

PLATELET-RICH FIBRIN AS A POSSIBLE AUTOGENOUS TRANSPORT MEDIUM FOR AVULSED TEETH: AN *IN VITRO* STUDY

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ABSTRACT

Objectives: Replantation of avulsed tooth depends on the viability of periodontal ligament cells. The aim of this study was to evaluate the survival of periodontal ligament cells (PDL) when placed in platelet-rich fibrin (PRF) transport media after an extra-oral dry time of 40 minutes.

Method: Forty human mandibular first premolar teeth with healthy periodontium and closed apices, previously planned for orthodontic extraction, were selected. The teeth were randomly divided into study group, PRF and control group, Hank's balanced salt solution (HBSS) as a reference medium. In both groups, the teeth were stored dry for 40 minutes, followed by 30 minutes immersion in the study and control media.

Results: There is a statistically significant increase in the number of viable PDL cells in PRF group as compared to reference medium group.

Conclusion: PRF demonstrated higher number of viable PDL cells and hence could be a suitable transport medium for avulsed teeth.

KEYWORDS: Platelet-rich fibrin, avulsed teeth

INTRODUCTION

Loss of teeth due to dento-alveolar trauma affects great part of the population especially the children; leading to significant negative functional, esthetic, and psychological problems on children.⁽¹⁾ Tooth avulsion is considered as the severe form of all traumatic dental injuries and it is characterized by complete displacement of the tooth out of its socket ⁽²⁾. The incidence of avulsion reaches about 16% of all traumatic injuries affecting permanent teeth ⁽³⁾. The most common age affected by injuries

to permanent teeth occur between 7 and 10 years old secondary to falls, traffic accidents, violence, and sports practices ⁽⁴⁾.

Replantation is widely accepted as an effective solution for avulsed teeth but the prognosis of avulsed tooth cannot be predicted and depends on various factors such as extra-oral dry time, the storage medium, state of pulp vitality and periodontal tissues, root development and the period of splinting ⁽⁵⁾.

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Different techniques were used for preserving the avulsed teeth until replantation. Immediate replantation of the avulsed tooth into the socket is the appropriate biological way, but this technique is not always possible⁽⁶⁾ due to the extra alveolar time interval before the patient arrives to the dentist. This leads to root surface dehydration, and increasing the risk of loss the vitality of the periodontal ligament (PDL) cells⁽⁷⁾. If the replantation is delayed more than 5 minutes; the avulsed tooth should be stored in a physiologic transport medium to maintain the vitality of the PDL, and pulpal cells as well as preservation of tissue viability, physiologic pH and osmolality similar to the surrounding tissues⁽⁸⁾.

Numerous media have been used for storage of avulsed teeth. Tap water has been shown to be almost as harmful to the periodontal ligament fibroblasts (PDLF) as dry storage⁽⁹⁾. Saliva and physiologic saline have been recommended for many years as storage media, but only for a short time and may result in PDLF death as saliva is very hypotonic and harbor different bacteria^(10,11). Other storage media as milk, Save-A-Tooth system and Via Span are also used but not readily available in all countries⁽¹²⁾. Egg white, powdered milk, Gatorade and Propolis have been recently studied and tested^(13,14).

If the extra-oral dry time of avulsed tooth is more than 20 minutes^(8,15,16), there is a considerable risk for ankylosis. However, the risk of pulp necrosis, root resorption, and ankylosis significantly increases with an extra-oral dry time of 60 minutes^(17,18) and the tissue regeneration is highly indicated.

Hank's balanced salt solution (HBSS) is the gold standard storage media with ideal pH of 7.2 and osmolality of 270-290 osmo/kg. It contains essential metabolites that necessary to preserve the vitality of PDL cells. It has been widely used as a reference media in studies on tooth avulsion^(19,20).

Platelet rich fibrin (PRF) is a second generation platelet concentrate. It is an autologous platelet concentrate and leukocyte. Alpha granules that

are released from the activated fibrin membrane enriched with platelets and growth factors play a crucial role in soft and hard tissue regeneration. Also, the enmeshed cytokine polypeptides in concentrate influence the extracellular matrix which allows migration, division and phenotypic change of endothelial cells, leading to angiogenesis⁽²¹⁻²³⁾. This autologous scaffold gives the highly needed biochemical mediators for enhancing the regeneration of the periodontium⁽²⁴⁾.

Although plenty of studies have been used for treatment of avulsed teeth, our *in vitro* study introduced the survival of periodontal ligament cells of avulsed teeth through the use of PRF as an autologous biologic medium compared with the gold slandered rejuvenating medium HBSS.

MATERIALS AND METHODS

Forty human first premolar teeth with healthy periodontium and closed apices, previously planned for orthodontic extraction (Fig. 1A), were selected. Atraumatic technique was performed for the extractions. Following extractions, the teeth were held with forceps by the crown, and the coronal 3 mm of PDL was scraped with a curette and rinsed with distilled water to remove cells that may have been severed during extraction. The teeth were randomly divided into two groups (n = 20).

Group 1 (study group): Platelet-rich fibrin (PRF) (Fig. 1B).

Group 2 (control group): Hank's balanced salt solution (HBSS) (Fig. 2A) as a reference medium.

In both groups, the teeth were stored dry for 40 minutes, followed by a 30 minutes immersion in the study and reference media (Fig. 1C; arrow and arrow head respectively). Written consent was obtained from all volunteers. The experimental protocol for analyzing surgical materials was reviewed and approved by the Ethical Board of Faculty of Dentistry, Tanta University.

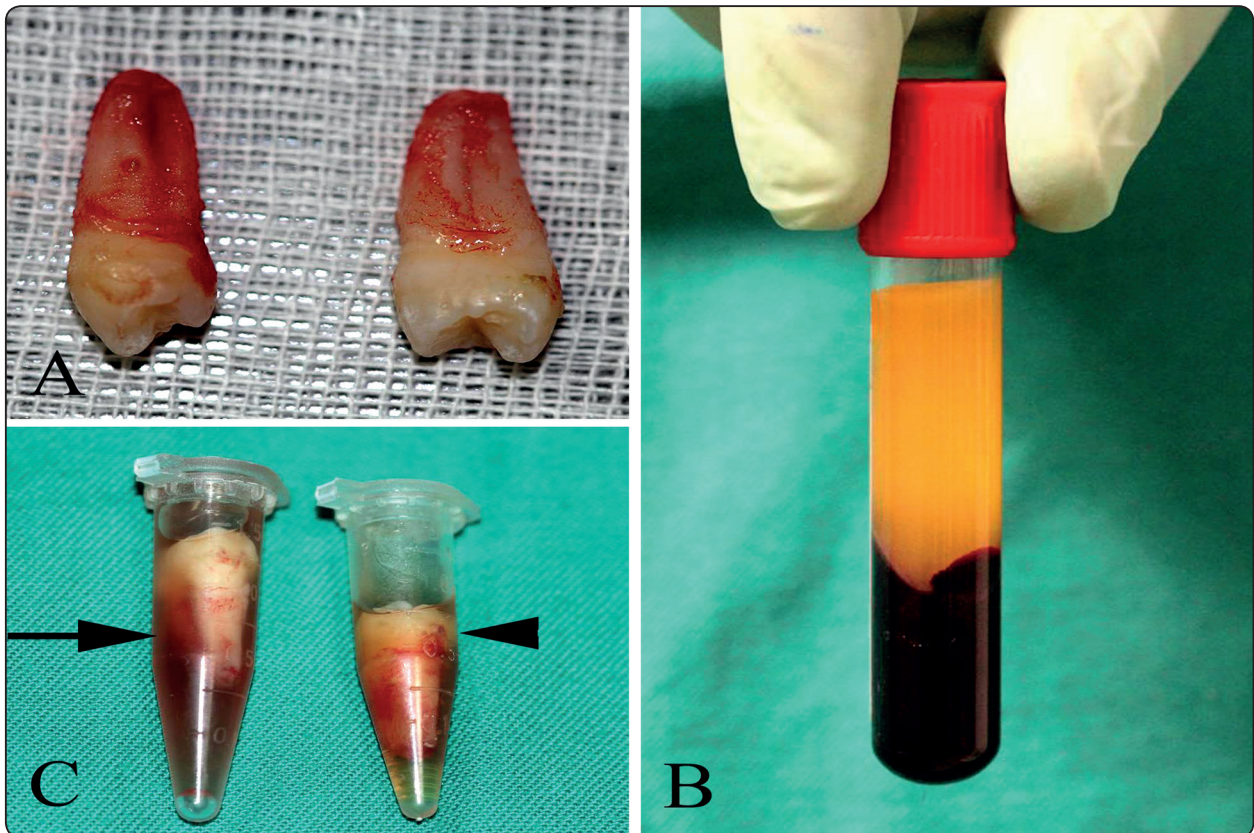


Fig (1) Extracted non-carious mature premolar teeth (A); preparation of platelet rich fibrin (B); Extracted teeth storage in study; (arrow) and reference media; (arrow head) (C).

Prior to teeth extraction, 5 ml of venous blood was collected from 20 healthy orthodontic patients from whom 40 teeth had been harvested for the study. PRF was obtained by centrifugation of collected blood at 705.6 g for 12 minutes with a PC-02 table centrifuge (REMI, Mumbai, India).

Each tooth in both groups was treated separately and incubated for 30 minutes in 15-ml falcon tubes with 2.4-mg ml⁻¹ solution of Dispase grade II (Fig. 2B) in phosphate-buffered saline (PBS) (Fig. 2C). The specimens were centrifuged for 8 min at 5000 rpm, and the supernatant was then removed with sterile micropipette; the cells were labeled with 0.4% trypan blue (Fig. 2D) (Lonza).

Trypan Blue stains non-viable cells blue and viable cells appear color-less or pink. Following

staining, the cells were observed with the help of hemocytometer with an optical microscope

Determination of the number of cells (total and viable):

The cells were viewed under a microscope at $\times 100$ magnification. The number of cells (total and non-viable) was determined by counting the cells overlying a 4 \times 1 mm² area of the counting chamber.

The viable cell percentage was calculated as:

$$\left[\frac{\text{Total cells} - \text{Stained cells}}{\text{Total Cells}} \right] \times 100$$

The number of total cells, nonviable (stained cells) and viable cells from both transport and reference media were collected, tabulated and statistically analyzed. A *P*-value < 0.05 was required for assessing the significance.

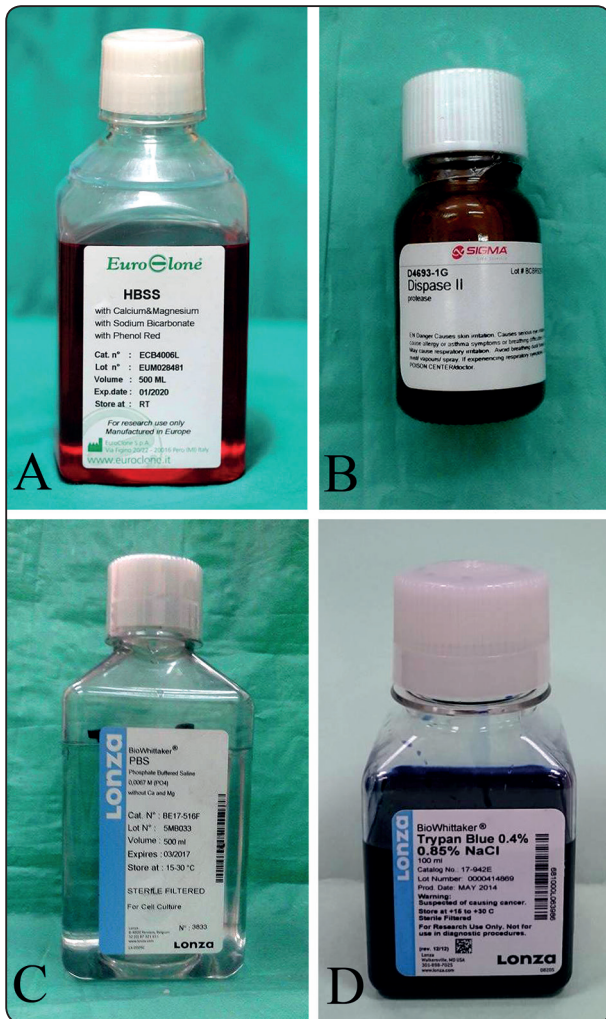


Fig. (2) Materials used in this study; Dispase grade II (A) Hank’s balanced salt solution (B); phosphate-buffered saline (C); Trypan blue (D).

RESULTS

On examination of PDL cells under the microscope, it was clear that some cells were stained blue and others showed clear cytoplasm. Non-viable cells were stained blue, whereas viable cells appeared colorless; (Fig. 3A, B; arrow head and arrow respectively).

Platelet-rich fibrin (PRF) group demonstrated higher number and the percentage of PDL viable cells was (116.05/ 80.2%); (Fig. 3A). The number of the PDL cells in Hank’s balanced salt solution (HBSS) group was markedly lower than that in the experimental group (88.35/ 72.8%); (Fig. 3B), table 1. The difference was significant (*P*-value < 0.05).

Groups	Periodontal ligament cells (Mean ± SD)			
	Total	Non viable	Viable	% of viable cells
PRF	144.6 ± 15.12	28.55 ± 8.58	116.05 ± 14.26	80.27214763
HBSS	121.6 ± 11.59	33.25 ± 7.19	88.35 ± 7.80	72.82418826

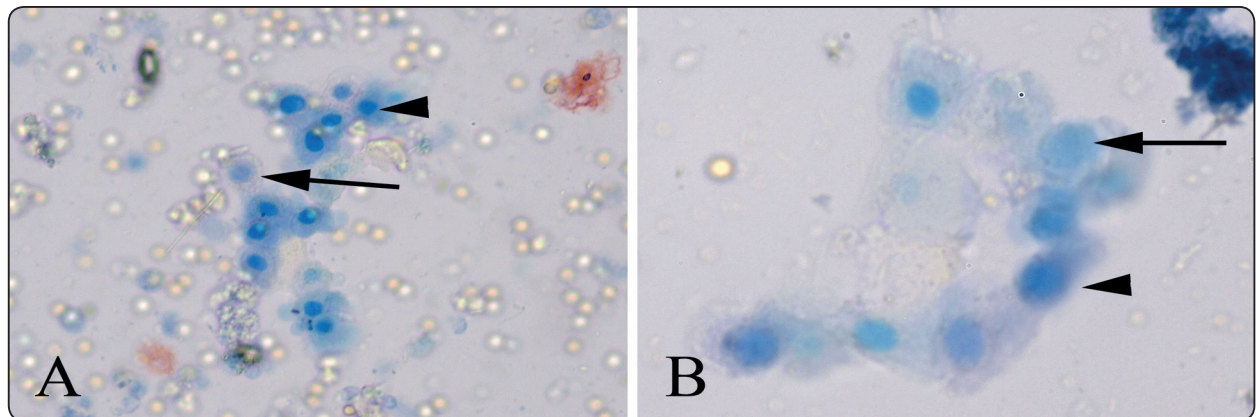


Fig. (3) Microscopic view of viable (arrow) and non-viable cells (arrow head) in PRF medium (A) and HBSS (B); (trypan blue; A, x-200 and B, x-400).

DISCUSSION

Tooth avulsion is the condition in which a tooth has been removed from its socket as a result of trauma⁽²⁵⁾. Clinical trials have revealed best prognosis and much higher reattachment success when avulsed teeth replanted within five minutes⁽²⁶⁾.

In addition, periodontal ligament necrosis is the fate of many replanted teeth due to difficulties in replanting teeth soon after the accident⁽²⁷⁾. Therefore, extra-oral dry time and storage media are essential factors affecting prognosis of avulsed teeth⁽²⁸⁾.

Moreover, survival of replanted teeth is correlated directly with amount of viable periodontal membrane⁽²⁹⁾.

Several transport media have been emerged to store avulsed teeth as immediate replantation is not possible such as HBSS, Viaspan, Eagle's medium, aloe vera and egg white. However, the disadvantages of most of these media are lack of availability and the high cost⁽³⁰⁾. Therefore finding a storage medium that is available and cheap is the target of many workers in the dental field. Hence, the current study was undertaken to evaluate effectiveness of PRF as an autogenous transport medium for avulsed teeth using HBSS as a reference medium as the latter has great potential to maintain PDL cells in a viable state after avulsion⁽¹⁹⁾.

PRF is a fibrin membrane enriched with platelets and growth factors⁽³¹⁾. It is readily available, cheap and safe as it can be prepared with little effort from patients' own blood. A number of studies revealed proliferation and differentiation of osteoblasts and gingival fibroblasts by using PRF⁽³²⁾.

In our study, the number of viable PDL cells in HBSS medium (88.35 ± 7.80 , 72.8%) is significantly lower than that in PRF medium (116.05 ± 14.26 , 80.2%). These findings could be attributed to the release of abundant amount of platelet-derived growth factors (PDGF) resulting in proliferation and survival of PDL cells⁽³³⁾.

In addition, PRF releases growth factors shortly after preparation and its slow release continues over a period of time⁽³⁴⁾. Moreover, Li *et al* demonstrated that PRF had significantly stronger effect on periodontal progenitor cell proliferation *in vitro*. These findings were attributed to different groups of cytokines trapped in PRF that are trapped in the fibrin mesh and released in a controlled manner⁽³⁵⁾.

It was demonstrated that fibroblast function was affected by age, trauma and inflammation⁽³⁰⁾. Therefore, sound teeth undergoing extraction for orthodontic treatment from young persons were selected in this study. In addition, PDL cells were shown to remain in non-compromised state up to 15 minutes of dry time⁽³⁶⁾.

In the current study, the 25 minutes extra-oral dry time was undertaken to simulate avulsion injury that most avulsed teeth usually subjected. After 40-minute dry time, teeth were placed in PRF and HBSS for 30 minutes. This period was very important as PDL cells were most susceptible to damage⁽³⁷⁾. So, keeping avulsed teeth in storage media may reduce damage.

Viable PDL cells were quantitated following treatment of root surfaces with dispase II protease that allows separation of PDL cells *in vitro*⁽³⁸⁾. In addition, trypan blue easily differentiate viable from non viable cells as chromophore present on cell membrane doesn't take up trypan blue stain unless the membrane is damaged. Hence, all viable cells did not pick up the stain⁽³⁹⁾.

Although HBSS medium has great potential to keep PDL cells in a viable state after avulsion, manipulation, cost and lack of availability make it less than ideal⁽¹⁹⁾. According to the present findings, PRF was shown to preserve PDL cells viability in avulsed teeth with an extra-dry time of 40 minutes in addition to its low cost, availability and ease of preparation.

In this context, PRF demonstrated higher number of viable PDL cells. Accordingly, it could be a possible effective autogenous storage medium for avulsed teeth.

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