

An Evaluation of Placentrex as a novel storage medium for avulsions: an Invitro study

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Abstract

Tooth avulsion is one of the common traumatic injury results in complete extrarticulation of tooth from its socket since resulting in damage to the periodontal tissues. Maintaining viability of the periodontal cells remains a challenge to the clinician to reimplant the avulsed tooth. Since various avulsion media are used in dentistry a novel medium Placentrex derived from 0.1 gm. of fresh term, sterilized, infection-free human placenta which has unique pharmacological effects like enhancement of wound-healing, anti-inflammatory, antioxidant and analgesic effect is compared with HBSS and Propolis media in freshly extracted, non carious, non restored, periodontal diseases free human teeth which are indicated for orthodontic treatment purposes were used. The extracted teeth were placed in the enzyme solution to facilitate detachment of the cells and after ten minutes teeth were removed and 1 ml of solution were collected through micropipette and centrifuged. The stained cells were counted in Neubauer's chamber under a light microscope at 10× magnification. Conclusion: Placentrex maintained PDL cell viability almost similar to Propolis and significantly better than HBSS.

Key Words : Avulsions, Avulsion media, HBSS, Reimplantation,

Introduction

Tooth avulsion is a complex traumatic injury characterized by the rupture of the neurovascular bundle and periodontal ligament (PDL) exposing the root of tooth to the outer environment¹. It has a reported incidence of approximately 1-16%^{2, 3}. The damage to the attachment apparatus during an avulsion injury is unavoidable, so maintaining the viability of the PDL cells attached to the avulsed tooth is critical. Thus, extra-alveolar period has been suggested as one among the important factors for the success of transplantation / replantation of avulsed teeth. Ideally, the tooth should be replanted immediately after the injury to preserve the viability of the PDL cells and minimize root resorption. Unfortunately, this occurs very rarely. If in any case, the immediate replantation of an avulsed tooth is not possible, storage conditions should be recommended to maximize the preservation of the PDL cells during extra-alveolar period⁴⁻⁷. A number of storage media have been evaluated till date. However none of these storage media could be adopted as ideal because of their mixed efficacy and other limitations. Hence, the search for an ideal storage media is still on.

Currently, no studies have suggested using Placentrex as a storage media. Each ml of Placentrex is derived from 0.1 gm. of fresh term, sterilized, infection-free human placenta. Placental extracts have been used for years as a wound healer and as a cosmetic in many countries. It has unique pharmacological effects like enhancement of wound-healing, anti-inflammatory, anti-oxidant and analgesic effect, etc^{8,9,10}. The variety of biological actions of human placental extract (HPE) is a matter of increasing interest. Hence we felt the

need to utilize Placentrex, with its multiple biological activities as a storage media in our study. Thus the present investigation was intended to evaluate the potential of Placentrex as a storage media to maintain the viability of the PDL cells in comparison with Propolis and Hank's Balanced Salt solution.

Materials and methods

Forty freshly extracted single rooted human teeth with closed apices were obtained for the study. The avulsion had been indicated for Orthodontic purpose, and teeth that had no caries, restorations, periodontal disease, or hypoplasia were selected. Extractions were performed as atraumatically as possible by oral surgery residents. Following extraction the teeth were held with forceps by the coronal region, and the coronal 3mm of PDL was scraped with a curette to remove cells that might have been damaged. The teeth were then randomly assigned to one of the three storage medium groups, with ten samples per group, namely group 1- Placentrex, group 2- Propolis, and group 3- HBSS.

Solid Propolis was ground into fine powder particles with a mortar and pestle. Propolis 50% was prepared with a 0.4% ethanol solution^{11,12}.

These teeth were dried for 30 minutes, inclusive of the time for curetting, followed by a 45 minute immersion in one of the three storage media. Ten teeth were allocated to the control groups. The positive control group comprised of five teeth that were neither dried nor stored in any solution, but rather they were assayed immediately for cell viability. The negative control group comprised of five teeth that were dried for 8 hours and then assayed. These comprised groups 4 and 5 respectively.

Blood and debris adhering to the roots were gently rinsed with phosphate buffer saline (PBS) and removed. The samples were then placed in an enzyme solution to isolate the cells from the periodontal ligament.

The enzyme solution consisted of (in 5 ml): 1 mg of Collagenase Type III, 0.8 ml (1.5 g per 100 ml) of Trypsin solution and 4.2 ml of PBS. Each PBS rinsed sample was immersed in 1 ml of this enzyme solution for 10 min in a sterile 15 ml Falcon tube. The solution was agitated for the last 2-3 min of immersion to facilitate detachment of the cells. The teeth were removed from the solution at the end of 10 min and 1 ml of solution was pipetted to a micro-tube. Ten microlitres of foetal bovine serum was added to it. The tube was then centrifuged at 1000 rpm for 4 min. The supernatant fluid was removed and the pellet was dissolved in 1 ml of PBS. The cells were labelled with 0.4% (w/v) trypan blue in a 1:1 ratio (100 µl of solution with 100 µl of dye). After 10 min, 10 µl of the stained solution was taken on a Neubauer's counting chamber and the cells were counted under a light microscope at 10× magnification^{13, 14}.

Results

The results were statistically analysed by One way analysis of variance and Pair-wise comparison of groups by Newman-Keuls multiple Post hoc procedure. The level of significance was 5%. The mean percentage of viable cells is represented in fig 1. Group IV (positive control) had the highest number of surviving cells, followed in rank order by Group II (Propolis), Group I (Placentrex), Group III (HBSS), and Group V (Negative control).

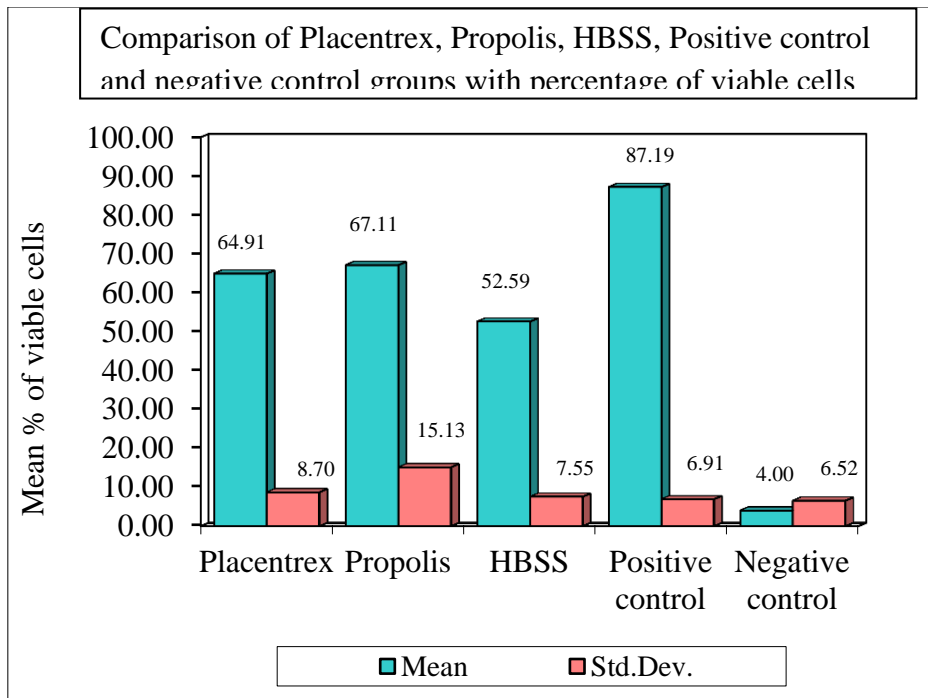


Fig 1: Mean % of viable cells in each group.

There was a statistically significant difference between Groups I and III (Placentrex and HBSS), and between Groups II and III (Propolis and HBSS). However there was no significant difference between Group I and II (Placentrex and Propolis). All experimental solutions were significantly lower than Positive control (Group IV) and higher than Negative control (Group V) (Table 1).

Table 1: Pair wise comparison of Placentrex, Propolis and HBSS groups with percentage of viable cells by Newman-Keuls multiple post hoc procedures.

	Placentrex	Propolis	HBSS	Positive control	Negative control
Mean	64.9060	67.1080	52.5880	87.1920	4.0000
Placentrex	-				
Propolis	0.6849	-			
	0.0283*	0.0281*	-		
Positive control	0.0007*	0.0008*	0.0002*	-	
Negative control	0.0001*	0.0002*	0.0001*	0.0001*	-

*p<0.05

Discussion

The two most critical factors affecting the prognosis of avulsed tooth after replantation are extra-oral dry time and the storage media in which the tooth is placed before treatment is rendered^{15,16}. The viability of the PDL cells is dependent on the storage media and the duration of storage¹⁷. Firstly a suitable storage medium should provide the best possible conditions to maintain the PDL cell viability. Secondly it should be available at the site of injury¹⁸.

In the present study, the teeth were dried for 30 minutes before being placed in the storage media representing a classic clinical scenario during which the avulsed tooth may remain dry before being placed into a storage media and also it permits for comparison with previous investigations^{11,19}.

Trypan blue exclusion was carried out in this study, as it is found to be the most sensitive assay. This may be attributed because of direct visualization of loss of membrane integrity, which leads to cell death². And it is quick and easily performed²⁰.

HBSS is considered as the gold standard and is accepted by the American Academy of Endodontics as an acceptable medium for avulsed tooth. Glucose, calcium and magnesium salts sustain and reconstitute the depleted cellular components of PDL cells. It has a long shelf life^{18, 21}. In the current study, HBSS maintained approximately 52% of viable PDL cells. This was in accordance with the previous studies^{21, 22}. However it is not readily available in pharmacies and thus not accessible at the site of injury.

Propolis maintained maximum number of viable cells among the experimental groups. Propolis (bee glue) is a resinous or sometimes wax-like bee hive product. The main chemical classes present in propolis are phenolic compounds like flavonoids, fatty acids, aromatic acids and esters, waxes, pollen proteins, vitamins, mineral salts (iron and zinc) and some strange materials. Flavonoids have antioxidant, antibacterial, antiviral, antifungal, and anti-inflammatory properties. Iron and zinc help in collagen synthesis^{23, 24}. Propolis is proved to be a superior transport medium than HBSS. This was depicted in the current study also. It has been shown that 50% Propolis to be not significantly different from 100% Propolis. 50% Propolis maintained a slightly higher number of viable PDL cells¹¹. Hence 50% propolis was used in the present study. However Propolis is not readily available and requires preparation, the chemical composition of Propolis varies in different geographical zones due to different plant sources; and it is not economical.

Placentrex maintained PDL cell viability almost similar to Propolis. Recent research studies reveal that HPE is a rich source of various bio-active substances which have biological and therapeutic activity. These bioactive substances include polydeoxyribonucleotides (PDRN), RNA, DNA, peptides, amino acids like glutamic acid, aspartic acid, glycine, tryptophan tyrosine etc, sugars, enzymes, fatty acids, trace elements, etc²⁵. The anti-inflammatory action of human placental extract is by inhibition of prostaglandin synthesis pathway or 5-HT release²⁶. The presence of diverse amino acids, monosaccharides, and fatty acids, suggests that it has possible anti-oxidant, immunomodulating effects.²⁶ It exhibits a wide range of immunosuppressive activities and has excellent wound healing properties^{27, 28}. Also it is readily available in nearby pharmacies or drug stores, economical and exhibits anti microbial action²⁹.

As there are limitations and variability's in any invitro study, the current study also presents with some limitations. The extractions were carried out by different clinicians. The variable trauma induced during extraction could affect the viability of PDL cells. This could lead to variability in the percentage of viable PDL cells during counting. The PDL cell counting was performed by a single observer to reduce the variability during the counting process. Despite these limitations, Placentrex maintained optimal amount of viable PDL cells.

Conclusion

Within the limitations, the present study concludes

1. Propolis maintained the maximum number of viable PDL cell among the experimental groups.
2. Placentrex also maintained PDL cell viability almost similar to Propolis and significantly better than HBSS

Hence we are of the opinion that Placentrex could be a promising novel storage media for avulsed teeth. However further researches on Placentrex as a storage media needs to be elucidated further.

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