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EVALUATION OF THE EFFICACY OF PROPOLIS AS STORAGE MEDIA FOR AVULSED TEETH

Nada Mohamed Abdelbaseer*, Nagwa Mohammed Ali Khattab** *and* Mona Nagy Mahmoud Hamdi***

ABSTRACT

Background: The best treatment for tooth avulsion is immediate replantation as it maintains the periodontal ligament (PDL) cell viability. If not possible, the tooth must be kept in a suitable storage medium before seeking dental treatment.

Aim of the study: Evaluation of the efficacy of propolis as storage media in maintaining periodontal ligament cell viability for different time periods, in comparison to milk.

Materials and Methods: Immortalized human skin fibroblasts were cultured and stored in the following media: Dulbecco's modified Eagle's medium (DMEM), pasteurized low fat content milk and two different concentrations of propolis (10%, and 20%) that were prepared by diluting stock solution of propolis with either Phosphate buffer saline (PBS) or DMEM or milk for time points of 1, 3, 6 and 12 hours. Cell viability was determined using the SRB assay.

Results: Propolis had a significantly higher percentage of viable cells survived in propolis 10%, 20% storage media in comparison to other storage media: milk, DMEM and PBS through different time periods.

Conclusion: Propolis could be a good naturally available storage media for avulsed teeth.

KEY WORDS: Avulsion, Cell viability, Storage media, Propolis, Milk

INTRODUCTION

Tooth avulsion is a complicated injury that results in loss of the periodontal ligament attachment and is characterized by complete displacement of the tooth out its alveolar socket as a result of traumatic dental injury [(1)]. Immediate replantation is the key to success. If immediate replantation is not possible, the avulsed tooth must be kept in an appropiate storage media to maintain periodontal ligament (PDL) cell viability[(2)]. The prognosis of an avulsed

* Dentist at Ministry of Health, B.D.S, Faculty of Dentistry, Minia University
** Professor of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Ain Shams University.
*** Lecturer of Pediatric and Community Dentistry, Faculty of Dentistry, Minia University.

tooth following replantation is determined by additional oral dry time and the storage media [4,3].

Many solutions have been suggested as a transportation medium for avulsed teeth including Hank's balanced salt solution (HBSS), Egg White, Saliva, Dulbecco's modified Eagle's medium (DMEM), milk and Coconut water[5]. Milk effectiveness to preserve PDL cell viability can be linked to a variety of factors, including the existence of nutritional proteins and growth factors, physiological osmolarity, a pH buffering mechanism and low bacteria [6].

Propolis was suggested as a potential alternative medium for preserving PDL cells[7]. Propolis, a honeybee extract, provides potent antimicrobial, antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral, and tissue regenerating properties. Because the constituents of propolis vary greatly depending on climate, season, and the location, therefore its chemical formula is unstable [8].

Therefore, the current study was conducted to assess the potential of propolis in preserving the PDL cell viability at different time periods compared to milk.

MATERIALS AND METHODS

Cell culture

The current study using cultured human skin fibroblasts was approved by the Ethics Research Committee of Faculty of Dentistry, Minia University number 254 in 2019. The methodology was adapted from that described by **Lee et al. (2018)** with modifications in the cell viability assay [9].

In a laminar flow tissue culture hood, all cell culture procedures were performed**.** The cells were kept in pre-warmed DMEM which supplemented with 10% fetal bovine serum (FBS) and antibiotics (2% Penicillin-Streptomycin (100 IU/ml), and 0.5 % Fungizone). Then cells were incubated at 37ºC with 5% CO2 and 95% air atmosphere for 24 hours. Cells were subcultured when they were in a semi confluent state.

Preparation of propolis

Propolis was commercially procured from Younis Apiaries, Egypt. The propolis stock solution was prepared by dissolving the propolis in dimethyl sulfoxide (DMSO). Propolis working dilutions of 10% and 20%, were prepared by diluting stock solutions with PBS, DMEM, milk

Storage media

The storage media tested in this study include 1) Propolis 10% with PBS or with DMEM or with milk, 2) Propolis 20% with PBS or with DMEM or with milk, 3) Commercially available low-fat content pasteurized milk, 4) DMEM and 5) Phosphate buffer saline (PBS) 6) Control group (untreated cells was added).

Cell Viability assay

The cultured cells were trypsinized, counted and seeded in 96-well culture plates at initial density of $1x10^4$ cells/well in 100 µl of culture medium. The plates were then incubated for 24 hours at 37°C in 5% CO2 and 95% air to allow cell attachment. Then cell culture media was removed from each well. Each of storage media were placed into a well in the amount of 100µl. Cells in the wells in which media was not removed were used as controls. The cells were placed in storage media at room temperature for time intervals of 1, 3, 6, and 12 hours. The plates were prepared for the SRB assay.

100 µl of ice-cold 10% (wt /vol) trichloroacetic acid (TCA) were added to each well of the 96-well culture plates, and plates were incubated at 4°C for 1 hour to fix the cells. After several washes, the cells were stained for 30 minutes at room temperature with 100 µl of SRB solution then SRB was removed from the plates. The washed plates were left to air dry then Tris-HCL were added to each well for 30 minutes to solubilize protein-bound dye. The

absorbance values was measured by a microplate reader at 540 nm wavelength and calculated as a percentage using the following equation:

The viability percentage = (mean absorbance of tested storage media at any time / mean absorbance of control group) × 100.

Statistical analysis

The student t-test was used to compare mean values between two groups, while One-way analysis of variance (ANOVA) was used to compare means between more than two groups. For all significant tests, a probability of less than 0.05 was used as a cutoff point, and all statistical tests were two-tailed. SPSS version 20 was used for all analyzes.

RESULTS

The results showed that there was statistically a difference in absorbance value among tested storage media at different time periods 1, 3, 6, and 12 hours (p< 0.001). Regarding absorbance value, the effectiveness of propolis was significantly higher than DMEM, PBS and Milk at 1, 3, 6, and 12 hours. In general, when two concentrations of propolis (10%, 20%) were compared, it was found that propolis 10% significantly higher than propolis 20% at 1, 3 and 6 hours (p value; 0.007, 0.002, 0.014 respectively); However, no statistically significant difference between them was found at 12 hours (p value 0.66) **(Table 1).**

When comparing different Propolis 10% preparations (with DMEM, PBS, milk) at different time intervals, there was a statistically significant difference between the three groups, except at 3hour, where there was no significant difference among them (p = 0.33). The highest viability was found in Propolis 10% + DMEM except at 12hours, Propolis 10%+ milk was more effective than other media. When different preparations of Propolis 20% were compared at different time intervals, Propolis 20% + milk was significantly higher than other groups (p < 0.001) **(Figures 1&2).**

TABLE (1): Mean absorbance of cultured fibroblasts at all periods in tested storage media.

Storage media	Mean absorbance ± SD				P value
	1 Hour	3 Hours	6 Hours	12 Hours	
Control	1.238 ± 0.137	1.213 ± 0.002	1.005 ± 0.011	2.111 ± 0.009	**<0.001***
DMEM	0.291 ± 0.011	0.286 ± 0.002	0.246± 0.006	0.293± 0.020	**0.679**
PBS	0.252 ± 0.003	0.257 ± 0.023	0.235 ± 0.001	0.403 ± 0.004	**<0.001***
Milk	0.311± 0.0005	0.307± 0.0017	0.419± 0.064	0.226 ± 0.006	**<0.001***
10% Propolis+ DMEM	1.149 ± 0.012	1.171 ± 0.081	0.927 ± 0.056	1.240 ± 0.024	**0.097**
10% Propolis+ PBS	1.095 ± 0.040	1.090± 0.057	0.785 ± 0.036	0.998 ± 0.071	**0.11**
10% Propolis + Milk	1.046 ± 0.0527	1.064 ± 0.103	0.882 ± 0.033	1.817 ± 0.137	**0.11**
20% Propolis + DMEM	0.782 ± 0.0023	0.784 ± 0.070	0.587 ± 0.008	1.173 ± 0.131	**0.028***
20% Propolis + PBS	0.718 ± 0.006	0.699 ± 0.005	0.529± 0.041	0.640 ± 0.074	**0.158**
20% Propolis + Milk	1.123 ± 0.0058	1.078 ± 0.052	0.909 ± 0.016	1.945 ± 0.045	**0.052**
P value	**<0.001***	**<0.001***	**<0.001***	**<0.001***	

; significant (p<0.05

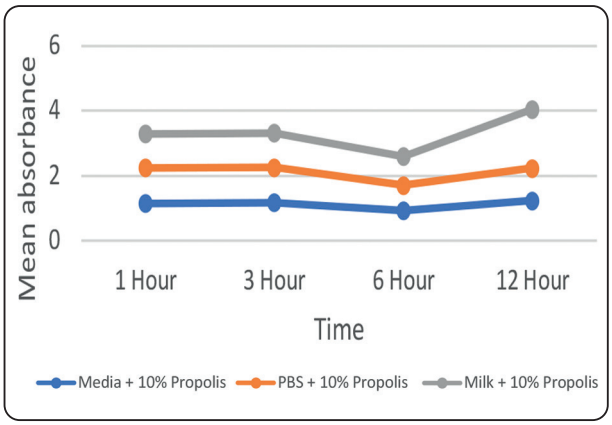

Fig (1): Line graph representing mean absorbance value of cultured fibroblasts at all periods in propolis 10% with different preparations.

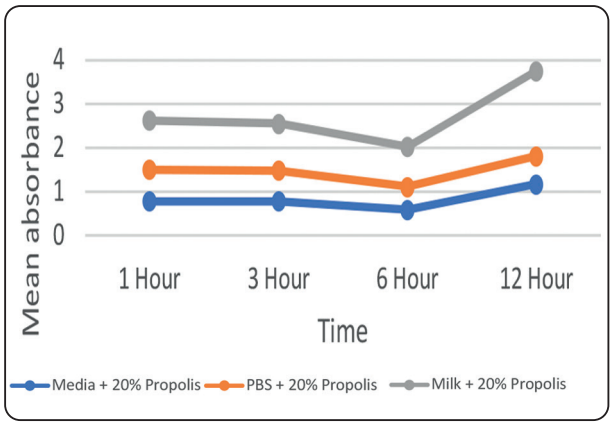

Fig (2): Line graph representing mean absorbance value of cultured fibroblasts at all periods in propolis 20% with different preparations.

DISCUSSION

The most serious type of traumatic dental injury is tooth avulsion. The preservation of viable PDL cells on the root surface is the most crucial factor impacting successful replantation. The immediate replantation of tooth into the socket, in these cases, is considered as the treatment of choice. If immediate replantation is not possible, the avulsed tooth must be kept in an appropriate storage media [10].

Great interest has arisen in the use of natural products for avulsed teeth. Therefore, the present study investigated the potential of propolis in preserving the viability of PDL cells for different time periods.

Propolis has certain characteristics that encouraged its testing as an effective storage media as it is rich in flavonoids, the most essential pharmacologically active element and potent antioxidant in propolis which explains its potential to maintain cell viability [11-13].

Chellammal et al., (2020) [15] reported that milk is the most recommended physiologic storage solution for an avulsed tooth so it was selected as a comparison medium in this study.

DMEM was selected in this study since it stimulates cell proliferation with reducing cell damage[15]. Also, PBS was selected in this study according to the recommendations of **Martins et al., (2016)**[16] who found that PBS is a non-toxic phosphate buffered solution similar to saline, with ideal osmolarity and pH, but no ions or glucose to preserve cell activity.

In terms of storage time periods, the present study was conducted in time frames of 1, 3, 6, 12 hours. These intervals were considered to be clinically relevant and allowed for comparison with prior studies [17-19].

The study was designed as in vitro cell culture study since one of the major challenges in conducting an in vivo study is that the method is difficult to standardize [20,21].

The SRB assay was selected for assessment since it is an independent of cellular metabolic activity for quantification of cell number, easy and sensitive [22,23].

The results of the present study revealed that propolis surpassed the other storage media used Milk, DMEM and PBS. This could be owing to its antibacterial, antioxidant, and anti-inflammatory characteristics. These results were in agreement

with several previous researches [8,24,28] who found that propolis more effective than milk. Despite these similar results it is important to address the contradiction with **De Souza et al., (2017)**[27] who showed that propolis less effective than milk. The reason for this conflict might be attributed to the difference in vehicles used to prepare the propolis and methodological differences.

The results of the current study also, revealed that reducing propolis concentration was associated with greater viability results. The results are in accordance with prior studies[24,25].

According to the results of this study, 10% propolis in DMEM ranked first among the media tested during all incubation periods. This might be attributed to the hypothesis that propolis has antibacterial, antioxidant, and anti-inflammatory characteristics and that DMEM provides as source of nutritional supplements. A finding that goes in accordance with **Özan et al., (2007), Saxena et al., (2011)**[5,25]. However, the results showed reduced cell viability in DMEM, when used as solo medium and this finding in agreement with previous studies [16,28].

There have been no previous investigations on the impact of the Propolis-milk mixture on cell viability. Milk and propolis, on the other hand, have scientific evidence that it can be used as a solo storage medium[5,29]. The viability of cells preserved in the Propolis-milk combination, on the other hand, was comparable to that of cells preserved in the Propolis-DMEM mixture under different storage times.

CONCLUSIONS

1. The effectiveness of propolis was higher than DMEM, PBS and milk at all incubation periods.
2. Propolis 10% was more efficient than propolis 20% in maintaining PDL viability.
3. Propolis-milk mix has the potential to maintain higher percentage of viable PDL cells at 12-hour period than each separately.
4. SRB assay is a reliable and valid method for assessing PDL cell viability.

LIMITATIONS OF THE STUDY

1. As with most substances for natural therapies, propolis has no standard recommended weight per volume of solution.
2. Cell viability assay should be augmented by complementary in vitro studies testing mitogenic and colonogenic capacities in addition to in vivo studies evaluating post replantaion response.

RECOMMENDATIONS

1. More researches are required to determine standard formulation for therapeutic usage of propolis.
2. Despite the promising results of this study for novel propolis-milk mix, further studies are required to determine the efficacy of this mix for storage media for avulsed teeth.
3. SRB assay is advocated as a reliable method for evaluating periodontal ligament cell viability.

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