A quantitative analysis of coconut water: a new storage media for avulsed teeth

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Objective. The purpose of this study was to use a Collagenase-Dispase assay to investigate the potential of a new storage media, coconut water, in maintaining viable periodontal ligament (PDL) cells on simulated avulsed teeth.
Study design. Fifty freshly extracted human teeth were divided into 3 experimental groups and 2 control groups. The positive and negative controls corresponded to 0 minutes and an 8-hour dry time, respectively. The experimental teeth were stored dry for 30 minutes and then immersed in 1 of the 3 media: coconut water (CW), Hank's balanced salt solution (HBSS), and milk. The teeth were then treated with Dispase grade II and Collagenase for 30 minutes. The number of viable PDL cells were counted with a hemocytometer and analyzed.
Results. Statistical analysis demonstrated that CW kept significantly more PDL cells viable compared to either HBSS or milk.
Conclusion. Within the parameters of this study, it appears that CW may be better alternative to HBSS or milk in terms of maintaining PDL cell viability after avulsion and storage. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:e61-e65)**

Clinical surveys indicate that traumatic dental injuries in children and adolescents are a common problem and studies have shown that prevalence of these injuries is increasing.[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.

In cases of tooth avulsions, the primary goal is to preserve the vitality of the periodontal ligament (PDL) cells attached to the root surface until appropriate treatment can be performed. This may bring about a favorable reattachment of the periodontal ligament. Soder et al.[2] showed that after avulsion, the number of viable cells on the root surface decreased with increased drying time and that after 2 hours it was not possible to demonstrate cell viability. Therefore, the ideal treatment of choice at the time of avulsion is immediate replantation, thus reestablishing the natural nutrient supply to the periodontal ligament cells, thereby minimizing further damage and enhancing the healing process. Unfortunately, some situations may occur that delay immediate replantation. Where such situations exist, the tooth should be stored in a medium that maintains periodontal ligament cell viability until definitive dental treatment can be accomplished.

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.[2,3] Various storage media such as tap water, saliva, saline, milk, culture media, Viaspan, and Hank's Balanced Salt Solution (HBSS) (marketed as Save-A-Tooth) have been employed. Dumsha[4] and Patil et al.[5] suggested storing the avulsed tooth in milk, HBSS, or saline.

The biologically pure, tender coconut water helps to replace fluids, electrolytes (potassium, calcium, and magnesium), and sugars lost from the body during heavy physical exercise. It is used as a blood plasma substitute as it is sterile and readily accepted by the body.[6] Taking these properties into consideration we hypothesized that this natural isotonic drink could be a viable storage medium in the transportation of an avulsed tooth. Currently no studies have suggested using coconut water as a storage medium.

This study was supported by a scientific research grant given by the Meenakshi University, Chennai, India, vide Grant No MAHER/517/2006. The authors thank M. Parthiban, PhD, Assistant Professor, Department of Animal Biotechnology, Tamilnadu University of Veterinary and Animal Sciences (TANUVAS), Chennai, India for his technical and collaborative support.
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Received for publication Mar 26, 2007; returned for revision Jul 20, 2007; accepted for publication Aug 1, 2007.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2007.08.003

Several techniques have been used to determine the viability of the PDL cells following avulsion; however, most of the experimental data that are available have been obtained using techniques in which the cells are cultured and/or trypsinized for longer periods of time. As the extracellular matrix has a high content of collagen and other proteins, it seems reasonable that the use of enzymatic desegregation would provide a greater number of cells within a shorter time frame.[7] Pileggi et al.[8] used a collagenase dispase assay to compare PDL viability of simulated avulsed teeth after storage in HBSS, milk, saline, or water. Both collagenase and dispase enzymes disrupt the extracellular matrix and cause the release of cells without excessive disruption and destruction of their own membrane. Therefore, these authors suggested that the use of these 2 enzymes may provide additional data regarding the viability of PDL cells after avulsion injury. Furthermore, this method may be more representative of the actual clinical situation because the cells are not subjected to long processing times to determine their viability status.

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

MATERIALS AND METHODS

Fifty-five human teeth with closed apices that were extracted for orthodontic purpose were obtained for this study. The average age of the patient was 24 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. Following 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 may have been damaged.

The teeth were then randomly divided into 1 of the 3 experimental storage solution groups: group 1, coconut water; group 2, HBSS; and group 3, milk. There were 15 samples per group. The positive and the negative control group consisted of 5 samples each. The teeth in the experimental groups were dried for 30 minutes (during which time the coronal PDL cells were curetted), followed by a 45-minute immersion in 1 of the 3 storage solution groups. The positive control teeth were neither dried nor were they stored in any solution, but rather 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 with a 2.5-mL solution of 0.2 mg/mL^{-1} of collagenase CLS II (Cooper Biomedical, Malvern, PA) and a 2.4 mg/mL^{-1} solution of dispase grade II (Gibco, Taastrup, Denmark) in phosphate-buffered saline (PBS). After incubation, 50 μL of fetal bovine serum (FBS) was added to each tube. All tubes were then centrifuged for 4 minutes at 110 × g. 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.[9] The number of viable protective least significant difference (PDL) cells was counted under a light microscope with a hemocytometer at ×20 magnification. Statistical analysis of the results was done by using Tukey-HSD multiple range test.

Table I. Number of viable cells (M ± SD) for the various test groups

Groups	*Number of samples*	*Mean number of viable cells*	*SD*
CW	15	525.00	9.51
HBSS	15	446.73	19.12
MLK	15	185.60	12.01
PC	5	3762.729	426.018
NC	5	38.700	5.16

CW, coconut water; *HBSS,* Hank's balanced salt solution; *MLK,* milk; *PC,* positive control; *NC,* negative control.

RESULTS

The teeth stored in coconut water demonstrated significantly the highest number of viable PDL cells followed in rank order by HBSS and milk.

There was also a significant difference in the number of viable PDL cells between HBSS and milk. All experimental solution groups were significantly lower than positive control and higher than negative control groups (Table I).

DISCUSSION

Avulsion is a traumatic injury leading to loss of attachment of periodontal ligament from the alveolar socket. The treatment of choice in such cases is to replant the tooth back into the socket. The importance of PDL cell viability before reimplantation was demonstrated by Hammer to prove that the length of survival of a reimplanted tooth is directly correlated with amount of viable periodontal membrane.[10] One of the sequelae following replantation of an avulsed tooth includes inflammatory or replacement resorption.[11] The development of inflammatory root resorption is directly related to damage of the periodontium at the time of the accident and the presence of bacteria within the root canals and dentinal tubules. The development of re-

placement resorption depends on both the degree of damage to the periodontium at the time of avulsion[12-14] and the extent to which the viability of periodontal ligament cells remaining on the tooth surface is maintained.[15] Hence, the prognosis of an avulsed tooth is largely dependent on the status of the periodontal ligament cells at replantation. Therefore, the predominant philosophy derived from the research of Andreasen and Hjørting-Hansen for the treatment of avulsed teeth is: *Replant the tooth immediately or as quickly as possible after the avulsion.*[16] But, certain situations, like presence of more severe injuries needing immediate medical attention or nonavailability of a dental hospital close by can lead to a delay in immediate replantation of the avulsed tooth. In such situations the teeth should be stored in a medium that can maintain the periodontal ligament cell viability until definite dental treatment is accomplished.

According to Andreasen and Hjørting-Hansen,[16] teeth that were replanted quickly (within 30 minutes) had a better success rate than those that were extraoral for longer periods of time before replantation. In the current investigation, a 30-minute dry time was chosen, as this seems to be a critical time at which damage has been done to many PDL cells, yet some cells remain for assessment. Also, 30 minutes represents a typical clinical scenario during which the avulsed tooth may remain dry before being placed into a storage medium.

Some experimental studies have indicated that storage media is a more critical prognostic factor than the extra-alveolar period.[2,17-19] Physiologic storage media such as milk, saliva, saline, HBSS, Propolis, and Viaspan have been used for preservation of viability of periodontal ligament cells.[8,20-22]

Blomlof et al.[15,18] has shown storage of avulsed teeth in tap water and saliva to be damaging to periodontal ligament cells causing increased root resorption.[23,24] Cvek et al.[25] found that avulsed teeth that were soaked in an isotonic saline solution for 30 minutes before replantation showed less resorption than those that were stored dry for 15 and 40 minutes. Recent studies have evaluated the use of 0.9% isotonic saline, milk, HBSS, and Viaspan as storage media for the preservation of cell viability.[20-22] HBSS was the most effective,[20,22] although milk and saline were suitable, provided the extraoral time did not exceed 2 hours.[21]

In the dental literature, various techniques have been used to quantitate the number of viable PDL cells. Reinholdt et al.[26] used a stepwise trypsinization procedure by exposing samples to trypsin 3 consecutive times for 20 minutes each. Soder et al.[27] used chromogenic stain to quantitate viable PDL cells. Patil et al.[28] used a stepwise trypsinization procedure and fluorescein diacetate as a new 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.[29] This procedure allowed rapid cell retrieval and maintained maximum cellular integrity, as was demonstrated by the positive control samples.

Coconut water has never been tested for its potential benefits on PDL cells of avulsed teeth. This study compared coconut water, HBSS, and milk in terms of PDL cell viability. The coconut water group demonstrated significantly more viable PDL cells than HBSS and milk, with the HBSS group showing significantly more viable PDL cells than milk.

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.[30]

Coconut water has a high osmolarity because of the sugars present, which are primarily glucose and fructose. It is also rich in many essential amino acids including lysine, cystine, phenylalanine, histidine, and tryptophan.[30] Coconut water is readily accepted by the human body and is a safe means of rehydration particularly in patients suffering from potassium deficiency.[31] In fact, coconut water has been shown to be just as effective as commercial electrolyte solutions in prolonging survival time in sick patients.[32]

Superior maintenance of viability of the PDL cells in the coconut water group may be due to the nutrients that are present in coconut water such as proteins, amino acids, vitamins, and minerals, which help in nourishing the cells and maintaining their viability (Table II). In a study conducted by Majundar,[33] coconut water was found to be sterile and nonhemolytic. Edirweera[6] used coconut water intravenously as a substitute for normal saline.

Blomlof[15] showed that the important factor in maintaining the viability is the osmolarity of the transport medium. Andreasen[17] found that, in contrast to tap 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

Table II. Composition of tender coconut water

Minerals, mg/100 mL		*Amino acids, % of total protein*		*Vitamins, value in μg/mL*	
Potassium	291	Alanine	2.41	Nicotinic acid	0.64
Sodium	42	Arginine	10.75	Pantothenic acid	0.52
Chlorine	75	Aspartic acid	3.60	Biotin	0.02
Calcium	44	Cystine	0.97-1.17	Riboflavin	0.01
Magnesium	10	Glutamic acid	9.76-14.5	Folic acid	0.003
Sulphur	58	Histidine	1.95-2.05	Thiamine	Trace
Phosphorus	9.20	Leucine	1.95-4.18	Pyridoxine	Trace
Iron (mg/100g)	06	Lysine	1.95-4.57		
Copper	26	Proline	1.21-4.12		
		Phenylalanine	1.23		
		Serine	0.59-0.91		
		Tyrosine	2.83-3.00		

more closely linked to the osmolarity of the solution than to its chemical composition. The water permeability of a cell is high, and therefore, in a hypotonic solution, the cells will swell and rupture, whereas in a hypertonic solution they will shrink because of water movement out of the cell. It has been reported that cell growth can occur at a range of 230 to 400 mOsm/L.[34] When measured in an osmometer, the osmolarity of the HBSS, milk, and coconut water was found to be 295 mOsm/L, 232 mOsm/L, and 372 mOsm/L, respectively. Blomlof[15] found that HBSS was slightly better for maintaining the cell integrity than milk, saliva, or saline. This was mainly because of its physiologic osmolarity. Milk was found to have the lowest osmolarity among the 3 but also within the range, but the inability of the cells to multiply and divide might be the reason that there was a drastic decrease in number of viable cells. The osmolarity of coconut water was found to be to be the highest and this might have enabled the superior maintenance of cell viability.

CONCLUSION

Within the parameters of this study, it appears that the ability of coconut water to maintain PDL cell viability is statistically superior to HBSS and milk.

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