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## Evaluation of the bio-physiological efficacy of tooth storage media based on normal physiological parameters

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### Abstract

An easily available tooth storage medium with correct bio-physiological properties is required to preserve the vitality of periodontal ligament (PDL) cells after avulsion. The aim of this study was to evaluate the bio-physiological efficacy of Hank's Balanced Salt Solution (HBSS), milk, and egg white as three storage media by comparing the osmolality, Na<sup>+</sup> and K<sup>+</sup> concentrations, and pH of these candidate media with physiological values. The result of this study indicated that the osmolality of egg white is better than that of HBSS and

milk for supporting optimal growth rate. The Na<sup>+</sup> concentration is very low in milk and egg white and the K<sup>+</sup> concentration is very high. Milk is slightly acidic and egg white is slightly alkaline. HBSS is used as a buffer in cell culture media and helps maintain optimum physiological properties for cell growth, but it is not available everywhere. As an easily available tooth storage medium, egg white has better physiological properties than milk for the preservation of PDL cells.

### INTRODUCTION

Dental avulsion is one of the most serious traumatic tooth injuries and is characterized by complete displacement of the tooth from its alveolar socket, causing severe damage to the supporting tissues and vascular and nerve structures (Gopikrishna et al., 2008). The ideal treatment is immediate re-implantation, but this is not always possible. The primary goal in cases of tooth avulsion is to preserve the vitality of the periodontal ligament (PDL) cells attached to the root surface until re-implantation can be performed (Gopikrishna et al., 2008). Consequently, an easily available tooth storage medium is required to preserve the vitality of PDL cells and prevent ankylosis and replacement resorption after avulsion (Gopikrishna et al., 2008).

Recent research has led to the development of storage media that closely mimic the conditions of the original socket environment, including adequate osmolality (cell pressure), pH, and nutritional metabolites (Marino et al., 2000; Melo et al., 2003). The Na<sup>+</sup> and K<sup>+</sup> concentrations are related to

osmolality and are important bio-physiological parameters of tooth storage media by helping maintain intracellular and extracellular activities (Hall et al., 2006).

The American Association of Endodontics has recommended milk as a tooth storage medium to preserve the viability of PDL cells (Krasner et al., 1992). Commercially available Hank's Balanced Salt Solution (HBSS) has been used as a tooth storage medium (Krasner et al., 1992), and Khademi et al. suggested that egg white is comparable to milk as a storage medium for avulsed teeth (Khademi et al., 2008).

The purpose of this study was to evaluate the bio-physiological efficacy of HBSS, egg white, and milk as storage media and their utility for reproducing normal physiological parameters.

### MATERIALS AND METHODS

HBSS, milk, and egg white were used as tooth storage media. The egg white was separated from egg yolk for experimental purpose. Normal physiological conditions were

obtained from the Textbook of Medical Physiology (Guyton et al., 2006). The measurements for each sample were done six times for every experiment.

### Osmolality measurements

The osmolality levels were measured using an osmometer (Osmo Station, OM-6060, ARKRAY Inc., Kyoto, Japan). The osmometer consists of an evaporation control cover A and an evaporation control cover B. A sample (tooth storage media) was placed in cup and set in the sample rack, the rack was placed in the instrument, then evaporation control cover B was closed. Evaporation control cover A was closed after the sample rack was set into the instrument.

### Na<sup>+</sup> and K<sup>+</sup> measurements

Na<sup>+</sup> and K<sup>+</sup> meters (LAQUAtwin's, HORIBA, Ltd. Kyoto, Japan) were used to measure the Na<sup>+</sup> and K<sup>+</sup> concentrations. Each was calibrated by immersing the tip in a standard solution and pressing the calibration button. Once the calibration was complete, the standard solution was washed off with distilled water, the meter was dried with a clean, soft tissue, and then the same volume of experimental storage media measured.

### pH measurements

pH indicator strips (MColorpHast, Merck KGaA, Darmstadt, Germany) were used to determine the alkalinity or acidity of the HBSS, milk, and egg white storage media.

## RESULTS

### The osmolality of different storage media compared to normal physiological osmolality. (Table-1)

Normal cellular physiological osmolality is in the range 230–320 mOsm/kg, and the osmolality of HBSS, milk, and egg white are within this range.

### The Na<sup>+</sup> and K<sup>+</sup> concentrations of different storage media compared to the normal physiological concentrations. (Table-2)

The concentration of Na<sup>+</sup> in HBSS is 160 mM, similar to the physiological concentration of 150 mM, whereas it is very low in milk (17 mM) and low in egg white (78 mM).

The HBSS and physiological concentrations of K<sup>+</sup> are both 5 mM, whereas the concentration in milk and egg white is high (38 mM and 30 mM, respectively).

### pH of the storage media compared to normal physiologi-

**Table-1** The osmolality of different media compared to the normal physiological range.

Medium	Osmolality (mOsm/kg)
Normal physiological range	230–320
HBSS	275–280
Milk	285–290
Egg White	295–300

**Table-2** Na<sup>+</sup> and K<sup>+</sup> concentration of different media compared to the normal physiological limit.

Medium	Na <sup>+</sup> (mM)	K <sup>+</sup> (mM)
Normal physiological concentration	150	5
HBSS	160	5
Milk	17	38
Egg White	78	30

**Table-3** pH of different media compared to the normal physiological range.

Medium	pH
Normal physiological range	7.35–7.45
HBSS	7.30–7.40
Milk	6.60–6.70
Egg White	7.15–7.65

### cal pH. (Table-3)

Normal physiological pH is in the range 7.35–7.45. The pH of HBSS is within this range (7.30–7.45). However, milk is slightly acidic (pH 6.60–6.70) and egg white is slightly alkaline (pH 7.15–7.65).

## DISCUSSION

Tooth storage medium must have specific physiological properties to support periodontal cell survival. The commercial tooth storage medium, HBSS, is used as a buffer with cell culture media and to help maintain physiological properties optimal for cell growth (Hanks et al., 1949). However, HBSS is not available everywhere.

The osmolality of a solution is determined by the concentration of the solute molecules and different types of ions. Osmolality increases as the concentration of water decreases (Remington et al., 2000) and decreases as the concentration of water increases (Blomlöf et al., 1981). The permeability of water is very high in cellular systems and thus cells act as “osmometers”, increasing their volume in a hypotonic medium and reducing their volume in a hypertonic environment

due to the osmotic movement of water. Both the increase and decrease in the concentration of water are critical for cells. Even short exposure to an external osmolality above 450 mOsm/kg results in cell death (Andreasen et al., 1981). The storage of cells in hypotonic solutions may cause irreversible damage to the cell membrane, depending on the length of exposure of the cells (Lindskog et al., 1981).

Waymouth measured the osmolality of different culture media and studied the growth of transformed mouse cells in these media. He suggested that these cells can grow in an osmolality range of 230–400 mOsm/kg and that the growth rate was optimal between 290–330 mOsm/kg (Waymouth, 1970).

Blomlöf et al. evaluated the osmolality of HBSS and milk and reported values of 275 mOsm/kg for HBSS and 250–270 mOsm/kg for milk (Blomlöf et al., 1981), broadly similar to our findings.

The osmolality of HBSS, milk, and egg white were 275–280 mOsm/kg, 250–270 mOsm/kg, and 295–300 mOsm/kg, respectively; all are within the normal physiological range, although that of egg white may be slightly high for supporting an optimal growth rate.

Earlier studies with other storage media found negative effects of hypotonic media or low osmolality. Tap water causes rapid cell lysis in the PDL because it is hypotonic (Ashkenazi et al., 2000; Ashkenazi et al., 1999; Blomlöf et al., 1983; Marino et al., 2000; Pearson et al., 2003). Saliva has much lower osmolality (60–70 mOsm/kg) and therefore boosts the harmful effects of bacterial contaminants (Blomlöf et al., 1981; Blomlöf et al., 1983; Blomlöf et al., 1980; Marino et al., 2000; Sonoda et al., 2008). Storage in saliva causes swelling of PDL cells (Blomlöf et al., 1980), indicating the importance of osmolality for maintaining PDL cell viability.

$\text{Na}^+/\text{K}^+$ -ATPase helps maintain the resting potential, affects transport, and regulates cell volume, and failure of the  $\text{Na}^+/\text{K}^+$  pump can result in swelling of the cell. A cell's osmolality is the sum of the concentrations of the various ionic species, proteins, and other organic compounds inside the cell. When this concentration is higher than the osmolality outside the cell, water flows into the cell through osmosis, potentially resulting in the cell swelling up and lysing. The  $\text{Na}^+/\text{K}^+$  pump helps maintain the right concentrations of ions (Hall et al., 2006).

$\text{Na}^+$  and  $\text{K}^+$  are very important for extracellular and intra-

cellular activity. The  $\text{Na}^+$  and  $\text{K}^+$  concentrations are well balanced in HBSS because it is an established cell and tissue culture medium. Milk and egg white have lower  $\text{Na}^+$  and higher  $\text{K}^+$  concentrations.

Although the  $\text{Na}^+$  and  $\text{K}^+$  concentrations of milk and egg white are outside the physiological ranges, their osmolalities are within range, indicating that other ions are responsible for bringing the osmolality within range. The satisfactory osmolality may make milk and egg white acceptable for short term tooth storage despite their low  $\text{Na}^+$  and high  $\text{K}^+$  concentrations.

The pH of HBSS was 7.30–7.40 and within the normal physiological range, whereas milk is slightly acidic (pH 6.60–6.70) and egg white is slightly alkaline (pH 7.15–7.65). Optimal cell growth occurs between pH 7.2–7.4, but cells can survive for a long period at pH 6.6–7.8 (Paul et al., 1970; Marino et al., 2000; Melo et al., 2003); therefore, short term storage in slightly acidic milk and slightly alkaline egg white tooth storage media may have little effect.

## CONCLUSION

The bio-physiological properties of egg white, makes it a suitable short term tooth storage media for avulsed teeth.

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