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ORIGINAL ARTICLE

Use of soymilk as a storage medium for avulsed teeth

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Abstract

Objective. Tooth avulsion is one of the most severe forms of dental trauma. In these cases, immediate reimplantation is ideal; however, it almost never happens. The purpose of this study was to evaluate the viability of cells stored in soymilk and compare with other several storage media. ***Materials and methods.*** The media tested were: long-shelf-life coconut water, long-shelf-life whole milk, long-shelf-life soymilk, Gatorade, egg white, and Hank's Balanced Salt Solution. Cells cultured in DMEM and distilled water served as positive and negative controls, respectively. Plates containing confluent 3T3 fibroblast were soaked in the various media for 2, 12 and 24 h. After incubation at 37°C, viability of the cells was determined using the MTS assay. Data were analyzed by using one-way ANOVA and complemented by Tukey test with a significance level of 5%. ***Results.*** Statistical analysis showed that DMEM, whole milk, HBSS and soymilk were the most effective media for maintaining cell viability at all tested times ($p < 0.05$), followed by coconut water, egg white and Gatorade. The least amount of viable cells was observed in the distilled water group. ***Conclusions.*** The present study shows that the efficacy of soymilk in maintaining the viability of 3T3 fibroblasts is similar to that of HBSS and milk. Therefore, it can be concluded that soymilk could be a suitable alternative storage medium for avulsed teeth.

Key Words: *Cell viability, fibroblasts, soymilk, tooth avulsion*

Introduction

Tooth avulsion is one of the main issues in dental traumatology because of its status as a severe dental injury. Due to the complexity of this injury, neurovascular supply is severely compromised in most cases, causing loss of pulpar vitality [1]. The main etiological factors are trauma after fighting and sports, as well as falls and bumps against hard objects or the floor [2]. The reported incidence of tooth avulsion is ~ 1–16% of all traumatic injuries to the permanent dentition [3,4].

During the extra-alveolar period, adherent cells on the root are subject to contamination and dehydration and might become necrotic [2,4,5]. Thus, it is recommended to replant the tooth as quickly as possible, preventing irreversible damage to the periodontal ligament (PL) [2–5]. Although immediate reimplantation is the treatment of choice [6], clinical experience has shown that most avulsed teeth are replanted only after an extended extra-alveolar time. Due to the unavailability of immediate reimplantation, it becomes necessary to choose a suitable storage medium for maintaining the viability of PL cells, avoiding further damage to the tooth.

Several experiments have been carried out in an attempt to find the ideal storage medium [1,5,7,8]. This ideal medium should be able to preserve cell vitality, adherence and clonogenic capacity [9] and should be readily available at the site of accident or be easily accessible [2,4,10]. Good medium examples are milk, Hank's Balanced Salt Solution (HBSS), Save-A-Tooth System and ViaSpan. Alternative media include egg white, isotonic solutions, propolis and green tea [1,5–10].

Soy is claimed to exhibit health benefits to consumers [11]. Soymilk, the water extract of soybean, is a rich source of high-quality protein and amino acid. It contains no cholesterol or lactose and very small amounts of saturated fatty acid [12]. In addition, it is also considered to have a potential role in the prevention of chronic diseases such as atherosclerosis,

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cancer, osteoporosis and menopausal disorders [13] and has been identified as an excellent culture media for cell growth and biochemical activities [14].

The purpose of this research was to evaluate *in vitro* the possibility of soymilk as a substitute for storage media for avulsed teeth. In addition, we estimated the fibroblast cell viability in different storage media during variant storage durations.

Materials and methods

Balb C 3T3 cells (American Tissue Type Collection; ATCC, Manassas, VA; passage 31) were cultured in Dulbecco modified Eagle medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Sigma Chemical Co, St. Louis, MO), 100 μg/mL of streptomycin, 100 mg/mL of penicillin at 37°C in a humidified incubator under ambient pressure air atmosphere containing 5% CO_2. Confluent cells were detached with 0.25% trypsin and 0.05% ethylenediaminetetraacetic acid (EDTA) for 5 min and aliquots of separated cells were sub-cultured. The MTS assay was used for cell viability evaluation, as described below.

Cell viability in the following storage media was evaluated: long-shelf-life coconut water (Taeq, Fortaleza, CE, Brazil), long-shelf-life whole milk (Parmalat, São Paulo, SP, Brazil), long-shelf-life soymilk (Ades, Belo Horizonte, MG, Brazil), isotonic (Gatorade®, São Paulo, SP, Brazil), egg white and Hank's Balanced Solution (Gibco BRL, Grand Island, NY). Cells cultured in DMEM and distilled water served as positive and negative controls, respectively. The pH of all solutions was measured with a digital pH meter (Hanna Instruments, Ann Arbor, MI) at room temperature. The osmolality was tested with an automatic cryoscopic osmometer (Osmomat 030, Gonotec, Berlim, Germany). These results are expressed in Table I.

The 3T3 cells were seeded into 96-well plates (Corning, Elmira, NY) at a concentration of 1×10^4 per well and incubated at 37°C for 24 h. Then the storage media were added to each experimental well.

Table I. pH and osmolality of different storage media.

Material	Osmolality (mOsmol/kg)	pH
Coconut water	378	4.7
Distilled water	3	8.0
Whole milk	288	6.7
Soymilk	267	7.3
Gatorate	407	3.0
Egg white	298	9.4
DMEN	312	8.1
HBSS	284	7.7

Following incubation for 2, 12 and 24 h, the cells were checked for the effect of each storage medium on their viability by using the MTS test (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI). In the MTS test, the cells in each well of the 96-well plate were incubated with 100 μl of culture medium and 20 μl of MTS reagent mixture for 4 h and MTS absorbencies were measured at 490 nm using a spectrophotometer (Urit 660, Urit, China) according to the manufacturer's instructions. Percentage cell viability was calculated by dividing the absorbance values of experimental wells by those of control wells and multiplying by 100. The data were analyzed by one-way analysis of variance (ANOVA) and follow-up comparison between the groups was made using Tukey multiple comparison test (at 95% confidence interval level, $\alpha = 0.05$). Data were analyzed using the statistical software SPSS® (SPSS, Inc., Chicago, IL).

Results

Statistical analysis showed significant differences between the tested storage media. Distilled water (negative control) showed the worst performance ($p < 0.05$) followed by egg white, coconut water and Gatorade®, which showed no statistical differences among them, in all tested time intervals. Among the tested media, DMEM, HBSS, soymilk and whole milk had the best results at all time intervals ($p < 0.05$), showing no statistical differences among them. Figure 1 shows the results of cell viability of 3T3 fibroblasts, obtained from the MTS assay.

Discussion

Avulsion injury, one of the most severe forms of dental trauma, is characterized by complete displacement of the tooth from its alveolar socket [7]. Extra-oral time and storage medium are the critical factors responsible for prognosis of avulsed tooth [1]. The longer the exposure of avulsed tooth to dry storage, the worse the prognosis for explantation. Therefore, many *in vitro* and *in vivo* studies aimed to evaluate the biological properties of various storage media to preserve the avulsed tooth [1,5,7–10]. *In vitro* studies are accepted as a routine method for the establishment of the cytotoxicity of storage media. Using *in vitro* methods, experimental factors can be well controlled. Some advantages of *in vitro* assays include simplicity, reproducibility and low cost. The present study used a 3T3 fibroblast cell line for easy preparation and handling. In addition, fibroblasts are routinely used for testing cytotoxic effects [15,16].

In the present study, cytotoxic effects of soymilk were compared with different storage media. Soymilk is an aqueous solution rich in protein, amino acids, vitamins and minerals essential for cell nutrition and

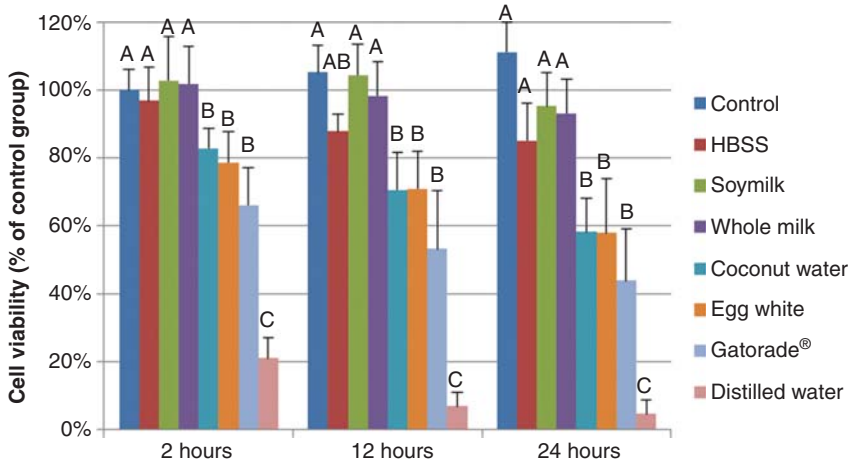

Figure 1. 3T3 fibroblasts cell viability, obtained from the MTS assay. Different letters mean statistically significant differences between tested groups in the same time point ($p < 0.05$).

maintenance. It also has a physiological pH and is gradually becoming present in population supply [11,12]. Several studies have shown the excellent biological properties of soymilk, indicating the same as the ideal replacement for people with milk lactose intolerance [17,18]. The great advantage of this milk is that, unlike cow's milk, is low in saturated fat and has no cholesterol. The authors have reported the excellent potential of soymilk as a storage medium and for proliferation of several cell types [13,14,18]. The results of this study showed similar cytotoxicity values between soymilk, whole milk, and HBSS solutions. These last solutions are described in the literature as the gold standard for the maintenance of traumatized teeth [15,16,19]. One reason for this result can be the excellent biological properties of soymilk. In this study, soymilk showed a physiologically compatible pH and osmolality. Both physiological osmolality and pH are important factors in preserving the viability of PDL cells. It has been reported that the growth of cells happen mainly at an osmolality of 230–400 mOsmol/kg and a pH of 6.6–7.8.

Similar cytotoxic results were found in the Moazami et al. [20] study, which showed that soymilk can be an excellent storage medium for avulsed teeth. In the previous study, cytotoxicity was observed using Trypan blue assay. This assay is less sensitive than the MTS assay because it does not characterize the true metabolic condition of cells not stained with Trypan blue. This means that the cell membrane is intact, although the cell might not have any kind of metabolic activity [21]. Thus, the present study further supports the results presented above, showing cytotoxicity values for soymilk similar to the widespread storage media.

Egg white, coconut water and Gatorade® showed lower cell viability values than soymilk. Several authors suggest that these products would be good alternatives in PL cells maintenance [7,19,22]; however, other authors have shown similar results to those observed in the present study, checking intermediate values for these solutions [16,23,24]. These results can be justified due to low pH, osmolarity, the storage time of these media [16,20,22] and the temperature at which they were used [16,19]. Therefore, in accordance with the present study, these solutions should only be used in the absence of whole milk, HBSS or soymilk.

According to the results of this study, it can be concluded that soymilk has been able to maintain cell viability at similar levels as solutions considered 'gold-standard' for avulsed teeth such as whole milk and HBSS, indicating its use in cases of dental trauma.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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