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Original Article

Dentistry Section

Efficacy of Natural Coconut Water, Pre-packaged Coconut Water, and Hank's Balanced Salt Solution as Storage Media in Maintaining Periodontal Ligament Cell Viability: An In-vitro Study

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ABSTRACT

Introduction: Avulsion of teeth is one of the most complex forms of dental injury, and the selection of an appropriate storage medium greatly influences the preservation of Periodontal Ligament (PDL) cell viability, which is crucial for the successful re-implantation of avulsed teeth. Therefore, identifying effective storage options such as natural coconut water and pre-packaged coconut water holds significant promise in improving outcomes for this challenging dental injury.

Aim: To evaluate the efficacy of natural coconut water, pre-packaged coconut water, and Hank's Balanced Salt Solution (HBSS) as storage media in maintaining PDL cell viability.

Materials and Methods: A total of 32 non-carious freshly extracted human premolars were randomly divided into four study groups (n=8) and stored in the following storage media respectively: Group I-Natural coconut water group, Group II-Pre-packaged coconut water group, Group III-Bench dry group, and Group IV-HBSS groups for 30 minutes. The PDL cells were collected and incubated in phosphate buffer saline for 30 minutes and then centrifuged at 800 rpm for five minutes. Following this, the cells were stained with trypan blue to observe their viability. The Analysis of Variance (ANOVA) with Tukey's post-hoc test was used for analysing the data.

Results: The mean percentage of viable cells in natural coconut water (80.6250) was higher than in HBSS (79.8750), pre-packaged coconut water (79.2500), and the bench dry group (6.1250). Meanwhile, the mean percentage of non-viable cells was highest in the bench dry group (93.8750), followed by the pre-packaged coconut water (20.7500), HBSS (20.1250), and natural coconut water (19.3750).

Conclusion: Natural coconut water and pre-packaged coconut water are equally effective in maintaining the viability of PDL cells. Therefore, pre-packaged coconut water can be used as a substitute for natural coconut water for tooth storage, depending upon availability.

Keywords: Alternative storage media, Natural remedies, Reimplantation, Tooth preservation, Transport media, Trauma

INTRODUCTION

The most common problems in children and adolescents are traumatic dental injuries [1]. As children grow older, they often experience unpleasant dental incidents that may result in injury. This type of trauma can lead to dento-alveolar fractures, displacement, or avulsion of teeth, impacting a child's appearance, psyche, and behaviour [2]. The most complicated and severe form of dental injury is avulsion, characterised by the total displacement of a tooth from its alveolar socket, affecting the Periodontal Ligament (PDL), pulp, alveolar bone, and gingival tissues [3]. Avulsion in permanent dentition occurs in 1%-16% of all traumatic injuries [4], while in primary dentition it ranges from 5.8%-19.4%, with 19.2% being luxation injuries only. Children aged two to four are most commonly affected, with boys affected 1.2-1.5 times more than girls [5].

Immediate replantation is the ideal procedure for maintaining the viability of PDL cells when avulsion occurs [6]. It has been reported that the success rate of immediate replantation is 85% to 97%, depending on the stage of root development and the healing of the periodontal ligament [7]. However, this is rarely achieved. The prognosis depends on the extra-alveolar dry time, how the tooth is stored, and how well the root part is preserved, all affecting the vitality of PDL cells [8]. Thus, selecting a suitable storage media and extra-oral dry time plays a pivotal role in maintaining PDL cell viability until replantation is performed [9].

Numerous storage media have been introduced, depending on their ability to preserve PDL cell viability [6]. The ideal storage media should be proficient at maintaining PDL cell viability, readily accessible, inexpensive, have clonogenic capacity, be antioxidant, and free from microbial contamination. Blomlof L et al., stated that the ideal requirement for storage media is 290-330 osmolality with a pH of 6.6-7.8, which aids in preserving the viability of PDL cells [10].

In 1995, the American Association of Endodontics [11] endorsed Hank's Balanced Salt Solution (HBSS) (SAVE-A-TOOTH) as a perfect storage medium to maintain periodontal cell viability, but the major drawback is that it is expensive and not readily available.

In 2008, Gopikrishna V et al., introduced natural coconut water as a storage medium. This safest soft drink and biologically pure water helps in replacing body fluids and electrolytes like potassium, calcium, and magnesium. Normally, it is present in a sterile form and promptly accepted by the body, so it can be used as a blood plasma substitute [12].

Currently, very few authors have suggested using coconut water as a storage medium [8,12]. However, to date, there is no study that has compared the efficacy of pre-packed coconut water with natural coconut water or HBSS. Thus, the aim of the present study is to evaluate the efficacy of natural coconut water, pre-packed coconut water, and HBSS as storage media in maintaining PDL cell viability.

MATERIALS AND METHODS

The current ex-vivo research was conducted in the Department of Pedodontics and Preventive Dentistry, in collaboration with the Department of Oral and Maxillofacial Surgery and the Department of Oral Pathology at Maitri College of Dentistry and Research Centre in Durg, Chhattisgarh, India, from November 2022 to January 2023. This study was reviewed and approved by the Institutional Ethical Committee {MCDRC/2022/DEC/1028(A)}.

Inclusion criteria: Those extracted premolar teeth which are non-carious with normal periodontium and closed apices were included in the study.

Exclusion criteria: The teeth with periapical pathology or periodontal issues, caries, or ones which were fractured , or those with developmental anomalies were excluded from the study.

Sample size calculation: Using G*power software version (3.1.9.4) at a 95% confidence interval, and the power of the study was kept constant at 80%. The total sample size was 32 (n=8).

Procedure

After atraumatic extraction, the teeth were rinsed with running water for 10 seconds to remove any blood or saliva from the crown. Subsequently, 3 mm of the Periodontal Ligament (PDL) was scraped off coronally using a 15-no BP blade to remove damaged cells that may have occurred during the extraction procedure. The teeth were then dried for 15 minutes after curettage, followed by 30 minutes of immersion. This was done because PDL cells are most susceptible to damage during this time, and preservation of the teeth in storage media reduced the damage [Table/Fig-1a]. The teeth were then randomly divided and placed into:

- Group-I: Natural coconut water
- Group-II: Pre-packed coconut water (Real active 100% tender coconut juice)
- Group-III: Bench dry (Negative control group)
- Group-IV: HBSS (Positive control group) (Lonza™ BioWhittaker™)

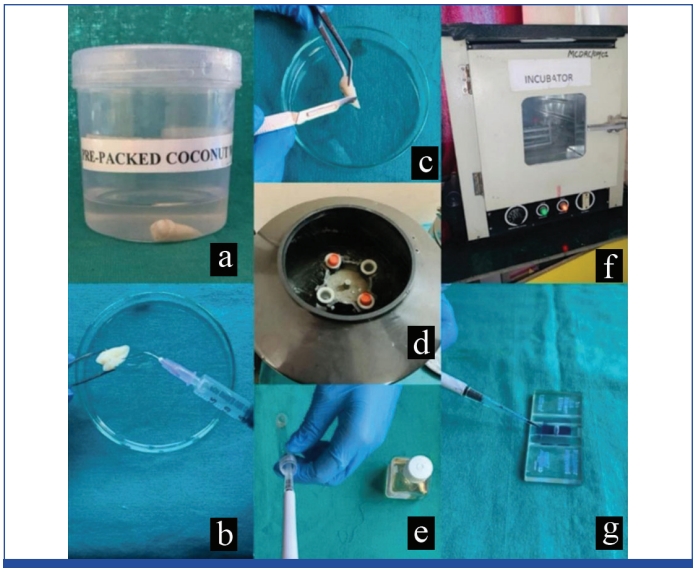
Following removal from the storage media, the teeth were rinsed with phosphate buffer saline (Himedia, 100 mL) [Table/Fig-1b]. PDL cells were then scraped from the apical two-thirds of the root with a sterile surgical blade [Table/Fig-1c]. The scraped-off PDL cells were collected in falcon tubes containing PBS and centrifuged for five minutes at 800 rpm to pelletize PDL tissue [Table/Fig-1d] [13]. After centrifugation, the phosphate buffer solution was discarded, and 0.5 ml of type-1 collagenase enzyme (Hi-media Labs, Mumbai, India) was added [Table/Fig-1e] and incubated for 30 minutes. Once the tissue was completely digested, the supernatant was discarded, and the collected residue was stained with 0.4% trypan blue dye (Loba), gently mixed, and incubated at room temperature for five minutes [Table/Fig-1f]. The suspension was loaded into a Neubauer haemocytometer [Table/Fig-1g], and the number of viable and non-viable cells was counted. The cells that appeared pink in colour were viable, whereas non-viable cells appeared blue in colour, which was observed under a light microscope at 10x magnification [Table/Fig-2] [13].

The cell count was done using this formula [14]:

$$\frac{\text{Total cells-stained cells} \times 100}{\text{Total cells}}$$

STATISTICAL ANALYSIS

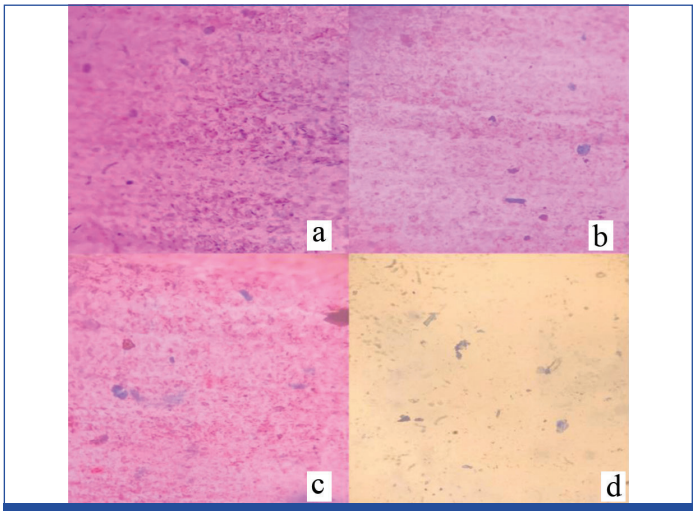
The collected data was entered into Microsoft Excel 2021, and data analysis was conducted using IBM Statistical Package for Social Sciences (SPSS) version 24.0. for Windows. For descriptive statistics, ANOVA with Tukey's post-hoc test was applied to analyse the data, and the level of statistical significance was set at a p-value of ≤0.05.



[Table/Fig-1]: a) Teeth stored in storage media; b) Rinsing of teeth with phosphate buffer saline after removing from storage media; c) PDL cells were scraped from the apical 2/3rd of the root with a sterile surgical blade; d) Centrifugation at 800 rpm; e) Addition of Type-1 collagenase enzyme before incubation; f) Incubation; g) Loading of suspension into Neubauer haemocytometer to count the number of viable and non-viable cells.

RESULTS

The mean percentage of viable cells in natural coconut water (80.6250) [Table/Fig-2a] was significantly higher (p=0.001) than in HBSS (79.8750) [Table/Fig-2b], pre-packed coconut water (79.2500) [Table/Fig-2c], and the bench dry group (6.1250). Conversely, the mean percentage of non-viable cells was highest in the bench dry group (93.8750) [Table/Fig-2d], followed by the pre-packed coconut water (20.7500), HBSS (20.1250), and natural coconut water (19.3750) when used as a storage media [Table/Fig-3].



[Table/Fig-2]: Microscopic view of viable and non-viable cells under 10x magnification a) Natural Coconut water; b) HBSS; c) Pre-packed coconut water; d) Bench dry.

A significant (p<0.05) difference between the bench dry group and the other groups was observed in both viable and non-viable cells. Natural coconut water was superior in preserving the viability of PDL cells compared to the pre-packed and HBSS groups. The efficacy of pre-packed coconut water in maintaining the viability of PDL cells was almost equal to that of natural coconut water and HBSS [Table/Fig-4].

DISCUSSION

The most dreadful form of traumatic dental injury is avulsion, characterised by the complete displacement of a tooth from its alveolar socket [15]. Hammer H stated that the prognosis and survival duration of a reimplanted tooth are directly related to the quantity of viable periodontal cells [16]. Our study concluded that the maximum percentage of viable cells was found in natural

Cell type	Group	Teeth evaluated	Mean	Std. Deviation	f-value	p-value
Viable cells	Coconut water	8	80.6250	4.06861	17.34	**0.001**
	Pre-packed coconut water	8	79.2500	7.20615		
	HBSS	8	79.8750	2.69590		
	Bench dry	8	6.1250	3.83359		
Non-viable cells	Coconut water	8	19.3750	4.06861	14.34	**0.001**
	Pre-packed coconut water	8	20.7500	7.20615		
	HBSS	8	20.1250	2.69590		
	Bench dry	8	93.8750	3.83359		

[Table/Fig-3]: Mean comparison of Group HBSS, coconut water and pre-packed coconut water.
Statistical test: ANOVA; (p<0.05- significant, CI=95%); bold p-values are significant

Cell type	Groups		p-value
Viable cells	HBSS	Coconut water	0.952
		Pre-packed coconut water	0.967
		Bench dry	**0.001**
	Coconut water	Pre-packed coconut water	0.849
		Bench dry	**0.001**
	Pre-packed coconut water	Bench dry	**0.001**
Non-viable cells	HBSS	Coconut water	0.56
		Pre-packed coconut water	0.24
		Bench dry	**0.001**
	Coconut water	Pre-packed coconut water	0.89
		Bench dry	**0.001**
	Pre-packed coconut water	Bench dry	**0.004**

[Table/Fig-4]: Intergroup comparison between all groups among viable cells.
Statistical test: Tukey's post-hoc test; (p<0.05- significant, CI=95%) ; bold p-values are significant

coconut water (80.62%), followed by HBSS (79.87%) and pre-packed coconut water (79.25%).

The management of an avulsed tooth involves replantation at the earliest possible time [6]. When immediate re-implantation is not possible, the tooth should be stored in a suitable storage medium that helps sustain PDL cell viability until treatment is given [15]. Andreasen JO et al., stated that replantation of teeth beyond five minutes of avulsion has been defined as delayed replantation [17]. Numerous transport/storage media have been proposed, such as HBSS, ViaSpan, propolis, milk, soya milk, honey, pomegranate juice, normal saline, and the patient's saliva, etc. Therefore, natural coconut water and pre-packed coconut water were chosen in the present study since they are easily available and cost-effective.

Natural coconut water (Cocos nucifera L.), often known as the "Tree of Life," is a drink that is organically manufactured and hermetically sealed in a sanitary manner without any contamination. It is highly rich in proteins, vitamins, and minerals. The electrolytic composition of coconut water resembles the intracellular fluid more than extracellular plasma. It is a hypotonic, sterile solution that is relatively more acidic than plasma, with a specific gravity of 1.020 and a pH of 4.1. It possesses regenerative and antioxidant properties and has an osmolality of 288 mOsm/kg. Coconut water at 100% concentration is more effective as a storage medium than coconut water at 50% [15].

Pre-packed coconut water (active real fruit) is a packaged version of 100% natural coconut water with a few percentages of preservatives and no additives. It possesses similar properties to natural coconut water and is easily available at a cheaper rate.

Age, trauma, and inflammation have all been recognised to have an impact on fibroblast function. Therefore, non-carious mature human premolar teeth, which were extracted for orthodontic purposes, were chosen. Pohl Y et al., stated that PDL cells remain uncompromised up to the 15-minute dry time [18]. Therefore, to maintain the extraoral dry period, 15 minutes were chosen to replicate avulsion injury and to guarantee that there are enough live PDL cells accessible for assessment, followed by immersion in storage media.

Type-1 collagenase was used to treat the PDL cells as it helps minimise cell exposure to active trypan and maintains maximum viability, aiding in cellular integrity. Meanwhile, the trypan blue staining technique is easy and quick to perform, so we have used this technique, which helps in the differentiation of viable cells from non-viable ones. Viable cells will have a clear cytoplasm, and non-viable cells will have a blue cytoplasm, as damaged cell membranes will allow trypan blue dye to pass into the cytoplasm [13].

Upon reviewing the literature, Omar SL et al., (2013) stated that coconut water, comparable to HBSS, showed more satisfactory results than milk and saline for maintaining the viability of PDL cells of avulsed teeth [19]. Gopikrishna V et al., conducted a study to compare the efficacy of HBSS, milk, propolis, and coconut water, and concluded that coconut water is superior to HBSS, propolis, and milk [20]. Thomas T et al., (2008) used a collagenase dispase assay II to determine the effectiveness of coconut water, HBSS, propolis, and milk in conserving the viability of PDL cells and observed that coconut water contains a greater number of viable PDL cells [21]. Sunil O et al., found that coconut water is a better storage medium than HBSS and milk [22].

HBSS, being a gold-standard storage medium commercially known as Save-A-Tooth, has a pH of 7.2 and is non-toxic, containing essential metabolites that are important for PDL cell viability. When kept in HBSS, the viability of PDL cells is maintained for up to 48 hours. It has been recommended to keep an avulsed tooth in HBSS for a maximum of 30 minutes before reimplantation. The major disadvantage of this storage medium is that it is not easily available and is expensive [23]. Similar studies from the literature comparing different storage media on PDL viability have been tabulated in [Table/Fig-5] [6,8,9,12,13,21,23,24].

Up to now, the in-vitro studies conducted have shown that coconut water maintains cell viability for a longer duration. However, our sample size is small; furthermore, more investigations are required.

S. No.	Author's name and year	Place of study	Sample size	Storage media compared	Parameters assessed	Conclusion
1.	Gopikrishna V et al., (2008) [12]	Chennai	70 premolars	Coconut water, propolis, HBSS, milk	Periodontal cell survival	Coconut water showed higher no viable PDL cells than propolis, HBSS and milk
2.	Thomas T et al., (2008) [21]	Chennai	50 premolars	Coconut water, HBSS, milk	Periodontal cell viability	Coconut water is better alternative to HBSS or milk
3.	Souza BD et al., (2010) [8]	Brazil	Plates of PDLF were soaked in storage media	HBSS, skimmed milk, whole milk, Save A Tooth system, industrialised coconut water and tap water	Effectiveness of storage media in maintaining the PDL cell viability at 5	Skimmed and whole milk had the greatest capacity to maintain PDLF viability when compared with others
4.	Al-Haj Ali S et al., (2011) [6]	Jordan	Sound permanent molars	Dulbecco's Modified Eagles's Medium (DMEM), coconut water	Evaluate the concentration and maturity of coconut water in maintaining the PDL cell viability	Avulsed teeth that are left dry for >30 minutes may benefit from soaking in 100% mature coconut water
5.	Moura CC et al., (2017) [9]	Brazil	70 mature dog's teeth	Powdered coconut water	Powdered coconut water at different osmolarities to maintain PDL cell viability	Powdered coconut water formulas, ACP-404-I and ACP-404-II, preserved viability for up to 24 hour

6.	Babaji P et al., (2017) [23]	Kerala	50 premolars	HBSS, Propolis, Aloe vera, Pomegranate juice	Pdl cell viability and best storage medium	Propolis showed more no viable cells followed by HBSS, Aloe vera and PJ
7.	Dhimole P et al., (2019) [13]	Jabalpur	90 Premolars	Milk, Neem, and turmeric	Pdl cell viability and best storage medium	Neem is as efficient as milk in maintaining PDL cell viability than others
8.	Sharma S et al., (2023) [24]	Indore	Freshly extracted premolars	Propolis, coconut water, aloe vera, and soy milk storage media	Assess the PDL cell viability	Higher viable PDL cells with propolis, followed by coconut water, aloe vera, morus rubra, and soy milk
9.	Present study	Chhattisgarh	32 freshly extracted premolars	Pre-packed coconut water, natural coconut water and HBSS	Assess the PDL cell viability	Pre-packed coconut water can be used as an alternative storage media

[Table/Fig-5]: Previous studies conducted by various authors on different storage media [6,8,9,12,13,21,23,24].

It is essential to carry out in-vivo investigations to evaluate the characteristics of effective replantation.

Limitation(s)

The limitations of our study are a small sample size and short-term evaluation.

CONCLUSION(S)

In the current study, we observed that the number of viable cells was significantly higher in natural coconut water than in HBSS, pre-packed coconut water, and the bench-dry group. The efficacy of pre-packed coconut water in maintaining the viability of PDL cells was almost equal to that of natural coconut water and HBSS. As both of these ingredients, i.e., pre-packed coconut water and natural coconut water, are readily available, cost-effective, and efficacious alternatives to HBSS, they can be used as alternative storage media for an avulsed tooth.

Longer-term clinical trials are needed to assess the sustained effect of the storage media on periodontal and cell viability. It is also essential to carry out in-vivo investigations to evaluate the characteristics of effective replantation.

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