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Comparative Evaluation of Aloe Vera, Green Tea, Histidinetryptophan-Ketoglutarate Solution, and Propolisstorage Media on Viability Of Periodontal Ligament Cell

Running title: Storage mediafor viability of periodontal ligament cell

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Abstract

Numerous storage media are advised toconserve the periodontal cells viability.This *in vitro*study was carried out to estimate the efficacy ofaloe Vera, green tea, Histidinetryptophan-Ketoglutarate Solution, and Propolis storage media on viability of periodontal ligament cell.The present study was conducted on 60freshly extracted teeth for orthodontic reasons were randomly categorized into 4 study groups.After centrifugation the cells from supernatant were colored with 0.4% trypan blue for determination of viability. Viable mean periodontal ligament cells in group I was 32.6 cumm, in group II was 26.2 cumm and group III was 17.6 cumm, 34.5 in group-IV. The dissimilarity was considerable ($p< 0.05$). Post Hoc test between unusualgroups revealed significant dissimilarity in mean periodontal cells ($P< 0.05$). Propolis, Histidinetryptophan-Ketoglutarate solution and aloe vera had higher pH and osmolality.This study found that propolis showed higher periodontal cells viability followed byHistidinetryptophan-Ketoglutarate solution,aloe vera and least in green tea. Propolis, Histidinetryptophan-Ketoglutarate solution, green tea and aloe vera media can be advocated as a storage media.

Key words: Aloe vera, green tea,Histidinetryptophan-Ketoglutarate solution, periodontal cells, propolis

Introduction

Tooth avulsion (tooth loss) can be defined as complete displacement of the tooth from its alveolar socket by dental traumatic injury. Avulsion is more frequently occurs in the maxillary anterior region than mandibular area. Itis one of the most severe forms of dental trauma affects esthetics of the patients. The incidence of tooth avulsions varies from 1% to 16% of all traumatic injuries occurring in permanent dentition. The consequences of these trauma affects neurovascular supply and usually results in loss of pulp vitality [1,2].

A vital periodontal membrane (PDL) is significant for the flourishing healing of replanted teeth. Instantaneous repositioning of the avulsed tooth may not be possible most of the time. In such a cases, avulsed tooth should be stored in an appropriate storage media to avoid dehydration and to preserve the vitality of tooth [2].The management includes instant re-implantation of tooth in its socket if the periodontal membrane is still vital. 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 ankyloses and replacement resorption [3].

There are different storage mediums available as natural media such as saliva, saline, milk, Aloe Vera, Pomegranate Juice, propolis, coconut water, and green tea and synthetic storage media such as; contact lens solution, Gatorade, Hanks balanced salt solution (HBSS), emdogain, viaSpan, and culture media[2-6]. Propolis is a muggy resin that leaks from the buds or bark of trees. It is made up of the: Resin (rich in flavonoids) (45-55%), waxes and fatty acids (23-35%), essential oils (10%), pollen proteins (5%), and additional organic components and minerals. Propolis has antibiotic, antiseptic, antifungal, antiviral, antibacterial, antioxidant, antithrombotic, anticarcinogenic, tissue regenerative and immunomodulatory properties [6].

The American Association of Endodontists (AAE) suggested HBSS as a storage medium of preference for management of avulsed teeth because of its ability to offer long-term maintenance of PDL cell viability [5].However, it is not easily available and expensive, hence other easily available inexpensive naturally occurring storage media have been tried by many researchers[2,3].

Histidine-tryptophan-ketoglutarate (HTK) cell culture medium is a preservation solution used for organ transplantation. The osmolality and physiological pH of this solution are known to enhance cell proliferation. The maximum cell viability was found in 50% HTK solution. HTK (Custodiol HTK; Koehler Chemi, Alsbach-Haihnlein, Germany) was developed in the 1970s by Bretschneider. It is composed of sodium (15), potassium (9), magnesium (4), caliucm (0.015), Ketoglutarate (1), Histidine (198), Mannitol 30 Tryptophan (2) in mmol/L. Histidine is comprised because of its considerable buffering ability. Tryptophan is involved in HTK as a membrane stabilizer, mannitol as an oxygen-free radical scavenger and ketoglutarate as an energy source.HTK solution is highly feasible because it is easy to store [7].

Aloe vera is a cactus like plant that fit in to family of Liliaceae. The inner gel of *Aloe vera* contains of more than 75 energetic ingredients. 98-99% of gel is composed of water and rest 1-2% consistsof active componentssuch as; aloe-emodin, aloesin, aloin, acemannan, aloemannan, naftoquinones, aloeride, flavonoids, methylchromones, sterols, saponin, amino acids and vitamins. *Aloe vera* gel has antibacterial, antioxidant, anti-inflammatory, immune-boosting; wound healing, cell proliferating potential and hypoglycemic properties because of its active components[6,8].

Green tea extract (GTE) has been described to have extraordinaryantioxidant, anti-inflammatory, and anticarcinogenic efficacy and to prolong allograft survivals properties. Hwang et al from their study found that green tea is equally effective as HBSS and better than milk for preservation of periodontal ligament cells of avulsed tooth[9].

Studies pertaining to natural storage media are scares, hence the present *in vitro*study was conducted to evaluate the efficacy of aloe Vera, green tea, Histidinetryptophan-Ketoglutarate Solution, and Propolis storage media for maintaining the viability ofperiodontal ligament cells.

Materials & Methods

The present study was conducted in the Department of Periodontics. Sixty extractedteeth due to orthodontic purposewith intact crown and close apices having a healthy PDL were used for the study. Samples size was calculated at 95% confidence interval and 90% power; hence the minimum sample size required was calculated as per group was 10 samples for 4 groups.Inclusion criteria were freshly extracted teeth without cracks and caries, no history of systemic conditions. Exclusion criteria includes, teeth extraction with periodontal and periapical pathology.

The teeth were randomly separated into four experimental storage groups with 15 in each. Group I: Histidinetryptophan-Ketoglutarate solution,group II: aloe vera, group III: green tea and group IV: Propolis. Commercially available Histidinetryptophan-Ketoglutarate solution and green tea were used in the study and Propolis and aloe vera were prepared manually.

Immediately after the extraction teeth were washed to remove blood and stored in each storage medium for 45 minutes. Teeth were moved to storage solution by grasping only crown part with extraction forceps and by avoiding tobotherthe viable cells on root surface. After drying and soaking of each experimental tooth, 2.5 ml of stock solution containing grade II dispase and collagenase were added to each tooth and incubated for 30 min at 37°C. After incubation, 50 µL of fetal bovine serum was added to each tube with help of micropipette. All tubes were then centrifuged for four minutes at 1000 rpm and supernatant was disinterested with sterile micropipettes. The cells from supernatant were colored with 0.4% trypan blue for determination of viability. The number of viable PDL cells was counted under light microscope with hemocytometer at 40x magnification.

Propolis preparation

Hard propolis was grounded into fine powderusing mortar and pestle. 50% Propolis was prepared withaddition of 50 mg ground propolis powder/250 ml to 0.4% ethanol solution. This solution was mixed thoroughlyfor 15 min to make a homogenous mix.

Aloe Vera Extract Preparation

The Aloe Vera leaf was cleaned carefully and added to 70% ethanol alcohol. Following aseptic procedure, the leaf was split opened and with the help of scalpel, the viscous gel was scrapped from the inner part of the leaf, followed by homogenizing and filtering by means of a 0.45 µm filter mesh.

Green tea preparation

10 grams of leaves of green tea was soaked in 100 ml of boiling distilled water for 5 min and filter sterilized by using a Whatman filter paper to obtain green tea solution.

pH level and osmolality determination

pH level of each tested storage media was evaluatedusing an Orion pH Meter model 720 A (Orion Research, Inc., Boston, MA). Osmolality measurements were determined with a Vapro model 5520 Vapor Pressure Osmometer calibrated from 100 to 500 mosm/kg (Wescor, Inc., Logan, UT).

Statistical analysis

The obtained data was statistically evaluated with SPSS package (20.0 version, Inc.; Chicago, IL) using Mann- Whitney test, ANOVA, and Post hoc test at P value less than 0.05 was believedconsiderable.

Results

The mean periodontal cell in group I was 32.6 cumm, in group II was 26.2 cumm, group III was 17.6 cumm, and 34.5cumm in group-IV (Table-1). 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 by Mann Whitney test was significant ($P< 0.05$). After 72 hours mean absorbance valueswas 34.5 cumm for group IV and 32.6 cumm in group I which was higher than other groups after 72 hours (Table-2). Post Hoc test between different groups' revealed significant difference in mean periodontal cells ($P< 0.05$) (Table-3). Table 4 indicates mean pH level and osmolality value of different storage media used.Histidinetryptophan-Ketoglutarate solution (7.4 pH) and Propolis (7.5) had higher pH and osmolality value (319 and 342 respectively) than others.

Discussion

Various media have been used for storage of avulsed teeth.The ideal storage media for an avulsed tooth should have a neutral pH, physiological osmolality, low bacterial content, with essential nutrients [2].

In our study we observed highest cell viability with Propolis, followed by Histidinetryptophan-Ketoglutarate solution, and aloe vera and least with green tea. We observed higher pH and osmolality with Propolis, Histidinetryptophan-Ketoglutarate solution and aloe vera, while green tea showedlesser pH. We have compared natural media, such as; propolis and aloe vera with artificial media, such as; Histidinetryptophan-Ketoglutarate solution. We have selected natural media as they have higher pH, easily available and inexpensive compared to artificial media. This article helps to save tooth using natural storage media.

Babaji et al assessed the effectiveness of Propolis, HBSS, pomegranate juice (PJ)and *Aloe vera*, in maintaining the viability of periodontal ligament (PDL) cells. They found higher cell viability with Propolis compared to aloe vera and HBSS. This is in accordance to our findings[2].

Badakhsh et al evaluated the efficiency of several concentrations of Aloe Vera in contrast to egg white and DMEM. They found aloe vera asmore effective than other media, and suggested that aloe vera at 10%, 30% and 50% concentrations can be used as a storage media[8].Abraham *et al*, evaluated the effectiveness of Aloe Vera, milk and HBSS in preserving the viability of PDL cells. They found highest viable ligament cells in HBSS fallowed by milk and aloe vera [10].Sharma et al evaluated aloe vera with egg white and milk and found aloe vera better than others [11].

Anegundi et al compared the milk, Gatorade, coconut water, saline, tap water, egg white, and contact lens solution for preservation of pdl cell viability and they found higher cell viability with milk fallowed by contact lens solution and least with Gatorade [12].Adeli et al from their study concluded that green tea showed better efficacy than HBSS on preservation of periodontal ligament cells[13].Bharath et al concluded that green tea is effective as a storage media but its cell viability level is lesser than tender coconut water, HBSS and Ringer's lactate solution[14]. Abedian et al concluded from their study that green tea and HBSS were uniformly effective from preserving periodontal ligament cell viability and superior to water [15].

In our study we used trypan blue cell staining method since it is simple and fast to

carry out and distinguishes viable cells from nonviable one. Viable cells will have a plain cytoplasm but a nonviable cell will have a blue cytoplasm. In presentstudy, PDL cells were treated with collagenase and dispase Grade II to protect maximum viability and to reduce exposure of cells to active trypan. It has been seen that collagenase and dispase will help in upholding utmost cellular integrity [2].

Efficacy of the storage media depends mainly on osmolality value and pH level of the medium. The optimal cellular proliferationhappens at pH level of 7.2-7.4 and an osmolality in range of 230-400 mosmol/kg. We got higher cellular viability in Propolis, Histidinetryptophan-Ketoglutarate solutionand aloe vera because of high osmolality and pH value and due to their antibacterial, tissue healing capacity. Aloe vera plant is familiar in wound healing supporter; and it consists ofnecessary nutrients for survival of cells[8].Jun Oh et al evaluated the Histidine-tryptophan-ketoglutarate Solution as a Storage Medium for the Avulsed Tooth and found highest cell viability in 50% HTK solution [7]. This result is in accordance to our findings.

The drawback of the study was; this study doesn't compare the result with HBSS storage media, sample size was small, it was *in vitro* study not an*in vivo*study, hence oral conditions was not achieved. Further long term *in vivo* studies are required to evaluate and compare with other storage media.

Conclusion

Different storage medium is available for restoring the viability of avulsed teeth. We found propolishad higher periodontal cells viability followed by, Histidinetryptophan-Ketoglutarate solution, aloe vera and least in green tea.Propolis, Histidinetryptophan-Ketoglutarate solution, aloe veraand green tea media can be used as a storage media.

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Legends for illustrations

Tables

Table 1: Mean periodontal cells comparison in each group

Group	Mean (cumm)	SD	P value
Group I	32.6	4.11	0.01
Group II	26.2	3.84	
Group III	17.6	4.52	
Group IV	34.5	3.72	

Test used- ANOVA significance: p<0.05

Table 2:At different time intervals mean absorbance values

Time	Group I	Group II	Group III	Group IV	P value
3 hours	29.4	24.6	15.4	30.5	0.05
6 hours	30.6	24.8	15.9	31.2	
24 hours	31.6	25.1	16.2	32.7	
48 hours	32.0	25.8	16.8	33.8	
72 hours	32.6	26.2	17.6	34.5	

Mann Whitney test p< 0.05

Table 3: Multiple comparison among the groups

Tukey HSD	Significant
Group I vs	
Group II	0.003
Group III	0.001
Group IV	0.228
Group II vs	
Group III	0.343
Group IV	0.001
Group III vs	
Group IV	0.001

Test used-Post Hoc test Significant at (p< 0.05)

Table 4: Mean pH level and osmolality value of tested storage media

Storage media	pH level	Osmolality (mosmol)
Histidinetryptophan-Ketoglutarate solution (Group I)	7.4	319
Aleo vera (Group II)	7.1	310
Green tea (Group III)	7.0	300
Propolis (Group IV)	7.5	342