Frontiers in Dentistry

Periodontal Ligament Fibroblast Cell Viability Following Treatment with Different Concentrations of Green Tea, Aloe Vera and a Mixture of their Extracts

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Article Info

Article type:
Original Article

Article History:
Received: 2 Apr 2022
Accepted: 29 Nov 2022
Published: 31 Dec 2022

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A B S T R A C T

Objectives: Various studies have identified green tea and Aloe vera as a suitable medium for avulsed teeth. The aim of this study was to evaluate and compare the viability of periodontal ligament (PDL) fibroblasts following treatment with the extracts of these two plants and their mixture.

Materials and Methods: Human PDL fibroblasts were purchased and treated with different concentrations of Aloe vera, green tea, and a combination of these two extracts. Hank's balanced salt solution and culture medium were employed as positive and negative controls, respectively. Viability was assessed using the MTT assay. Two-way ANOVA and post-hoc tests were used for statistical analysis ($P<0.05$).

Results: There was a significant difference in PDL fibroblast viability between different concentrations of the extracts. Higher concentrations of green tea and the combination of the two extracts significantly increased cell viability. Higher concentrations of Aloe vera had the least positive effect on maintaining the viability of these cells.

Conclusion: If confirmed by further studies, the combination of Aloe vera and green tea extracts might be considered as a suitable media for different purposes like storing avulsed teeth.

Keywords: Fibroblasts; Periodontal ligament; Aloe; Tea; Cell survival

➢ **Cite this article as:** Alavi SH, Rezvani G, Esfahani MN, Nobakht Lahrood F. Comparison of Periodontal Ligament Fibroblast Cell Viability in Different Concentrations of Green Tea, Aloe Vera and their Mixture. *Front Dent*. 2022:19:40.

INTRODUCTION

The periodontal ligament (PDL) is the connective tissue located between the tooth and alveolar bone. This ligament absorbs pressure from chewing and prevents bone damage [1]. One of the cells that constitute the PDL are fibroblasts [2]. After an injury, PDL remodeling occurs to a limited extent. To help improve the regeneration of this tissue, it is necessary to protect the remaining stem cells so that they could easily differentiate and form PDL cells. [3]. Various media have been introduced to help maintain the viability of PDL cells, in case of injury [4]. Low bacterial levels, physiological osmolarity, normal pH, essential nutrients, increased survival of periodontal fibers, decreased antigen-antibody response, sterility, and antimicrobial properties are some of the features that an ideal dental

storage environ-ment should possess [5]. The effects of various substances such as Hank's Balanced Salt Solution (HBSS), milk, saliva, saline, tap water, and propolis on PDL fibroblasts have been investigated [6]. HBSS is the standard solution for the maintenance of these cells, but its high cost and limited access are considered as disadvantages [7].

Green tea belongs to the *Camellia synesis*, family which is one of the most commonly consumed beverages in the world after water [8]. Past studies have shown that green tea has significant anti-inflammatory, antioxidant and anti-cancer properties [9,10]. The natural com-pounds found in green tea extract exceeds 400 phytochemicals, mainly poly-phenols, which are proven to be active against *P. nigrescens*, *P. intermedia*, and *P. gingivalis* [11,12]. Moreover, EGCG (epigallocatechin-3-gallate), a component of the extract, is known to inhibit bone resorption in periodontal diseases by blocking the expression of matrix metalloproteinase-9 in osteoblasts and pre-venting osteoclast formation [13].

Aloe vera is a cactus-like plant with green and cone-shaped leaves, filled with a transparent viscose gel [6] and has over 75 types of nutrients that exert favorable pharmacological effects such as anti-inflammatory, antimicrobial, and antioxidant activity [14,15].

There have been many studies on the properties of Aloe vera and green tea, but few of them have investigated the effect of these two substances on the viability of PDL fibroblasts [6,9] Also some research has been done on the effect of the combination of these two substances on gingival inflammatory indices and reduction of surgery-related pain. The present study aimed to determine the effect of Aloe vera, green tea and the combination of their extracts on the viability of PDL fibroblasts. Our results may be helpful in the future establishment of a suitable medium that while supporting PDL fibroblast survival, could be used for storage of avulsed teeth in cases of dental emergencies.

MATERIALS AND METHODS

PDL fibroblasts were purchased from the National Iranian Genetic Resource Center (IBRC: C11326).

Preparation of Aloe vera extract:

The Aloe vera plant was washed with normal saline and disinfected with 70% alcohol. Its pulp was extracted by filtration of 100% soluble polystyrene extract [6], after which the extract was freeze dried.

Preparation of green tea extract:

A total of 10g green tea was boiled in 100ml distilled water for 5 minutes and was then sterilized by filtration [9] followed by freeze drying.

Preparation of different concentrations of the extracts:

Fifty milligrams of each of the extracts was combined with 2.5ml water and 2.5ml DMSO (dimethyl sulfoxide) to obtain a concentration of 10mg/ml. Afterwards 1ml of this solution was combined with 9ml water to obtain a total concentration of 1mg/ml of each extract. Different percentages of each of the extracts were obtained by combining these concentrations with culture medium. For the combined extract, two concentrations of each extract were used in equal amounts. HBSS and FBS-free culture medium were employed as positive and negative controls, respectively.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay:

Cells were cultured at a density of 5×10^3 in 96-well plates and incubated for 24 hours at 37◦C before being exposed to the test extracts for 24 hours. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed as described previously [6,12]. In brief, the culture medium was removed and 10µL MTT solution was added to each well and incubated for 4 hours. The MTT solution was then evacuated and 100 µl DMSO was added to each well. Plates were measured after 20 minutes by an ELISA reader with a wavelength of 570nm and the optical density (OD) value was considered as proportional to cell viability.

Statistical analysis:

Data were analyzed using SPSS software. Two-way analysis of variance and post-hoc Tukey's test were used to compare the variables. $P<0.05$ was considered statistically significant.

RESULTS

Our MTT findings showed significantly different (P<0.05) cell viability among green tea, Aloe vera and their mixture at different concentrations and time points (Table 1). Higher concentrations of green tea and the mixture of green tea and Aloe Vera extract demonstrated the highest viability for a longer duration.

Table 1. Fibroblast cell viability comparison between different time points in the study groups

Media	Dilution (%)	Viability %		
		6h	12h	24h
Aloe vera 1mg/ml	25	89.37	93.35	113.96
	50	105.84	108.31	47.52
Aloe vera 10mg/ml	25	19.71	38.49	20.48
	50	12.79	40.40	18.06
Green tea 1mg/ml	25	33.41	122.17	24.28
	50	26.98	72.97	23.52
Green tea 10mg/ml	25	119.83	178.19	89.07
	50	105.45	278.14	141.96
Mixture 1mg/ml	25	94.88	135.66	26.29
	50	38.58	58.17	24.15
Mixture 10 mg/ml	25	86.98	165.44	77.73
	50	92.15	199.45	191.19
HBSS	---	85.03	123.83	72.20
Negative control	---	100	100	100

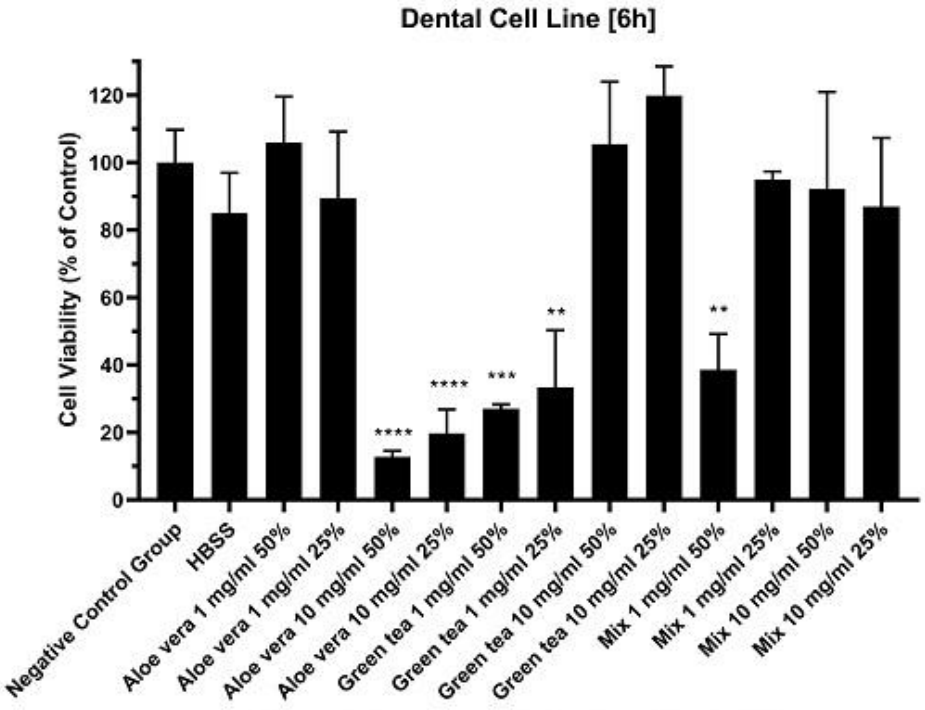
HBSS: Hank's balanced salt solution

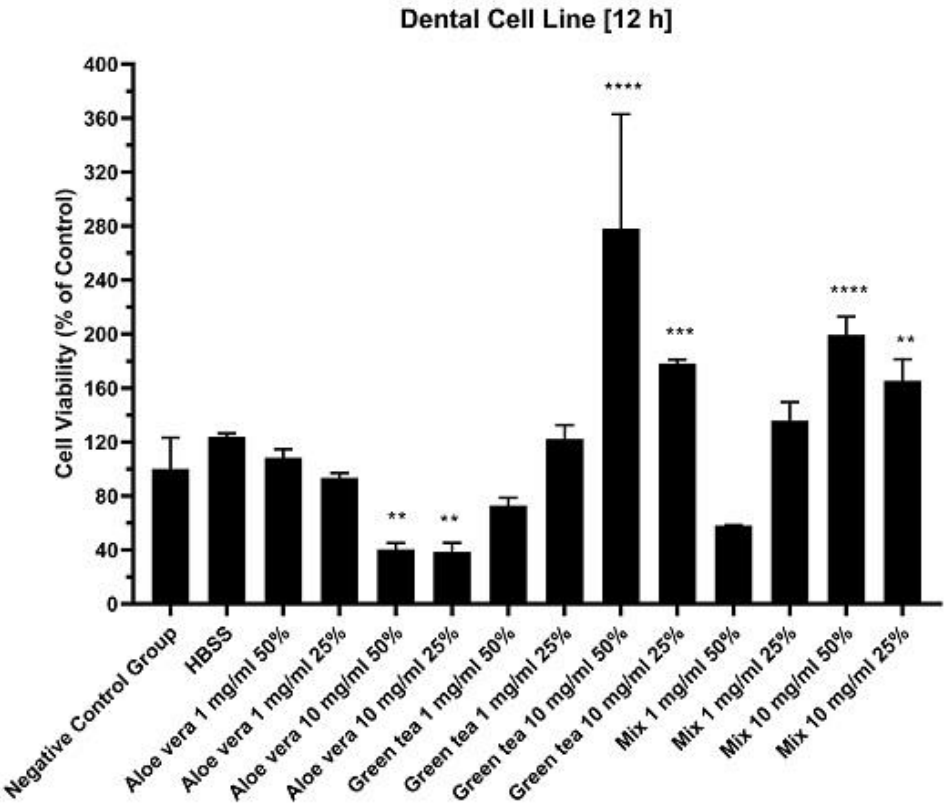
As shown in Figure 1, green tea and the mixture of green tea and Aloe vera extract in higher concentrations had the greatest ability to preserve cell viability after 12 and 24 hours (time:dilution interaction; P=0.003 and P=0.002 respectively; two-way ANOVA followed by Tukey HSD test).

Results in the first 6 hours showed that 10mg/ml green tea with 50% (P=0.88) and 25% (P=0.99) dilutions, and 1mg/ml Aloe vera with 50% dilution (P=0.99) showed the highest viability but differences were not statistically significant (time:dilution interaction P=0.002; two-way ANOVA followed by Tukey HSD test). Also, 10mg/ml Aloe vera with 50% and 25% dilution showed the lowest cell viability compared to the other groups, which was statistically significant (P<0.0001, for both) (time:dilution interaction P<0.001; two-way ANOVA followed by Tukey HSD test).

Our findings at 12 hours post-treatment showed that 10mg/ml green tea extract with 50% (P<0.0001) and 25% (P=0.002) dilutions and 10mg/ml combination of green tea and Aloe vera extract, with 50% dilution (P<0.0001) had augmenting effects on the viability of PDL fibroblasts in comparison to the negative controls, which were statistically significant (time:dilutions interaction P=0.003 and P<0.001, respectively; two-way ANOVA followed by Tukey HSD test). Aloe Vera extract at the concentration of 1 mg/ml with dilutions of 25% (P=0.99) and 50% (P=0.99) had a considerable effect on cell viability, which was not statistically significant (time:dilutions interaction P<0.001 and P=0.006 respectively; two-way ANOVA followed by Tukey HSD test). When using concentrations of 10mg/ml, Aloe vera extract with 50% (P=0.0068) and 25% (P=0.0048) dilutions showed the least cell viability and the differences were statistically significant (time:dilutions interaction P<0.001 and P=0.003, respectively; two-way ANOVA followed by Tukey HSD test).

After 24 hours of exposure, our results showed that the combination of Aloe vera and green tea extract (10mg/ml) with 50% dilution had the most significant increasing effect (P<0.0001) on fibroblast cell viability (time:dilutions interaction P<0.001; two-way ANOVA followed by Tukey HSD test). Green tea at a concentration of 10mg/ml and dilutions of 25% and 50%, was able to increase cell viability (time:dilutions interaction P<0.001 and P=0.002, respectively; two-way ANOVA followed by Tukey HSD test). Results also showed that all concentrations of Aloe vera extract except for 1mg/ml concentration with 25% dilutions had the lowest ability to


Dental Cell Line [6h]
Cell Viability (% of Control)
0
20
40
60
80
100
120
****
****
***
**
**
Negative Control Group
HBSS
Aloe vera 1 mg/ml 50%
Aloe vera 1 mg/ml 25%
Aloe vera 10 mg/ml 50%
Aloe vera 10 mg/ml 25%
Green tea 1 mg/ml 50%
Green tea 1 mg/ml 25%
Green tea 10 mg/ml 50%
Green tea 10 mg/ml 25%
Mix 1 mg/ml 50%
Mix 1 mg/ml 25%
Mix 10 mg/ml 50%
Mix 10 mg/ml 25%


Dental Cell Line [12 h]
Cell Viability (% of Control)
0
40
80
120
160
200
240
280
320
360
400
**
**
****
***
****
**
Negative Control Group
HBSS
Aloe vera 1 mg/ml 50%
Aloe vera 1 mg/ml 25%
Aloe vera 10 mg/ml 50%
Aloe vera 10 mg/ml 25%
Green tea 1 mg/ml 50%
Green tea 1 mg/ml 25%
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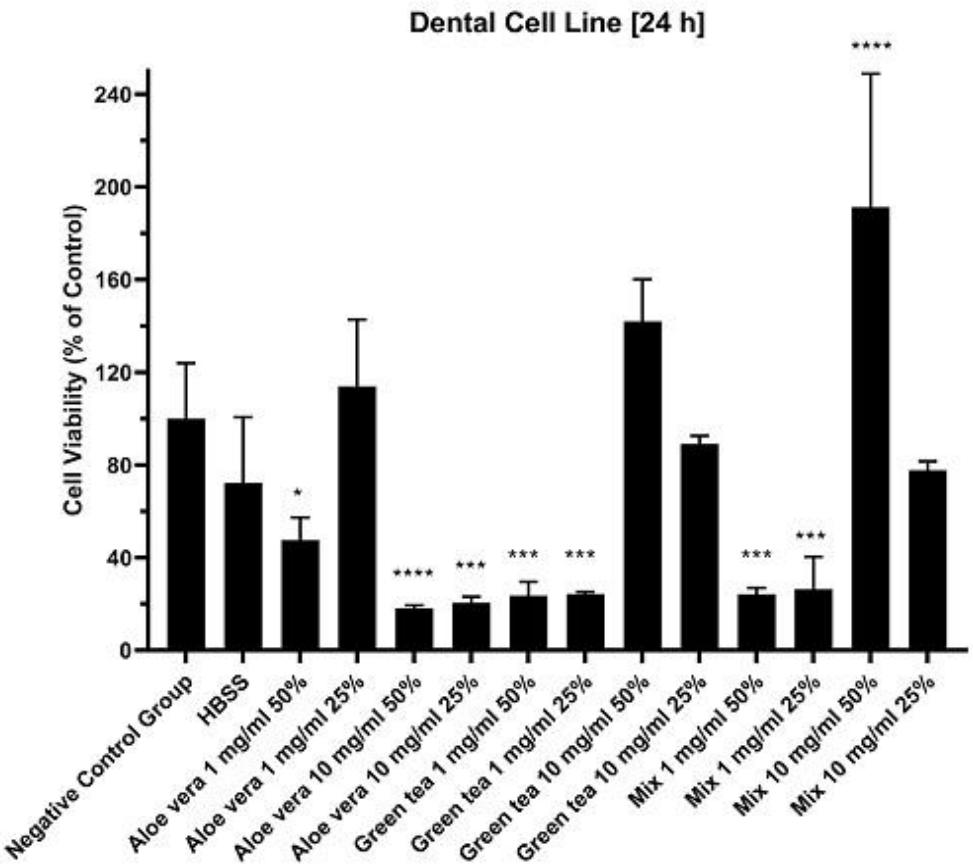

Figure 1: Cell viability comparison after treatment with different concentrations and dilutions of the extracts at 6-, 12-, and 24-hour time-points. Statistical analysis: two-way ANOVA; error bars: mean±standard deviation. **** P-value<0.0001, *** P-value between 0.0001-0.001, **P-value between 0.001-0.01, *P-value between 0.01-0.05 in comparison to the negative control group. Negative control: culture media without FBS; HBSS: Hank's Balanced Salt Solution (positive control); Mix: mixture of Aloe vera and green tea extract.

increase viability of fibroblast cells within 24 hours, with all results being statistically significant (P=0.0241, P<0.0001 and P=0.0001, respectively).

DISCUSSION

Fibroblasts are the most common cells in connective tissues that originate from mesenchymal cells. Their function is to form and maintain connective tissue components and substances [16].

The periodontal ligament is a unique connective tissue that lies between the tooth cementum and alveolar bone [17]. In this tissue, there are fiber strands, and blood vessels, in addition to large numbers of fibroblasts, osteoblasts, and cementoblasts [18]. This ligament has an important role in protecting, supporting and transmitting occlusal forces to the alveolar bone. It also has an essential function in repairing damage caused by periodontal diseases and mechanical trauma [19]. Considering the importance of these cells and their possible role in regeneration and repair, their maintenance would be essential in cases of tooth-related accidents like avulsion. Hence our search for a suitable, economic, and available substance that could preserve the viability of these cells as long as possible.

Our results showed that green tea at a concentration of 10mg/ml with both 25 and 50% dilutions can provide a suitable medium for fibroblast cell growth [20]. At these

specifications, the best viability was achieved at 12 hours of incubation. Green tea extract also showed good results in comparison with the control group at 24 hours when using a concentration of 10mg/ml and a dilution of 50%.

Various studies have shown that components of green tea have positive effects on fibroblasts and can help control and treat periodontitis [13,21]. One of the most effective green tea phenolic components are catechin derivatives, which include catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG) [8]. EGCG is the most common constituent of green tea and possesses a variety of health-promoting features including anti-cancer, antimicrobial, and antioxidant effects [22]. Various studies have suggested that green tea can help prevent systemic diseases such as heart problems and cancer. In dentistry, regular consumption of green tea has been shown to assist in reducing probing depth, attachment loss, and bleeding on probing (BOP) [23]. A clinical study of 940 Japanese men aged 49-59 years revealed an inverse correlation between the intake of green tea and periodontal parameters including mean probing depth and BOP [20]. Furthermore, green tea extract is regarded to be safe, considering that daily intake of high amounts of EGCG (338 mg/day) has shown to be of no harm to human health [24].

It seems that the positive effects of green tea on fibroblast cell viability and proliferation are due to the presence of effective phenolic components including EGCG. Results of a study by Adeli et al. [25] showed that green tea extract can have superior at 2, 4, and 24 hours compared to HBSS. Also, Hwang et al. [9] demonstrated that green tea extract can be a suitable medium for PDL cell growth compared to HBSS. According to Desjardins and Grenier [23] epigallocatechin-3-gallate in green tea has beneficial effects on the viability of gingival fibroblasts. Shin et al. [26] conducted a study to investigate the effect of green tea extract on preventing L-arginine toxicity on human mesangial cells and reported that green tea can protect these cells against the damaging effects of L-arginine [26].

Based on the findings obtained in the present study, the effect of 1mg/ml Aloe vera extract, with both 25% and 50% dilutions, on PDL fibroblast proliferation was similar to HBSS at 6 and 12 h. However, when using a concentration of 10mg/ml, both dilutions of Aloe vera extract demonstrated the least positive effect on cell proliferation compared to the other groups.

Studies have shown antimicrobial, antibiotic and anticancer properties of Aloe vera [27]. Aloe vera contains 20 amino acids needed for protein synthesis. This plant also contains a variety of simple sugars such as glucose and fructose, which along with phenolic compounds like emodin, can provide the nutrients needed for cell proliferation [28]. According to previous investigations, Aloe vera can be used as a constituent of mouthwash to treat recurrent aphthous stomatitis and lichen planus [29]. It has been applied in surgical wound healing, as an analgesic agent in intra-canal lining, and as a coating agent for dental implants to act as an anti-inflammatory agent. In the process of wound healing, Aloe vera can increase fibroblast activity and collagen proliferation by increasing blood flow and oxygen in the wound site [27]. Also, Aloe vera extract was reported to increase cell viability in dental pulp fibroblasts [30]. The favorable results obtained from lower concentrations of Aloe vera are probably due to the presence of different substances including a variety of amino acids, minerals, vitamins, and enzymes [31]. Emodin found in Aloe vera is one of the most abundant and effective therapeutic components that can improve cell viability and proliferation. Badakhsh et al. [6] observed that 10, 30, and 50% dilutions of Aloe vera extract had favorable effects on fibroblast cell proliferation, but dilutions of 100% did not have the same impact and showed the least positive effect on cell growth compared to other media. Curto et al. [32] evaluated the wound healing effect of Aloe vera on primary corneal epithelial cells and fibroblasts and found that lower concentrations of this substance might be associated with accelerated wound healing.

The combination of green tea and Aloe vera has been studied in mouthwashes. Sargolzaie et al [33] showed a higher amount of reduction in plaque and bleeding indices after using an Aloe vera+green tea mouthwash in

comparison to 0.2% chlorhexidine. Also Yaghini et al. [10] demonstrated favorable effects of an Aloe vera+green tea mouthwash on plaque, gingival, BOP, and stain indices and suggested that it could be considered as a suitable replacement for chlorehexidine. In another study, Tafazoli Moghadam et al [34] concluded that the Aloe vera+green tea mouthwash significantly decreased pain related to postoperative periodontal pocket surgery.

Our results indicated that a combination of green tea and Aloe vera extracts in both 25 and 50% dilution of 10mg/ml had a promising effect on viability and proliferation of fibroblast cells after 12 hours and significantly increased cell proliferation. Also, 50% dilution of 10mg/ml concentration of this extract at 24 hours had the most significant positive effect on cell proliferation compared to all other groups. Mixed extract with 1mg/ml concentration and 25% dilution at 6 and 12 hours had a positive effect on cell proliferation, but with time and after 24 hours these effects decreased significantly. Contrary to the positive effect of other dilutions of mixed green tea and Aloe vera extract, 1mg/ml concentration of 50% dilution did not affect cell proliferation in any of the time points.

Favorable results obtained from the combination of Aloe vera and green tea extract with higher concentrations are probably due to the beneficial and additive effects of the phenolic components to the other constituents present in these two plants. Green tea contains a variety of catechin derivatives and Aloe vera possesses diverse amino acids, sugars, vitamins, and enzymes, which may provide necessary components for cell viability and proliferation. In future studies, it is necessary to compare the effects of different constituents of these two plants on each other and determine their effects on various cells.

In order to complement the findings of this study, it is recommended that the effective components of each extract be examined individually and other, more sophisticated methods be applied to assess cell viability and proliferation.

CONCLUSION

According to the results obtained in the present study, green tea extract and the combination of green tea and Aloe vera extracts with higher concentrations could be suitable environments for preserving viability of PDL fibroblast cells. It may be hypothesized that low concentrations of Aloe vera extract can provide an acceptable medium for proliferation of these cells. Therefore, if confirmed by further studies, these extracts can be used for transfer of avulsed teeth.

CONFLICT OF INTEREST STATEMENT

None declared.

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