**Journal of Advanced Medical and Dental Sciences Research**

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Index Copernicus value = 85.10

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

# Original Research

## Assessment of efficacy of Aloe vera, Propolis, and HBSS in maintaining the viability of periodontal ligament cells- An *in-vitro* study

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***ABSTRACT:***
**Aim**: The present study was conducted to assess effectiveness of, Propolis, Aloe vera and Hank's Balanced Salt Solution (HBSS) in maintaining the viability of periodontal ligament cells. **Materials & Methods**: The present study was conducted on 90 freshly extracted teeth that were randomly divided into four experimental storage solution groups. Group I: Stored in Propolis, Group II: Stored in Aloe vera and Group III: Stored in Hank's Balanced Salt Solution (HBSS). **Results**: The percentage of viable PDL cells was found to be higher in teeth stored in aloe vera (82%) as compared to those preserved in propolis (68%) and HBSS (66%). The difference was significant ($p< 0.05$). The mean absorbance value at 3 hours, 6 hours, 12 hours, 24 hours, 48 hours and 72 hours in different groups as calculated by Mann Whitney test was significant ($P< 0.05$). Post Hoc test between different groups revealed significant difference in mean periodontal cells ($P< 0.05$). **Conclusion**: Aloe vera as a storage media was found to show higher periodontal cells viability followed by 50% Propolis and HBSS.
**Keywords:** Aloe vera, HBSS, Propolis, Storage media

Received: 15 September, 2020 Accepted: 20 November, 2020

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**This article may be cited as:** Sepolia S, Verma P. Assessment of efficacy of Aloe vera, Propolis, and HBSS in maintaining the viability of periodontal ligament cells- An *in-vitro* study. J Adv Med Dent Scie Res 2020;8(12):83-87.

### INTRODUCTION

Dental tissues have the capability for regeneration this attributes to their uniqueness in comparison to most other body tissues.[1] Traumatic injuries to the pulp and PDL may sometimes regenerate or alternatively show repair with fibrous scar tissue or bone. Understanding the circumstances leading to repair and regeneration in oral tissues has been a formidable challenge. In this regard, damage to PDL, cementum, alveolar bone, gingival, and pulp tissues releases a variety of signals that induce neighbor cell populations to respond by proliferation, migration, or differentiation. Migration of the specialized cell populations (pulp or PDL cells) with regenerative potential at the site of injury free from contamination contributes to the healing process. [2, 3]

Avulsion of the permanent teeth is a real emergency situation that requires immediate intervention and often affects esthetics of the patients. The reported incidence of tooth avulsions ranges from 1% to 16% of all traumatic injuries occurring in permanent dentition. The consequences of this trauma are that it affects neurovascular supply and usually results in loss of pulp vitality. The prognosis depends on the measures taken at the place of accident or the time immediately after the avulsion because the preservation of the cell viability would be significantly higher. [4]

The management includes immediate re-implantation of tooth in its socket if the periodontal membrane is still vital. However, it is difficult to maintain the viability of periodontal ligament (PDL) cells of avulsed tooth. If the tooth needs immediate reimplantation it is gently washed under tap water or sterile saline in order to remove the gross debris.If the immediate reimplantation is not possible, it should be placed in an appropriate storage medium until it can be reimplanted. Dry time must be avoided. Storing of

avulsed tooth is of paramount importance. Two of the most critical factors affecting the outcome of an avulsed tooth after replantation are extra oral dry time and the storage medium in which the tooth is placed. However, the ability of a storage/transport medium to support cell viability is more important than the extra oral time to prevent ankylosis and replacement resorption. [5]

The most important complication after the replantation of the avulsed tooth is external root resorption, and ankylosis. Defects in the PDL/cementum interface smaller than $4mm^2$ show more complete healing or transient ankylosis which is later resorbed and repaired. In contrast, defects larger than $4mm^2$ are more likely to develop permanent ankylosis. [6, 7]

Ideal properties of a good storage media include: Maintains viability of periodontal fibres, Clonogenic and mitogenic capacity, Physiological osmolarity an pH, No antigen antibody reaction, Less risk of root resorption, Effective under various conditions, Antimicrobial, Sterile, easily available.[8]

Various storage mediums that can be used are: Saliva, Normal saline, Milk, Aloe Vera, Hanks balanced salt solution (HBSS), Pomegranate Juice, Propolis, Coconut water, and green tea.

HBSS has been proposed as the storage medium of choice for treatment of avulsed teeth by the American Associations of Endodontists. HBSS is found to be effective in preserving the viability of periodontal ligament cells for up to 24 hours at $4^o$ c and at room temperature. However, the major disadvantage of HBSS and many of the other aforementioned media is that they are not easily available at places where these traumatic injuries occur. Hence, there is a need to identify a storage medium that will be readily available, yet effective. [9]

Propolis is a resin obtained from conifer trees used by bees for constructing the hives. It is supposed to have anti-inflammatory, anti-bacterial, antioxidant, anti-fungal and tissue regenerative properties. [10]Aloe vera has recently gained popularity in the medicinal field because of its antidiabetic, anticancer, and antibiotic properties. It has anti-inflammatory properties and is easily available. [11, 12, 13]

The present study was conducted to assess effectiveness of Aloe vera, Propolis, and HBSS in maintaining the viability of periodontal ligament cells.

## MATERIALS & METHODS

Ninety freshly extracted non carious teeth for orthodontic purpose with normal periodontium, intact crown and closed apices were included in this study. These teeth were equally divided into four experimental storage solution groups with 30 in each. Group I: Stored in Propolis, Group II: Stored in Aloe vera and Group III: Stored in Hank's Balanced Salt Solution (HBSS). Propolis was made into 50% concentration using 0.4% ethanol solution. Propolis 50% was prepared by adding 50 mg ground propolis per 250 ml of the 0.4% ethanol solution. Before submersion of teeth in propolis, solutions were shaken for 15 minutes.

Aloe vera gel was prepared from the freshly collected aloe vera leaves. The outer spikes of Aloe vera leaves were cut using a sharp knife and the the inner gel was extracted and transferred into a blender. The contents were blended thoroughly and filtered through a piece of muslin cloth and were placed into a glass jar with a tight fitting lid. [11]

After atraumatic extractions, the teeth were held with forceps from the coronal region, and the coronal 3 mm of PDL was scraped with a curette to remove cells that might have been damaged. The scrapped cells were subsequently incubated in Falcon tube for 30 minutes in a mixture containing 2.5 mL of phosphate buffer and 0.5 mg of type I collagenase. Following incubation, the Falcon tubes were centrifuged for five minutes at 800 rpm. The supernatant was discarded and the centrifuged residue was collected. The cells were labeled with 0.4% trypan blue for determination of viability. Trypan Blue stains non-viable cells blue and viable cells appear colorless or pink. The number of viable PDL cells was counted under light microscope with hemocytometer at 40× magnification. [11]

The obtained data was statistically evaluated with SPSS package (21.0 version, Inc.; Chicago, IL) using Mann- Whitney test, Post hoc test at P value less than 0.05 was considered significant.

## RESULTS

Table I indicated the distribution of teeth in different storage media. The percentage of viable PDL cells was found to be higher in teeth stored in aloe vera (82%) as compared to those preserved in propolis (68%) and HBSS (66%).(Table II) The difference was significant ($p< 0.05$). The mean absorbance value at 3 hours , 6 hours, 12 hours, 24 hours, 48 hours and 72 hours in different groups was calculated using Mann whitney test ($P< 0.05$).(Table III) Post Hoc test between different groups revealed significant difference in mean periodontal cells ($P< 0.05$).

**Table I Distribution of teeth in different medium**

| Groups | Group I | Group II | Group III |
|---|---|---|---|
| Medium | Propolis | Aloe vera | HBSS |
| Teeth | 30 | 30 | 30 |

**Table II Comparison of viable and non viable periodontal cells in each group**

| Groups | Total no. of PDL cells | Viable cells | Non viable cells | % Viable cells |
|---|---|---|---|---|
| Propolis | 150±7.3 | 102±6.8 | 48±6.5 | 68% |
| Aloe vera | 145±7.4 | 120±8.2 | 25±6.9 | 82% |
| HBSS | 149±9.8 | 99±7.8 | 50±7.1 | 66% |

**Table III Mean absorbance values at different time intervals**

| Time | Group I | Group II | Group III | P value |
|---|---|---|---|---|
| 3 hours | 27.5 | 30.1 | 16.6 | 0.05 |
| 6 hours | 27.7 | 31.5 | 16.7 | |
| 24 hours | 28.3 | 32.1 | 17.2 | |
| 48 hours | 28.2 | 32.2 | 17.4 | |
| 72 hours | 28.1 | 32.3 | 17.5 | |

Mann Whitney test $p < 0.05$

## DISCUSSION

The long term success of re-implanted tooth depends on the availability of the viable PDL cells. The ability for a PDL to heal normally and return to its proper form and function is dependent on viable PDL cells at the time of replantation. Viability of the remaining periodontal ligament root surface, integrity of root cementum and minimal bacterial contamination determines the prognosis of replanted tooth along with extra-alveolar time, type of storage medium used after avulsion and root surface morphology. [14, 15]

There are various storage media available that can maintain the viability of PDL cells. The ideal storage media for an avulsed tooth must have following characteristics: Neutral pH, physiological osmolarity, antimicrobial properties and easy availability. However, the main disadvantages of these media are high cost and lack of availability. Therefore, there should be an effort to find a storage medium that is easily available and cheap.

Propolis is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various other substances including organic debris. [16, 17]Propolis has been considered as a efficient storage medium that maintains the cellular viability of the PDL. It is known to possess anti-microbial, anti-inflammatory, and anti-oxidant properties. [18,19]

Aloe vera gel contains 99% water and over 75 essential nutrients, that include minerals, amino acids and vitamins. It is thought to promote healing; therefore it can be used in surgical wounds, in root canal treatment as an analgesic dressing, and around dental implants to control inflammation. Aloe vera has been used as an anesthetic, anti-bacterial, antifungal, antiviral, anti-inflammatory, and antioxidant as well as in healing process of surgical wounds. Moreover aloe vera is being recently employed as a root canal analgesic medicament and as an anti-inflammatory agent at the site of dental implants. [20, 21, 22]

HBSS is commonly used to maintain the growth of various cell types including PDL cells. This solution is nontoxic; it is biocompatible with PDL cells, pH balanced at 7.2, and has an osmolality of 320 mOsm/kg. It contains ingredients, such as glucose, calcium, and magnesium ions, which can sustain and reconstitute the depleted cellular components of the PDL cells. [3,23,24]

The present study was conducted to assess effectiveness of Propolis, Aloe vera, and HBSS in maintaining the viability of periodontal ligament cells. In the present study it was found that the percentage of viable cells was higher in aloe vera gel as compared to that of propolis and HBSS storage media. These findings were in accordance to the following studies:

Fariborz Moazzami et al evaluated the viability of periodontal ligament cells using soy milk, HBSS, milk, and aloe vera extract. It was found that no significant difference in cell viability was seen among aloe vera, soymilk, and HBSS- stored teeth and concluded that aloe vera extract can be recommended as a suitable storage media for avulsed teeth. [22]

Meenakshi Sharma et al evaluated the viability of periodontal ligament cells using three storage medium (milk, aloe vera and egg white). Teeth stored in aloe vera showed the highest percentage of viable cells, followed by egg white and milk.[11]

The maintenance of PDL cell viability in aloe vera gel is mainly due to the presence of essential nutrients such as vitamins, amino acids, minerals, and sugars.[25] Various studies have been conducted that have assessed the wound healing capacity of aloe vera in different fields of medicine and found it a stimulant for fibroblast activity and collagen proliferation due to increase in vascular supply and enhanced oxygenation.[26,27,28]

Fibroblast cell viability and proliferation is a critical factor in the prognosis of avulsed teeth in avulsion injuries. Thus, aloe vera might be useful in the replantation of avulsed teeth due to its fibroblast stimulating substances. [22]

Ahmed Osmanovic et al compared the efficacy of different storage media used for the survival of PDL cells of avulsed teeth in the in vitro setting. It was found that for storage up to 2h, HBSS, DMEM, milk, 10% propolis, 20% propolis, and Viaspan conserved

more than 80% of PDL viability. For storage at 24h, Viaspan showed best cell survival at 88.4%, followed by DMEM (70.9%) and 10% propolis (68.3%). Milk and HBSS showed similar PDL survival at 24h (57.2% and 57.3%, respectively).It was further concluded that milk remains the most convenient, cheapest and readily available solution for maintain the PDL cell viability.[29]

Babji et al compared orthodontically extracted sound teeth with healthy PDL and teeth and found that Propolis showed more viable PDL cells followed by HBSS, Aleo vera, and Pomegranate juice. Sinpreechanon et al stated that low-fat cow's milk in maintaining the viability and proliferation of PDLFs, and in enhancing the process of collagen production. [30]

Mahesh et al conducted a study to compare the viability potential of four storage mediums: Electoral solution, Ringer's lactate, Oral rehydration salt liquid and Coconut water. It was found that Ringer's lactate maintained highest PDL cell viability followed by coconut water, electoral solution, and ORS-L at various time intervals. [31]

Blomlof L et al in their study found that green tea extract also showed higher number of viable cells compared to milk, which was used as a positive control group. [32]

Aloe vera is easily available, less expensive and hence can be used as a tooth storage media. This study helps to choose the appropriate storage media to save avulsed tooth in practice of primary care. Further long term studies are required to evaluate and compare other storage media.

## CONCLUSION

Avulsed tooth requires immediate reimplantation provided that the tooth has viable PDL cells at the time of reimplantation. Storage media provides the best option to preserve its viability of PDL cells until re-implantation is possible. In the present study it was found that teeth stored in aloe vera demonstrated highest PDL cell viability followed by propolis and HBSS.

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