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A COMPARISON OF THE EFFECTIVENESS OF PACKAGED COCONUT WATER AND UHT MILK AS A STORAGE MEDIA FOR AVULSION TOOTH

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ABSTRACT : Avulsed tooth ought to be replanted quickly, because the vitality of periodontal ligament predetermines the success rate of the procedure, and the tooth storage media is an important factor for maintaining its vitality. In addition, there is a possibility for utilizing packaged coconut water and UHT milk as storage media, based on its isotonic mineral content and the commercial availability. To compare the effectiveness of packaged coconut water and UHT milk as a storage medium for avulsed tooth. Twenty-seventh freshly extracted Caviacobaya teeth were divided into 1 treatment and 2 control groups, where each was further divided into 3 subgroups, based on the immersion time of 30, 60, and 240 minutes. In addition, the periodontal ligament fibroblasts were cultured with versen trypsin solution and labeled using 0.4% trypan blue, and subsequently counted with a hemocytometer, on a light microscope. Furthermore, the data were analyzed with SPSS version 24, using ANOVA analysis. The amount of fibroblast obtained in UHT milk group was significantly higher than the value obtained for mineral and also packaged coconut water groups in 30, 60, or 240 minutes ($p \leq 0.05$). It was established that UHT milk is more effective than bottled coconut water as a storage medium of avulsed teeth, at a value of up to 240 minutes.

Key words : Tooth avulsion, fibroblast, storage media, coconut water, UHT milk.

INTRODUCTION

Clinical survey has been implicated in dental trauma as a common problem in adolescences and adults (Arrizza and Ramadhan, 2010). In addition, there are different types of teeth luxation, which include concussion, subluxation, lateral luxation, intrusion, extrusion and avulsion (Berman *et al*, 2007).

The degree of periodontal tissue damage in avulsion and the viability maintenance of its ligament cells on the surface of the vital root ultimately determine the success rate of the avulsed teeth replantation process. Moreover, it has been established that the period where it was outside the socket, as well as the condition of storage influences the vitality of the periodontal ligament cells (Kuswandari, 2004). Prior studies reported the occurrence of an intact condition within 2 hours of exposure (Riyanti, 2010).

Furthermore, an incidence of severe medical wounds or the absence of a clinic at reach are capable of causing late replantation, thus demanding the use of a storage media, in an attempt to maintain the vitality of periodontal ligament cells before replantation. This practice is meant to preserve the avulsed tooth, especially on instances where it is not possible to conduct the process immediately (Jacobsen and Andreasen, 2003), *e.g.*, through the journey to the dental clinic (Sigalas *et al*, 2004). The ideal media is expected to preserve the lifespan, adherence and capacity of celclonogenity and also the accessibility (Gomes *et al*, 2009).

Furthermore, the following have been adopted as extra oral storage media, including milk, isotonic solution, water, saliva, culture media and *Hanks Balanced Salt Solution* (HBSS), which is specifically recommended by the American Endodontic Association as the best. This is because of its biocompability towards the periodontal ligament cells, and also due to the fact that it contains every electrolyte and glucose needed to maintain the normal metabolism and that it possesses a stable pH (McIntyre *et al*, 2009; Ram and Cobenca, 2004). Ideally, beside the previously mentioned abilities, it is known to be easily obtained and also economical.

Several experimental studies have shown other alternatives to HBSS, including saline solution, saliva, and coconut water (Ram and Cobenca, 2004; Endah, 2005), which specifically has a balanced sterile pH, which eliminates the tendency to destroy red blood cells,

subsequently enhancing its acceptability by the body. In addition, coconut water is known to contain electrolytes that are similar to the intracellular liquids of the body, and has also been reported to have high osmolarity values because of the sugar, glucose and fructose content (Gopikrishna, 2008). A study demonstrated the propensity of adopting coconut water as a fibroblast cell storage media, where the percentage alive reached 102/97% (Khairina, 2015). This provides further proof on the capacity of applying, it as an alternative media for avulsed tooth storage. In recent times, advancements of packaging in aseptic containments are readily available in the market, enhancing the ease of procurement when needed for emergency situations. However, UHT milk is comparably easier to obtain in some areas.

The American Association of Endodontics reported milk as the solution to maintain the vitality of the periodontal ligament cells of an avulsed tooth (An Nisaa, 2015). This treatment, as an extra oral storage media confers the advantage of appropriate osmolarity, the provision of few metabolite substances and sufficient glucose necessary for the physiology of the ligament cells, as well as the ease of accessibility. Furthermore, it has frequently been recommended for use within 6 hours (Gomes, 2009). Nowadays, numerous kinds have been identified in the market, including those processed using Ultra High Technology (UHT), which is mostly produced and very easy to obtain. This is particularly an advantage in contrast with HBSS solution, with availability that is limited to the confines of of the laboratorium environments, although it is the best media. In addition, previous studies have showed the possibility of utilizing UHT milk as storage for fibroblast cell, with percentage living composition that reached 93.4% (Rahardian, 2013).

Based on the similarity in composition and function recorded between packed coconut water and UHT milk with HBSS, they have collectively been easier to obtain. This study was, therefore, conducted to provide information on substitutes to HBSS solution, including packed coconut water and UHT milk, present in the market. This was conducted to estimate the effectivity of use as an avulsed tooth storage media, through periodontal ligament cell vitality test, performed on the teeth of rodents. Furthermore, the consideration that both media were easy to obtain and economic in value consequently served as an advantage in relation to the extra oral time, which does not exceed 30 minutes. Also, the possibility of ease of access to the society, including children, thus, reduces the risk of replantation failure. This study was then differentiated into two samples, which include packed coconut water and UHT milk.

MATERIALS AND METHODS

Laboratory experimental studies with post-test only control group design was conducted in the biochemistry laboratory of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. In addition, the preparation of the rodent as the test animal, and its periodontal ligament cell was conducted in the Pharmacian Veterenary Centre PUSVETMA, Surabaya, within October-December 2016. Furthermore, a total of 27 Caviacobaya rodents were collected as study sample, based on the fact that they fulfilled the sample criteria. This was inclusive of males aged 2-3 months, with body weight of 200-300 grams, in a healthy physical condition. Also, they were assessed to be caries-free and devoid of periodontal teeth problems, raised in the same place and condition, and also fed with similar food.

The materials used include needle holder, syringe, scalpel, mini glass lab, incubator, falcon tube, micropippete, pippete, centrifuge, light microscope, hemocytometer, counter, timer, digital camera, packed coconut water, UHT (Ultra High Temperature) milk, mineral water, rodent, ether, povidone iodine, Versen Trypsin (VT) solution, PBS (Phosphate Buffer Saline) solution, Bovine serum solution, 0,4% Trypan Blue solution.

The selection of rodents was based on the sample criteria, and they were allowed to adapt for one week. Furthermore, 27 Caviacobaya teeth were extracted and divided into 3 groups, consisting of 1 experimental and 2 controls, where each was further split into 3 subgroups, based on the length of immersion time, encompassing 30, 60 and 240 minutes. In addition, each sample was placed in a 5 ml sterile folcon tube, and the fibroblast cell on the periodontal ligament was subsequently cultured with Versen trypsin liquid, and labelled using 0.4% trypan blue. Furthermore, the count for vital and non-vital cells was conducted under the light microscope, using the hemocytometer with 20x magnification.

Therefore, the distribution of each group was analyzed using Kolmogorov-Smirnov normality test, while the homogenity was evaluated with the Levene test. Also, the significance of each group was estimated using ANOVA, while Tukey HSD test was used to compare the result between groups.

RESULTS

The result showed the vital fibroblast cell count on the treatment group that was immersed in the UHT milk in all time variations to be higher, when compared with that of coconut and mineral waters.

The normality test on the data from each group was conducted using the Kolmogorov Smirnov test, with the

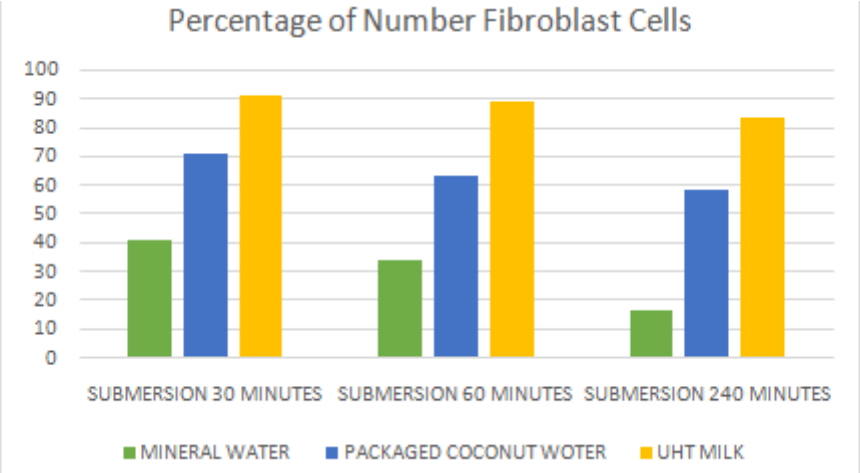

Fig. 1 : Precentage of fibroblast cells submersion in mineral water, packaged coconut water, and UHT milk for 30 minutes, 60 minutes and 240 minutes.

aim of viewing its distribution, based on significance value. In addition, the results showed a normal distribution of vital fibroblast cell percentage in all sample groups with the significant value >0.05.

Furthermore, parametric study using ANNOVA test was performed, in an attempt to analyze the difference between the control and treatment group. The result obtained showed a significant value at 0,000, which was less than 0.05, indicating a substantial variation between the percentages of vital fibroblast cells on the sample group.

The Tukey HSD test conducted on the mineral water group showed a significance value of packed coconut water and UHT milk in 30, 60 and 240 minutes observation that was less than 0.05.

DISCUSSION

An ideal storage media must be able to maintain the vitality of periodontal ligament cell, in order to ensure mitosis and subsequently replace the fibroblast present on the damaged forms (Badarudin *et al*, 2013). Furthermore, other characteristics include its ability to be clonogenic, the antioxidant properties, and the absence of or the presence of low level microbial contamination, with pH and osmolarity that is similar to the body, high availability, easy accessibility and low economy cost (Wilson, 2013). Therefore, the maintenance of metabolism and physiology of normal cells requires that the media possesses an optimal osmolarity, sufficient nutritient and ideal pH. This occurs between the range of 280-300 mOsm/l (isotonic) and a pH range of 7.2 on fibroblast cells (Balogh and Fehrebanch, 2006), while the osmolarity is attained as a standard isotonic solution, alongside the balanced movement of substances in and out of the cell. In addition, placing it in a hypertonic solution leads to the movement of intra-cell liquid to the extracellular space, thus causing depletion (Waterhouse and Farmery, 2015).

Several studies have been conducted on storage media for avulsed teeth and the most ideal is on the tissue culture media, where the best and proven form is the Hank's Balanced Salt Solution (HBSS) (Krasner, 2004). This is not readily available in numerous places where traumatic incidents occur often, including schools, houses, camping places and sports arenas, where people are physically active and its use is unknown to the society. A study placed Eagles media as the second best, based on its ability to preserve the fibroblast cell up to 72 hours (Khademi and Abbas, 2008). However, its media is not usually packed to be marketed for individual use, and the cost is relatively high, in contrast with others (Krasner, 2004).

Prior studies conducted on milk and egg-white media towards the healing of periodontal ligament showed result after the tooth immersion for 3 to 6 hours (Khademi and Abbas, 2008). The best form of milk for this purpose is the freshly pasteurized variety, stored in a cold temperature, making it difficult to obtain. Therefore, an alternative form (UHT milk) was assessed in this study, based on the ease of acquisition and its reasonable price (Badarudin *et al*, 2013).

Milk is known to maintain the life, mitogenity and cell clonogenic capacity of the periodontal ligament in storage for up to 24 hours, at the temperature of 4°C, with osmolarity of 350 mOsm/l (Suradi, 2001). Furthermore, the pH evaluation on UHT milk provided a value of 6.7, although the optimal requirement for fibroblast cells varied between 7.2-7.4 and a few tough cells possibly tolerate at 6.6-7.8 (Aparna *et al*, 2012). Thus, the immersion of UHT milk demonstrated a higher

result in contrast with the data obtained for packed coconut and also mineral waters. In addition, another study demonstrated the capacity for coconut water to confer similar effectivity with HBSS in maintaining the vitality of BHK-21 fibroblast cell, during the immersion time between 15 minutes and 2 hours (Thomas, 2008). This process shows a balanced level of substance movement in and out, thus conferring protection against damage (Krasner, 2004).

Furthermore, the assessment of packed coconut water shows a satisfactory percentage (58.5733%) of vital fibroblast cells, through the immersion time of 240 minutes, although at 30 minutes (71.3467%), which was quite high. However, the vitality maintenance capacity of was not as high in previous records, probably due to the presence of other ingredients, and preservatives. Moreover, the pH assessment of the current study showed a value of 4.1, which proved significant level of acidity, which is capable of destroying the process of cell metabolism. Despite the recorded physiologic osmolarity and the presence of carbohydrate that that is capable of providing energy for periodontal ligament cells, the acidity of packed coconut water has been identified to immensely reduce their defense capacity (de Borba *et al*, 2013). This is the possible reason for the comparably higher vital cell percentage in the UHT milk media.

The vitality percentage in the control group, which is the immersion in mineral water, was observed to not be as high, in comparison with others. However, viable fibroblast cells were identified in the 240 minutes immersion, which is possibly due to the maintenance of humidity, and the presence of several minerals. Meanwhile, water is easily accessible in places where events frequently occur, although it possesses the capacity of cause necosis of the periodontal ligament and vast radicular resorption, due to its hipotonic characteristic, acid pH, chlorine composition and the incident of microorganism contamination (de Borba *et al*, 2013).

CONCLUSION

The ability of UHT milk and packed coconut water to maintain the viability of fibroblast cells and their ready availability in stores and supermarkets at an affordable economic value enhances the possibility of adoption as an alternative media for avulsed tooth storage. However, the weakness of this study was identified in the parameter of osmolarity for UHT milk and packed water, which was not conducted, thus, further investigation ought to be performed. Furthermore, it was established that the isotonic concept of UHT milk and packed coconut water promoted the balance and equivalence between the concentrations of the extracellular environment and the condition in the cytoplasm.

In conclusion, the UHT milk is reported to be more effective in comparison with the packed coconut water as an alternative media for avulsed tooth storage within the period of 30 to 240 minutes. Furthermore, it is hoped that the ideal media confers healing after the replantation process.

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