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Evaluation, using extracted human teeth, of Ricetral as a storage medium for avulsions – an *in vitro* study

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Abstract – The prognosis of teeth replanted following avulsion is determined by the extra-alveolar time and storage medium used. This study was undertaken to determine the efficacy of an oral rehydration solution 'Ricetral', in retaining the vitality of periodontal ligament cells when used as a storage medium for avulsed teeth prior to replantation. The study consisted of a comparative evaluation between Ricetral and two currently recommended solutions, Hank's balanced salt solution (HBSS) and milk. Thirty extracted teeth were dried for 30 min and soaked in the respective storage media for 45 min. The periodontal ligament cells were isolated by an enzyme treatment with collagenase and trypsin. The cells were evaluated for vitality by trypan blue staining and number of vital cells counted in a hemocytometer. Statistical analysis revealed that cell vitality was high with Ricetral and HBSS, but poor with milk.

Tooth avulsion or exarticulation is the loss of a tooth, following trauma. It results in attachment damage and pulp necrosis. Attachment damage as a direct result of avulsion cannot be avoided. However, considerable additional damage can occur to the periodontal ligament in the time that the tooth is out of the mouth (1). Treatment strategies should, therefore, aim at limiting the extra-oral dry time. The treatment of choice for an avulsed tooth is immediate replantation. If doubt exists that the tooth can be replanted immediately, the tooth should be stored in a storage medium.

Various materials have been studied for their potential to serve as a storage medium for avulsed teeth, such as water, saline, milk, and culture media, with differing degrees of success. However, none of the currently used media is ideal and the search for a medium that can overcome the limitations of these materials continues. Hence, this study was undertaken to assess the potential of a commercial oral rehydration formulation (Ricetral), in comparison with the currently recommended materials, Hank's balanced salt solution (HBSS) and milk, as a storage medium for avulsed teeth. The main objective was to assess the ability of the different media to preserve the vitality of periodontal ligament cells in simulated avulsed teeth for extended periods of time.

Materials and methods

The study was conducted in the Molecular Biology Department of Amrita Institute of Medical Sciences, Kochi, in collaboration with the Amrita School of Dentistry. The methodology followed in the study was adapted from that described by Martin and Pileggi (2), and Nonnenmacher et al. (3).

Freshly extracted human teeth were taken for the study. The teeth had been extracted for orthodontic purposes. Thirty healthy single-rooted teeth that had no caries, restorations, periodontal disease, or hypoplasia were selected. Following extraction, the teeth were held with forceps by the coronal region and the coronal 3 mm of the periodontal ligament was scraped with a curette to remove cells that may have been damaged during extraction. The teeth were then randomly assigned to one of the three storage medium groups, with 10 samples per group.

Group I – Ricetral
Group II – HBSS
Group III – Milk

These teeth were dried for 30 min, inclusive of the time for curetting, followed by a 45-min immersion in one of the storage media: Ricetral, HBSS, or milk, respectively.

Ten teeth were allocated to the control groups. The positive control group comprised of five teeth that were neither dried nor stored in any solution, but assayed immediately for cell vitality. The negative control teeth (five samples) were dried for 8 h, with no follow-up storage solution time, and then assayed. These, respectively, comprised Groups IV and V.

Group IV – Positive control

Group V – Negative control

Each sample was gently rinsed in phosphate buffer saline (PBS) to remove blood and debris adhering to the roots. Isolation of the cells from the periodontal ligament was carried out enzymatically, using Collagenase Type III (Gibco BRL, Taastrup, Denmark) and Trypsin (Sigma-Aldrich, Taufkirchen, Germany).

The enzyme solution for the experiment consisted of (in 5 ml):

Collagenase Type III (lyophilized powder, 152 U mg^{-1})	1 mg
Trypsin solution (1.5 g per 100 ml)	0.8 ml
PBS	4.2 ml

Each PBS-rinsed sample was immersed in 1 ml of this enzyme solution for 10 min in a sterile 15-ml Falcon tube. The solution was agitated using a micropipette for the last 2–3 min of immersion, to facilitate detachment of cells. At the end of 10 min of enzyme treatment, the teeth were removed from the solution and 1 ml of the solution was pipetted to a microtube. Ten microliters of fetal bovine serum was added to it. The tube was then centrifuged at 90 ***g*** for 4 min. The supernatant was removed, and the pellet was dissolved in 1 ml of PBS. This was stained using 0.4% (w/v) trypan blue (Cell culture – tested; Sigma-Aldrich) in a 1:1 ratio (100 μl of solution with 100 μl of dye). After 10 min, 10 μl of the stained solution was taken on a Neubauer's counting chamber and the cells were viewed under 10× magnification.

Results

The mean number of vital cells in each group is represented in Fig. 1. Group IV (positive control) had the highest number of surviving cells, followed by Group II (HBSS), I (Ricetral), and III (milk) in decreasing order. The number was lowest in Group V, the negative control group. The number of cells in the positive control group was significantly higher than any of the test groups, just as the number of cells was significantly low in the negative control group.

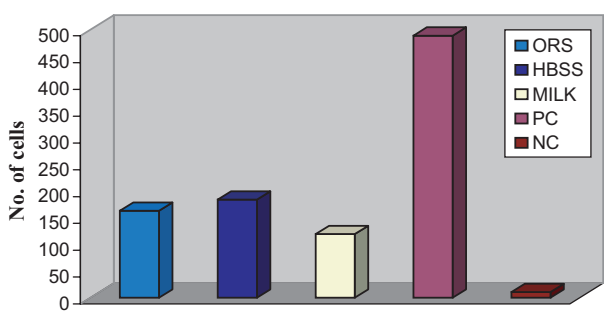

Fig. 1. Mean number of vital cells in each group.

Table 1. Mann–Whitney *U*-test for comparison between individual groups

Groups	Asymp. sig. (two-tailed)	SE	Significance
I and II	0.290	13.229	Not significant
I and III	0.014	13.229	Significant
II and III	0.001	13.229	Significant

Statistical analysis of the data was carried out using nonparametric tests, Krusker–Wallis *H*-test, and Mann–Whitney *U*-test. The tests were carried out to determine the differences among the groups, and significance level was set at 5%.

There was a statistically significant difference between Groups I and III (Ricetral and milk), and also between Groups II and III (HBSS and milk). However, there was no significant difference between Groups I and II (Ricetral and HBSS) (Table 1).

Discussion

The ideal outcome after replantation of an avulsed tooth is regeneration of the periodontal ligament, which is possible by retaining the viability of its fibroblasts. The cells that remain on the root after exarticulation are deprived of their blood supply and begin to immediately deplete their stored cell metabolites. To maintain optimal cell metabolism, these depleted cell metabolites must be replaced within 2 h (4). Hammer (5) demonstrated that the length of survival of a replanted tooth is directly correlated with the amount of viable periodontal ligament. Since then, many materials have been evaluated for use as a storage medium that can retain cell vitality over extended periods of time.

Milk effectively maintains the vitality of periodontal ligament (PDL) cells (6–10). It is usually readily available at or near the accident site (11), has a pH and osmolarity comparable to vital cells, and is relatively free of bacteria. However, concern has been raised over its state of sterility and temperature.

Cell culture media in specialized transport containers, such as HBSS (12, 13) or Viaspan, have shown superior ability to maintain the vitality of the periodontal ligament cells for extended periods (14–16). In the West, HBSS has been popularized by Krasner and is available as part of the Save-A-Tooth kit designed for safe transportation of avulsed teeth. Such systems are limited in availability and are largely inaccessible to the layman, across the world.

The limitations of the currently used storage media have prompted much research in this field in recent years. Sigalas et al. (13) compared two brands of contact lens solutions and Gatorade with milk, culture media, and HBSS, but found Gatorade and the contact lens solutions to be inferior to HBSS and milk. Martin and Pileggi (2) introduced propolis as a storage medium, with more efficacy than HBSS, milk, and saline. A very recent

study reported that the water of tender coconut kept significantly more PDL cells viable compared with propolis, HBSS, or milk and that coconut water can be used as a superior transport medium for avulsed teeth (17). However, these findings have been refuted by another study that reports milk to be better than coconut water (18). They also suggest the addition of sodium bicarbonate to coconut water to improve its efficacy. Studies on rehydrating solutions such as sports drinks (Gatorade) have also been conducted, but have not yielded positive results (13, 19–21).

ORS solutions are used to combat dehydration in cases such as diarrhea. They consist of essential cell nutrients, such as glucose and vital salts, in concentrations deemed adequate for the cell metabolism to remain unhindered. They are capable of maintaining the body hydration, as well as replenishing the lost fluids by enhanced absorption from the intestine. ORS solutions are readily available to the common man over the counter at any pharmacy and are economically feasible. Another advantage regarding these formulations is that they are marketed in sealed sterile pouches.

In previous research on rehydrating solutions such as tender coconut water and sports drinks, the results have been varied. The results of the present study are in accordance with those supporting the use of such rehydrating solutions.

As with any *in vitro* study, limitations and variability do exist, even in the present study design. The use of extracted teeth to simulate avulsed teeth has been recommended in previous studies (2, 22). In this investigation, the extractions were performed by different clinicians, which may have induced variable trauma during the actual extraction. This could translate into variability of PDL cell vitality counts. The counting of vital cells was performed by a single observer to minimize variability in the counting process, strictly adhering to the standard recommendations when using a hemocytometer.

Trypan blue testing has been used to assess cell viability in many studies, but this stain only assesses vitality of the cell and not the actual physiologic health or metabolic capabilities of the cell. Further, it does not distinguish between necrotic and apoptotic cells.

The health status of a viable PDL cell is likely critical to the prevention of resorptive sequelae after replantation. Although culture studies can help give an insight into the health state of the cells, the actual end result of any treatment can be precisely measured only by clinical trials.

Ricetral, an ORS solution conforming to the WHO recommendations, may be substituted by any commercially available ORS solution of a similar composition. These solutions address only one of the concerns in successful replantation of an avulsed tooth, namely drying and cell metabolite depletion. However, other major issues such as contamination and the regenerative capacity of the surviving fibroblasts also need to be considered. The addition of an antibacterial component to counter possible contamination of the avulsed tooth may be beneficial. Similarly, an agent to minimize resorption would be desirable.

Conclusion

Within the limitations of this study, it is seen that the ability of Ricetral to retain PDL cell vitality is similar to HBSS and both these solutions are better than milk.

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