

Comparative Evaluation of Efficacy of Platelet-Rich Fibrin and Hank's Balanced Salt Solution as a Storage Medium for Avulsed Teeth: An *In Vitro* Study

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ABSTRACT

Objective: To compare the efficacy of platelet-rich fibrin (PRF) and Hank's balanced salt solution (HBSS) in the preservation of the periodontal ligament (PDL) cells viability of avulsed teeth.

Methods: A total of 30 non-carious third molars with healthy periodontium, indicated for extraction for orthodontic reasons or chronic pericoronitis, were selected for the study. Samples were divided into four groups: one standard group and one experimental group in addition to two control groups (positive and negative). The positive and negative control group corresponded to immediate and 2-hour dry time respectively. The experimental teeth were bench dried for 40 minutes and then immersed in one of the two storage media: HBSS (standard storage media) and PRF (experimental storage media) for 45 minutes. The teeth in each group were treated with dispase II and collagenase for 30 minutes and later centrifuged for 4 minutes at 1000 rpm. The supernatant was removed with sterile micropipette, the cells were labeled with 0.4% trypan blue, and the number of viable PDL cells was counted with a hemocytometer under a light microscope. One-way Kruskal–Wallis test and Mann–Whitney U test with Bonferroni correction were used for statistical analysis.

Results: Results did not demonstrate any statistically significant differences in the viability of PDL cells between the groups with standard and experimental storage media. Group 1 showed a statistically significant difference of mean compared to Groups 2, 3 and 4. When Group 2 was compared with Groups 3 and 4, a P -value > 0.05 suggested no statistical significance.

Conclusion: Within the parameters of this study, HBSS and PRF demonstrated a similar number of viable PDL cells. Hence, PRF could be used as a good substitute of HBSS as a storage media for avulsed teeth.

Keywords: Avulsion, HBSS, platelet-rich fibrin, PDL cell viability, storage media

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HIGHLIGHTS

- An avulsed tooth can successfully be replanted, provided it is kept in storage media that can retain the viability of PDL cells.
- PRF, prepared from the same individual, matches the pH and osmolality of blood.
- PRF can be used as a good alternative to HBSS in preserving the PDL cell viability.

INTRODUCTION

The prognosis of an avulsed tooth depends on the status of periodontal ligament (PDL) cells at the time of replantation (1). The best biological approach is immediate replantation of the avulsed tooth into the socket, but this is rarely done at the time of traumatic injury (2). This leads to loss of PDL cells, which results in external root resorption.

A similar pH value, blood osmolality, easy availability, and low cost are four major factors considered when selecting a storage media. Hank's balanced salt solution (HBSS) is the most studied storage solution for avulsed tooth, and it has been considered a standard solution by many authors. HBSS is a pH balanced salt solution containing essential metabolites and glucose necessary for the cell maintenance. It can preserve cells and tissues for 24 hours. Both the pH (7.4) and the osmolality (280 mOsmol kg⁻¹) are ideal for the survival of PDL cells (3-5). A HBSS disadvantage is that it may not be readily available in many places where tooth avulsions are likely to occur (6).

Platelet-rich fibrin (PRF), as described by Choukroun et al. (7), is a second-generation platelet concentrate that allows one to obtain fibrin membranes enriched with platelets and growth factors, after an anticoagulant-free blood harvest. Warunee et al. (8) reported that PRF releases growth fac-

tors rapidly after preparation, and its release continues slowly over a period of time. Since PRF is prepared from patients own blood easily by centrifugation of blood without any anticoagulant, it is readily available and cost effective; it also matches the pH and osmolality of blood.

Hence the aim of the *in vitro* study was to compare the efficacy of PRF and HBSS in the preservation of viability of PLD cells of an avulsed tooth.

MATERIALS AND METHODS

A total of 30 freshly extracted human third molars without extensive carious lesions or periodontal disease were collected from patients aged 18–35 years with informed consent. Teeth with open apex, root-canal-treated, or fractured teeth were discarded. Transalveolar extractions where burs were used during the extraction were not considered for the study. A written ethical clearance was obtained from the human volunteer’s research and ethics committee of The Oxford Dental College (IEC no. 431/2015-16).

Following extraction, coronal 3 mm of the PDL were scraped with a sterile scalpel while tooth was held by the crown with forceps. This was done to exclude the traumatized cells during the forceps extraction.

Teeth were rinsed with distilled water to remove damaged cells and debris from the cervical area. Samples were then randomly divided into four groups: Group 1–positive control; Group 2–negative control; Group 3–standard storage group; and Group 4–experimental storage group.

Immediate periodontal cell extraction was carried out without any bench drying or storage for positive control teeth. Teeth in Group 2 (negative control) were bench dried for 2 hours on a sterile gage in ambient temperature before PDL cell extraction. Teeth in both standard storage and experimental storage group (Groups 3 and 4) were bench dried for 40 minutes and then immersed in HBSS and PRF, respectively, for 45 minutes before the extraction of PDL cells.

A total of 8–10 ml of blood were obtained from each patient for the preparation of PRF before extraction. Collected blood was centrifuged in a table centrifuge at 705.6g for 12 minutes to obtain PRF.

Each tooth was incubated separately for 30 minutes in a 15 ml Falcon tube with 2.5 ml solution of 0.2 mg/ml collagenase and an equal volume of 2.4 mg/ml solution of dispase Grade II in phosphate buffered saline. 20 µl of fetal bovine serum was added to inactivate further enzyme activity. Tubes were

centrifuged for 4 minutes at 1000 rpm. The supernatant was removed using sterile micropipettes. The tooth was removed from the test tube. The cells were labeled for the trypan blue dye exclusion test using trypan blue at 1:1 concentration, and they were incubated for 5 minutes to determine the cell viability. Following incubation, 20 µl of cell suspension mixed with trypan blue was loaded onto a hemocytometer (Neubauer chamber, Precision Scientific Instruments Corporation, Delhi, India). Cells were examined at 40X magnification under a light microscope, and the total number of viable cells (unstained) was counted in four different fields.

Statistical analysis

Data were analyzed using the one-way Kruskal–Wallis test and Mann–Whitney U test with Boneferroni correction. The statistical calculations were performed using the SPSS for Windows (Statistical Presentation System Software, SPSS Inc. 1999, New York) version 21.0. A P-value <0.05 was considered statistically significant.

RESULTS

From the values obtained from each sample, the mean of each group was calculated and shown in Table 1. Group 1 showed the maximum mean of 99.520 cells/mm³, whereas Groups 2, 3, and 4 showed a mean of 18.432, 76.800, and 79.072 cells/mm³, respectively. The standard deviation of each group was 3806, 3910, 4727, and 7570 cells/mm³, respectively. The mean of each group is presented in a bar diagram in Figure 1.

The one-way Kruskal–Wallis test (Table 2) and Mann–Whitney U test with Boneferroni correction (Table 3) with P<0.05 were

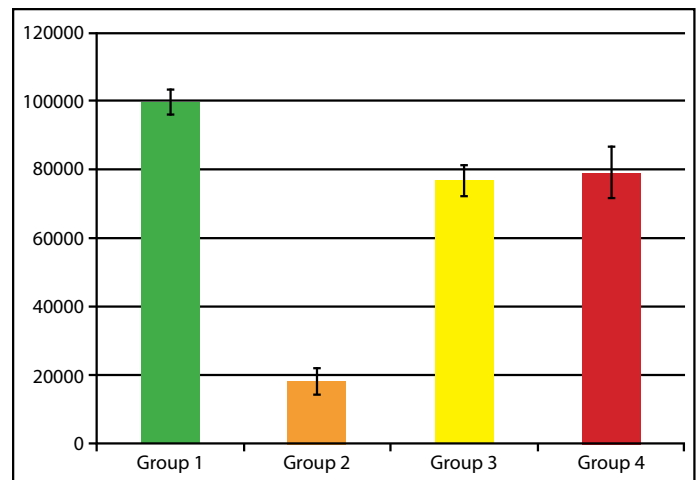


Figure 1. Mean and error bars showing comparison among the four groups

TABLE 1. Descriptive details of the study groups

Group	Number of samples	Total cells Per mm ³ (mean)	SD	Minimum	Maximum
Group-1 (+ ve control)	5	99520.00	3806.521	93760	104000
Group-2 (– ve control)	5	18432.00	3910.028	12160	22400
Group-3 (HBSS group)	10	76800.00	4727.151	69760	85120
Group-4 (PRF group)	10	79072.00	7570.253	69440	92160

CC: Cells per WBC chamber, SD: Standard deviation

TABLE 2. Comparison of the four groups

Group	Number	Mean	SD	P
Group 1	5	99520.00	3806.521	0.001*
Group 2	5	18432.00	3910.028	
Group 3	10	76800.00	4727.151	
Group 4	10	79072.00	7570.253	

*Significant at the 0.05 level, SD: Standard deviation

TABLE 3. Pair-wise Comparison of the four groups

(I) value	(J) value	P
Group 1	Group 2	0.001*
	Group 3	0.001*
	Group 4	0.001*
Group 2	Group 3	0.001*
	Group 4	0.001*
Group 4	Group 3	0.807

*Significant at the 0.05 level

carried out. This demonstrated no statistically significant difference in the number of viable PDL cells between the standard and experimental storage.

In this study, a pairwise comparison of the groups with Bonferroni correction (Table 3) was carried out. When Group 1 was compared to all other groups, the P-value obtained was <0.05. Group 1 showed a statistically significant difference of mean compared to Groups 2, 3 and 4. When Group 2 was compared with Groups 3 and 4, a P-value <0.05 was also obtained in both the cases. The statistical analysis showed a significant difference. But when Groups 3 and 4 were compared, the P-value remained >0.05, suggesting no statistical significance.

DISCUSSION

Dental avulsion is a common consequence of orofacial trauma, denoted by complete dislodgement of the tooth from its socket. The rate of occurrence varies between 0.5% and 16% of all oro-facial traumatic injuries (9-11). Certain pre-disposing factors include protruded maxillary incisors and insufficient lip closure, which may affect the extent of the dental trauma. In adults, traumatic injuries are more likely related to car accidents, fights, and sports (9).

The fate of the avulsed tooth depends on the measures taken immediately after the avulsion. Replantation of the tooth back into the socket is the treatment of choice, but it cannot always be carried out immediately due to lack of professional support at the site of injury. Hammer (12) reported that the chance of survival of a replanted avulsed tooth is directly correlated with the volume of viable periodontal membrane. In subsequent studies, it is shown that the highest possibility of periodontal healing takes place when the avulsed tooth is replanted immediately (13).

The extra-alveolar time includes an episode of dry period, starting immediately after exarticulation, and a wet period, if any storage medium is used, until the tooth is replanted. Andreassen in his study found a significant increase in the replacement re-

sorption after 60 minutes of dry storage and no viable cells after 120 min (13). Hjorting-Hansen reported that teeth replanted within 30 minutes had a better survival rate (14). In the present study, an extra-oral dry time of 40 minutes was chosen to exceed the critical time frame of 30 minutes for the standard storage group (HBSS) and the experimental storage group (PRF).

Various attempts were taken to modify the extra-alveolar conditions by storing the tooth in a physiological transporting or storing medium until professional help could be arranged. Studies have shown that an avulsed tooth can be replanted successfully 1–3 hours after exarticulation when a suitable transport or storage media is used (13). Matsson et al. (15) demonstrated that dry tooth stored for more than 15 minutes shows lesser frequency of ankylosis when immersed in an isotonic solution, that is, HBSS for approximately 30 minutes prior to replantation. Few other studies also were carried out with the time frames of 30 minutes (16, 17) or 45 minutes (2, 18). Thus, the time frame of 45 minutes was chosen for the current study as it allows comparison with previous similar investigations.

Two major factors that regulate the efficacy of a storage medium are osmolality (mOsm kg⁻¹) and pH. The cell membrane acts as a semi-permeable membrane for animal cells. This means that cells behave as osmometers, swell in hypotonic media, and shrink in hypertonic media (13). Although cell growth may occur between the pH level of 6.6 and 7.8, the most favorable pH for the PDL cell survival is considered to be 7.2 to 7.4 is pH (19). This coincides with the pH of human blood. The PDL membrane is expected to exert pH-dependent effects on membrane curvature in intact cells. Under low-pH conditions, there is either increased sodium (Na⁺) efflux or decreased influx or a combination of both. This leads to a severe ionic imbalance in cells (18).

In a study by Krasner (20), HBSS was found to be the most suitable solution for storing avulsed teeth. It has a shelf life of 2 years and does not require refrigeration. This solution is successful in preserving PDL cells, reviving degenerated PDL cells, and maintaining a high success rate when avulsed tooth is soaked in them for 30 minutes. The major two disadvantages of HBSS are, first, that it may not be readily available in all places where tooth avulsions are likely to occur, and second, its high cost, which may not be affordable by all socioeconomic groups of patients.

Several techniques for fractionating and concentrating human blood have produced a number of autologous-platelet-derived materials that possess potential to improve regenerative grafting outcomes. Platelet α -granules contain numerous growth factors, such as transforming growth factors (TGF β 1, TGF β 2), platelet-derived growth factor (PDGF), insulin-like growth factors (IGF-I, IGF-II), etc. (21, 22). PRF, first described by Choukron et al. (7) in 2000 is obtained from a patient's blood by centrifugation without anticoagulants or chemical agents. During the initial period of bone healing, the PDGF from PRF induces the mesenchymal cell migration, activation, and proliferation, and angiogenesis and chemotaxis of acute inflammatory cells (23). When growth factors were incorporated in the storage media, an increase in the mitogenicity of PDL fibroblasts for 20%–37% was reported by Ashkenazi et al. (24).

Zhao et al. (25) revealed that the PRF promoted effective periodontal healing, represented by the regeneration of PDL-like tissues and a reduction of inflammation and ankylosis. A case report on replantation of avulsed maxillary incisors using PRF demonstrated favourable results after 6 months of follow-up. According to the author, the osteoconductive and osteoinductive properties of PRF were responsible for the success (26).

In various *in vitro* studies, several techniques have been used to determine the number of viable PDL cells (3, 4, 6, 17, 24). However, most of the authors had carried out techniques in which the cells were cultured and/or enzyme treated. Since extracellular matrix consists of high content of collagen and other proteins, it seems rational that the use of enzymatic de-segregation would provide a greater number of cells within a shorter time frame. In the current study, to minimize the exposure of cells to active trypsin and to preserve maximum cell viability, the root surface was treated with collagenase and dispase Grade II, as performed by Hiremath et al. (5).

The results of this study suggest that immediate replantation is the best remedy for the management of avulsed tooth where dry storage of 2 hours definitely affects the viability of the PDL cells and leaves very few of them. When the efficacy of the HBSS was compared to PRF, there was no statistical difference in the preservation of PDL viability.

This study has some limitations that need to be addressed, and further research needs to be carried out. In our study, we had chosen third molars for extracting PDL cells. The root size and morphology varies in third molars. Although *in vitro* studies are very helpful for determining the biologic effects of PRF on periodontal cells, they cannot simulate clinical conditions. Preparing PRF also needs technical facilities, and it also involves additional time to prepare it, and this will further increase the extra-oral dry time of the avulsed teeth. Further research with varying time intervals of extra-oral dry time and larger sample size is required.

CONCLUSION

Within the limitations of this study, the PRF behaved similar to HBSS in preserving the PDL cell viability. It can be a potential alternative as it is cost effective and can be easily prepared in medical and dental institutions.

Disclosures

Conflict of interest: The authors declared no conflict of interest.

Ethics Committee Approval: Ethical committee approval was received for this study from the Institutional Ethical Committee of The Oxford Dental College, Rajiv Gandhi University of Health Sciences, Karnataka & Recognised by Dental Council of India, New Delhi (REF # 431/2015-16).

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