



A NEW STORAGE MEDIUM FOR AN AVULSED TOOTH - EFFECT OF ALOE VERA ON SURVIVAL OF PERIODONTAL LIGAMENT CELLS

Dental Science

M Swarna latha Senior lecturer, MNR Dental College and Hospital, Sangareddy- 302294, Telangana, India.

Narender Reddy M Reader, Department of Conservative Dentistry & Endodontics, S.V.S. Institute of Dental Sciences, Mahabubnagar Dist - 509 060, Telangana, India.

Aisha Habeeb* Post Graduate Student, Department of Conservative Dentistry & Endodontics, S.V.S. Institute of Dental Sciences, Mahabubnagar Dist - 509 060, Telangana, India
*Corresponding Author

Srikanth Pasari Reader, Department of Conservative Dentistry & Endodontics, S.V.S. Institute of Dental Sciences, Mahabubnagar Dist - 509 060, Telangana, India.

ABSTRACT

Tooth avulsions occur commonly due to dento-facial trauma. An ideal storage media is needed until the teeth are replanted. This study aimed to evaluate the ability of Aloe vera (AV) and Mc-carey kaufman (MK) medium as storage media in comparison with Coconut water (CW) and HBSS in maintaining the periodontal ligament (PDL) cell viability. Freshly extracted premolars were divided into 6 groups. These were stored dry for 30 mins and soaked in one of the 4 storage media i.e., HBSS, CW, Aloe vera pulp (AV) and MK medium for 45mins. Lastly they were treated with collagenase II and dispase grade II enzymes for 30 mins. The number of viable PDL cells were counted with the help of hemocytometer. CW and AV showed significantly more viable cells in comparison to HBSS and MK ($p < .05$). Thus it was concluded that AV had the potential of maintaining the PDL cell viability.

KEYWORDS

Storage medium, Aloe vera, Coconut water, MK medium, Collagenase, Dispace.

INTRODUCTION

Nearly 0.5%-16% of traumatic dental injuries result in tooth avulsion [1-4]. According to World Health Organization's (WHO) classification of traumatized teeth, "avulsion or ex-articulation is the complete displacement of a tooth from its alveolar socket due to traumatic injury"[5].

Immediate replantation is the ideal treatment strategy for an avulsed tooth. It results in repair of the PDL by 85.0% - 97.0% (8). However immediate replantation is usually impossible due to the lack of familiarity and understanding of replantation at the accident site.

The ability of a storage medium to support cell viability is an important factor to prevent ankylosis (9). Various storage media such as patient's buccal vestibule, saline, milk, milk substitutes, eagle's medium, viaspan, propolis, culture media, contact lens solutions, oral rehydration fluids, coconut water (CW), egg albumen, Hank's balanced salt solution (HBSS) have been tried.

HBSS is commercially available under the name save-a-tooth solution and recommended by the American Association of Endodontics (AAE) as the medium of choice for the avulsed teeth (5, 9)

Aloe vera (AV) has curative and therapeutic properties. It is reported to have anti-diabetic, wound healing, anti-cancer, antioxidant, antigenotoxic, chemopreventive, anti-inflammatory, angiogenic, antimicrobial and hepato-protective properties (10).

McCary-Kaufman medium (MK medium) was introduced in 1974 as a simplified method of short-term corneal preservative to maintain its viability until transplantation (11).

This study evaluated the ability of AV and MK medium as storage media for simulated avulsed teeth in comparison with CW and HBSS in maintaining the PDL viability.

MATERIALS & METHODS

Sample selection

Seventy freshly therapeutically extracted premolars were included in the study. The teeth with moderate to severe periodontal disease and caries were excluded.

Sample preparation

Post extraction, 3mm of PDL was removed below the cemento-enamel junction with a sharp curette. To eliminate extraction induced damaged PDL cells. The teeth were then dried for 30mins, to simulate the clinical situation as in avulsion.

Storage of teeth

Teeth were stored randomly into 4 groups, i.e. HBSS group (HiMedia Laboratories, Secunderabad, India), CW group, AV group and MK group (Sarojini Devi Eye Hospital) for 45mins.

Preparation of enzymatic solutions:

Both the collagenase II and dispase grade II solutions (HiMedia Laboratories, Secunderabad, India) were freshly prepared before the procedure.,

Then the samples were incubated for 30mins in a test tubes containing 2.5ml solution of collagenase II and dispase grade II solutions. To each sample test tube 50 μ l of fetal bovine serum (HiMedia Laboratories, Secunderabad, India) was added. The cellular suspension in each test tube was transferred to another test tube and the teeth were discarded. These test tubes were centrifuged for 4mins at 1000rpm. Supernatant obtained was discarded to obtain sediment.

One drop of sediment was placed on to a glass slide to which a drop of trypan blue dye was added. The number of viable protective least significant difference cells in a grid of Neubauer's chamber was counted under a light microscope (Olympus) at 20x magnification with a hemocytometer using the following formula:

$$\text{Total count} = \frac{\text{No. of cells counted} \times \text{dilution factor}}{\text{Volume of the chamber (Area} \times \text{depth)}}$$

Post extraction positive control teeth were directly treated with collagenase and dispase. The negative control teeth were bench dried for 8hrs, and directly placed in dispase and collagenase enzymatic solutions.

Statistical Analysis

Statistical analysis of the results was carried out using kruskal-wallis and mann-whitney with a significance level of 0.05 ($p=0.05$).

RESULTS

The mean number of viable PDL cells in different storage media are

presented in Table 1. of all the groups tested, positive control group exhibited highest number of viable cells and negative control the least. When comparison was made between the four groups, the groups CW and AV gave the highest mean viable cells and were significantly different, in comparison with groups HB and MK ($p < .05$).

TABLE 1: Means and standard deviations of all test groups

DISCUSSION

Avulsion, is the complete displacement of the tooth from the alveolar (12). After avulsion, if PDL cells are viability, replantation would occur with minimal destructive inflammation. Excessive drying of PDL cells will elicit a severe inflammatory response causing large area of damage were cementoblasts cannot cover the entire root surface. As a result bone will attach directly on the root surface. Which is termed osseous replacement or replacement resorption or ankylosis (13).

To avoid such complications, avulsed tooth should be replanted immediately or stored in suitable media until replanted. Andreassen (14) and Doyle et al (15) showed that a dry period of 2hrs results in necrosis of almost all of PDL cells. Andreassen et al (16) showed that replantation of teeth within 30mins has 90% chance of success when compared to 2hrs which has a negligible chance of almost 5%.

Ideally the storage medium should be readily available at the site of accident. It should have an osmolarity of 290-300 mosmol/kg to support cell growth.

This study uses a collagenase assay, which minimizes the exposure of cells to active trypsin and to preserve the maximum cell viability (9). Because the extra-cellular matrix has a high content of collagen and other proteins, the use of the enzymes collagenase and dispase (or neutral protease) cause the release of greater number of cells within a shorter period of time (17) and releases cells without excessive disruption and destruction to their membrane (9).

The results of this study demonstrated that AV maintained PDL cell viability. The reason might be that the parenchymal tissue (inner pulp) of AV contains proteins, lipids, amino acids (Essential- lysine, threonine, valine, leucine, iso-leucine, phenylalanine, methionine and non-essential amino acids such as histidine, arginine, hydroxyproline, aspartic acid, glutamic acid, proline, glycine, alanine, tyrosine) vitamins (B1, B2, B6, C, choline, folic acid, β -carotene, -tocopherol), enzymes(cyclo-

Group	N	Mean no. of viable cells	SD
HBSS	15	425.66	74.01
CW	15	540.66	162.38
AV	15	590	179.75
MK	15	412.6	67.81
Positive control	5	3010.00	265.61
Negative control	5	21.00	6.52

oxygenase, oxidase, amylase, catalase, lipase, alkaline phosphatase, carboxypeptidase) inorganic compounds(Ca, Na, Cl, Mn, Zn, Cr, Cu, Mg, Fe) and small organic compounds minerals, sugars, lignin, saponins, salicylic acids. It has as an optimal pH range above 5 (10, 18).

It is generally believed that last pathways of cell injury undergo free radical reactions caused by ischemia. These free radicals further initiate lipid peroxidation of cell membrane. Suppressing the generation of free radicals may improve the effectiveness of organ preservation and prevent lipid peroxidation. Hence presence of anti-oxidants in storage media is necessary for inhibiting the generation of free radicals thereby minimizing cell damage (19).

Sandstrom et al in 1998 found that the presence of catalase in storage medium for avulsed teeth can prevent cell apoptosis (20). The osmolarity of AV pulp is 280-300 mosm/lit. As it was reported by Lindsog (21) that cell growth can occur at a range of 230-400mosm/lit, it might also be a possible reason for its ability to maintain cell viability.

In this present study, CW was better compared to HBSS and MK in maintaining cell viability. The nutrients in CW such as proteins, amino acids, vitamins and minerals, help in nourishing the cells and maintaining their viability. It has high osmolarity of around 372

mosm/lit due to the presence of sugars such as glucose and fructose (9). HBSS, inspite of being the medium of choice, did not exhibit PDL cell viability as good as AV. The reason might be, because in addition to various nutrients present in HBSS, AV contains carbohydrates, chromones, organic compounds, lipids, non-essential amino acids, proteins, saccharides, vitamins and most importantly anti-oxidants which are known to inhibit the generation of free radicals thereby minimizing cell damage (19).

CONCLUSIONS

Within the limitations of this study, AV maintained the PDL cell viable when compared to CW, HBSS and MK medium. Although, AV exhibited maximum cell viability when compared to CW and there was no statistically significant difference between the two storage media. Hence depending on the availability, either AV or CW can be used as a suitable storage media for avulsed teeth as an alternative to HBSS.

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