Assessment of the viability of human periodontal ligament cells in black tea, lime juice, and passion fruit concentrate – A comparative in vitro study

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Abstract

Background: Tooth avulsion is considered as a severe form of dental trauma, causing damage to the periodontium. Hence, the preservation of healthy periodontal ligament (PDL) cells in the storage medium are pivotal for the success of replantation.

Aim and Objective: The aim of this study is to assess the viability of human PDL cells in black tea, lime juice, and passion fruit concentrate.

Methods: Human periodontal cells were cultured and stored in three experimental media – black tea, lime juice, and passion fruit concentrate and subjected to 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay for 1 h and the cell viability was determined. Mean and standard deviation were statistically analyzed using one-way analysis of variance to identify the significant groups.

Results: The human PDL cells showed 100% viability in lime juice and passion fruit concentrate, followed by 98% viability in black tea.

Conclusion: Black tea, lime juice, and passion fruit concentrate can be used effectively as storage media for maintaining PDL cells viability in avulsed teeth, with 100% viability exhibited by lime juice and passion fruit concentrate.

Keywords: Avulsion; black tea; lime juice; passion fruit; storage media; viability

INTRODUCTION

An avulsion is defined as the complete displacement of a tooth from its socket in alveolar bone owing to trauma.1 Periodontal ligament (PDL) tissues begin to dehydrate the following avulsion, and its vitality plays a pivotal role in the successful healing of replanted teeth,2,3 for which various storage media have been suggested.4-6 Yet, there is a continuous search for an ideal and accessible storage media.

Hence, the present study aimed to assess the viability of human PDL cells in black tea, lime juice, and passion fruit concentrate as alternatives to already available and previously proposed storage media for the avulsed tooth.

METHODS

The viability of human PDL cells were assessed in vitro, using L929 fibroblast cell line in three different media, namely, Black tea, Lime juice, and Passion fruit concentrate and were compared.
Cell line culture
The cell line (L929 fibroblasts) was propagated in Dulbecco modified minimum essential medium in a humidified atmosphere of 5% CO₂ and 95% O₂ at 37°C with antibiotic and anti-mycotic solutions, to prevent contamination and were incubated for 48 h and then visualized under inverted microscope.[7]

Sample preparation
The black tea solution was prepared by boiling the tea powder (Brookbond 3 roses, Hindustan Unilever Limited, India) in 50 ml of water at 100°C for 10 min.[8] The solution was allowed to cool and filtered. Lime juice was prepared by squishing five lemons, and the solution was filtered. Passion fruit concentrate was prepared by crushing two fruits in a mixer, and then the solution was filtered. All three test solutions were then transferred to small plastic containers of 5 ml capacity filled in five containers for each sample.

Cell viability analysis
The test samples were divided into the following three groups, which were tested against Control containing only cells in the well with no added test solutions.

- **Group 1** – Black tea solution
- **Group 2** – Lime juice
- **Group 3** – Passion fruit concentrate.

The test solutions were then transferred and inoculated into the 96-well culture plates containing the fibroblasts cells and placed in an incubator of 5% CO₂ at 37°C for 24 h and subjected to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT substrate was then prepared in a physiologically balanced solution, added to cells in culture, at a final concentration of 0.2–0.5 mg/ml, and incubated for 1 h. The quantity of formazan (presumably directly proportional to the number of viable cells) was measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer.[9] The percentage of viable cells for each test sample was then calculated at the end of 1 h.

The mean and standard deviation were estimated from the triplicate values of results that were statistically analyzed (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA.) using one-way analysis of variance to identify the significant groups at P ≤ 0.05.

RESULTS
The cell viability of human periodontal cells at different concentrations with three experimental transport media is shown in Table 1. The difference in the cell viability among the 10% concentration of lime, black tea, passion fruit and control is statistically significant (P < 0.05). No statistically significant difference has been found in the cell viability among the other concentrations of lime, black tea, passion fruit, and control.

The results show that the human PDL cells showed 100% viability in lime juice and passion fruit concentrate, followed by 98% viability in black tea.

DISCUSSION
The most severe type of traumatic tooth injury is avulsion because it causes damage to several structures and also results in the complete displacement of the tooth from its socket in the alveolar bone.[10] Hence, the ideal solution is to replant an ex-articulated tooth immediately after an avulsion, because the extra-oral time is one of the determinant factors for treatment success and for a good prognosis. This statement is in agreement with Lekic et al., who confirmed in their study that the in vitro clonogenic capacity of PDL cells is temporarily associated with the ability of PDL progenitor cells to attach and recolonize the root surface after replantation. The critical period for rapid loss of cell viability begins 15 min after tooth storage notably.[11] Moreover, an extra-oral dry time of 60 min makes the survival of the periodontal cells unlikely, and literature supports moist storage to be a more productive approach to optimize their survival.[12] Furthermore, the choice of storage medium for preserving traumatically avulsed teeth is important for the success of future replantation.[13]

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

| Concentrations (%) | Media                | Mean  | Standard deviation | P value for ANOVA |
|---------------------|----------------------|-------|--------------------|-------------------|
| 10                  | Lime juice           | 79.78 | 4.13               | 0.002             |
|                     | Black tea            | 88.09 | 1.53               |                    |
|                     | Passion fruit concentrate | 94.79 | 2.41               |                    |
|                     | Control              | 100   | 0.00               |                    |
| 5                   | Lime juice           | 82.48 | 4.09               | 0.120             |
|                     | Black tea            | 86.68 | 4.39               |                    |
|                     | Passion fruit concentrate | 95.69 | 9.83               |                    |
|                     | Control              | 100   | 0.00               |                    |
| 2                   | Lime juice           | 96.99 | 3.30               | 0.121             |
|                     | Black tea            | 88.88 | 4.91               |                    |
|                     | Passion fruit concentrate | 97.19 | 5.55               |                    |
|                     | Control              | 100   | 0.00               |                    |
| 1.25                | Lime juice           | 98.19 | 4.12               | 0.116             |
|                     | Black tea            | 94.79 | 2.41               |                    |
|                     | Passion fruit concentrate | 100.09 | 6.37       |                    |
|                     | Control              | 100   | 0.00               |                    |
| 0.625               | Lime juice           | 95.69 | 9.83               |                    |
|                     | Black tea            | 95.69 | 9.83               |                    |
|                     | Passion fruit concentrate | 100.60 | 3.83       |                    |
|                     | Control              | 100   | 0.00               |                    |

The difference in the cell viability among the 10% concentration of lime, black tea, passion fruit, and control is statistically significant (P < 0.05). ANOVA: Analysis of variance.
In the present study, the cell viability was evaluated using the MTT assay, which 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.\[17\]

Tea is the second most consumed beverage in the world, and the ease of its availability made black tea to be tested as one of the samples. Commercially available green tea was tested for its avulsion tooth storage properties and advocated it as an alternate due to superior osmolality, easier availability, and cost-effectiveness.\[9\] The PDL cell viability in Black tea might be attributed to various properties shared by both green and black tea. The presence of similar catechins and flavonoids in green and black tea might contribute to the proven anti-oxidant and anti-inflammatory effect. Certain tea infusions are also known to have high fluoride levels, the presence of which is appreciated in this scenario. Chatterjee et al. concluded that both green and black tea leaves possess a marked anti-inflammatory effect against in vitro denaturation of the protein.\[18\]

*Citrus limon* (Lime), a potential source of vitamin C is known for its antibacterial potential, anti-oxidant and anti-cancer activities.\[19\] Its *in vitro* anti-cariogenic potential has also been proved.\[20\] Lime juice exhibited 98% viability of cells, and this reduction in its viability as compared to the other two samples, might be attributed to the acidic pH range, and also the absence of water (concentrated solution). Also, a minimal concentration of 0.625% passion fruit concentrate exhibited highest viability compared to 10% lime juice which showed lowest viability [Figure 1]. It has been documented for the presence of nonnutritive phytochemicals, carotenoids, and polyphenols within the fruit with anti-oxidant properties.\[21\] Septembre-Malaterre et al. characterized the ability of polyphenol-rich fruit extract of passion fruit to protect cells against free radical damage and high concentration of Vitamin C.\[24\]

**CONCLUSION**

Under the limitations of this study, it can be concluded that Lime juice, Black tea, and passion fruit concentrate...
can be used as effective alternatives for avulsed tooth storage, because of their ability to maintain high viability. Furthermore, black tea and lime juice are easily accessible popular beverages around the world. While passion fruit is not very accessible, its desirable anti-oxidant and high viability properties, make it a choice, which can be marketed commercially for usage as a storage medium.

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**Conflicts of interest**
There are no conflicts of interest.

**REFERENCES**

1. Bastone EB, Freer TJ, McNamara JR. Epidemiology of dental trauma: A review of the literature. Aust Dent J 2000;45:2-9.
2. Flores MT, Andersson L, Andreassen JO, Bakland LK, Malmgren B, Barnett F, et al. Guidelines for the management of traumatic dental injuries: I. Fractures and luxations of permanent teeth. Dent Traumatol 2007;23:66-71.
3. Panzarini SR, Gulinelli JL, Poi WR, Sonoda CK, Pedrini D, Brandini DA. Treatment of root surface in delayed tooth replantation: A review of literature. Dent Traumatol 2008;24:277-82.
4. 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;34:987-9.
5. Caglar E, Sandalli N, Kuscu OO, Durban MA, Pisiriciler R, Caliskan EA, et al. Viability of fibroblasts in a novel probiotic storage media. Dent Traumatol 2010;26:383-7.
6. Malhotra N. Current developments in interim transport (storage) media for periodontal ligament cell viability, mitogenicity, and clonogenic capacity to plating efficiency and vital dye staining of human periodontal ligament cells: Implications for tooth reimplantation. J Periodontal Res 1996;31:294-300.
7. Goewami M, Chatra T, Chaudhary S, Manuja N, Sinha A. Strategies for periodontal ligament cell viability: An overview. J Conserv Dent 2011;14:215-20.
8. Ashkenazi M, Sarnat H, Keila S. In vitro viability, mitogenicity, and clonogenic capacity of periodontal ligament cells after storage in six different media. Endod Dent Traumatol 1999;15:149-96.
9. Khademi AA, Saei S, Mohajeri MR, Mirkhestiti N, Ghassami F, Torabnia N, et al. A new storage medium for an avulsed tooth. J Contemp Dent Pract 2008;9:25-32.
10. Malhotra N, Cyriac R, Acharya S. Clinical implications of storage media in dentistry: A review. Endodontic Pract Today 2010;4:179-88.
11. Ghabanchi J, Moattari A, Darafshi R, Andisheh Tadbir A, Khorsandi H, Shakib M. Effects of three commercial mouth rinses on the cultured fibroblasts: An in vitro study. J Dent [Shiraz] 2013;14:84-7.
12. Chowdhary KY, George JP, Gowda P, Rao JA. Human periodontal ligament fibroblast response to mPDGF-BB application on periodontally diseased root surfaces in vitro. Growth Factors 2013;31:130-8.
13. Chatterjee P, Chandra S, Dey P, Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative in vitro study. J Adv Pharm Technol Res 2012;3:136-8.
14. Kawai S, Tomono Y, Katase E, Ogawa K, Yano M, Koizumi M, et al. Quantitative study of flavonoids in leaves of citrus plants. J Agric Food Chem 2000;48:3865-71.