Extra-Alveolar Storage Media For Tooth Autotransplants And Replants

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Abstract

The maintenance of the vitality of the cells of the periodontal ligament and cementum is essential for the long-term success of a transplant or replant. The tooth is surgically transferred from its initial position, without traumatizing the cells of the periodontal ligaments and cementum, into a new socket. An appropriate storage medium should maintain or improve the vitality of the cells during the extra-alveolar period; when the new socket is being prepared or the tooth being transported for replantation after an avulsion.

Storage media proposed in the literature include Hank's Balanced Salt Solution (HBSS), patient's own serum, isotonic saline, tap water, saliva and pasteurized milk. The most favoured ones are HBSS, patient's own serum, Eagle's culture medium, pasteurized milk and isotonic saline.

INTRODUCTION

Tooth autotransplantation is the process in which tooth, usually impacted, is surgically transferred to correct position, or to replace another tooth, in the same alveolus ([1]). This involves removing the tooth, creating a new socket in the same alveolar bone and repositioning the tooth. The tooth is stored in an appropriate storage medium to preserve the periodontal membrane and cementum of the root during the extra-alveolar period when the new socket is being prepared.

The third mandibular molars and the maxillary canines are the two most frequently misplaced or impacted teeth in the human dentition. They are therefore frequently autotransplanted ([2,3,4,5]).

Moss ([6]) gave three reasons for the frequent autotransplantation of maxillary canines:

1. The upper canine is the most frequently misplaced tooth in the anterior part of the mouth.
2. Orthodontic alignment of the tooth can be difficult and protracted. According to Andreasen et al (7), tooth autotransplantation has recently become a method of treating orthodontic problems.
3. Patients are often unaware that the canine is misplace until they are in their late teens and early twenties, and at this age, orthodontic treatment is less acceptable to many patients on aesthetic and social grounds.

An impacted or developing third molar may also be autotransplanted to the position of a first or second molar indicated for extraction.

Intentional and accidental replants may also need to be stored in a medium. An intentional replant is a tooth extracted then replaced after treatments, such as root canal therapy; while an accidental replant is a tooth replaced after being knocked out accidentally ([1]) An accidental replant will need to be stored in an appropriate medium to keep the periodontal membrane and cementum vital before getting the tooth to a dentist for replantation. Loss of vitality of the cementum and periodontal membrane could lead to root resorption of the replant or transplant ([5,8,9,10]).

This article intends to review the various storage media for tooth transplants and replants in the literature and to also point out the most favoured storage media.

STORAGE MEDIA

Various storage media have been proposed in the literature for tooth transplants during the time in which the new socket

is being prepared. The storage medium is expected to maintain or improve the vitality of the cells of the periodontal membrane and cementum, since it is generally accepted that a vital periodontal ligament is essential for the long-term survival of a transplant or replant ([2,5,11,12,13,14]).

Thonner ([15]) proposed the use of the patient's serum as a storage medium, He claimed that the histological picture of the periodontium of a freshly extracted tooth showed that the cementum and periodontal tissue present over the root are well vascularized. The histology picture after the tooth had been preserved in serum for about one hour showed that the tissues still stained well, indicating that serum can maintain the vitality of the periodontal membrane during the critical extra-alveolar period.

Martin and Pileggi ([16]) investigated the potential of a new storage medium, Propolis, in maintaining viable periodontal ligament (PDL) cells on simulated avulsed teeth. The experimental teeth were stored dry for 30 minutes and then immersed in one of the five media (Hank's balanced salt solution (HBSS), milk, saline, Propolis 50%, and Propolis 100%) for 45 minutes. The teeth were then treated with dispase grade II and collagenase for 30 minutes. The number of viable PDL cells were counted with a haemacytometer and analyzed. It was found that both Propolis groups kept significantly more PDL cells viable compared to milk, saline or HBSS. It was concluded that Propolis appeared to be a better alternative to HBSS, milk or saline in terms of maintaining PDL cell viability after avulsion and storage.

Various authors have also used isotonic saline as a storage medium for transplants. Altonen, et al ([17]) used it in their autotransplantation of completely developed maxillary canines, so also did Burley and Crabb ([18]). Andreasen ([19]) also used isotonic saline as a storage medium for mature permanent incisor replants in monkeys, during his analysis of the topography of surface and inflammatory root resorption on these replants. The authors believed that isotonic saline could maintain the vitality of the periodontal membrane.

Various culture media have been used for storing teeth for later replantation. Culture medium 199 containing 700 units of penicillin G and 0.7mg of streptomycin, to prevent bacterial growth, was used as a storage medium by Nasjlet, et al ([20]).

Eagle's culture medium has also been used for storing teeth by many authors. These include Andreasen et al ([21]). Litwin, et al ([22]) and Thomsson et al ([23]). They believed that the Eagle's culture medium allowed the proliferation of vital parts of the periodontium to cover areas of the root surface denuded of periodontal membrane or areas covered by necrotic periodontal membrane. The Eagle's culture medium contains a number of amino acids and vitamins, and bicarbonate that acts as a buffer.

Pohl et al ([24]) investigated the suitability of specially composed cell culture media for storage of extracted teeth for up to 48 hours. Auttoradiographic investigations revealed that the proliferative activity of periodontal ligament (PDL) cells of teeth stored in cell culture medium for up to 48 hours increased with storage time. Immunohistochemical investigations with markers for cell proliferation revealed that pulp cells of extracted immature teeth showed numerous proliferation after storage for up to 24 hours in a special cell cultured medium, but few proliferation after storage in Hank's Balanced Salt Solution (HBSS). The investigation indicated that a special culture medium could preserve cell viability of PDL cells adhering to extracted teeth for at least 48 hours.

The human saliva has also been suggested as a potential storage medium. Andreasen ([5]) compared tap water, normal saline and human saliva as potential storage media before replantation. Monkey's incisor teeth were stored either in the storage media or dried before replantation. 8 weeks post-operative histology examination revealed a significant relationship between the frequency of root resorption, extra-alveolar period and storage medium. This was especially significant after dry storage. Teeth stored in tap water, saline or saliva showed about the same frequency of resorption which increased slightly with increased extra-alveolar period. Ankylosis (replacement resorption) was rarely found among teeth stored in saline or saliva whereas it was significantly increased amongst teeth store in tap water. The author concluded that normal saline and saliva were suitable for storage during he extra-alveolar period.

Similarly, in a study of 380 freshly extracted anterior teeth and premolars stored for periods ranging from 30 to 360 minutes in different types of media and subsequently the residual vitality of PDL cells was assessed by means of the fluorescein diacetate reaction, Pongsiri et al ([25]) found that after 90 minutes of dry storage, 88% of the periodontal cells were devitalized, whereas after 180 minutes of storage in saliva or normal saline solution, the loss of vitality was 40% or 37% respectively. Storage in Alpha Minimal Essential

Medium (Alpha MEM) or UHT milk for 360 minutes, 52% or 51% of the PDL cells showed loss of vitality.

Blomlof and Otteskog ([26]) compared the survival of human periodontal ligament fibroblasts in milk and saliva. The cells were incubated in the media for varying periods of time, at different temperatures, and analyzed for viability. Milk was found to be superior to saliva as storage medium with respect to number of viable cells, cell size and ability to recover.

Long shelf-life milk, which has the advantage of not requiring refrigeration, is as effective a storage medium for avulsed teeth as regular pasteurized milk and more effective than Save-A-Tooth medium ([27]). Similarly, in their study to determine the efficacy of several milk substitutes: reconstituted powdered milk, evaporated milk or one of two baby formulas: Similac or Enfamil; compared to whole milk, in maintaining the viability of human PDL cells on avulsed teeth, Pearson et al ([28]) reported that Enfamil, which is supplied in powdered that does not require special storage and has a shelf-life of 18months, is a more effective storage medium for avulsed teeth than pasteurized or whole milk for at least 4 hours.

However in another study to determine the periodontal ligament cell vitality from extracted teeth stored in saline or milk using fluorescein dictate as a staining medium, Patel et al ([9]) found that there was no statistically significant difference in the number of viable cells on the root surfaces of teeth after 2 hours of storage in either milk or saline.

Newly extracted teeth from monkeys were stored in saline, milk or saliva for 1 to 3 hours by Blomlof et al ([29]). Frozen sections were made and the vitality of the periodontal ligaments determined histochemically. Cells seemed to survive better in milk than saliva and saline. The reason for this, according to the authors, may be that milk contains important nutritional substances such as amino acids, carbohydrates and vitamins. Also, the commercially available milk is pasteurized, which may inactivate enzymes that are potentially harmful to the periodontal ligament. Saliva on the other hand contains potentially harmful substances such as enzymes, bacteria and their products.

Huang et al ([30]) exposed cultured PDL cells from healthy extracted human teeth to milk, Alcon Optic – Free contact lens solution, K- Mart contact lens solution, saline and Hank's balanced salt solution (HBSS). The appearance and rate off loss of the cells from the culture dishes were recorded over time at both room temperature (20 degrees Centigrade) and 4 degrees Centigrade. The results indicated that saline was superior to either of the contact lens solutions in its ability to maintain the vitality of the PDL cells. Milk at 4 degrees provided good short-term viability. The study supports milk as a good short-term storage medium for maintaining the viability of PDL cells in vitro.

Layug et al ([31]) proposed that milk packed in ice seems to be the best alternative amongst; Modified Eagle's Medium, HBSS, saline and saliva, for the temporary storage of avulsed teeth due to its wide availability and the minimal detrimental effect it has on the PDL cells.

Lekic et al ([32]) examined the relative dependence of cell membrane integrity, attachment and clonogenic capacity (ability to produce similar cells asexually) of human PDL cells on the temperature and duration of the extra-alveolar period and the type of storage medium. Twenty-four premolars were maintained at 4 degrees and 23 degrees Centigrade for 15, 30, 60 or 120 minutes in either milk or dry condition. Cell membrane integrity was determined by BCECF/AM dye inclusion. Plating efficiency was determined by measurement of cell attachment at 3 and 6 hours. The clonogenic capacity of progenitor cells was estimated by limiting dilution and colony counts. For all assays, teeth stored in milk at 4 degrees Centigrade showed the highest percentage of BCECF attached cells with clonogenic capacity. Increased storage time (15 – 120 minutes) was associated with a 50% relative reduction of BCECF staining and a 5-fold relative reduction of cell attachment regardless of storage condition. Hence, in vitro assays of clonogenic capacity are much more sensitive to extra-oral storage time and storage conditions than dye inclusion or cell attachment.

Ashkenazi et al ([33]) evaluated the effectiveness of six different media: culture medium, alpha minimal essential medium (alpha MEM), HBSS, Via Span and conditioned medium (CM) to preserve cultured periodontal ligament fibroblasts (PDLF). The PDLF were obtained from explants of human healthy extracted teeth. Plates with confluent PDLF were soaked in the various media for 2, 8 and 24 hours at 4 degrees Centigrade. A control group was incubated with culture medium at 37 degrees. After incubation, cell viability was determined by trypan blue exclusion test. Viable cells were then analyzed for mitogenic (with thymidine) and clonogenic capacity (by culturing one cell/ well). Vitality of PDLF after 2,8 and 24 hours was

highest when stored in milk or HBSS (91% - 97%) and lowest when stored in Via Span or CM (82% - 93%). The highest mitogenicity was found in PDLF stored in milk or HBSS and the lowest in CM or Via Span. Milk and HBSS were the most effective media in preserving the clonogenic capacity. It was concluded that HBSS and milk were the most effective media for preserving the viability, mitogenicity and clonogenic capacity after storage for up to 24 hours at 4 degrees C.

Similar results were reported by Ashkenazi et al ([34]) in their in-vitro study of viability, mitogenicity and clonogenicity capacity of periodontal ligament cells after storage in four media: HBSS, CM, alpha MEM and Via Span, at room temperature. Growth Factors, IGF - 1 and PDGF – BB when added to the storage media showed that HBSS and alpha MEM–S were the most effective media for preserving the viability, mitogenicity and clonogenic capacity of PDLF stored for 24 hours at room temperature. However, according to Ashkenazi et al ([35]), for short periods of storage (2 hours and 8 hours), HBSS and alpha MEM–S without Growth Factors were preferable.

HBSS and culture medium were also found to be suitable culture media by Sigalas et al ([36]). They exposed human PDL cells for 1 hour to culture media: milk, HBSS, SoftWear, Opti Free and Solo Care contact lens solutions, Gatorade and tap water, at room temperature and on ice. The number of viable cells was counted using the trypan blue exclusion technique immediately after exposure (0 hour) and at 24 and 48 hours, to test the proliferative capacity of the cells after treatment.

It was found that water had a detrimental effect on the cells, whereas culture medium and HBSS preserved significantly more viable cells than the other experimental solutions. It was concluded that within the parameters of this study, it appeared that HBSS was the optimal storage medium for avulsed teeth.

According to Schwartz et al ([37]), the temperature (above 0 degree Centigrade) of the storage medium is of importance only for dry storage and in such a situation only for shorter extra-alveolar periods i.e. for 60 minutes storage and not for 120 minutes, where extensive destruction of the PDL always takes place. It was suggested that the temperature effect of 4 degrees Centigrade could be related to less evaporation from the PDL and thereby less damage to the PDL cells or a strict temperature effect upon cell metabolism

The tooth storage media that are therefore mostly favoured in the literature include: HBSS, patient's own serum, Eagle's culture medium, pasteurized milk and isotonic (normal) saline. HBSS significantly preserved the viability of the PDL cells during the crucial extra-alveolar period ([33,34,35,36]). Serum also maintained the vitality of the periodontal membrane during the extra-alveolar period ([15]) while Eagle's medium allowed the proliferation of vital parts of the periodontium to cover area of root surface with denuded or necrotic periodontal membrane ([21,22,23]) Pasteurized milk contains important nutritional substances such as amino acids, carbohydrates and vitamins, which are useful to the periodontium ([26,29,30,38]) while isotonic saline also maintained the vitality of periodontal membrane during the critical extra-alveolar period ([17,18,19]).

The medium most often used at the Department of Oral and Maxillofacial Surgery, Obafemi Awolowo University, Ile-Ife is the patient's own serum as proposed by Thonner ([15]).

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