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Assessment of post-traumatic PDL cells viability by a novel collagenase assay

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Abstract – Both length of extra-alveolar time and type of storage media are significant factors that can affect the long-term prognosis for replanted teeth. Numerous studies have examined various media in an attempt to determine the ideal material for storage of the avulsed tooth. The purpose of this study was to compare the number of viable periodontium ligament (PDL) cells in different storage media using a collagenase assay. Thirty-three freshly extracted human teeth were divided into four experimental and two control groups. The positive and negative controls corresponded to 0 min and an 8-h dry time, respectively. The experimental teeth were stored dry for 30 min and then immersed in one of four media (Hank's balanced salt solution (HBSS), milk, saline, water) for 45 min. The teeth were then treated with dispase grade II and collagenase for 30 min. The number of viable and nonviable PDL cells was counted with a hemocytometer and analyzed. An ANOVA demonstrated no statistically significant differences in the viability of PDL cells among saline, HBSS and milk. Within the parameters of this study, it appears that milk or saline is an equally viable alternative to HBSS for storage of avulsed teeth.

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Traumatic injuries are a common occurrence that require both expedient and informed management by the practitioner. Andreasen and Andreasen (1990) predicted that the incidence of these injuries may eventually surpass the incidence of dental caries (1). Avulsion injury, one of the most severe forms of dental trauma, is characterized by complete displacement of the tooth from its alveolar socket. Due to the complexity of this injury, the neurovascular supply is severely compromised and usually results in a loss of pulpal vitality.

According to Andreasen et al. (3), the factors that play a role in healing of the periodontal ligament (PDL) after avulsion injuries are primarily the amount of physical damage to the root surface and the type of medium in which the exarticulated tooth is stored (2, 3). The greatest success of a replanted avulsed tooth occurs when it is immediately replanted, which is not always feasible. Several methods have been suggested to preserve the vitality of the PDL cells. Axhausen (4), suggested placing the tooth under the patient's tongue in cases in which immediate replantation was not possible. However, Dumsha (10), and Patil et al. (18) suggest storing the avulsed tooth in milk, HBSS or saline.

Several techniques have been used to determine the viability of the periodontal cells following avulsion. However, most of the experimental data that is available has been obtained using techniques in which the cells are cultured and/or trypsinized for longer periods of time. Because 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.

Both collagenase and dispase enzymes disrupt the extracellular matrix and cause the release of cells

Table 1. Analysis of variance

Vitality	Sum of squares	d.f.	Mean square	*F*	Significant values
Between groups	23320.34	5	4664.07	80.15	$P < 0.01$
Within groups	1571.16	27	58.191		
Total	24891.50	32			

without excessive disruption and destruction of their own membrane. Therefore the use of these two 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 purposes of this study were:

1. To investigate the efficacy of a novel and potentially faster method to quantitate the number of viable PDL cells after a simulated avulsion injury.
2. To determine which storage medium maintains the greatest number of viable PDL cells after 30 min of dry time followed by a 45-min storage period in commonly accepted media.

Material and methods

Thirty-three freshly extracted caries-free human teeth with normal periodontium and closed apices were used for this study. The extraction was performed as traumatically as possible, by one oral surgery resident. Following extraction, the teeth were held with forceps by the coronal region and the coronal 3 mm of PDL was scraped with a curette, rinsed with distilled water to remove cells that may have been damaged during extraction. The teeth were divided into six groups after rinsing.

The teeth in the experimental groups were stored dry for 30 min, followed by a 45-min immersion in one of four experimental media (Hank's balanced salt solution (HBSS), milk, saline, water). The positive control teeth after extraction were immediately treated with dispase and collagenase. The negative control teeth were bench dried for 8 h followed by treatment in dispase and collagenase.

Each tooth in all the groups was treated separately and incubated for 30 min in 15 mL Falcon tubes with a 2.5-mL solution of 0.2 μg/mL of collagenase CLS II (Cooper Biomedical, PA, USA) and a 2.4-μg/mL solution of dispase grade II (Gibco, Taastrup, Denmark) in PBS. The specimens were then centrifuged for 5 min at 800 r.p.m. and the cells labeled with 0.4% Trypan Blue (Gibco BRL) for determination of viability according to Polverini and Leibovich (5).

The number of viable and nonviable PDL cells was counted under light microscopy with a hemocytometer and statistically analyzed with an ANOVA and a post hoc Scheffe's Test. An alpha of 0.05 was used for statistical analysis.

Results

Teeth stored in milk demonstrated the highest number of viable PDL cells followed in rank order by HBSS, saline and water. The analysis of variance demonstrated a significant difference among the groups (Table 1). Table 2 presents the mean and SD of all groups. A post hoc Scheffe's test indicated that water was statistically significantly different than saline, HBSS, milk and both control groups (Fig. 1). The negative control group was statistically significantly

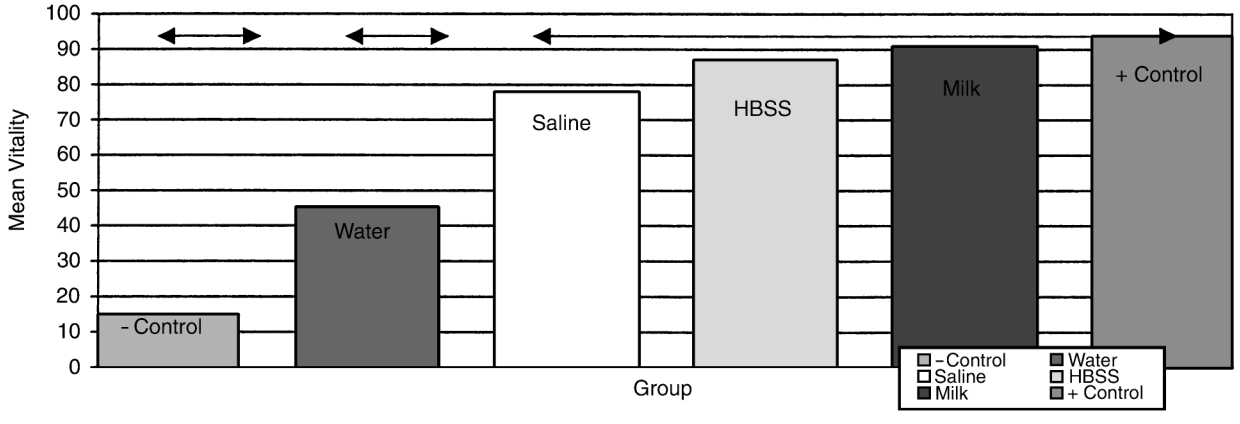

Fig. 1. Mean value of viable cells.

Table 2. Mean and standard deviation table

Groups	Mean	SD	SE
Water	45.17	12.03	4.54
Saline	77.80	2.92	1.19
Milk	90.59	3.77	1.53
HBSS	87.04	5.70	2.32
+Control	93.92	1.76	0.88
–Control	15.04	11.78	5.89

different than each of the experimental groups and the positive control group.

Discussion

Clinical surveys indicate that traumatic dental injuries in children and adolescents are a common problem, and several studies have shown that the prevalence of these injuries is increasing (6). During the 1950s and 1960s, it was reported that more than 2000 teeth were replanted (7).

Long-term prognosis of replanted teeth is dependent in part, upon immediate treatment following injury and PDL cell viability (8–11). Unfortunately, immediate treatment is not always feasible and it has been reported that only one in five children are seen immediately following a traumatic injury (12).

The importance of PDL cell viability prior to replantation was first addressed in 1955, when Hammer demonstrated that the length of survival of a replanted tooth is directly correlated with the amount of viable periodontal membrane (13).

After an avulsed tooth is replanted, the damaged PDL may undergo cell necrosis, macrophage activation, and removal of the necrotic area (8). The activation of osteoclasts may result in inflammatory root resorption. In cases of large areas of damage to the PDL, competitive wound healing begins between cells destined to form bone and PDL-derived cells which formed PDL fibers and cementum. This competition can lead to transient or permanent ankylosis. These sequelae may be minimized if either the appropriate storage media is utilized or immediate replantation is performed.

Numerous studies have attempted to determine which storage media provide the greatest percentage of viable PDL cells in avulsed teeth (13–18). In 1983, Lindskog et al. in an *in vivo* study with monkeys, compared saliva with milk and concluded that saliva was less suitable than milk due to its low osmolality and higher risk for bacterial contamination (19). Hiltz and Trope demonstrated that Viaspan was the most effective storage medium when compared with milk and HBSS. After 168 h of storage, 37.6% of human lip fibroblast stored in Viaspan was still viable (20). However, from a practical standpoint, Viaspan is highly questionable.

In the dental literature, various techniques have been utilized to quantitate the number of viable cells. Reinholdt et al. (1977) used a stepwise trypsinization procedure by exposing samples to trypsin three consecutive times for 20 min each (21). Soder in 1977 utilized chromogenic stain to quantitate viable PDL cells (22). In 1992, Patil et al. used a stepwise trypsinization procedure and fluorescein diacetate as a new staining technique for determining the viability of PDL cells in simulated avulsion injuries (18).

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. This procedure allowed rapid cell retrieval and maintained maximum cellular integrity based on the positive control teeth.

The results of this investigation demonstrated no statistically significant difference in the number of viable cells among saline, milk and HBSS after a 30-min dry time. The results of this *in vitro* study suggest that milk is comparable to both saline and HBSS as a storage media when teeth are stored dry for up to 30 min. This is in agreement with Trope (1995), who suggested that milk is considered the best storage medium when compared with HBSS for uncomplicated avulsion cases due to its practical availability (11).

The fact that saline, milk, and HBSS were comparable with respect to PDL cell viability may be a function of the amount of dry time and/or the storage media. This is also in part supported by the results of Hiltz et al. (20) in that all lip fibroblasts had comparable viability values after 6 h of storage in milk, HBSS, and Viaspan.

Since our study only examined avulsed teeth with 30 min of dry time, further investigations are in progress to determine the consequences of longer periods of dry time with respect to storage media and PDL cell viability.

Conclusions

1. Within the parameters of this study, it appears that milk is comparable to saline and HBSS for storage of avulsed teeth when dry time does not exceed 30 min.
2. The collagenase and dispase assay appears to be a viable method for evaluating PDL cell viability.

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