

# Comparison of Coconut Water, Propolis, HBSS, and Milk on PDL Cell Survival

Velayutham Gopikrishna, MDS, Parvinder Singh Baweja, BDS, Nagendrababu Venkateshbabu, BDS, Toby Thomas, MDS, and Deivanayagam Kandaswamy, MDS

## Abstract

Coconut water is biologically pure and sterile, with a rich presence of amino acids, proteins, vitamins, and minerals. The purpose of this study was to use a collagenase-dispase assay to investigate the potential of a new storage medium, coconut water, in comparison with propolis, Hank's balanced salt solution (HBSS), and milk in maintaining viable periodontal ligament (PDL) cells on simulated avulsed teeth. Seventy freshly extracted human teeth were divided into 4 experimental groups and 2 control groups. The positive and negative controls corresponded to 0-minute and 8-hour dry times, respectively. The experimental teeth were stored dry for 30 minutes and then immersed in 1 of the 4 media (coconut water, propolis, HBSS, and milk). The teeth were then treated with dispase grade II and collagenase for 30 minutes. The number of viable PDL cells was counted with a hemocytometer and analyzed. Statistical analysis showed that coconut water kept significantly more PDL cells viable compared with propolis, HBSS, or milk. Coconut water can be used as a superior transport medium for avulsed teeth. (*J Endod* 2008;34:587–589)

## Key Words

Avulsed teeth, coconut water, collagenase dispase assay, propolis, transport media

From the Department of Conservative Dentistry and Endodontics, Meenakshi Ammal Dental College, Maduravoyal, Chennai, India.

Address requests for reprints to Dr Velayutham Gopikrishna, Reader, Department of Conservative Dentistry and Endodontics, Meenakshi Ammal Dental College, Alapakkam Main Road, Maduravoyal, Chennai 600 095, India. E-mail address: [hi\\_gopikrishna@hotmail.com](mailto:hi_gopikrishna@hotmail.com).

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The reported incidence of complete avulsion ranges from 1%–16% of all traumatic injuries to the permanent dentition (1). Avulsion injury, one of the most severe forms of dental trauma, is characterized by complete displacement of the tooth from its alveolar socket. Because of the complexity of this injury, the neurovascular supply is severely compromised and usually results in loss of pulp vitality (2). When avulsion occurs, the avulsed tooth should be immediately replanted at the site of the accident to prevent further damage to the periodontal ligament (PDL) cells from desiccation. However, immediate repositioning of teeth is not always possible under certain conditions. In such a case, storage medium is used to preserve PDL cell viability. The choice of such storage medium for maintenance of maximum PDL cell survival until replantation is very important (3).

Two of the most critical factors affecting the prognosis of an avulsed tooth after replantation are extraoral dry time and the storage medium in which the tooth is placed before treatment is rendered (2). However, the ability of a storage/transport medium to support cell viability can be more important than the extraoral time to prevent ankylosis and replacement resorption (4, 5). The American Association of Endodontics has recommended Hank's balanced salt solution (HBSS) as the storage medium of choice for avulsed teeth (6). Dumsha (7) and Patil et al. (8) suggested storing of an avulsed tooth in milk, HBSS, or saline.

Propolis, a substance made by the honeybee, is a potent antimicrobial, antioxidant, and anti-inflammatory agent. The main chemical classes present in propolis are flavonoids (9), phenolics, and various aromatic compounds. Flavonoids are well-known plant compounds that have antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties. Martin and Pileggi (2) and Özan et al. (3) found propolis to be a superior transport medium to HBSS or milk in terms of maintaining PDL cell viability after avulsion and storage.

The biologically pure, tender coconut water is readily accepted by the body because it is sterile and thus used as a blood plasma substitute. It also helps to replace fluids, electrolytes (potassium, calcium, and magnesium), and sugars lost from the body during heavy physical exercise (10). Gopikrishna et al. (10) found that coconut water was superior to HBSS or milk in terms of maintaining PDL cell viability after avulsion and storage. However, no study to date has compared the ability of both these natural mediums; coconut water and propolis, in terms of maintaining PDL cell viability after avulsion and storage.

Hence, the purpose of this study was to evaluate the efficacy of a new storage medium, coconut water, in comparison with propolis, HBSS, and milk in maintaining the viability of PDL cells by using a collagenase-dispase assay.

## Materials and Methods

Seventy human teeth with closed apices that were extracted for orthodontic purpose were obtained for this study. The average age of the patient was 21 years. Teeth extracted from patients with moderate to severe periodontal disease or with extensive caries were excluded. Extractions were performed as atraumatically as possible by an oral surgeon. After extractions, the teeth were held with forceps by the coronal region, and the coronal 3 mm of PDL was scraped with a curette to remove cells that might have been damaged.

The teeth were then randomly divided into 1 of the 4 experimental storage solution groups, namely group 1, coconut water; group 2, propolis 50%; group 3, HBSS; and group 4, milk, with 15 samples per group. The positive and negative control groups consisted of 5 samples each.

Solid propolis was ground into fine particles with a mortar and pestle. Propolis was then made into 50% concentration within a 0.4% ethanol solution. Propolis 50% consisted of 50 mg ground propolis per 250 mL of the 0.4% ethanol solution. Before submersion of teeth in propolis, the solutions were shaken for 15 minutes.

The teeth in the experimental groups were dried for 30 minutes (during which time the coronal PDL cells were curretted), followed by a 45-minute immersion in 1 of the 4 storage solution groups. The positive control teeth were not dried and were not stored in any solution, but instead, they were immediately treated with dispase and collagenase. The negative control teeth were bench-dried for 8 hours, with no follow-up storage solution time, and then placed in the dispase and collagenase.

Each experimental tooth, after drying and soaking, was incubated for 30 minutes in 15-mL Falcon tubes (BD Biosciences, San Jose, CA) with a 2.5-mL solution of 0.2 mg/mL<sup>-1</sup> of collagenase CLS II (Cooper Biomedical, Malvern, PA) and a 2.4 mg/mL<sup>-1</sup> solution of dispase grade II (Gibco, Taastrup, Denmark) in phosphate-buffered saline. After incubation, 50 µL of fetal bovine serum was added to each tube. All tubes were then centrifuged for 4 minutes at 1000 rpm. The supernatant was then removed with sterile micropipettes, and the cells were labeled with 0.4% trypan blue for determination of viability, according to Polverini and Leibovich (11). The number of viable protective least significant difference (PDL) cells was counted under a light microscope with a hemocytometer at 20× magnification. The results were statistically analyzed with analysis of variance and multiple range by using Tukey honestly significant difference. The level of significance was 5% ( $P < .05$ ).

**Results**

Statistical analysis showed a significant difference among the groups. Tukey honestly significant difference test showed that coconut water group demonstrated a significantly higher number of viable PDL cells than propolis 50%, HBSS, and milk. There was no significant difference between propolis 50% and HBSS groups. However, both propolis 50% and HBSS groups demonstrated a significantly higher number of viable PDL cells than milk. All experimental solution groups were significantly lower than positive control and higher than negative control (Table 1).

**Discussion**

An avulsion injury results in complete displacement of the tooth from the socket, which leads to loss of attachment of the PDL and loss of vascularization to the pulp (2). The success of post replantation is dependent on the immediate treatment (7, 12). According to Hammer

(13), length of survival of a replanted tooth is directly related to the amount of viable periodontal membrane. Successful replantation of avulsed teeth is dependent on the prevention or limitation of inflammatory and replacement root resorption (14, 15). Damage of periodontium at the time of accident and presence of bacteria within the root canals and tubules are directly related to inflammatory resorption. Damage to periodontium at the time of avulsion and the extent to which the viability of the PDL remains on tooth surface are the reasons for replacement resorption (16–18). Hence, prognosis of an avulsed tooth is mainly based on status of the PDL. Thus, extraoral time and storage medium are the critical factors responsible for prognosis of avulsed tooth (2).

Various techniques have been used to quantitate the number of viable PDL cells. A stepwise trypsinization procedure by exposing samples to trypsin 3 consecutive times for 20 minutes each was reported by Reinholdt et al. (19). Chromogenic stain has been used by Soder et al. (4) to quantitate viable PDL cells. Patil et al. (8) used a stepwise trypsinization procedure and fluorescein diacetate as a staining technique for determining the viability of PDL cells in simulated avulsion injuries. In the current study to minimize the exposure of cells to active trypsin and to preserve maximum cell viability, the root surface was treated with collagenase and dispase grade II as was performed in the work by Pileggi et al. (20). This procedure allowed rapid cell retrieval and maintained maximum cellular integrity, as was demonstrated by the positive control samples (20). This method is more representative of the actual clinical situation because the cells are not subjected to long processing times to determine their viability status.

Milk, saliva, saline, HBSS, propolis, Viaspan, and recently coconut water have been used as storage media. HBSS is a standard saline solution that is widely used in biomedical research to support the growth of many cell types (21). It is nontoxic and pH balanced and contains many essential nutrients (22, 23). Matsson et al. (24) examined extracted dogs' teeth stored dry for 15, 30, or 60 minutes and then replanted versus teeth stored dry for the same dry times but also soaked in HBSS for 30 minutes before replantation. These researchers found significantly less resorption in the HBSS-soaked teeth for all dry times. HBSS has been commercially available as Save-A-Tooth (Save-A-Tooth Inc, Pottstown, PA) (21) as a storage medium for avulsed teeth, although it is not yet widely available in pharmacies or drug stores.

Propolis has been recently used as storage medium for avulsed teeth (2, 3) and as an intracanal medicament (25). According to Martin and Pileggi (2), propolis might be a better alternative transport medium compared with HBSS, milk, or saline in terms of maintaining PDL cell viability after avulsion and storage. They also proved that 100% propolis was not significantly different from 50% propolis, indicating that former concentration was not more toxic to the PDL cell. Hence, in our study we used 50% propolis as one of the test groups. The performance of propolis 50% in our study was similar to that of HBSS in the maintenance of PDL cell viability. These results are in contrast to those of the studies by Martin and Pileggi (2) and Özan et al. (3). This could be attributed to the variability in the constituents of propolis, which varies because of climate, season, and location (26, 27). Moreover, the chemical formula of propolis is not stable (26, 27).

Coconut (*Cocos nucifera L.*), popularly known as “tree of life,” is a natural drink produced biologically and hermetically packed inside the coconut in a hygienic way without any contamination. The electrolyte composition of coconut water resembles intracellular fluid more closely than extracellular plasma. The predominant cations are potassium, calcium, and magnesium. Sodium, chloride, and phosphate are found in much lower concentrations. It is a hypotonic solution that is more acidic than plasma and has a specific gravity of approximately 1.020, comparable with blood plasma (28). Coconut water can be given

**TABLE 1.** Means and Standard Deviations of All Test Groups

Group	No. of Samples	Mean no. of Viable Cells	Standard Deviation
Coconut water	15	532.8	9.7
Propolis	15	443.0	18.5
HBSS	15	434.2	6.0
Milk	15	183.3	13.0
Positive control	5	3062.7	417.0
Negative control	5	28.7	4.7

HBSS, Hank's balanced salt solution.

to patients with potassium deficiency as a means of rehydration because it is readily acceptable by human body (29).

From the results of our study, coconut water (532.80) statistically showed better viability of cells than propolis (443.07), HBSS (434.27), and milk (183.33). This might be due to the nutrients present in coconut water such as proteins, amino acids, vitamins, and minerals, which help in nourishing the cells and maintaining their viability. The primary sugars present in coconut water are glucose and fructose, which are responsible for the high osmolarity of coconut water. It is also rich in many essential amino acids including lysine, cystine, phenylalanine, histidine, and tryptophan (28). Blomlof (30) showed that the important factor in maintaining the viability is the osmolarity of the transport medium. Andreasen (14) found that in contrast to water, both physiologic saline and saliva, with their differences in chemical composition but similar osmolality, were able to decrease the incidence of root resorption. This suggests that the viability of the PDL is more closely linked to the osmolarity of the solution than to its chemical composition.

Coconut water obtained from the fruit of coconut palm is grown in more than 93 countries around the world, with a very high growth density in South Asian countries (India and Sri Lanka), South East Asian countries (Vietnam, Thailand, Indonesia, Malaysia, Philippines), and Pacific nations (Western Samoa, Vanuatu, Fiji). It also has a significant presence in Jamaica and Mexico (31). Thus, coconut water, which is natural, hygienic, and easily available in these geographical locations, can be advocated as a superior transport medium for avulsed teeth.

## Conclusion

Within the limitations of our study, coconut water maintained better PDL cell viability than 50% propolis, HBSS, and milk.

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