

**Comparative Evaluation of Post-Traumatic Periodontal
Ligament Cells Viability using Four Different Storage Media
- An In Vitro Study**

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CERTIFICATE

This is to certify this dissertation titled “COMPARATIVE EVALUATION OF POST-TRAUMATIC PERIODONTAL LIGAMENT CELLS VIABILITY USING FOUR DIFFERENT STORAGE MEDIA - AN IN VITRO STUDY” is a bonafide record work done under my guidance by Dr.L. OMAR SUNIL, during his postgraduate study period between 2002-2005.

This dissertation is submitted in partial fulfillment for the award of the degree of Master of Dental Surgery in Branch III - Conservative Dentistry of The Tamil Nadu Dr. M.G.R. Medical University.

It has not been submitted (partially or fully) for the award of any other degree or diploma.

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INTRODUCTION

Traumatic injuries are a common occurrence that requires both expedient and informed management by the practitioner. Andreasen and Andreasen (1990) predicted that the incidence of these injuries might eventually surpass the incidence of dental caries. Avulsion injury, one of the most severe form of dental trauma constitute as much as 16 % of all traumatic injuries to permanent anterior teeth.⁴⁷ Avulsion 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. Successful treatment of an avulsed tooth by replantation is dependent upon the prevention of progressive root resorption by minimizing damage to the periodontal ligament and timely endodontic treatment.^{20,47} In addition, it is important to preserve the vitality of the periodontal ligament tissue remaining on the root surface (Hammer 1955).

The greatest success of a replanted avulsed tooth occurs when it is immediately replanted, which is not always feasible. Periodontal ligament cell viability can be preserved by replantation of the tooth within 15 -20 minutes after avulsion or by immersing the tooth in a suitable storage medium until it can be replanted.^{42,47} According to Andreasen et al (1995)³⁶, the factors that play a role in healing of the periodontal ligament 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.

Several methods have been suggested to preserve the viability of the periodontal ligament cells. Previous studies have tested a variety of storage media for their ability to maintain periodontal ligament cells viability including water, saliva, Hank's balanced salt solution (HBSS), cell culture media, and ViaSpan. The media were compared for their effectiveness in preserving the viability of periodontal ligament cells as well as prevention of replacement resorption. Although there are some differences, in general, the results of those studies showed that milk was better than saliva or water. HBSS and ViaSpan were better than milk. ViaSpan was equal to, or better than, HBSS in preserving periodontal ligament cells. Unfortunately, despite its effectiveness in maintaining periodontal ligament cells viability, ViaSpan and HBSS are expensive and are not readily available. Therefore, it is of interest to identify an effective, readily available and economically favorable storage media that can be as good as ViaSpan and HBSS, to maintain the periodontal ligament cells viability so that the percentage of successful replantation is high.

This study evaluates the effectiveness of Tender Coconut Water as a storage medium, an alternative to HBSS or ViaSpan in maintaining the periodontal ligament cells viability.

REVIEW OF LITERATURE

- 1. Soder PO, Otteskog P et al (1977)⁴⁵** studied the effect of drying time on viability of periodontal membrane of extracted mature human and monkey teeth. Following 0,30, 60, 90 and 120 minutes of dry storage, cell viability of periodontal ligament was examined with following methods

(1) culturing teeth with subsequent trypsinization and counting the viable cells (2) culturing the teeth with subsequent vital staining of the root surface with neutral red and estimation of the vital stained periodontal membrane area in percentage of the total root surface. Both methods revealed that the number of viable cells decline very rapidly with an increase in drying time and after 2 hours, it was not possible to demonstrate cell viability using tissue culture technique.

2. Blomlof L, Otteskog P (1980)¹¹ compared the effect of milk and saliva on human periodontal ligament cells. The cells were stored in milk or saliva for 60, 120 and 180 minutes at temperatures of 4°C, 20°C and 37°C. The stored cells were analyzed for cell viability, cell swelling, cell recovery and wound healing. They discovered that most of the periodontal ligament cells were viable after prolonged storage in milk. According to the authors, the viability of periodontal ligament cells is dependent upon the type of storage media, the temperature of the storage media, the duration of storage, and that the osmolarity of milk seems to be the most important factor.

3. Andreasen JO (1981)³ studied the effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. The extra-alveolar period before replantation was 0, 18, 30, 60, 90 and 120 min. The storage media for the extracted teeth were tap water, physiologic saline, saliva or dry storage. The replanted teeth were examined histometrically for surface resorption, inflammatory resorption, replacement resorption, periapical inflammatory changes, the extent of vital pulp and downgrowth of pocket epithelium. A significant relationship was found between the

frequency of root resorption, extra-alveolar period and storage medium.

They concluded that

- i) Surface resorption was found with approximately the same frequency irrespective of extra-alveolar period and storage media.
- ii) Inflammatory root resorption was especially common after dry storage and was related to the length of the extra-alveolar period. After 30 minutes of dry storage, this resorption type was very prominent. Teeth stored in tap water, saline or saliva showed about the same frequency of inflammatory resorption, which increased slightly with increased extra-alveolar periods.
- iii) Replacement resorption showed a strong relationship to dry storage and became very prominent after 60 minutes. Replacement resorption was rarely found among teeth stored in saline or saliva; whereas it was significantly increased among teeth stored in tap water.
- iv) Saline and saliva offer good protection against root resorption during the extra-alveolar period.

4. *Blomlof L, Otteskog P, Hammarstrom L (1981)*¹² studied the effect of storage in media with different ion strengths and osmolarities on human periodontal ligament cells. The effect on cultured periodontal ligament cells of saliva and milk was compared with some reference media such as tap water or saline by means of 3H - uridine leakage test. Cells stored in milk for 60-180 minutes showed about the same leakage as cells stored in saline or Hanks balanced salt solution. Osmolarity measurement showed that saliva was hypotonic and when the osmolarity was increased by addition of Sodium chloride, the leakage decreased to the level of cells stored in 0.9% saline or milk.

5. *Lindskog S, Blomlof L (1982)*²⁸ studied the influence of osmolarity and composition of saline, milk, physiologic sucrose solution and saliva as storage media on human periodontal ligament cells. They found that

physiologic media, such as milk, saline and physiologic sucrose solution, preserved cell viability and membrane morphology equally well. They concluded that a hypotonic osmolarity seemed to cause more damage.

6. **Blomof L et al (1983)¹³** studied the storage of experimentally avulsed monkey teeth in milk or saliva prior to replantation. It was found that the teeth stored for 2 or 6 hours in milk or for 2 hours in saliva showed periodontal healing almost as good as that of immediately replanted teeth. It was concluded that milk is superior to saliva as a storage medium for exarticulated teeth prior to replantation. They also said that milk does not revitalize dead cells.
7. **Oikarinen KS, Seppa ST (1987)³⁴** studied the effect of preservation media on proliferation and collagen biosynthesis of cultured human periodontal ligament fibroblasts. Proliferation of cells was studied after 60 minutes incubation in Dulbecco's Modified Eagle's Medium (DMEM), milk, saliva and tap water. They concluded that milk and saliva were superior to water in maintaining these cell functions.
8. **Hiltz J, Trope M (1991)²¹** studied the vitality of human lip fibroblasts in milk, Hank's balanced salt solution and ViaSpan storage media. The fibroblasts were grown to confluent monolayers in petridishes. The growth medium was replaced by one of the storage media and average number of cells that each plate contained at confluence was counted at time zero. At times ranging from 2 to 16 hours the average number of vital cells remaining was measured using the trypan blue exclusion test. The results showed that
 - i) The groups stored in milk maintained a high percentage to vital cells for 6 hours (68.2%). At 12 hours milk's effectiveness had dropped to 43.4% vital cells and it was not effective at all at 48 hours (0.024% vital cells).

- ii) Hank's balanced salt solution was extremely effective for 24 hours with 71.3% vital cells remaining. At 48 hours, the percentage of vital cells dropped to 38.0% and by 120 hours no cells survived.
- iii) ViaSpan was the most effective storage medium at all observation periods and at 168 hours still had 37.6% vital cells present.

9. **Gamson EK, Dumsha TC, Sydskis R (1992)¹⁸** studied the effect of drying time on periodontal ligament cell vitality. They studied various dry storage time ranging from 0 to 120 minutes followed by 45 minutes storage in milk.

A dual labeling fluorogenic labeling techniques was utilized in order to discriminate between viable and non-viable cells. It was found that at 30 minutes of dry storage, there was a statistically significant decrease in the number of viable periodontal ligament cells when compared to 10 and 20 minutes of dry time. They concluded that milk is a suitable storage media for maintaining periodontal ligament cell viability.

10. **Nordenvall KJ (1992)³³** reported a clinical case in which milk was used as a storage medium for exarticulated teeth following trauma. He reported the presence of functional periodontal ligament despite an extra oral period of more than twelve hours.

11. **Trope M, Friedman S (1992)⁴⁶** studied histologically the periodontal healing and root resorption of replanted dog teeth stored in ViaSpan for different time periods and compared these healing patterns to those after storage in milk or Hank's balanced salt solution. The extracted teeth were divided into four groups comprising 4 teeth each and placed in vials containing ViaSpan or milk for 6, 12, 24 and 36 hours and ViaSpan or Hank's balanced salt solution for 36, 48, 72 and 98 hours, after which they were replanted. Two months later the teeth were examined

histologically for healing of the supporting tissues. They concluded that for ViaSpan neither replacement nor inflammatory root resorption was seen after 6 and 12 hours storage. A statistically significant rise in the incidence of replacement resorption was seen at 24, 36 and 48 hours, which decreased again at 72 and 96 hours to levels, equal to storage for 6 and 12 hours. The occurrence of inflammatory root resorption was low and significantly increased only at 48 hours after which it decreased significantly again. ViaSpan proved superior to milk as a storage medium. Teeth stored in Hank's balanced salt solution showed healing results similar to those stored in ViaSpan.

12. *Patil S, Dumsha TC, Sydiskis RJ (1994)*³⁷ evaluated periodontal ligament cell vitality from exarticulated teeth stored in saline or milk using fluorescein diacetate stain. It was concluded 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 in saline.
13. *Alaoam T (1996)*¹ studied Lactate dehydrogenase activity in periodontal ligament cells stored in Hank's balanced salt solution, Custodiol (an organ storage medium) and sterile saline. This study measured lactate dehydrogenase on the root surfaces of extracted teeth for evaluating the results of breakdown and necrosis of periodontal ligament cells. They concluded that both Hank's balanced salt solution and Custodiol appeared to be suitable transport media for maintaining cell viability, whereas saline solution was not.
14. *Lekic P, Kenny D (1996)*²⁶ studied the relationship of clonogenic capacity to vital dye staining of human periodontal ligament cells. The cell vital dye staining (BCECF / AM dye inclusion) was done to determine the cell membrane integrity. They found that extracted teeth

stored in milk as transport medium exhibit improved cell viability compared with dry conditions. They also concluded that in vitro assays of cell viability based on dye inclusion are not as closely related to clinical survival of replanted teeth

as are assays of clonogenic capacity, since these dye inclusion assays provide only a measure of membrane integrity.

15. Harkacz OM, Carnes DL, Walker WA (1997)¹⁹ conducted a study on determination of periodontal ligament cell viability in the oral rehydration fluid Gatorade and milks of varying fat content. They concluded that

- i) Gatorade yielded cell viability data similar to the negative control, tap water, indicating that oral rehydration fluid was not suitable as a temporary storage medium for the avulsed tooth.
- ii) The fat content of milk had an effect on cell viability, suggesting that milks with lower fat content may be more appropriate for maintaining periodontal ligament cell viability than milks with higher fat content.

16. Olson BD, Mailhot JM et al (1997)³⁵ compared milk, Save-A-Tooth (HBSS), HBSS supplemented with platelet derived growth factor -BB (PDGF) and Gatorade as transport media on human periodontal ligament cell viability. The results showed that milk and Save-A-Tooth with PDGF are suitable as transport medium for avulsed teeth. The addition of PDGF to Save-A-Tooth may enhance its ability to maintain periodontal ligament cell viability. They also suggested that Gatorade was unsuitable as a storage medium.

17. Pettiette M, Hupp J et al (1997)³⁸ studied periodontal healing of extracted dogs teeth air-dried for extended periods and soaked in Hank's balanced salt solution, ViaSpan and Conditioned Medium for 30 minutes before replantation. It was concluded that an avulsed tooth that has been

left dry for 30 minutes should be replanted immediately without soaking. However, teeth that have been dry for 45 or 60 minutes would benefit from soaking for 30 minutes in ViaSpan.

18. Doyle DL, Dumsha TC, Sydiskis RJ (1998)¹⁷ evaluated the effect of soaking in Hank's balanced salt solution or milk on periodontal ligament cell viability of human teeth stored dry for 30,60 and 90 minutes. Trypan blue dye exclusion test was used to assess the vitality. The results of this study demonstrated no significant difference in the number of viable cells with or without soaking in Hank's balanced salt solution or milk at any of dry storage time. In addition, there was no significant difference in the periodontal ligament cell viability between the 30 and the 60 minutes dry periods.

19. Lekic PC, Kenny DJ, Barrett EJ (1998)²⁷ studied the influence of the storage media and extra-alveolar time on the clonogenic capacity of periodontal ligament. The extra-alveolar time tested was 30 and 60 minutes and the various storage media used were saliva, milk, Hank's balanced salt solution and Eagle's medium (α MEM). The reduction in clonogenic capacity was determined. Periodontal ligament cells stored in α MEM showed the least reduction between 30 and 60 minutes and it was greatest when stored in saliva. It was concluded that immediate storage of avulsed teeth in autologous saliva, followed by transfer to chilled milk, preserves the presence of sufficient progenitor cells and warrant the possibility of periodontal ligament healing at 60 minutes extra alveolar duration.

20. Ashkenazi M, Sarnat H, Keila S (1999)⁷ studied in vitro viability, mitogenicity, and clonogenic capacity of periodontal ligament cells after storage in six different media. The media evaluated were culture

medium, alpha minimal essential medium, milk, Hank's balanced salt solution, ViaSpan and conditioned medium. They concluded that Hank's balanced salt solution and milk were the most effective media for preserving the viability, mitogenicity and clonogenic capacity after storage for upto 24 hours at 4°C.

- 21. *Kitzis GD, Miller P (1999)***²⁵ reported reimplantation of an avulsed tooth after prolonged storage. This report demonstrates that if an intact avulsed tooth is retrieved, stored carefully and treated, it can be re-implanted successfully, even after more than 42 hours outside the alveolus.
- 22. *Pohl T, Tekin U (1999)***⁴¹ made investigations on a cell culture medium for storage and transportation of avulsed teeth for up to 48 hours. Auto radiographic investigations revealed that the proliferative activity of periodontal ligament cells of teeth stored in cell culture medium for up to 48 hours increased with storage time.
- 23. *Ashkenazi M, Marouni M, Sarnat H (2000)***⁸ studied in vitro viability, mitogenicity and clonogenic capacity of periodontal ligament cells after storage in Hank's balanced salt solution, culture medium, alpha minimal essential medium and ViaSpan at room temperature. They concluded that culture medium, followed by Hank's balanced salt solution and ViaSpan, was the most effective media for preserving the viability, mitogenicity and clonogenic capacity of periodontal ligament fibroblast stored for up to 24 hours at room temperature.
- 24. *Marino TG, West LA (2000)***³⁰ evaluated the ability of long shelf-life milk as a storage media compared with pasteurized milk and Save-A-Tooth. The results showed that at 8 hours, periodontal ligament cells viability in regular pasteurized milk and long shelf-life milk were significantly greater than in Save-A-Tooth. There was no significant

difference between regular pasteurized milk and long shelf-life milk at any time period. These results suggest that long shelf-life milk, which has the advantage of not requiring refrigeration, is as effective a storage medium as regular pasteurized milk and more effective than Save-A-Tooth.

25. Ashkenazi M, Marouni M, Sarnat H (2001)⁹ studied in vitro viability, mitogenicity and clonogenic capacities of periodontal ligament fibroblasts (PDLF) after storage in four media supplemented with growth factors. The evaluated culture media were ViaSpan, Hank's balanced salt solution (HBSS), alpha minimal essential medium (α MEM) and α MEM supplemented with fetal calf serum and antibiotic (α MEM-S). They concluded that the mitogenic and clonogenic effects of growth factor were observed only after 24 hours of storage at room temperature. HBSS and α MEM-S supplemented with growth factor were the most effective media for preserving the viability, mitogenicity and clonogenic capacity of PDLF stored for 24 hours at room temperature. For short periods of storage (2 and 8 hours), HBSS and α MEM-S without growth factor were preferable.

26. Pileggi R, Dumsha TC (2002)⁴⁰ assessed post traumatic periodontal ligament cells viability stored in Hank's balanced salt solution, milk, saline and water using Collagenase and Dispase treatment. The viable cells were assessed using trypan blue exclusion test. It was concluded that following 30 minutes dry storage and 45 minutes storage in transport media, milk appeared comparable to saline and Hank's balanced salt solution for storage of avulsed teeth. They also suggested

that Collagenase and Dispase assay appeared to be a viable method for evaluating periodontal ligament cell viability.

27. Schwartz O, Andreasen FM, Andreasen JO (2002)⁴⁴ studied the effect of temperature, storage time and media on periodontal and pulpal healing after replantation of incisors in monkeys. It was concluded that

- i) Storage in saliva at 37°C showed a similar amount of normal periodontal ligament compared to saline storage for both 60 and 120 minutes.
- ii) Saline storage for 60 or 120 minutes showed no difference in the effect of normal periodontal ligament when storage was compared at 37,22 and 4°C, but at -18°C it resulted in significantly less normal periodontal ligament.
- iii) The temperature of the storage medium is of importance only for dry storage.

28. Buttke TM, Trope M (2003)⁴¹ studied the effect of Catalase supplementation in storage media for experimentally avulsed dog teeth. The media used were Hank's balanced salt solution and ViaSpan. They showed that the roots stored in media containing antioxidant activity undergo less surface resorption, and also low levels of H₂O₂ in storage media may adversely affect the periodontal ligament cells.

29. Pearson RM et al (2003)³⁸ studied human periodontal ligament cell viability in milk and milk substitutes. The milk substitutes used were reconstituted powdered milk, evaporated milk, one of two baby formulas (Similac or Enfamil). They concluded that

- i) At 1 hour there was no difference in the effect on periodontal ligament cell viability between any of the material and whole milk.
- ii) At 2 hours, Enfamil and Similac performed significantly better than whole milk, where as evaporated milk performed worse.
- iii) At 4 hours, Enfamil performed better than whole milk, whereas all milk substitutes performed worse.

iv) At 8 hours, all substitutes performed worse than whole milk.

It was suggested that Enfamil, which is supplied in powder form, that does not require special storage and having a shelf life of 18 months is a more effective storage medium for avulsed teeth than pasteurized milk for at least 4 hours.

30. Martin MP, Pileggi R (2004)³¹ made a quantitative analysis of Propolis, a new storage media following avulsion. Collagenase - Dispase assay was used to assess the storage media. They showed that within the parameters of the study, it appeared that Propolis may be better alternative to Hank's balanced salt solution, milk or saline in terms of maintaining periodontal ligament cell viability after avulsion and 45 minutes storage.

SUMMARY

The study evaluated the post-traumatic periodontal ligament cell viability following storage in four different media. The media assessed were

Hank's balanced salt solution

Milk

Saline

Tender Coconut Water

The specimens were 48 intact human mandibular first premolars divided into 6 groups of 8 each. The teeth in each experimental media were dried for 30 minutes and were immersed in any one of the storage media for 45 minutes. The teeth stored dry for 8 hours served as negative control and teeth in positive control were subjected to the study immediately.

Collagenase Type IA and Protease Type IX were used for enzymatic desegregation and Trypan blue exclusion staining technique was followed.

The number of viable cells were counted under light microscope (45x magnification), using hemocytometer.

The mean number of viable periodontal ligament cells was statistically analyzed by One-Way ANOVA, and Scheffe's test.

As per the results of this study, Hank's balanced salt solution was the most effective media in maintaining viability of periodontal ligament cells, followed in order by Tender Coconut Water, Saline and Milk.

CONCLUSION

Expedient and immediate replantation is the best treatment with guaranteed success for avulsed tooth. When this is not possible, the prognosis of an avulsed tooth is largely dependent on the status of the periodontal ligament cells at the time of replantation. Storage media helps in preserving the viability of the periodontal ligament cells when immediate replantation is not possible.

This study evaluated the post-traumatic periodontal ligament cells viability following storage in 4 different media.

With in the parameters of this study Hank's balanced salt solution is the most effective media for maintaining the viability.

Tender Coconut Water maintains viability less than HBSS but greater than Milk and Saline. But further detailed studies are required to validate the results, before it is recommended for clinical use.

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