis showed a steady decline in the viability as the time intervals increased. A study by Hwang et al conducted (2011) evaluated the effect of Green tea extract on avulsed-teeth preservation. Findings were comparable to the present study and confirmed that Green Tea extract could maintain PDL cell viability of extracted tooth and is able to postpone the period of tooth storage.[10] A similar study was done by Ghaesmpour et al (2015)[12] to evaluate the potential of Green Tea Extract in periodontal ligament cells preservation in cases of avulsion. The viability was assessed with Trypan Blue dye exclusion test. The specimen were assessed for 1, 3 and 4 hours. No significant difference in viability was noted at 1 hour and 3 hours time intervals whereas with respect to the present study a significant difference was seen. It was revealed that green tea and HBSS are equally effective in maintaining PDL cell viability.

CONCLUSION

From this study, it was observed that the five herbal study media can be used as efficient transport media for avulsed teeth. Vaccinium macrocarpon had the ability to preserve viable cells even when the extraalveolar period is increased and showed a least reduction in viability of 24.7 % from 15 mins to 3 hours time intervals as compared to other study media. It maintained relatively constant cell viablility at 15 mins and 30 mins time intervals. Punicia granatum has an ability to preserve more number of viable cells at the initial time intervals and the viability declined as the time intervals increased. Prunus domestica and Psidium guajava leaves showed increase in cell viability at 15 mins-30 mins and 1 hour to 3 hours time intervals respectively suggesting that these 2 media might have a proliferative capacity or it could be a procedural error. Camellia sinensis like Punicia granatum showed a steady decline in the cell viability.

RECOMMENDATIONS

- Vaccinium macrocarpon has shown promising results in terms of its efficacy to maintain PDL cell viability and can be used as a suitable storage medium.
- The other four study media also maintained a relatively good cell viability and hence can be used shorter extraalveolar period of upto 30 mins to 1 hour.
- Long term research might be needed in assessment of the efficacy of these herbal media.
- The polyphenolic compounds of the herbal media can be segregated and assessed for its efficacy as potential storage media.
- The common man should be made more aware regarding the potential benefits of the herbal media which are accessible, cost effective and feasible.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 22-01-2016; **Published online**: 12-02-2016