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Original Article

Dentistry Section

Addition of L-dopa to HBSS in enhancing the maintenance of cell viability of Periodontal Ligament (PDL) cells: An In-Vitro Study

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ABSTRACT

Aim: To investigate the viability of PDL cells in an avulsed tooth using a storage medium with and without L-dopa.

Materials and Methods: Twenty freshly extracted non-impacted human teeth with closed apices and no caries beyond the CEJ were obtained for this study. The teeth were then randomly divided into 1 of the 4 experimental storage solution groups, namely group 1: deionized water; group 2: deionized water with L-dopa (1μg/μl); group 3: HBSS; and group 4: HBSS with L-dopa (1μg/μl). After storing the teeth in different test solutions for 45 minutes collagenase assay was performed and the number of viable PDL cells were counted under a light microscope using a hemocytometer at 20X magnification.

Results: The mean number of viable PDL cells was highest in group 4, followed by group 3 and group 2. Group 1 shows the least number of viable PDL cells.

Conclusion: The synergistic effect of L-dopa and HBSS paves way for its usage as an effective storage medium for an avulsed tooth

Keywords: Avulsed tooth, Cell viability, HBSS, L-dopa, Storage medium

INTRODUCTION

Avulsion or exarticulation is a traumatic injury that results in the complete dislodgement of a tooth from its alveolar socket [1]. In permanent dentition, the incidence of avulsion ranges from 1 to 16% of all the traumatic injuries [2]. In avulsion, the anterior tooth is most commonly affected [3]. Children in the age group of 7-11 y predispose to avulsion with a greater prevalence towards males [4,5]. When a tooth gets avulsed, it should be replanted immediately to prevent further injury to periodontal ligament (PDL) cells in the future. After avulsion, the number of viable cells on the root surface decreases with increased drying time and that after two hours it was not possible to demonstrate cell viability. Therefore, the ideal treatment of choice at the time of avulsion is immediate replantation, thus reestablishing the natural nutrient supply to the periodontal ligament cells, thereby minimizing further damage and enhancing the healing process [6]. However, immediate replantation is seldom achieved due to the emotional stress of patients and lack of knowledge of appropriate first-aid measures to manage the problem at the site of injury. In these conditions the tooth should be maintained in a suitable medium until it is replanted by a dentist as soon as possible [2]. The prognosis of the replanted tooth depends largely upon the extra-oral time of the avulsed tooth and the medium used to preserve the tooth before replantation [7].

The ability of a storage/transport medium to support cell viability is more important than the extraoral time to prevent ankylosis and replacement resorption [6,8]. Various storage media such as tap water, saliva, saline, milk, culture media, Viaspan, Hank's Balanced Salt Solution (HBSS) and tender coconut water have been employed. Hank's balanced salt solution (HBSS) has been recommended as the storage medium of choice for avulsed teeth by the American Association of Endodontics [9]. Unfortunately HBSS is not commonly used in India since it is not readily available. In addition to preserving the cell vitality, a storage media which has the potential to increase the cell viability of PDL cells is highly desired. L-dopa is a new storage media which has been developed along these lines.

Levodopa (L-dopa) a precursor of central nervous system catecholamines is a drug with possible mitogenic effects. In the human body, L-dopa changes to dopamine, which stimulates anterior part of hypophysis to secrete growth hormone which is a promoter of healing process [10]. L-dopa has been successfully used in the treatment of Parkinson's disease and for managing fractured long bones with delayed healing or nonunion healing [10,11]. L-dopa also has an effect on endochondral bone repair and intramembranous bony repair in the mandibular defect of rats [12]. Mandona et al., observed the effect of levodopa on human PDL fibroblasts and concluded that L-dopa due to its mitogenic activity can be used for preserving viable cells [13]. Hence, the aim of this study is to investigate the viability of PDF cells in an avulsed tooth as a storage medium with and without L-dopa.

MATERIALS AND METHODS

Twenty freshly extracted non-impacted human teeth with closed apices and no caries beyond the CEJ were obtained for this study. The average age of the patient was 21 yrs. Teeth extracted from patients with moderate to severe periodontal disease or with extensive caries were excluded. Extractions were performed as atraumatically as possible by an oral surgeon. After extractions, the teeth were held with forceps by the coronal region, and the coronal 3mm of PDL was scraped with a curette to remove cells that might have been damaged. All the teeth were left to dry for 30 minutes. This is to simulate the clinical situation where the teeth may be left dry for atleast 30 minutes (extra-oral time period) before any definitive action being taken in the dental orifice.

The teeth were then randomly divided into 1 of the 4 experimental storage solution groups, namely group 1, deionized water; group 2, deionized water with L-dopa (1mg/ul); group 3, HBSS; and group 4,HBSS with L-dopa(1mg/ul), with 5 samples per group. The positive and negative control groups consisted of 5 samples each, with negative control being group 1 and positive control being group 3.

Group	No of viable PDL cells
Group 1(Deionised water)	29.8
Group 2(Deionised water with L-dopa)	69.6
Group 3(HBSS)	441
Group 4(HBSS with L-dopa)	508.4

[Table/Fig-1]: Total number of viable PDL cells for all the groups

The teeth were then stored in the different test solutions for 45 minutes. The collagenase assay was then performed as follows: From each group, the teeth were then incubated for 30 minutes in a test tube containing 2.5ml solution of 0.2mg/ml collagenase. After incubation 50µl of fetal bovine serum was added to the test tube and centrifuged for 4 minutes at 1000 rpm. The supernatant fluid was removed with a sterile micropipette and the cells were labeled with 0.4% trypan blue for determination of cell liability. The number of viable PDL cells was counted under a light microscope using a hemocytometer at 20X magnification.

RESULTS

The mean number of viable PDL cells was highest in group 4, followed by group 3 and group 2. Group 1 shows the least number of viable PDL cells [Table/Fig-1].

DISCUSSION

The main objective of treating the avulsed teeth is directed towards preservation and vitality of periodontal ligament elements, which consists mostly of fibroblasts [14]. Only teeth from healthy individuals without periodontal disease were used in this study since fibroblast function can be affected by age, trauma and inflammation. In the current study, a 30-min dry time was chosen, where damage has been done in many PDL cells, yet some cells remain for assessment. Also, 30 min represents a typical clinical scenario during which the avulsed tooth may remain dry before being placed into a storage medium.

There are two methods for evaluating the efficacy of different storage media in preserving the viability of dental fibroblast cells. In one method the fibroblasts are removed from the root surface and added to a storage medium for culturing. The main benefit of this method is that only fewer teeth are needed for the study, since large number of fibroblasts can be derived from those teeth. The biggest drawback of this method is that it doesn't replicate clinical scenario because cells in the proliferative phase are placed directly in the medium which is not rich in nutrients [15]. In the second method, the extracted tooth is placed directly in the storage medium. After a pre-determined time the PDL cells are isolated using enzymes and the tooth is taken out of the medium to evaluate cell viability [16]. This method is identical to primary cell culture. This method closely replicated the actual clinical situation. In the current study, to preserve maximum cell viability, the root surface was treated with collagenase. This resulted in rapid cell retrieval and maintained maximum cellular integrity [17].

HBSS as a storage medium has an excellent ability in maintaining the vitality of the periodontal cells and also prevent the morphological distortion of the cells. HBSS can preserve the viability of 70% of fibroblasts for 96 h. In this study, HBSS, deionized water and L-dopa were tested for the effect of maintaining the viability of PDL cells. From the results of the study [Table/Fig-1], it can be inferred that the number of viable PDL cells is maximum in group IV followed by group III and is the least in group I. Addition of L-dopa to deionised water (group II) results in the increase of viable PDL cells when compared to group I. This may be due to the mitogenic effect of L-dopa on PDL cells [13]. Addition of L-dopa to HBSS (group IV) results in a marked increase in the viability of PDL cells when compared with HBSS (group III). This may be due to the synergistic effects of HBSS which has all nutrients, pH and osmolarity for cellular growth and L-dopa which contributed in the mitogenic activity of these viable PDL cells.

CONCLUSION

Within the limitations of this study, it can be concluded that the synergistic effect of L-dopa and HBSS paves way for its usage as an effective storage medium in increasing the viability of PDL cells for an avulsed tooth. Further clinical research is essential before its wider application in clinical dentistry.

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