ABSTRACT

Several studies shows that a wide range from 3-16% of dentoalveolar traumatic injuries result in avulsion. The ideal treatment for avulsion would be the reimplantation of the tooth. Thus, it is recommended to replant the tooth as quickly as possible. However, immediate repositioning of teeth is not always possible, so the choice of a suitable storage medium for maintenance of Periodontal Ligament cell viability is of extreme importance for the success of replantation. At the present article are discussed some storage medium and the results published in the international literature.

KEYWORDS
Avulsion, Dental trauma, Periodontal ligament fibroblasts, Storage media.

RESUMEN

Diversos estudios demuestran un porcentaje que varía del 3-16% de las lesiones traumáticas dentoalveolares resultan en avulsión. El tratamiento ideal de la avulsión sería la reimplantación del diente. Por lo tanto, se recomienda reimplantar el diente lo más rápido posible. Sin embargo, el reposicionamiento inmediato de la pieza involucrada no siempre es posible, por lo que la elección de un medio de almacenamiento adecuado para el mantenimiento de la viabilidad celular es de extrema importancia para el éxito de la reimplantación. En el presente artículo se discuten algunos medios de almacenamiento y los resultados al respecto de la presente línea de investigación publicados en la literatura internacional.

PALABRAS CLAVE
Avulsión, Trauma dental, Fibroblastos del ligamento periodontal, Medio de almacenamiento.

In permanent dentition, almost 16% of dentoalveolar traumatic injuries result in avulsion (1). The direction and the intensity of the force, combined with a susceptible periodontal ligament (PDL), predispose this type of trauma (2), in which part of the periodontal ligament remains inserted in the alveolar socket, while another retains into the root surface.

Recently, Andreasen et al. (3) establishes that about a 0.3-3% of dental trauma results in avulsion. On the other hand, in a research of the
During the period that the tooth remains outside the alveolar socket, the cells adhered to the socket remains viable in its natural environment. However, there is a concern with the cells adhered to the root because, in addition to mechanical trauma, shortly after avulsion they tend to suffer dehydration and are subject to contamination and might become necrotic (5). Andreasen (6) reported the occurrence of severe damage in the cells of periodontal ligament of teeth that remained for 30 min in dry conditions. Studies showed that a 2h dry-period results in necrosis of substantially all periodontal ligament cells. The replantation of teeth with committed periodontal ligament results in inflammatory resorption (2,6) or ankylosis and consequent replacement resorption (1).

Thus, it is recommended to replant the tooth as quickly as possible. However, immediate repositioning of teeth is not always possible, so the choice of a suitable storage medium for maintenance of PDL cell viability is of extreme importance for the success of replantation. Ideally, the storage medium should maintain normal physiological conditions of cells, presenting an osmolality between 230 - 400 mOsm and a 6.6 - 7.4 pH, and provides essential ions such as Ca 2+ and Mg 2+.

Andreasen in 1981 (6) demonstrated that the storage medium for maintenance of PDL cells might be more important than the period of time that remains dry outside the alveolar socket. An inadequate medium conservation increased the percentage of necrotic cells and, consequently, the resorption rate. Moreover, the temperature of the storage medium affects the viability of PDL cells; studies showed that a lower temperature has a positive effect on cellular viability maintenance.

Several experiments were conducted to find the ideal medium that achieve physiologic pH and osmolality and ideally, that provide essential ions to the cells until the tooth can be replanted.

Among the most studied storage mediums may be highlighted water, saliva, saline, solutions for contact lenses, energy-sports drink, Eagles medium, Viaspan®, propolis, coconut water, milk and Hank’s balanced salt solution.

Water is a storage medium that we must avoid, because of its low osmolality and the presence of chlorine, studies have shown that water is an inadequate medium to maintain the viability of the PDL cell. Due to its poor performance, water has been used as a negative control in several cell culture studies that evaluate storage mediums (7).

Moreover, saliva although slightly more effective than water, it is not an appropriate storage medium, because in addition to be hypotonic, is a source of bacterial contamination for periodontal cells. Thus isn’t yet recommended by dental trauma specialists.

Physiological saline (sodium chloride 0.9%) has physiological pH and osmolarity, but provides no essential ions or glucose to the cells. Therefore, it is considered an acceptable storage medium for 2 to 3h, then, from these periods, the possibility of cell necrosis increases significantly.

Sports drinks like Gatorade®, due to its low pH, revealed disappointing results when compared with water. However when cooled (0 ° C), preserved a significantly greater number of cells than water and is recommended as a medium of storage for up to 1h.
Natural coconut water is sterile and has a 93% water and 5% sugar composition, which gives it a high osmolality. It is rich in proteins, vitamins and minerals such as potassium, calcium and magnesium (5,7). Even that a research stated that this product was better than HBSS and milk in maintaining human fibroblast viability, our several cell culture studies revealed differing results. In a previous research of our team (5), we tested different storage medium, as natural coconut water is not readily obtained, industrialized coconut water was used. Both types of coconut water results were disappointing. It could be hypothesized that its low pH value (4.7) and the presence of other products in its composition, such as acidulants, antioxidants and preservatives, interfered with its performance.

Eagle’s medium is one of the most widely used synthetic media for cell culture. It has glucose, vitamins, amino acids, and antibiotics, also is supplemented with fetal bovine serum, and is a product rich in growth factors and high content of gamma globulin. Eagle was superior to other mediums tested and would be an excellent storage medium for preserving PDL cells if were easily obtained. Viable fibroblasts with differentiate capacity were observed after 1 year of storage in Eagle’s medium supplemented with nutrients, buffers and antimicrobial solutions (8). Even after a drying time of 60 min, the monkeys teeth with a pre-soaking in Eagle’s medium for 5, 7 or 14 days, improved periodontal condition, decreasing the percentage of inflammatory resorption after replantation (3).

Viaspan® is used mostly during preservation of organs for transplantation; with an osmolality of 320 mOsm/kg and 7.4 pH, the Viaspan® proved been optimal in preserving the viability of periodontal cells for a long period of time. Unfortunately its use is unfeasible due to the high cost, short shelf life (few months) and difficult access.

Thus, milk has been intensively studied due to be easily obtained and of low cost, and has gained wide acceptance as a storage medium. It has been suggested that its effectiveness can last from 3 to 48h and that milk with a lower fat content might be more appropriate in maintaining PDL cell viability than milk with a higher fat content (5). Milk has a physiological osmolality and pH (230–270 mOsm/kg and 6.5–6.8, respectively) and provides nutrients and growth factors to the cells. In ours previous studies, skimmed and whole long-life milk preserved significantly more viable PDLF than any other experimental solution at every time period (7). When skimmed and whole milk were compared, skimmed milk was found to be better than whole milk only at 120h (5). Although the results obtained with the milk are satisfactory, its main disadvantage is that did not reconstitute the lost cellular metabolites, thus we conclude that milk must delays cell death, but does not restore the normal morphology of cells, nor their ability to differentiate and mitosis. According to Krasner (9) when a tooth is kept in a medium that allows cell reconstitution, their periodontal ligament remains physiologically healthy.

Hank’s balanced salt solution (HBSS), can be commercially available as Save-A-Tooth® (Shartlesville, PA, USA), and has been recommended as the storage medium to maintain PDL cell viability (American Association of Endodontists 1995). Several experiments have shown that it is an effective medium for the storage of avulsed teeth for periods varying from 3 to 72 h. A disadvantage of HBSS is that it may not be readily available in many locations in which tooth avulsions are likely to occur. However, several studies were in disagreement regarding Save-A-Tooth® been the ideal storage medium (5,7,10,11). Save-A-Tooth® manufacturers stated that this product and HBSS had the same components, however our results and research experience showed a low performance.
revealed by Save-A-Tooth® in relation to a newly lab manufactured HBSS. This may have been due to the storage time (6 months) or by differences in the concentrations of these components, which are not revealed by the manufacturer of the Save-A-Tooth®. Our results showed that Save-A-Tooth® was less effective than skimmed milk, whole milk and coconut water for all time periods. At 3, 24 and 48 h, Save performed similarly to HBSS. After 72 h, it was found to be similar to tap water (5). Other studies carried out with this product revealed similar results.

Another variable that has to be considered among storage medium is the storage temperature of the solution. Our research and other studies revealed that a lower temperature has a positive effect on cellular viability maintenance. Thus, we indicate that cold UHT Skimmed milk is more effective than regular milk at room temperature.

In my experience, the ideal of establishing this type of Research line would be focus in an epidemiological approach. Ideally, every city or country must apply the research and methodology available in international literature in order to analyze which could be the ideal medium for their respective area. The establishment of a Dental Trauma clinic at dental faculties enriches research, quality of dental education for alumni, clinical procedures and an epidemiological approach for all types of attention. With a dental trauma clinic faculty would have the backboard of previous research background, clinical experience in this sub-specialized, multidisciplinary area and can transmit all that knowledge through a national campaign regarding the care and protocols on attending Dental Trauma cases. Nowadays, all data, case reports and research develop in dental trauma clinics are in exponential growth, and enrich international literature, which have a direct profound effect on clinical protocol establishment worldwide.

![Figure 1](image1.png)

Figure 1. PDL cell culture is normally obtained from the periodontal ligament of extracted human tooth (A). After the cell line establishment, several experiments can be performed in order to obtain results of an ideal storage medium (B).

![Figure 2](image2.png)

Figure 2. A. PDL cell culture in physiological medium shows elongated shape named usually in international literature as fibroblast-like morphology. B. PDL cell culture in saliva showed the loss of usual morphology turning spherical. C. PDL cell culture in milk displays optimal physiological morphology.
REFERENCES


Beatriz Mendes De Souza¹

¹. Faculty of Dentistry, Federal University of Santa Catarina, Brasil.

E-mail: dentbia@gmail.com