Evaluation of Aloevera Gel as a Storage Medium in Maintaining the Viability of Periodontal Ligament Cells - An *in Vitro* Study

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Objective: *To investigate the effectiveness of aloevera gel as a new storage medium in maintaining the viability of periodontal ligament cells.* ***Study design****: Premolars extracted for orthodontic reason were obtained. Confluent monolayers of fibroblasts were grown by cell culture method from the PDL cells isolated from the extracted teeth. One ml of this cell suspension was transferred to wells of culture plates, incubated for 24 hrs, followed by exposure to the three experimental media, Hank's balanced salt solution (HBSS), aloevera gel, and packaged drinking water. These plates were then assessed for viable cells using trypan blue dye exclusion test with haemocytometer after 15, 30, 60, 90 and 120 mins. The results obtained were statistically analysed using one-way analysis of variance (ANOVA).* ***Results:*** *At 15 min, HBSS presented maximum mean percentage of viable PDL cells (89%), followed by aloevera at 81% and packaged drinking water at 10%. Aloevera demonstrated 71%, 59%, 57% viable cells at 30, 60, 90 mins respectively. At 120 min, HBSS presented 57% viable cells followed by aloevera gel (45%) and packaged drinking water (3%). No statistical significant difference was observed between HBSS and aloevera gel.* ***Conclusions:*** *Within the parameters of this study, both aloevera gel and HBSS were effective in maintaining the viability of PDL cells. Hence, aloevera gel could be used as a storage media for avulsed tooth in situations where availability of HBSS is in question.*

Key words: PDL viability, storage media, cell culture, HBSS, Aloevera gel.

INTRODUCTION

Traumatic injuries are a common occurrence that requires both expedient and informed management by the practitioner. Andreasen and Andreasen (1990) predicted that the incidence of these injuries might eventually surpass the incidence of dental caries[1]. Avulsion injury constitutes about 0.5 to 16 % of all traumatic injuries in school going children [2]. It is characterized by complete displacement of the tooth from its alveolar socket. Successful treatment of an avulsed tooth by reimplantation is dependent upon the prevention of progressive root resorption and preservation of the viability of the periodontal ligament cells remaining on the root surface[3]. Immediate reimplantation of an avulsed tooth is advised but not always possible. Immersing the tooth in a suitable storage medium like Hanks Balanced Salt Solution(HBSS), till reimplantation has been found to improve its prognosis. HBSS is a commercially available storage medium. But, despite its effectiveness in maintaining the viability of periodontal ligament cells, it is not readily available.

Various species of aloevera are widely cultivated thorough out the world[4]. Early records of aloevera use regard it as one of nature's most revered therapeutic herbs due to its healing properties [5].

Thus, the present study was aimed to evaluate the efficacy of indigenously prepared aloevera gel as a storage medium in maintaining the viability of periodontal ligament cells.

MATERIALS AND METHOD

The periodontal ligament cell viability was evaluated in the following storage media a) Hank's balanced salt solution (Himedia, India), b) Aloevera gel and c) Packaged drinking water (Oxyrich, Dhariwal Industries Ltd. India). Approval for conduction of the study was obtained from the institutional ethical committee. (Ref. No. DMIMS (D.U.)/IEC/2010-11/104)

Fresh leaves of aloevera *Barbadensis* were obtained. Lower one inch of the leaf base, the tapering point of the leaf top and the short, sharp spines located along the leaf margins were removed with Bard Parker (B.P) blade no.15 (Lister, India). With the help of the same blade, the inner gelatinous part between the top and bottom rind was removed and the inner gel was collected into a test tube.

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Premolars extracted for orthodontic reasons were obtained from the department of oral surgery, following patient consent. Soon after extraction, cell isolation was carried out as follows. The teeth were washed in sterile Normal Saline (NS), and the gingival attachment was carefully removed using a sharp B.P blade no.15(Lister, India). The crown was then dipped in a 5% sodium hypochlorite solution for two minutes to reduce contamination. The teeth were rinsed in three changes of sterile normal saline[6]. The teeth were then transferred to a test tube containing 5ml Dulbecco's Modified Eagle's Medium (DMEM) with 100 U/mL penicillin, 100 mg/mL streptomycin. Under sterile conditions, the PDL tissue was mechanically removed from the teeth by scraping the middle third of the root surface with a B.P blade no.15(Lister, India). The isolated tissue explants were placed in 25 cm^2 tissue culture flasks containing Dulbecco's Modified Eagle's Medium with 100 U/mL penicillin, 100 mg/mL streptomycin, supplemented with 10% heat-inactivated fetal bovine serum. These flasks were incubated at 37°C in humidified air with 5% $C0_2$ for 2-4 weeks. The medium was replaced every 2-3 days until sufficient cell proliferation was obtained[7]. Trypsin incubation was used to harvest the cells, which were transferred into different 25 cm^2 flasks for continued growth[8]. All tissue manipulations were done in a sterile class I laminar airflow cabinet (SteriClean Air Systems Pvt. Ltd.,India).

From the flasks, 1 ml of cell suspension was distributed to each well of the five culture plates (Tarsons Products pvt. ltd.,India) with twenty-four wells and placed in an incubator containing 5% CO_2 at 37°C for 48 hours, to form a complete monolayer. The number of cells in each of the wells were standardised by obtaining the total cell count in one of the wells, which was 2.3 X 10^3 cells/cm. In each group, the cells were exposed for a period of 15min, 30 min, 60min, 90 min and 120 min. For each experimental subgroup, eight wells were assigned. After 48 hours, the DMEM was discarded from all wells using a pipette and 1 ml of each experimental group was added to wells of the culture plates.

Trypan blue exclusion staining technique was used to count the viable cells using a hemocytometer. The number of cells in a grid of Neubauer's chamber was counted under a light microscope. The total numbers of cells were calculated using the following formula

$$\text{Cell count} = \frac{\text{No. of cells counted x dilution factor (2)}}{\text{Volume of the chamber (Area x depth)}}$$

The percentage of viable PDL cells were calculated as follows

$$\text{Viable cells (\%)} = \frac{\text{Total number of viable cells per ml of aliquot}}{\text{Total number of cells per ml of aliquot}} \times 100$$

The results obtained were analysed statistically using PASW statistics software (vr.18.0, Predictive Analytics Software, Polar Engineering and consulting, IBM, Chicago, IL).

Table 1: Percentage of viable PDL cells along with mean and standard deviation

Time	HBSS		Aloevera		Water	
	Mean	SD	Mean	SD	Mean	SD
15	89%	±4%	81%	±4%	10%	±3%
30	80%	±3%	71%	±3%	9%	±1%
60	72%	±3%	59%	±4%	5%	±3%
90	63%	±3%	57%	±4%	5%	±3%
120	57%	±3%	45%	±4%	3%	±3%

Table 2: Analysis of variance

Source of variation	Sum of Squares	Df	Mean Square	F	Significance
Between Groups	14910	3	4971	37.03	0.000 Significant p<0.05
Within Groups	2148	16	134.2		
Total	17060	19			

Table 3: Tukey's Multiple Comparison Test

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
HBSS	Aloevera	9.700	2.474	.100 p>0.05	3.28	16.12
	Water	66.200*	2.474	.000 p<0.05	59.78	72.62
Aloevera	HBSS	-9.700	2.474	.100 p>0.05	-16.12	-3.28
	Water	56.500*	2.474	.000 p<0.05	50.08	62.92

*. The mean difference is significant at the 0.05 level.

RESULTS

Table 1 represents the mean percentage of viable PDL cells along with standard deviation among different storage media. The intergroup comparison was done using one-way analysis of variance (ANOVA)(Table 2). The mean percentage of viable PDL cells between groups showed statistically significant difference, ($p<0.05$).

To investigate the significance of all possible mean differences between all groups, multiple comparison tukey test (Table 3) was done. It was observed that no statistical significant difference ($p>0.05$) was present between HBSS and aloevera. On the contrary, packaged drinking water showed a statistically significant difference when compared with all the other groups ($p<0.05$).

Intergroup comparison, HBSS demonstrated that maximum percentage of viable PDL cells followed by aloevera over a period of 120min.

DISCUSSION

Immediate reimplantation is the ideal treatment for the re-establishment of the supply of nutrients to the cells in the periodontal ligament on the root surface. In some situations when reimplantation may be delayed, the tooth should be stored in a humid environment to maintain the viability of the cells in the periodontal ligament. It has been observed that complete necrosis of PDL cells result when exposed to a dry period of even 2 hours [9]. Hence, to avoid such complications, avulsed tooth should be reimplanted immediately or stored in a suitable media until reimplantation.

An ideal storage media should be capable of preserving the viability of cellular periodontal ligament, so that the cells could go through mitosis and form clones of the periodontal ligament fibroblasts and its generating cells. This is essential for the surface of the root to be repopulated by fibroblasts, thus avoiding the adherence of osteoclasts in this area[8, 10]. The pH and the osmolarity of the storing environments must be physiologic, as both these factors interfere in the survival of cells of the periodontal ligament[11]. The way in which the tooth is transported also affects significantly the degree of success. The container used for the transport of avulsed teeth must be unbreakable, non-toxic, leak proof and easy to handle, with internal walls made of soft, sterile material besides making the removal of the tooth easy[12].

Hank's balanced salt solution is a commercially available, non toxic, standard saline solution that is widely used in biomedical research as it aids in maintaining the optimum physiological pH for cellular growth.[12, 13]. This solution has a pH of 7.2 and osmolarity of 320 mOsm/kg[8, 12] which makes it biocompatible with periodontal ligament cells. HBSS, as a storage media has the capacity to maintain the viability of cells of periodontal ligament without any morphological distortion[14].Trope has suggested that the chances of success of a reimplanted, avulsed teeth with less than 60 min extra oral dry time is high, provided the tooth is soaked in HBSS for about 30 mins [15]. Additionally, teeth that have been stored dry for 15, 30 and 60 min and then reimplanted have shown more resorption when compared with the same dry time but also soaked in HBSS for 30 min before reimplantation[16]. Due to properties of HBSS like preservation and renewal of the degenerated periodontal ligament cells of avulsed teeth and maintenance of superior success rate[12], it has been recommended by the American Association of Endodontics to be the ideal storage medium[17, 18]. Hence, it was selected as a positive control in the present study.

Aloevera is a very popular plant used in alternative medicine. Among the many species of aloevera, aloevera *Barbadensis* is of medicinal value[5]. The parenchymatous cells of the plant contain a transparent mucilaginuous jelly that is referred to as aloevera gel[5]. It has been reported to have anti-viral, anti-diabetic, wound healing, anti-cancer, antioxidant, antigenotoxic, anti-inflammatory, angiogenic, antimicrobial and hepato-protective properties [5]. The Ayurvedic Pharmacopoeia recommends the use of freshly extracted juice of aloevera leaves (aloevera gel) for its medicinal usage [19]. In dentistry, aloevera has shown to enhance defence mechanisms and accelerate healing process in periodontal diseases by slowing or inhibiting the synthesis of thromboxane [19]. It also has been used in treatment of recurrent apthous stomatitis[20], lichen planus[21] as mouth rinse[22] and tooth gel[23] with promising results.

Despite the existence of better quality storage media such as HBSS, the lack of availability of these products at the place and moment of accident makes their recommendation questionable [14]. On the contrary, aloevera is a commonly available plant and is widely used in alternative medicine. Hence, the chances of availability of aloevera near the site of injury could be relatively high. Thus, in the present study, the need to prepare an easily available plant gel was considered and emphasis was given to evaluate the efficacy of aloevera gel as a storage media.

In the present study, the efficacy of different storage media in preserving the viability of dental fibroblasts was evaluated by cell culture, wherein fibroblasts from the root surfaces were removed and added on to a storage media for culturing [6]. The cells were then made to grow using growth media and once the confluence of cells were obtained, the number of cells could be determined per ml. These cells were then seeded on to the culture wells or a flask to which the experimental group was added. This method has been found to provide a much more accurate analysis[3]. In this study, cell viability was assessed after 15, 30, 60, 90 and 120 min intervals as detection of viable cells was difficult after 2 hours. Moreover, number of necrosed PDL cells have always increased with increased extra oral dry time[24].

In the present study, results have demonstrated that highest percentages of viable cells were found in HBSS, followed by aloevera over a period of 120 min, with no statistically significant difference between these groups. At 15 min, aloevera presented 80.63% viable cells, which dropped to 71.5% at 30 min, but with no statistically significant difference compared to HBSS. At 60, 90, 120 min also aloevera showed comparable results to HBSS. These results acknowledge the previous studies by Ashkenazi et al.[10], which stated that HBSS was the most effective medium for preserving viability of periodontal ligament cells.

The results of our study demonstrated that aloevera maintained PDL cells which could be attributed to the parenchymal tissue (inner pulp) of aloevera which contains proteins, lipids, amino acids and other vital nutrients along with optimal pH. The number of viable cells in aloevera group might also be because of the presence of catalase enzyme, an antioxidant enzyme that converts hydrogen peroxide (H_2O_2) to water and oxygen and suppression of the generation of these free radicals may improve the effectiveness of cell preservation and prevent lipid peroxidation. Hence, presence of antioxidants in storage media is necessary for inhibiting the generation of free radicals thereby minimizing cell damage. Buttke *et al* also suggested that reimplantation success may be increased by storing avulsed teeth in medium containing one or more antioxidants[25]. The osmolarity of aloevera was found to range from 280-300 mOsm/L and as normal cell growth occurs at a osmolarity range of 230 to 400 mOsm/L, the possibility of maintaining cell viability is high with aloevera[4,5,26].

Packaged drinking water showed the least number of viable cells. This may be due to the non-nutritive and hypo-osmotic environment to which the cells are exposed when stored in packaged drinking water, leading to rapid cell death.

Based on the favourable results obtained in this study, aloevera gel could be recommended as one of the suitable storage media for avulsed teeth. Ease of availability of aloevera and additional knowledge of properties of the plant in dentistry could ensure commercial

and clinical success of the gel. However, long term *in vivo* studies need to be conducted to evaluate the efficacy of aloevera gel in maintaining the healthy periodontium.

CONCLUSION

From this study, indigenously prepared pure alovera gel has exhibited PDL cell viability comparable to HBSS inspite of the latter being the medium of choice. Thus, within the parameters of this study, it can be concluded that pure aloevera gel could be used as an alternative storage media for avulsed tooth where availability of HBSS is questionable.

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