STORAGE MEDIA FOR AVULSED TEETH: A REVIEW

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Dental avulsion is a common type of trauma that results in the complete displacement of tooth from the alveolar socket. Although the ideal treatment would be the immediate re-implantation of the tooth at the site where the trauma took place, this may not be practically possible in every case. Hence, the avulsed tooth may have to be placed in an appropriate storage or transport medium until it is re-implanted. The biological properties of the storage medium have significant impact on the success of re-implantation, as it must be capable of preserving the vitality, clonogenic and mitogenic ability of the PDL cells for successful re-implantation.

KEY WORDS: Dental trauma, Avulsed teeth, storage media, PDL cell viability.

CLINICAL RELEVANCE: Avulsion of teeth is relatively common and adequate knowledge regarding acceptable storage media may help preserve the PDL cells on the avulsed tooth, improving the prognosis of re-implantation.

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INTRODUCTION

Avulsion is defined as the complete displacement of tooth out of its socket. This kind of dental trauma causes the periodontal ligaments to be severed with or without fracture of the alveolus. (1, 2) Reported incidence of dental avulsion is 1-11% of all dental injuries to the permanent dentition, with the maxillary central incisor being the most frequently involved tooth. The age group of 7-10 years appears to be most affected. (3) Detrimental consequences for the avulsed tooth as a result of this type of trauma include a severed vascular and nerve supply, resulting in the death of the pulp, especially in a tooth with a closed apex. More importantly, the complete separation of the tooth from the socket results in tearing of the periodontal ligaments, leaving viable periodontal ligament cells on the root surface. (4) The periodontal ligament cells, a quarter of which comprise of PDL fibroblasts, are responsible for the re-attachment of the avulsed tooth once it is re-implanted into its socket. Although immediate re-implantation is the treatment of choice as it results in PDL healing in up to 85% of mature teeth (5), it is not always possible to immediately re-implant the avulsed tooth. Therefore, an intermediate storage or transport media is essential for storage of such a tooth until the time it is re-implanted. The prognosis for success of re-implantation attachment is greatly dependent on the extra-oral dry time to which the avulsed tooth is exposed and placement in a storage medium that is capable of maintaining the viability of the PDL cells. (6) Numerous properties of the ideal storage media have been described in literature (7) (Table 1), and the pursuit for the best

| Maintains viability of periodontal fibres |
| Clonogenic and mitogenic capacity |
| Physiological osmolarity and pH |
| No antigen antibody reaction |
| Less risk of root resorption |
| Effective under various conditions |
| Antimicrobial |
| Sterile |
| Long Shelf life and easy availability |
| Inexpensive |

Table 1. Properties of the ideal storage media

storage medium has yielded a vast array of research on various substances. This article attempts to provide an overview of the multiple types of storage media reported in literature with a brief account of their suitability as storage or transport medium. The storage media are listed according to their origin and composition.

1. Tap water:

   Use of tap water to store avulsed teeth is not
recommended as it is not compatible with PDL cells because of its hypotonic osmolarity which causes cell lysis, and is reported to causes replacement resorption in avulsed teeth when they are place in it. (8) It is considered the least desirable storage medium.

2. **Saliva:**

Saliva is a readily available, natural storage medium. Despite this fact, due to the presence of substances like enzymes and bacteria and its non-physiologic osmolarity, which can exert harmful effects on the PDL cells, this can at best be used as an interim storage medium (no longer than 30 minutes).(8, 9) If stored for more than 60 minutes, a significant decrease in functional capacity of PDL cells occurs. (10)

3. **Milk and Variants:**

The use of milk as storage media has gained much popularity due to its ease of availability, cost effectiveness and physiologic osmolarity. Studies in literature have investigated various forms of milk including whole milk, skimmed milk, low fat content milk, baby formula and long shelf life milk. With a pH of 6.5-7.2 and the presence of essential nutrients important for maintaining the viability of PDL cells, milk can be considered as acceptable storage medium in most situations.(11), increasing the life of the PDL cells on the root surface.(12) Milk can maintain the viability of PDL cells from 2hrs(13) to 6 hours.(8, 10, 14-17) Milk that has been refrigerated or which has a lower fat content demonstrated better results as storage media in various studies.(10, 15-18) Its clonogenic and mitogenic capacity for PDL cells is considered equivalent to Hank's Balanced Salt Solution [HBSS].(19, 20) Some of the drawbacks of using milk as storage medium are the presence of antigens that could interfere with the process of reattachment of PDL cells when the tooth is re-implanted.(7) Also the milk needs to be fresh and refrigerated.(21) Sour milk should not be used as it is considered to be harmful.(10) Milk also has no proven role in assisting cell mitosis in PDL cells.(8, 14)

4. **Soy Milk:**

Soy milk, the water extract of soybean, contains no cholesterol or lactose and very small amounts of saturated fatty acid .(22) It is considered an excellent culture medium for cell growth and biochemical activities. Recent studies have shown that soy milk in contact with periodontal ligament cells promoted good cell viability, comparable to HBSS and milk and hence is recommended as a storage medium.(22, 23)

5. **Saline:**

Although isotonic saline has been used in various studies for its effect as a storage medium, it is unable to maintain the metabolism of PDL cells. It has comparable osmolality to the PDL cells, but lacks nutrients. It is considered acceptable to place an avulsed tooth in isotonic saline rather than storing it dry(24)although in another study, no significant difference was found in the development of ankylosis between teeth kept dry or placed in normal saline.(25) It is acceptable to place avulsed teeth in this storage medium for not more than 10 minutes.(26)

6. **Oral Rehydration Solutions:**

Ricetral is a commercially available oral rehydration formulation, consisting of essential nutrients like glucose and vital salts which help in maintaining cell metabolism. They are marketed in sealed sterile pouches and easily available over the counter in addition to being economical. It does not promote cell mitosis and regenerative capacity of the PDL fibroblasts. Its ability to maintain PDL cell viability was demonstrated to be equal to HBSS in a study, both retaining PDL vitality better than milk.(27)

7. **Hank’s Balanced Salt Solution:**

HBSS is a salt-solution which is pH balanced and contains essential metabolites needed for viability of PDL cells. It is considered a gold standard for storage media used in transport of avulsed teeth and it is used to compare the efficacy of other storage media. Its ability to maintain the vitality, clonogenic and mitogenic capacity of PDL cells for up to 48 hours has been proven.(8) It is considered superior to many other media in this regard (11, 20, 28) and can be used to store the avulsed tooth for at least 24 hours. It has also shown to replenish metabolites which have been depleted from PDL cells.(13) Hence, it has been recommended to place avulsed teeth in HBSS for 30 minutes before re-implantation in order to replenish the PDL cells, even if the avulsed teeth have been stored in an appropriate storage medium. HBSS is not readily available to public as its use is more in research laboratories, although in some countries it is available in emergency kits [Save-A-Tooth, PA, USA]. This kit comprises a small basket.
to hold the avulsed tooth while it is submerged in HBSS, until the tooth is re-implanted. Lack of availability and cost are considered the major drawbacks for this storage medium.

8. Propolis:
Propolis is a resin obtained from conifer trees. This sticky material is used by bees for constructing and maintaining their hives. It is a non-toxic biological material with anti-inflammatory, anti-bacterial, anti-oxidant, anti-fungal and tissue regenerative properties. Its chemical composition can be highly variable due to the different variety of plants the honey bees can visit while collecting this material. Recent studies have shown 10% propolis to be an effective storage media when compared with milk, HBSS, tap water and DMEM. Due to the fact that propolis is not readily available, its utility as a storage media is diminished when compared to other ready available materials.

9. Coconut Water:
Coconut water is a biological liquid which is pure, sterile and rich in nutrients like amino acids, proteins, vitamins and minerals. Readily available in tropical countries, it is an isotonic solution which can be obtained fresh directly from coconuts or commercially in packages and bottles. When compared with other media like HBSS, propolis and milk, it was found that coconut water was the most effective in maintaining viability of PDL cells. The same study found the combination of coconut water with sodium bicarbonate to be more effective but some studies have also demonstrating contradicting results. Since the pH of coconut water is 4.1, it has harmful effects on cell metabolism until sufficiently neutralized. Further research in this regards needs to be undertaken before coconut water can be used effectively as a storage medium.

10. Egg white:
This medium has not been found to be significantly different than HBSS in some studies in terms of cell viability and demonstrates greater PDL healing when compared with milk. It can be used to store avulsed teeth for up to 10 hours. One study found no difference between milk, egg white and artificial saliva. Although it is easily available, its major setback is impracticality of use.

11. Salvia officinalis:
Salvia officinalis is a perennial, evergreen shrub with blue to purplish flowers. It is a member of the family Lamiaceae and is native to the Mediterranean region; it has a long history of medicinal and culinary use. The extract from this plant has been used as spasmodylic, antiseptic and astringent. This extract has been proposed as a storage medium for avulsed teeth because of the anti-oxidants effects caused due to the presence of its phenolic components like rosmarinic acid, camosic acid, salvianolic acid and derivatives. These antioxidants help to prevent root resorption by inhibiting the effect of osteoclastic cells. Studies have shown that Salvia extract at 2.5% helps maintain PDL cells viability over longer periods of time when compared with HBSS, phosphate buffered saline and tap water. It has also demonstrated anti-microbial and anti-inflammatory properties. Thus salvia officinalis can be recommended as suitable storage media for avulsed teeth.

12. Morus rubra:
Morus rubra [red mulberry] belongs to the Moraceae family and active components include flavonoids, alkaloids an polysaccharides. In a study, Morus rubra juice at 4% concentration was found to be superior to HBSS for maintaining PDL cell viability for up to 12 hours.

13. Epigallocatechin-3-gallate [EGCG]:
Epigallocatechin-3-gallate [EGCG] is a major polyphenol of green tea, is known to have various biological effects such as anti-oxidative, anti-carcinogenic, anti-mutagenic, anti-inflammatory, anti-microbial, and anti-viral activities. Recently, research has been conducted to determine its role as an adequate storage medium. Greater viability of PDL cells of guinea pig and Beagle dog has been maintained when placed in EGCG. A study on extracted human teeth showed that EGCG can be used adequately as a storage medium, with a higher potential than HBSS and milk to promote favorable re-implantation, with less risk of root resorption and ankylosis.

14. CaseinPhosphopeptide:
Casein phosphopeptides [CPP] are derived from casein, which account for 80% of the total protein in bovine milk. They can form soluble organophosphate
salts and may function as carriers for different minerals, especially calcium. Their role in preventing demineralization and aiding in remineralization has been demonstrated. In a study which investigated the use of different concentration of commercial CPP-amorphous calcium phosphate as storage media by observing morphological changes in fibroblast cells, it was found that cell apoptosis did not occur when very dilute concentrations of CPP-ACP [10-12] was used as storage medium and further research was deemed necessary to determine the ideal concentration for preserving PDL cell viability.

15. Conditioned medium:
This medium is derived from supernatant of human gingival fibroblasts grown in culture. Since this medium contains stimulatory growth factors produced by the gingival fibroblasts, it is believed to have a beneficial effect on the PDL cells and their proliferation. This medium is also not readily available for general use, which limits its practicality.

16. Culture media:
Culture media can include Eagle's medium, alpha-Minimum Essential Media and alpha-MEM-S (with addition of foetal calf serum and antibiotics). Eagle's medium contains many nutrients like amino acids, vitamins and bicarbonates considered essential for maintaining the viability and proliferative capacity of PDL cells for longer time periods when compared with other storage media. The addition of growth factors like platelet derived growth factor, insulin-like growth factor, epidermal growth factor and many others, helps to enhance the clonogenic and mitogenic capacity of PDL cells for longer periods of time. Recently, research has demonstrated that the use of special cell culture medium (SCCM), which has been formulated especially to be used as a storage medium for avulsed teeth, is better at maintaining PDL cell viability than HBSS for time period of longer than 24 hours. Another variation of Eagle's Modified Essential Medium (EMEM) is Dubelco's modified Eagle’s Medium (DMEM) which contains a greater concentration of vitamins and amino acids as well as glucose compared to the EMEM. Despite their excellent properties, due to their need for refrigeration and lack of availability, all these culture media are not considered as practical for use as storage medium for avulsed teeth.

17. Custodial:
This medium is the registered trademark of Dr. Franz. It contains a histidine-tryptophan ketoglutarate solution containing high flow properties and low potassium content. It is basically an organ transport medium, also used for perfusing and flushing donor organs prior to their removal. In a study it was reported that custodial was comparable to HBSS in terms of cell viability. Similar to other organ storage medium, it is not available to public which limits its practicality as a storage medium for avulsed teeth.

18. Via Span:
This is a medium which was formulated for use in transplant procedures. It is used for cold storage of organs when they are removed from a donor. It is clear to light yellow in color, sterile and non-pyrogenic, with a pH of 7.4. It has shown to maintain the viability of PDL cells effectively, while maintaining cell morphology. Drawbacks of using Via Span include the need for refrigeration, high cost and inaccessibility.

19. Dentosafe:
Dentosafe (Miradent, Germany) is the commercial name of a tooth rescue box containing Special Cell Culture Medium (SCCM) which is a combination of amino acids, vitamins and glucose. In the USA it is marketed as EMT Tooth Saver (Phoenix, USA). It has demonstrated the maintenance of vitality of PDL cells for 48 hours at room temperature. If unopened, this medium has a shelf-life of 3 years. A study by Pohl et al showed that avulsed teeth placed in Dentosafe solution showed functional healing and recommended that Dentosafe should be included in all first aid kits, especially in locations prone to tooth avulsion injuries like schools, sports ground and facilities, public pools as well as emergency units like hospitals and ambulances. The use of this system is self-explanatory and simple to understand for lay persons. Although effective, this medium is still not easily available in many countries.

20. Contact lens solutions:
Since contact lens solutions are basically saline solutions, there use as storage media has been researched in some studies. However when compared with other storage media, they were deemed to be harmful and thus are not recommended for storing and transporting avulsed teeth.
avulsed teeth.(14)

21. Gatorade:
This drink was originally developed for athletes in order to replenish electrolytes lost during exercise and physical activity. Compared to tap water, the use of Gatorade as storage medium yielded better results for PDL cells survival.(16) Although it is relatively easily available at sporting events, where avulsion injuries to teeth tend to occur, its osmolarity causes cells destruction and hence it is not recommended for long term storage of avulsed teeth.(21)

CONCLUSION
Although research has been undertaken on a wide variety of materials to be used as storage media for the transport of avulsed teeth, lack of availability and high cost limit the use of majority of these media. Therefore, because of its acceptable performance, ease of availability and lesser cost, milk remains the storage medium of choice in cases where avulsed teeth cannot be immediately re-implanted. This knowledge should be made part of a public awareness program, in order to properly handle and save a large number of avulsed teeth, the prognosis of which can greatly improve if placed in an appropriate storage medium.

REFERENCES
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