Novel Herbal Storage Media for Exarticulated Teeth

Rajesh T. Anegundi¹, Shukla Nisha Paritosh², Anand Tavargeri³, Shruthi Patil⁴, Vijay Trasad⁵, Prashant Battepati⁶

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. 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 sinensis², Punicia granatum³, Vaccinia macrocarpon⁴, Prunus domestica and Psidium guajava leaf extract⁵ and research says that they help prevention of adhesion of streptococcus 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.² 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 37⁰ 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 media:

¹Professor and Dean, Patient Care Committee, ²Postgraduate, ³Professor and HOD, ⁴Professor, ⁵Associate Professor, ⁶Assistant Professor, Department of Pediatric Dentistry, SDM College of Dental Sciences and Hospital, Sattur, Dharwad, Karnataka, India.

Corresponding author: Dr Rajesh T. Anegundi, Department of Pediatric Dentistry, SDM College of Dental Sciences and Hospital, Sattur, Dharwad, Karnataka- 580009, India

media for 15 mins, 30 mins, 1 hour and 3 hours time intervals.
Group 1: Vaccinium macrocarpon (Cranberry)
Group 2 : Punica 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 macrocarpon, Punica granatum and Prunus domestica were prepared. The psidium guajava leaf extract was prepared by soaking 400 g of leaves in 200 ml of distilled water. Camellia sinensis extract was prepared by soaking 10 g of leaves in 100 ml of boiling distilled water for 5 minutes.

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 onto each 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 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, Punica granatum 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:”

“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** |
| Punica granatum | Bactericidal, stimulant, refrigerant, astringent, styptic, 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. |
| 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. |
| Prunus domestica | Another variant, Prunus mume used in chinese medicine, antimicrobial and anti-inflammatory properties of prunus mume against oral pathogenesis. |
| Camellia sinensis | Antiinflammatory, antioxidiant and anticarccinogenic effects, used for allografts survival. |

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. Punica granatum also showed a steady decline in the cell viability as the time intervals increased. A similar
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. 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 hour to 3 hour time intervals indicating 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.

### 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 viability 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.

### REFERENCES

4. Yoo S, Murata RM, Duarte S. Antimicrobial traits of Tea- and Cranberry Derived Polyphenols against Strep-

---

**Table 1:** Mean Optical Density and Standard Deviation

<table>
<thead>
<tr>
<th>Groups</th>
<th>15 min Mean</th>
<th>15 min SD</th>
<th>30 min Mean</th>
<th>30 min SD</th>
<th>01 Hrs Mean</th>
<th>01 Hrs SD</th>
<th>03 Hrs Mean</th>
<th>03 Hrs SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.753</td>
<td>0.035</td>
<td>0.643</td>
<td>0.024</td>
<td>0.545</td>
<td>0.024</td>
<td>0.441</td>
<td>0.027</td>
</tr>
<tr>
<td>2</td>
<td>0.592</td>
<td>0.015</td>
<td>0.364</td>
<td>0.014</td>
<td>0.534</td>
<td>0.019</td>
<td>0.492</td>
<td>0.021</td>
</tr>
<tr>
<td>3</td>
<td>0.505</td>
<td>0.010</td>
<td>0.515</td>
<td>0.009</td>
<td>0.481</td>
<td>0.012</td>
<td>0.415</td>
<td>0.016</td>
</tr>
<tr>
<td>4</td>
<td>0.598</td>
<td>0.017</td>
<td>0.446</td>
<td>0.043</td>
<td>0.379</td>
<td>0.030</td>
<td>0.483</td>
<td>0.020</td>
</tr>
<tr>
<td>5</td>
<td>0.585</td>
<td>0.017</td>
<td>0.554</td>
<td>0.008</td>
<td>0.511</td>
<td>0.014</td>
<td>0.472</td>
<td>0.020</td>
</tr>
</tbody>
</table>

**Table 2:** Intergroup comparison

<table>
<thead>
<tr>
<th>Groups</th>
<th>15 mins</th>
<th>30 mins</th>
<th>1 hour</th>
<th>3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>p=0.004*</td>
<td>p=0.004*</td>
<td>p=0.2623</td>
<td>p=0.0104</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>p=0.004*</td>
<td>p=0.004*</td>
<td>p=0.040*</td>
<td>p=0.193</td>
</tr>
<tr>
<td>1 vs 4</td>
<td>p=0.004*</td>
<td>p=0.004*</td>
<td>p=0.040*</td>
<td>p=0.0250*</td>
</tr>
<tr>
<td>1 vs 5</td>
<td>p=0.004*</td>
<td>p=0.004*</td>
<td>p=0.0250*</td>
<td>p=0.00656</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>p=0.0040*</td>
<td>p=0.0040*</td>
<td>p=0.0040*</td>
<td>p=0.0040*</td>
</tr>
<tr>
<td>2 vs 4</td>
<td>p=0.5218</td>
<td>p=0.0040*</td>
<td>p=0.0040*</td>
<td>p=0.4712</td>
</tr>
<tr>
<td>2 vs 5</td>
<td>p=0.5218</td>
<td>p=0.1282</td>
<td>p=0.0656</td>
<td>p=0.2002</td>
</tr>
<tr>
<td>3 vs 4</td>
<td>p=0.0040*</td>
<td>p=0.0104</td>
<td>p=0.0040*</td>
<td>p=0.0040*</td>
</tr>
<tr>
<td>3 vs 5</td>
<td>p=0.0040*</td>
<td>p=0.0040*</td>
<td>p=0.0065</td>
<td>p=0.0040*</td>
</tr>
<tr>
<td>4 vs 5</td>
<td>p=0.00202</td>
<td>p=0.0040*</td>
<td>p=0.0040*</td>
<td>p=0.4712</td>
</tr>
</tbody>
</table>

**Figure 1:** Intragroup comparison
32. Friedman M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res. 2007;51:116-34.

Source of Support: Nil; Conflict of Interest: None
Submitted: 22-01-2016; Published online: 12-02-2016