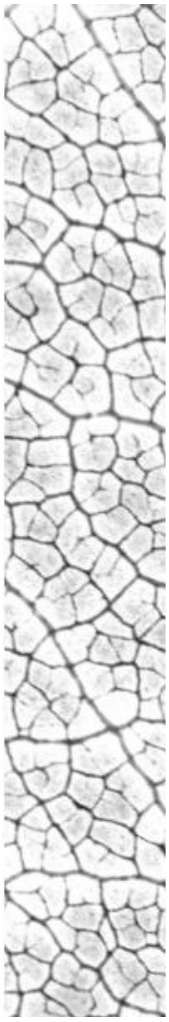

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The effect of aloe vera on apoptosis of periodontal ligament cells

Maryam Roayaei Ardekani [1*], Bahar Houshmand [2], Seyed Mohammad Razavi [3]

[1] Department of Pediatric Dentistry, School of Dentistry, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, IRAN
[2] Department of Pediatric Dentistry, University of Connecticut School of Dental Medicine, Farmington, CT, USA
[3] Department of Oral and Maxillofacial Pathology and Dental implant Research Center, School of Dentistry, Isfahan University of Medical Sciences Isfahan, IRAN
*Corresponding author: ma_roayaei@yahoo.com

Abstract
Aloe Vera is a medicinal plant with anti-inflammatory and anti-microbial effects. There is not enough data regarding application of Aloe Vera as storage media for periodontal ligament (PDL) preservation. The aim of this study was to evaluate the effects of this material at different concentrations and different time points on PDL cell vitality and apoptosis. In this study PDL cells were treated at different concentrations of Aloe Vera (25%, 50% and 100%), HBSS and water in- vitro. Cell Vitality was studied by Tetrazolium-based colorimetric MTT assay at 2, 6,12, 24 and 48 hours. Apoptosis assay was performed at 12, 24 and 48 hours. There was no significant difference in the number of viable and apoptotic cells in 100% Aloe Vera group compared to HBSS. The minimum number of viable cells and maximum number of apoptotic cells were observed in cells exposed to 25% Aloe Vera.

Keywords: aloe vera, storage medium; periodontal ligament cell, apoptosis

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INTRODUCTION

Tooth avulsion or exarticulation is complete displacement of a tooth from its socket which causes damage to periodontal ligaments. It`s more common in 7-9 years old children. Epidemiologic studies have shown that traumatic injuries cause 0.5-16% avulsion in permanent dentition (Andreasen et al. 2019).

Severity of injury to PDL cells, pulp status and patient age determine the type of repair occurs after avulsion. Repair with normal PDL occurs if root surface cells and internal alveolar socket cells remain vital after avulsion. PDL repair of avulsed teeth depends on repair potential of involved cells. Therefore, maintaining the vitality of these cells increase the treatment prognosis of avulsed teeth (Andreasen et al. 2019).

If avulsed teeth replant immediately it has positive effects on vitality of the cells and PDL repair (Andreasen et al. 2019). In many instances immediate replantation is not possible. Hence, keeping avulsed teeth in a physiologic storage media can help maintaining cell vitality which is critical for repair process

Extra alveolar period and storage media are important determinants of vitality and proliferation capacity of remained PDL cells (Oswald et al. 1980). Experimental studies have shown that storage media has a more significant effect on prognosis compared to extra alveolar period (Andreasen et al. 2019).

Ideally, storage media features should closely resembles the alveolar socket environment. Therefore, evaluation of nutritional metabolites, physiologic PH and osmolality should be considered when choosing a storage media (Hiltz and Trope 1991, Lindskog and Blomlof 1982). In addition, storage media must be accessible.

Many studies have investigated the effects of different storage media on avulsed teeth. American Association of Endodontics (AAE) recommends the use of Hanks Balanced Salt Solution (HBSS) as the selective storage media (American Association of Endodontics 1995). HBSS is a sterile and non-toxic salt solution with desired PH and osmolality. Although HBSS has many advantages, it is not usually available at injury location. Therefore, many studies are being conducted to find an available and affordable storage media with anti-bacterial and anti-fungal characteristics.

It`s been shown that water is not a suitable storage media. Water is hypotonic and cause rapid cell lysis (Lindskog and Blomlof 1982, Moazami et al. 2012).

Aloe Vera, a tropical plant from Liliaceae family, has traditional medical use (Evans 2002, Hedegard and

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Stalhane 1973). Currently, this plant is grown and sold in Iran and many other countries and is one of the most recognizable plants in the world. Aloe Vera is a non-toxic plant with anti-inflammatory, anti-viral, anti-fungal, anti-diabetic, anti-cancer and immunomodulatory properties (Evans 2002, Gontijo et al. 2013).

It has been shown that Aloe Vera gel stimulates growth and proliferation of fibroblasts and cellular regeneration (Yagi et al. 1997, Yao et al. 2009). In addition, it is a biocompatible material that induces tertiary dentin formation when used directly on exposed pulps (Gala-García et al. 2008).

Necrosis and apoptosis are two main reasons for cell death (Chamorro et al. 2008). Necrosis results from acute cellular injury and cause functional host defects. On the other hand, most of body cells die due to programmed cell death called apoptosis (Hetts 1998, Kerr et al. 1972). Apoptosis is a cell-intrinsic cell suicide program to remove unnecessary or potentially harmful cells in an organism. Apoptosis has important effects on many physiological and pathological processes. Destructive cell environment and mild harmfull stimulations can cause irreversible DNA damage which result in cell autolysis (Cohen et al. 1992, Mitchell et al. 2007).

Chamorro et al. (2008) has shown that apoptosis is the main reason for fibroblast death after exposure to a sport drink. These cells were not necrotic but lost their viability and proliferation potential. Jabarifar et al. (2015) by using the apoptosis method has reported that ORS is an appropriate storage media for PDL cells. In most of previous studies and in all study that evaluated aloe vera (to our knowledge), only necrosis of PDL cells of avulsed teeth and not apoptosis have been investigated when evaluating a suitable storage media. However, this method is not evaluated the apoptotic cells that initiating the programmed death.

To investigate the potential use of Aloe Vera as storage media for avulsed teeth, this study evaluated its effects on apoptosis and necrosis of human PDL cells for the first time to our knowledge. Results were compared to PDL cells exposed to water and HBSS.

METHODS AND MATERIALS

Cell Culture

In this study periodontal ligament cells were isolated from freshly fully erupted and clinically healthy premolar or molar from young adult. The teeth had been extracted for orthodontic purposes.

After the extraction, the teeth were placed in tubes containing 15% FBS and antibiotic (penicillin and streptomycin 0.05%) and were immediately transferred to the laboratory. In the laboratory, under sterile conditions, the teeth were held from the crown by forceps and PDL tissue was mechanically isolated from the middle third of the root surface by a sharp surgical knife.

Table 1. pH and Osmolality mean values of different tested media

Maintenance environments	Osmolality (mOsm/Kg)	pH
Aloe Vera 100%	295	7
Aloe Vera 50%	258	6.9
Aloe Vera 25%	242	6.6
Drinking water	3	7.3

The isolated tissue, after being washed, was first cut into small size pieces by magnesium-free and neutralized phosphate-buffered saline solution (PBS). Then, it was subjected to type I collagenase until the tissue was completely digested. The resulting liquid was centrifuged twice at 2000 rpm for 5 minutes.

The obtained cells were placed in flasks containing DMEM, FBS 15%, and Pens/Trep, and were incubated at 37 ° C, 5% CO2, and 95% humidity. Each day, the cell culture medium of flasks containing the cells was replaced with fresh medium until sufficient numbers of cells were obtained. Passage 4 cells were used in this study. For each test, 10^8 cells were placed in each well, and 4 wells were assigned to each tested substance at any given time.

Storage Media (SM)

The SM tested in this study consisted of 3 different concentrations of Aloe Vera gel (100%, 50% and 25%), Hanks' Balanced Salt Solution (HBSS) (Sigma Co., Germany), and tap water.

Aleo Vera gel was made of Aleo Vera leaves at Barij-Essence Pharmaceutical company, Kashan, Iran. Leaves were washed with distilled water and dried. The outer leaf of the Aleo vera plant was peeled and parenchyma gel was removed. Gel was centrifuged for 5 minutes at 2500 rpm after being blended using a homogenizer. This gel was used as 100% gel in experiments. 25% and 50% concentrations were made by diluting 100% gel in distilled water. PH and Osmolality of all samples were measured with PH meter (Metron,Germany) and Osmometer (Osmometer 3320, Germany) respectively (**Table 1**).

Tetrazolium Salt-based Colorimetric (MTT) Assay

The MTT assay was used to evaluate cell viability. The procedure is as follows. Initially, the culture medium in the wells was replaced by 1ml of the tested SMs. These wells were kept at 25 ° C and were subjected to MTT assay at 2, 6, 12, 24, and 48 hours. So that, at these times, the SMs in the pits were replaced by [3-(4, 5 – dimethythazol – 2-y1)-2,5-diphenyl tetrazolium bromide] (Sigma Co., Germany), the plates were placed in the incubator, and the MTT solution was replaced by 100 ml of dimethyl sulfoxide (DMSO) solution 4 hours later.

Table 2. Mean percentage and standard deviation (±SD) values of PDL cells based on MTT assay

Storage Media	2H	6H	12H	24H	48H
Aloe 100%	99.5±0.577	95.75±1.70	90.75±3.94	82.25±6.94*	69.75±5.67
Aloe 50%	96.00±1.41*	78.50±6.02*	71.25±2.98*	65.25±3.77*	60.50±4.20*
Aloe 25%	82.50±2.08*	77.75±2.06*	73.75±3.50*	69.25±4.34*	64.00±7.65
HBSS	99.75±0.50	98.50±0.577	92.00±1.63	85.50±1.00	70.75±3.40
Tap Water	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00	00.00±00.00

* Statistically significant difference (p≤0.05) when compared to HBSS

Table 3. Mean percentage and standard deviation (±SD) values of apoptotic cells

APP	12H	24H	48H
Aloe 100%	3.00±1.41*	4.00±1.63*	8.75±4.42
Aloe 50%	6.75±1.50	10.25±1.70*	14.25±1.70*
Aloe 25%	12.25±0.50*	14.25±0.95*	19.00±0.816*
HBSS	6.00±2.44	7.00±1.82	8.25±2.061

* Statistically significant difference (p≤0.05) when compared to HBS

Cell viability was determined by measuring the optimal density at 750 nm on an ELIZA Plate Reader (Awareness Technology Stat Fax 2100, USA).

Apoptosis Assay

After keeping cells exposed to the SMs tested at 12, 24 and 48 hours, the SMs were aspirated from the wells, and after washing the cells with flow cytometry-specific rinsing solution (containing magnesium-free PBS, 1% physiological serum, and 0.058% EDTA), they were fixed for 30 minutes by neutral buffered formaldehyde 4%, and Anti Bcl2-L13 Antibody (BCl2 - DAKO-Germany), which binds directly to the secondary fluorescence antibody, was incubated for 30 min. Again, the cells were fixed with paraformaldehyde and examined by Becton Dickinson apparatus (Mountain View, CA, USA) and WinMDI software, and the percentage of apoptotic cells was determined.

Data were analyzed by SPSS software (version 16 IBM, NY, USA) using ANOVA and Post hoc (Duncan) tests. P ≤ 0.05 was considered significant.

RESULTS

Isolated fibroblasts were treated at different concentrations of Aloe Vera (25%, 50% and 100%), HBSS and water in-vitro. Cell Vitality was studied by Tetrazolium-based colorimetric MTT assay at 2, 6,12, 24 and 48 hours. Apoptosis assay was performed at 12, 24 and 48 hours (**Tables 2** & **3**). There were no viable cells in fibroblasts treated with water. There was a statistically significant difference in vitality of cells treated with water compared to all the other samples (P<0.05). Therefore apoptosis assay was not performed for this group of cells.

MTT assay results showed that samples treated with 25% gel had the least number of viable cells 2 hours after treatment. Number of viable cells were higher in 100% gel and HBSS groups compared to 25% and 50% gel (**Table 2**).

Maximum amount of apoptosis was observed in cells treated with 25% gel (**Table 3**). Analysis of cell number at different time intervals revealed that average number of viable cells in each group decreases and amount of apoptosis increases by time. Decrease in number of viable cells in the group treated with 50% gel was significant after 48 hours.

DISCUSSION

It has been shown that prognosis of tooth replantation is directly affected by the number of viable cells attached to the root surface (Andreasen et al. 2019). Keeping cells in a nutritious storage media with physiologically compatible PH and osmolality is of great importance in cell survival and replantation prognosis consequently (Hiltz and Trope 1991, Lindskog and Blomlof 1982).

Colins and Colins (1935) were pioneer in publishing the first report of Aleo Vera application in modern medicine in 1935. Their study, like similar studies which have been performed later, was on the effects of Aleo Vera on treatkment of radiation injury.

Aleo Vera plant contains more than 75 ingredients including sugar, minerals, vitamins, water, enzymes, amino acids, salisilic acid, polysaccharides and etc, (Danial and Korsmeyer 2004). This plant has anti-microbial activity which does not exist in many current recommended storage medias (Fani and Kohanteb 2012, Yao et al. 2009). It has been shown that anti-inflammatory effect of this plant is beneficial for periodontal ligament regeneration (Evans 2002, Hamman 2008). This material increases collagen synthesis, cell proliferation and regeneration of fibroblasts (Yao et al. 2009). Currently Aleo Vera is grown and sold in Iran and many other countries and has many applications in food, cosmetic and Pharmaceutical industries.

Most studies conducted on the evaluation of plants on avulsed teeth have reported positive effects of plant treatment on PDL cells, however, there is still no consistent answer to the question of whether these studied plants can be recommended for clinical management of teeth with avulsion (Resende et al. 2019). So far, several studies have examined the effect of Aloe Vera as storage media. In these studies, different methods of examining cells, including Trypan blue and MTT, have been used.

Sharma et al. (2015) have shown that Aleo Vera gel is more effective on the viability of PDL cells than milk and egg white. Fulzele et al. (2016) found no significant difference between Aloe Vera gel and HBSS. Badakhsh et al. (2014) also showed that diluted Aloe Vera gel did

not differ significantly from culture media and was better than egg white. While, in the study by Abraham et al. (2019), Aloe Vera retained less PDL cells than HBSS and milk, and Babaji et al. (2017) showed that Aloe Vera gel retains fewer living cells than Propolis and HBSS, but it can still be used as storage media.

In the present study, for more accurate evaluation of the usefulness of Aloe Vera gel as storage media, in addition to investigating 3 different concentrations of Aloe Vera gel as a storage medium, apoptosis of cells after storage in these three concentrations was also investigated. Apoptosis appears to provide a more appropriate assessment of cell viability (Gjertsen et al. 2011). We also examined the effect of Aloe Vera gel over longer periods, and the results showed that Aloe Vera gel at 5% concentration had no significant difference with HBSS ($P \leq 0.5$). In addition, fewer apoptotic cells were observed ($P \leq 5$). However, the results for the concentrations of 50% and 5% Aloe Vera gel were not very favorable. Interestingly, if only the results of cell viability and the lack of necrosis are taken into account, although these two concentrations retained fewer viable cells than HBSS, but because more than 1% of the cells were apparently viable after 48 hours, it seems that these materials can also be used as storage media. However, by examining the cells more closely and by specifying apoptotic cells, it can be concluded that many of the apparently viable cells have suffered a programmed cell death, so that slightly less than 1/5 of cells in the 25% Aloe Vera group and about 1/6 of cells in the 50% Aloe Vera group were apoptotic after 48 hours. Therefore, it can be safely concluded that these two concentrations of Aloe Vera cannot be recommended as storage media.

Chamorro et al. (2008) showed that apoptosis plays an important role in the cell death of periodontal ligament cells exposed to Gatorade (a sports drink). These authors suggested that the mild damage to the membrane caused by the low pH of this substance (PH = 3) that induces apoptosis may not be severe enough to cause cell necrosis. This substance is also hypertonic and can also cause cellular dehydration and stimulate apoptosis. In recent studies, it has been suggested that pH and osmolality should be the first parameters for evaluating media (Resende et al. 2019). Optimal cell growth occurs at a pH ranging from 7 to 7.4 and an osmolality of 320-280 mosm/kg. In the case of the unfavorable results of 25% Aloe Vera concentration and less favorable results of 50% Aloe Vera concentration, the less than ideal pH of these two substances can be noted. The osmolality of these two substances was also less than ideal, which, like the osmolality of Gatorade, seems to stimulate further apoptosis in the cells.

Blonelef et al. (1981) showed that the cells exposed to saliva, which is a hypotonic medium, develop swelling and membrane damage.

Unlike the present study, Badakhsh et al. (2014) showed that Aloe Vera gel at 100% concentration was not a suitable medium for retaining PDL cells, whereas Aloe Vera at 10%, 30% and 50% concentrations had no significant difference with culture media. As mentioned, we showed in this study that many apparently viable cells could have suffered a programmed death, and the Badakhsh study has examined only the first 9 hours.

Tudose et al. (2009) also showed in their study that the growth of skin cells treated with different concentrations of Aloe Vera syrup was directly proportional to its concentration, and more cell growth was observed at higher concentrations.

According to the results of this study, we recommend Aloe Vera 100% gel as a suitable storage medium. This substance, in addition to preserving the majority of cells and being fully comparable to HBSS, has all the factors of availability and affordability, ease of use, and storage.

However, it should be noted that although studies on the culture medium may provide insights into the health of cells, but the actual results of each treatment should be carefully examined by clinical trials. Laboratory studies should also be performed under sterile conditions, which itself may be another reason for limiting the generalization of these results to clinical conditions. Given the favorable properties of Aloe Vera and desirable results in laboratory tests, it is suggested that this substance should also be evaluated in clinical trials. It is anticipated that the antimicrobial and anti-inflammatory properties of this substance can have favorable effects after tooth replantation in clinical cases. This adds to other desirable properties of Aloe Vera as a transitional material and this requires clinical examination.

REFERENCES

Abraham B, Kumaran P, Varma B, Xavier A, Kumar SJ (2019) Comparative Evaluation of The Efficacy of Aloe Vera Gel with Milk and Hank's Balanced Salt Solution in Maintaining the Viability of PDL Cells in Avulsed Teeth. Journal of Clinical & Diagnostic Research 13(3).

American Association of Endodontics. (1995). Treatment of the avulsed permanent tooth: recommended guidelines of the American Association of Endodontics. Dent Clin North Am 39(1): 221-5.

Andreasen JO, Andreasen FM, Tsilingaridis G (2019) In: Andreasen JO, Andreasen FM, Andersson L, editors. Dental text book and color atlas of traumatic injuries to the teeth.5rd ed. UK: Wiley Blackwell: 486-520.

Babaji P, Melkundi M, Devanna R, Suresh BS, Chaurasia VR, Gopinath PV (2017) In vitro comparative evaluation of different storage media (hank's balanced salt solution, propolis, Aloe vera, and pomegranate juice) for preservation of avulsed tooth. European journal of dentistry 11(1): 71.

Badakhsh S, Eskandarian T, Esmaeilpour T (2014) The use of aloe vera extract as a novel storage media for the avulsed tooth. Iranian journal of medical sciences 39(4): 327.

Blomlöf L (1981) Milk and saliva as possible storage media for traumatically exarticulated teeth prior to replantation. Swed Dent J Suppl 8: 1-26.

Chamorro MM, Regan JD, Opperman LA, Kramer PR (2008) Effect of storage media on human periodontal ligament cell apoptosis. Dent Traumatol 24(1): 11-6.

Cohen JJ, Duke RC, Fadok VA, Sellins KS (1992) Apoptosis and programmed cell death in immunity. Annu Rev Immunol 10: 267-93.

Collins CE, Collins C (1935) Roentgen dermatitis treated with Fresh Whole Leaf of Aloe vera. Am. J. Roentgenol 33: 396-397.

Danial NN, Korsmeyer SJ (2004) Cell Death Critical Control Points. Cell 116: 205–219.

Evans WC (2002) Trease and Evans pharmacognosy. 5th ed. *China: WB* Saunders: 210-211

Fani M, Kohanteb J (2012) Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. Journal of Oral Science 54(1): 15-21.

Fulzele P, Baliga S, Thosar N, Pradhan D (2016) Evaluation of Aloevera gel as a storage medium in maintaining the viability of periodontal ligament cells-An in vitro study. Journal of Clinical Pediatric Dentistry 40(1): 49-52.

Gala-García A, Teixeira KI R, Mendes LL, Sobrinho APR, Santos VR, Cortes ME (2008) Effect of Aloe vera on Rat Pulp Tissue 46(5): 302-308

Gjertsen AW, Stothz KA, Neiva KG, Pileggi R (2011) Effect of propolis on proliferation and apoptosis of periodontal ligament fibroblasts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 112(6): 843-8.

Gontijo SML, Gomes ADM, Gala-García G, Sinisterra RD, Cortés ME (2013) Evaluation of antimicrobial activity and cell viability of Aloe vera sponges. Electron. J. Biotechnol. [online] 16(1): 1-10. http://dx.doi.org/10.2225/vol16-issue1-fulltext-2.

Hamman JH (2008) Composition and applications of Aloe vera leaf gel. Molecules 13(8): 1599-1616.

Hedegard B, Stalhane I (1973) A study of traumatized permanent teeth in children aged 7-15 years. Part 1.Swed Dent J 66: 431-50.

Hetts SW (1998) To die or not to die: an overview of opoptosis and its role in disease. Jama 279: 300-7.

Hiltz J, Trope M (1991) Vitality of human lip fibroblasts in milk, Hanks balanced Salt solution and Viaspan storage media. Dental Traumatology 7: 69–72.

Jabarifar SE, Razavi SM, Haje Norouzali Tehrani M, Roayaei Ardekani M (2015) The effect of Oral Rehydration Solution on apoptosis of periodontal ligament cells. Dental Traumatology 31(4): 283-7.

Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26: 239-57.

Lindskog S, Blomlof L (1982) Influence of osmolality and composition of some storage media on human periodontal ligament cells. Acta Odontol. Scand 40: 435-444.

Mitchell RS, Kumar V, Abbas AK, Fausto N (2007) Robbins basic pathology.8th ed. Philadelphia: Saunders: 19-22.

Moazami F, Mirhadi H, Geramizadeh B, Sahebi S (2012) Comparison of soymilk, powdered milk, Hank's balanced salt solution and tap water on periodontal ligament cell survival. Dent traumatol 28(2): 132–135.

Oswald RJ, Harrington GW, Van Hassel HJ (1980) A postreplantation evaluation of air-dried and saliva stored avulsed teeth. J Endod 6: 546-51.

Resende KK, Faria GP, Longo DL, Martins LJ (2019) In vitro evaluation of plants as storage media for avulsed teeth: A systematic review. Dental Traumatology.

Sharma M, Sharma S, Reddy YG, Mittal R, Agarwal V, Singh C, Singh A (2015) Evaluation of periodontal ligament cell viability in three different storage media: an in vitro study. Journal of dentistry (Tehran, Iran) 12(7): 524.

Tudose A, Celia C, Cardamone F, Vono M, Molinaro R, Paolino D (2009) Regenerative properties of aloe vera juice on human keratinocyte cell culture. Farmacia 57(5): 590-597.

Yagi A, Egusa T, Arase M, Tanabe M, Tsuji H (1997) Isolation and characterization of the glycoprotein fraction with a proliferation-promoting activity on human and hamster cells in vitro from aloevera gel. Planta Medica 63: 18-21.

Yao H, Chen Y, Li S, Huang L, Chen W, Lin X (2009) Promotion proliferation effect of a polysaccharide from Aloe barbadensis Miller on human fibroblasts in vitro. Int J Biol Macromol 45(2): 152-6.