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STUDY OF STORAGE MEDIA FOR AVULSED TEETH

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ABSTRACT

Dental avulsion is a traumatic injury characterized by the complete displacement of the tooth from its socket, with damage to the periodontal ligament, cementum, alveolar bone, gingival and pulp tissues. The best way to conduct this type of dental trauma is reimplantation. The prognosis of the tooth reimplantation depends on the existence of viable cells in the periodontal ligament and also depends on those which are able to proliferate on the damaged areas of the root. This can be achieved by the immediate reimplantation or through the storage of the tooth in an appropriate environment for further reimplantation. The aim of this study was to perform a literature review on methods of storage for avulsed teeth and its effectiveness in the maintenance of cellular vitality. Tap water, amongst those environments which were studied, was the worst storage medium, because it causes rapid cell lysis, similar to dry storage. Saliva, for a small period of time is effective, but contains microorganisms and it has low osmolality, which adversely affects the viability of cells in the periodontal ligament. Saline solution does not have essential ions and does not provide cell nutrients. As for *Gatorade*®, its low pH prevents cell growth and, its hypertonicity may cause dehidratation of cells. Contact lenses solutions showed no advantages over other means. Propolis and White egg albumen need more studies, and Propolis is not easily found and, as for White egg albumen, considering its availability, is a promising storage medium. *Emdogain*® is not effective in the regeneration of periodontal ligament. Milk is significantly better than other storage media, but not as efficient as Hank's balanced salt solution, *ViaSpan*® and Eagle's environment, as it restores and/or restores the vitality of periodontal ligament cells damaged, ie Milk only prevents cell death but does not restore the normal cell morphology. However, because both *ViaSpan*® and Eagle's medium are expensive solutions and they were not better than Hank's balanced salt solution, this is, therefore, the ideal environment for storage of avulsed teeth.

Keywords: Dental reimplantation, dental trauma, storage media.

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1 INTRODUCTION

Dental avulsion is a traumatic injury characterized by the complete displacement of the tooth from its socket, with damage to the periodontal ligament, cementum, alveolar bone, gingival and pulp tissues [2, 11, 21, 41, 55]. The most indicated procedure for this kind of dental trauma is reimplantation, which is a surgical technique consisting of reinserting a tooth in its socket after it has been extracted on purpose or accidentally [34, 36, 53].

Immediate assistance is crucial for the maintenance of the tooth inside the dental arch. Many teeth which have suffered from avulsion are ignored, specially due to lack of knowledge of its treatment. When knowing the correct procedure under certain circumstances, it helps offering the correct permanence of the tooth for a long time, contributing for a better quality of life for the patient [62].

The prognosis of the tooth reimplantation depends on the existence of feasible cells in the periodontal ligament and also depends on those which are able to proliferate on the damaged areas of the root [2, 4, 6, 27, 37, 47]. The portion of the periodontal ligament attached to the alveolar wall remains alive and does not need treatment [36, 23], whilst that attached to the tooth relates to the repairing process after reimplantation [5].

The vitality of the periodontal ligament on the surface of the root, increases the probability of reinsertion of dental fibers with the alveolar ones, when reimplantation is immediate; that is, when it is done up to one hour after avulsion [24].

Besides the promptness of reimplantation and the storing medium in which the tooth is placed is also a determining factor to extending the life of the tooth. The maintenance of vitality of periodontal ligament attached to the tooth is lower in a dry environment. The tooth should, necessarily, remain in a humid place [2, 7, 9, 16, 17, 18, 20, 21, 24, 25, 30, 32, 34, 36, 41, 48, 51, 54].

There are solutions which are capable of preserving the life of cells from the periodontal ligament during the time they are out of their alveolar socket. These solutions must be used when immediate reimplantation can not be done [5, 12, 41].

The aim of this study is to perform a review on literature concerning the possible ways of storing avulsed teeth and its effectiveness in the maintenance of cellular vitality.

2 LITERATURE REVIEW

The ideal storage medium should be capable of preserving the feasibility of cellular periodontal ligament, so that the cells could go through mitosis and form clones of the damaged fibroblasts of the periodontal ligament and its generating cells. This is essential so that the nude surface of the root be repopulated by fibroblasts, thus avoiding the adherence of osteoclasts on this area [7, 8, 9, 10]. An ideal storage medium should preserve the majority of the functional capacities of the cells of the periodontal ligament [7].

All the previous procedures of dental reimplantation should focus mainly on the maintenance of the vitality of the remaining periodontal ligament [5, 23]. Thus, is it indispensable the creation of a medium that simulates, the best way possible, the dental alveolus [23, 35].

The way which the tooth is transported also affects significantly the degree of success. The dental root should not be touched, for each time this happens, the cells of the periodontal ligament are damaged and they die [36].

The container for the transport of avulsed teeth must be unbreakable, non-toxic, leaking proof, and of easy handling, with internal walls made of soft material, sterile and it must protect the tooth during transport, besides making the removal of the tooth easy without any traumas [36].

2.1 Hydrogenionic Potential (pH)

The acid or basic characteristic of a solution is determined based on the concentration of molar ions of hydrogen (H^+). The higher the concentration of H^+, the lower will be the pH of the solution [39].

The pH of most cells of the organism are found around 7.0 which means that the concentration of H^+ is approximately the same as the concentration of OH^-. Because of that, even small variations on the concentration of both, may be harmful to the cells and their functioning [60].

Almost all the biological processes depend on the pH. A small variation on the pH of the medium produce a big variation in the speed of most of biological processes, which develop in this same environment. That is not only true for many reactions in which the H^+ ion takes direct participation, but also for those in which there is no apparent role of these ions [39].

2.2 Osmolality

The terms osmolality and osmolarity refer to the osmotically active molecules of a solute in a specific solution. Incase they are not used with criterium, they may cause some confusion [52].

The word osmolarity refers to the number of molecules osmotically active of a solute contained in 1 liter of solution. Osmolality refers to the number of molecules of an osmotically active solution present in 1 kilogram of solvent which, almost always, is water. The osmolality has a relation of weight/weight and its measuring unit is mOsm/kg, while the osmolarity has a relation of weight/volume and its measuring unit is mOsm/L [52].

The osmolality of a solution is determined by the concentration of the molecules non-ionized dissolved and in last analysis responsi-

ble for the "osmotic pressure". The osmolality increases when there is lack of water [52] and diminishes with excess of water [14].

In cellular systems, the permeability of water is very high. This means that the cells act as "osmometers", rising the volume in a hypotonic medium and reducing its volume in a hypertonic environment, due to the osmotic movement of the water. Both the rise and the reduction of the quantity of water are critic for the cells. An external osmolality bigger than 450 mOsm/kg results in cell death even when it is kept for a short period of time [5].

According to Marino et al. [44], Melo [46] the pH and the osmolality of the storing environments must be physiologic, for both interfere in the surviving of cells of the periodontal ligament. Authors have reported that the cellular growth may occur between 290 and 330 mOsm/kg. The pH must be between 7.2 and 7.4, but growth may occur between 6.6 and 7.8.

The storage of cells in hypotonic solutions may cause irreversible damage to the cell membrane depending on the time of exposure of the cells to these solutions [43].

2.3 Types of Storing Environments

There are many types of storing environments for avulsed teeth; amongst them we can find:

2.3.1 Tap water

Amongst the studied environments, tap water has shown to be the one with the least desirable results, though it protect the tooth from dehydration – for being a hypotonic medium – it causes rapid cellular lise of the periodontal ligament, similarly to a dry storage [7, 9, 12, 44, 50].

2.3.2 Saliva

Saliva can be used as a storing medium for a short period of time, for it can damage the cells of the periodontal ligament if used for longer than an hour [15, 22, 29, 36, 38, 40]. Its osmolality is much lower than the physiologic (60-70 mOsm/kg), thus, it boosts the harming effects of bacterium contamination. Its only advantage is its availability [12, 13, 44, 56].

2.3.3 Saline solution

The saline solution provides osmolality of 280 mOsm/kg and despite being compatible to the cells of the periodontal ligament, it lacks essential nutrients such as magnesium, calcium and glucose; necessary to the normal metabolic needs of the cells of the periodontal ligament [1, 13, 15, 36].

Blomlöf [15], Courts et al. [22], Krasner [36] have stated that saline solution was harmful to the cells of the periodontal ligament in avulsed teeth if it is used for longer than two hours.

2.3.4 Gatorade®

Gatorade®, according to Harkacz et al. [29], did not turn to be an adequate storing medium for avulsed teeth due to its pH around 2.91 and its osmolality of 407 mOsm/kg.

According to Chamorro et al. [19] when cells are exposed to *Gatorade*®, it is possible that the delicate cellular membrane gets damaged because of the low pH, which makes it impossible for the cell to grow. As for osmolality, because of *Gatorade*® is hypertonic, it can make cells lose water.

2.3.5 Contact lenses solutions

The solutions for keeping contact lenses were worse than saline solution, milk and Hank's Balanced Salt Solution [19, 31]. The presence of preservatives in its formula was harmful to the cells of the periodontal ligament [54].

2.3.6 Propolis

Özan et al. [48] have analysed the properties of propolis as a storing medium for the maintenance of cellular viability of the periodontal ligament of avulsed teeth. Authors have concluded that it can be considered as favorable storing medium for the fact that it has anti-microbiotic properties. For this reason, it maintains the cellular viability of the periodontal ligament, besides being anti-microbiotic, anti-inflammatory and anti-oxidant.

2.3.7 Emdogain®

According to Ashkenazi and Shaked [10] *Emdogain*® diminishes the percentage of fibroblasts of the periodontal ligament with capability of forming colonies and that lowers the capability for the fibroblasts to repopulate the dental radicular surface after dental avulsion. The diminishing can happen due to the lack of an adherent surface or the increase on the difference of fibroblasts, which grow in its presence. *Emdogain*® increased the difference on the cells, in cells of mineralized tissues. In cases of periodontitis, *Emdogain*® has improved the periodontal recovering after surgeries. In contrast, the post-traumatic periodontal healing needs contrary differentiation. *Emdogain*® can delay, but not stop the development of replacement resorption, one of the worst complications of dental trauma. *Emdogain*® on its own is not efficient in the regeneration of injured periodontal tissues of the avulsed tooth.

2.3.8 Egg white

Khademi et al. [33] have compared milk and egg white as solutions for storing avulsed teeth, and the results have shown that teeth stored in egg white for 6 to 10 hours had a better incidence of repair than those

stored in milk for the same amount of time ($p < 0,05$). The osmolality of the egg white is between 251 and 298 mOsm/kg.

Sousa et al. [57] has microscopically analyzed the human periodontal ligament attached to the extracted tooth after one hour of extra-alveolar time, compared to milk, egg white and artificial saliva. The results of teeth stored in milk and egg white were similar concerning the organization of collagen fibers and the number of cells. Artificial saliva had an inferior result. The authors suggest that egg white can be the perfect medium for storing avulsed teeth.

2.3.9 Milk

The American Association of Endodontics indicates milk as a solution for avulsed teeth, for keeping the viability of the human cellular periodontal ligament (36, 40, 48, 49].

Milk is significantly better than others solutions for its physiological properties, including pH and osmolality compatible to those of the cells from the periodontal ligament; the easy way of obtaining it and for being free of bacteria [14, 15, 22, 44, 48, 50], but it is important that it is used in the first 20 minutes after avulsion [26].

The favorable results of milk probably occur due to the presence of nutritional substances such as aminoacids, carbohydrates and vitamins [9, 13, 14, 22, 44, 50]. The pasteurization of milk is responsible for diminishing the number of bacteria and bacteriostatic substances [9, 15, 22, 44, 50], also for the inactive presence of enzymes, which could be potentially harmful to the fibroblasts of the periodontal ligament [9, 13].

Blomlöf et al. [12]; Trope and Friedman [59] recommend milk as an excellent storing solution for 6 hours, however, milk can not revive the degenerated cells. An avulsed tooth which has remained in a dry medium and later has been put into milk before reimplantation, will probably have as undesirable prognosis as that which has been into a dry medium and has undergone reimplantation [12, 15].

2.3.10 Hank's balanced salt solution

Hank's balanced salt solution is a standard saline solution that is widely used in biomedical research to support the growth of many cells types [35, 48]. This solution is non-toxic; it is biocompatible with periodontal ligament cells, pH balanced at 7.2 and has an osmolality of 320 mOsm/kg [9, 35, 36].

It is composed of 8 g/L sodium chloride; 0.4 g/L of D-glucose; 0.4 g/L potassium cloride; 0.35 g/L sodium bicarbonate; 0.09 g/L sodium phosphate; 0,14 g/L potassium phosphate; 0.14 g/L calcium chloride; 0.1 g/L magnesium chloride and 0.1 g/L magnesium sulfate (Biological Industries, Beit Haemek). It contains ingredients such as glucose, calcium and magnesium ions which can sustain and reconstitute the depleted cellular components of the periodontal ligament cells [9, 35, 36].

In accordance to Krasner [36], Hank's balanced salt solution is the best solution for storing avulsed teeth. It does not require refrigeration and it can be kept on the shelf for 2 years and it has been recommended and used successfully as a storage medium by clinicians and researchers. This solution is effective in preserving periodontal ligament cells of avulsed teeth, renew the degenerated periodontal ligament cells and maintain a superior success rate if an avulsed tooth is soaked in them for 30 minutes.

In accordance of Ashkenazi et al. [9] Hank's balanced salt solution was the most effective medium for preserving viability, mitogenicity and clonogenic capacities of periodontal ligament cells for up to 24 hours at 4°C, when compared with culture medium (Eagle's medium supplemented with 15% fetal calf serum and antibiotic solution [100 UI/mL Penicillin, 50μg/mL Gentamicin and 0,3μg/mL Fungizone]), Eagle,'s medium, milk, *ViaSpan*® and condicioned medium.

Hank's balanced salt solution is commercially available as Save-A-Tooth™ (Save-A-Tooth™, Inc., Pottstown, PA), with ideal osmolality and pH. It has an inner net to receive the avulsed tooth and to minimize cells trauma during transport.

2.3.11 ViaSpan®

The *ViaSpan*® (Belzer VW-CSS, Du Pont Pharmaceuticals, Wilmington, DE, USA) is a medium used for the transportation of organs which are going to be transplanted and it has been very effective for storing avulsed teeth [7, 9].

ViaSpan® has osmolality of 320 mOsm/kg, which enables excellent cellular growth. Its pH is around 7,4 at room temperature; ideal for the cellular growth [7, 8, 9, 30].

Hiltz and Trope [30] have compared the vitality of lip fibroblasts, at room temperature which were stored in milk, Hank's balanced salt solution and *ViaSpan*®. The *ViaSpan*® was the best storage medium observed at all times, and after 18 hours, there was still 37,6% of living cells.

Ashkenazi et al. [9] have evaluated the effectiveness of 6 different storage media for avulsed teeth: culture medium (Eagle's medium with 15% of livestock fetal serum and antibiotics [100 UI/mL Penicilina, 50μg/mL Gentamicina and 0,3μg/mL Fungizone], Eagle's medium, milk, Hank's balanced salt solution, *ViaSpan*® and conditioned medium for the observation of fibroblasts of the periodontal ligament.

Interestingly, the clonogenic capacity of the stored cells kept in *ViaSpan*® for 8 hours was high and comparable to Hank's balanced salt solution and superior to milk. This capacity has diminished in 65% after 24 hours when compared to the control, and inferior to milk

and Hank's balanced salt solution. The cells of the periodontal ligament stored in *ViaSpan*® for 24 hours, at 4°C, have shown low vitality. The biggest mitogenic capacity was found in the fibroblasts kept in milk or in Hank's balanced salt solution and the lowest were found in conditioned medium or *ViaSpan*®.

Ashkenazi et al. [7] have compared the effectiveness of the four storage media (Hank's balanced salt solution, culture medium, Eagle's medium and *ViaSpan*®) concerning the preservation of fibroblasts of the periodontal ligament at room temperature (22°C). *ViaSpan*® and culture medium, followed by Hank's balanced salt solution were the most effective in keeping the clonogenic capacity of the fibroblasts of the periodontal ligament after 24 hours, at room temperature (22°C). The culture medium, followed by Hank's balanced salt solution and *ViaSpan*® were the most effective in keeping vitality and mitogenicity.

The small functional capacity of the fibroblast of the periodontal ligament kept in *ViaSpan*®, its high cost (USD 300 per liter), its short vitality expiration date (a couple of months) and the difficulty to find it (only some drugstores and hospitals have it), make it difficult to find and use this storage medium [7, 8, 9, 44, 50].

2.3.12 Eagle's medium

Eagle's Minimal Essential Medium contains 4 ml of L-Glutamine; 10^5 IU/L of Penicillin; 100μg/mL of Streptomycin, 10μg/mL of Nystatin and calf serum (10% v/v) [14, 15].

Many studies demonstrated that the cell culture medium (Eagle's medium at 37°C) can preserve periodontal ligament fibroblasts for extended periods before dental reimplantation [7, 8, 9, 30].

In accordance with Ashkenazi et al. [9] Eagle's medium had relatively high viability, mitogenic and clonogenic capacity up to 8 hours of storage at 4°C. When the storage time was up to 24 hours, Eagle's medium was less effective than milk or Hank's balanced salt solution, which could be attributed to the low temperature (4°C) of storage at the study. This low temperature may have induced aggregation and thus lowered the cell's functional capacity. Authors suggest that additional studies are needed to evaluate the effectiveness of this medium at higher temperatures.

Ashkenazi et al. [7] concluded that the lower functional capacities were encountered on periodontal ligament fibroblasts stored in Eagle's medium when compared to Hank's balanced salt solution, culture medium and *ViaSpan*®.

Ashkenazi et al. [8] evaluated *ViaSpan*®, Hank's balanced salt solution, Eagle's medium and Eagle's medium supplemented with growth factors and antibiotics. In this study Hank's balanced salt solution supplemented with growth factors was the medium that preserved better the viability, mitogenicity and clonogenic capacity of periodontal ligament fibroblasts. Eagle's medium supplemented with growth factors was the second better medium in preserving the viability, mitogenicity and clonogenic capacity after 24 hours at room temperature (24°C). Eagle's medium supplemented with growth factors had better results than *ViaSpan*® or Ealge's medium without growth factors. The addition of growth factors to the storage media are only for long time of storage (more than 24 hours). Before any clinical conclusions, *in vivo* studies must be conducted, because there are many differences between *in vivo* and *in vitro* studies, like temperature, air, environment and the presence of other biological factors. And also, the role of fibroblasts in repopulating denuded root surfaces is not completely clear and the addition of growth factors on storage media need to be manufactured and distributed.

CONSIDERING

The prognosis of dental reimplantation is directly related to the viability of periodontal ligament cells that remain on the root surface of the avulsed tooth [2, 4, 6, 27, 37, 47] or, the success of dental reimplantation depends on the existence of viable cells on the periodontal ligament, which are able to proliferate on the denuded areas of the root surface. This can be reached through immediate reimplantations taking place within up to 30 minutes or with the storage of the tooth in an adequate medium for dental reimplantation [15].

Root re-absorption is a frequent complication after dental reimplantation [3, 58, 61] and this disease can occur because of the leak of periodontal ligament or part of it, where the bone tissue goes to the root surface, being straight to it and making anchilosys. In consequence of fusion, will occur replacement re-absorption, where the tooth is substituted by bone tissue when there is no infection [28].

Other kind of root re-absorption that can occur in consequence of avulsion is inflammatory re-absorption, related to infected pulp tissue [2].

Dental reimplantation has been considered a temporary treatment because many teeth have root reabsorption. However, in many related cases teeth reimplanted were in function for 20 years or more with normal periodontal ligament. These cases show that reimplanted teeth, under some conditions can maintain its integrity and function [45].

However, for this procedure to have success, it is fundamental that some factors be considered, like the time that the tooth is out of the socket, the medium in which the tooth is stored and the conditions of the periodontal ligament at the moment of dental reimplantation.

When immediate dental reimplantation is not possible, the tooth must to be in some storage medium. The reason why it is necessary to put the tooth in some storage medium is for the maintenance of the

viability of periodontal ligament cells on root surface for extended time and in some cases it even stimulates its proliferation [2], and it is available at the moment of dental avulsion.

The storage medium must simulate periodontal ligament cells of avulsed teeth a normal biological condition, like physiologic osmolality and pH [12, 15, 42, 43]. Periodontal ligament cells with normal anatomy and physiology present osmolality of 320 mOsm/Kg and pH of 7.2 [15].

Tap water, saliva and salt solution must be avoided for the storage of avulsed teeth, because they do not offer any benefit to the healing. Contact lens solutions and *Emdogain*® also do not present good results and they do not have advantage over other media.

Propolis and egg white need additional studies, furthermore, propolis is not easily available in emergency situations.

Egg white, in accordance with Khademi et al. [33]; Sousa et al. [57] is a promising storage medium for avulsed teeth, considering its availability. However, egg white needs additional studies.

Milk is better than saliva, tap water or dry storage, but it is not so efficient as Hank's balanced salt solution as storage medium for avulsed teeth for the maintenance of vitality and proliferative capacities of periodontal ligament fibroblasts [22], because milk does not re-establish and/or rebuilds the vitality of damaged periodontal ligament cells [12, 35]. The storage of avulsed teeth in milk must occur within 6 hours, because at this time the storage was as efficient as tooth immediate reimplanted [12, 59].

Hank's balanced salt solution can preserve periodontal ligament cells *in vitro* for 120 hours (5 days) and *in vivo* for 96 hours (4 days). This is the best solution for the storage of avulsed teeth, because it does not need refrigeration, it has two years of shelf-life and it has been recommended and used with success for clinicians and researchers [35].

Hank's balanced salt solution presented the best effectiveness to preserve the viability of periodontal ligament fibroblasts, mitogenicity and clonogenic capacity for up 24 hours at 4°C, when it was compared to milk, *ViaSpan*® and Eagle's medium. Milk was the second more effective storage medium at the condition of the study [9].

Hank's balanced salt solution was so effective as *ViaSpan*®, with the same aim and methodology of the last study, in 24 hours at 22°C [7]. *ViaSpan*® is a medium used for the storage and transport of transplanted organs before its transplantation. Its osmolality (320 mOsm/kg) and pH (7.4) are ideal for cell growth [30]. However, its high price, the short shelf-life and the difficult access make ViaSpan a not so practical storage medium [7, 8, 9].

Eagle's medium can preserve periodontal ligament fibroblasts for extended periods before dental reimplantation [7, 8, 9, 30]. However, *ViaSpan*® and Eagle's medium were not superior to Hank's balanced salt solution [8, 9].

Even though Hank's balanced salt solution is superior to milk, because of the availability of milk at the moment of the accident, it is relatively free of bacteria [22] and 6 hours are enough for the patient to find treatment, milk can be the storage medium of choice. Nevertheless, milk only prevents cell death, it does not restore its morphology or its capacity of differentiation and mitosis [23].

Hank's balanced salt solution, *ViaSpan*® and Eagle's medium allow cells reconstitution, thus, they could be the ideal media. However, because of *ViaSpan*® and Eagle's medium high price, Hank's balanced salt solution can be the ideal storage medium. Hank's balanced salt solution is commercially available in many countries, but unfortunately it still isn't in Brazil.

It is fundamental to have a storage medium easy and commercially available, for more avulsed teeth could be reimplanted, with better prognosis.

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