



AN OVERVIEW OF STORAGE MEDIA USED FOR STORAGE OF AVULSED TOOTH

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ABSTRACT

The choice of storage medium for preserving traumatically avulsed teeth is important for the success of future replantation. storage media is a significant factors that can affect the long-term prognosis of replanted teeth. Numerous studies have examined various media in an attempt to determine the ideal material for storage of the avulsed tooth. Various storage media can be used for avulsed tooth such as Hank's balanced salt solution (HBSS), milk, saliva, Visapan, saline, etc., HBSS, a pH preserving fluid, is best used for traumatized tooth. It is biocompatible with the tooth periodontal cells and keep the cells viable for 24 h due to its ideal pH and osmolality. Prolonged extraoral storage of an avulsed tooth before replantation will lead to total necrosis of periodontal ligament and will affect the outcome of treatment. In this article we will discuss about the various storage media used for storage of avulsed tooth and about their properties.

KEYWORDS : Avulsed Tooth, Storage Media, HBSS, Milk, Propolis

INTRODUCTION:

The most important phase during the management of an avulsed tooth is the handling of the tooth from the time of injury till the tooth is replanted back into its socket. A periodontal ligament with viable cells capable of proliferating over denuded areas on the root and of re-establishing a normal attachment apparatus of the tooth to the bone is of utmost importance.

Yet another important factor is the storage of the tooth during this time. An **Ideal storage medium** would be one that is capable of preserving the viability, mitogenicity and clonogenic capacity of the damaged PDL in order to facilitate repopulation of the denuded root surface thereby preventing further root resorption. The storage medium should have a physiological osmolality, pH and should be maintained at an appropriate temperature to allow optimal cell growth or survival. Finally, the ideal storage media should be readily available for use in emergency situations. The type of storage medium used following avulsion affects the prognosis of tooth replantation.⁽¹⁾

TAP WATER is the most easily available medium at any accident site is tap water but is the least favorable due to its hypotonicity. It is not compatible with PDL cells and causes rapid cell lysis. **Blomlof (1981)⁽²⁾** reported that water is damaging to PDL cells and is not a good storage medium at any time. It has a pH of 7.4 to 7.79 and an osmolality of 30 mOsm/kg. As it is hypotonic, it causes lysis of the cells⁽¹⁾. Although some studies have suggested that it may be accepted as a storage medium for very brief periods when there are no alternatives, it should be remembered that it is the least desirable storage medium available and its use leads to ankylosis and replacement resorption.⁽³⁾

SALIVA is useful in spasmolytics, antiseptics and astringents. Human saliva (buccal vestibule) is used as a storage medium due to its availability, but it has unfavorable characteristics, such as non-physiological pH and osmolality, high microbial contamination and hypotonicity. Studies have shown that saliva is inefficient to maintain cell viability, but it is preferable to use it rather than keep the tooth in dry conditions because the effects of resorption become more severe with time. There is evidence that artificial saliva is also inappropriate to receive avulsed teeth.

Sousa et al. (2010)⁽⁴⁾ found disorganization of collagen fibers, altering therefore the PDL quality. Saliva officinalis

extract can be used as a storing medium for a short period of time, as it can damage the cells of the periodontal ligament if used for longer than an hour.⁽¹⁾

NORMALSALINE provides osmolality of 280mOsm/kg and despite being compatible to the cells of the periodontal ligament, it lacks essential nutrients such as magnesium, calcium and glucose. They are necessary to the normal metabolic needs of the cells of the periodontal ligament. **Blomlof⁽²⁾, Courts et al.⁽⁵⁾** have stated that saline solution was harmful to the cells of PDL in avulsed teeth if it is used for longer than two hours. Saline may be easily encountered in a variety of places; with this wide availability, it is more convenient to use them for short periods than let the tooth dry out.⁽³⁾ **Moreira Neto et al.⁽⁶⁾** evaluated the viability of cultured cells and found 55% of living cells after 4 hour storage and evaluated the PDL cells viability when maintained in this medium for 45 min and resulted in only 20% mortality. Using a similar methodology, **Martin and Pileggi⁽⁷⁾** found that saline had a worse behavior compared with HBSS and milk. Evaluating cell viability by the Tripan blue method, **Ozan et al.⁽⁸⁾** concluded that water was worse than saline. Consequently, saline is not an adequate medium, but it may be employed for short periods of time.

Cvek et al.⁽⁹⁾ found that a tooth stored in normal saline for 30 min showed less resorption than a tooth stored dry for between 15 and 40 min. This contrasts with a human study where Trope reported little difference in the development of ankylosis between teeth stored in normal saline and teeth kept dry.

MILK-

The American Association of Endodontics indicates milk as a solution for preserving avulsed teeth, to keep the viability of the human cellular periodontal ligament⁽¹⁰⁾. Milk, as storage medium is the most practical transport medium for the short-term storage of avulsed teeth because of its ready availability in almost all situations⁽¹¹⁾.

Milk is significantly better than others solutions for its physiological properties, including pH and osmolality compatible to those of the cells from the periodontal ligament; the easy way of obtaining it and for being free of the favorable bacteria⁽³⁾ but it is important that it is used in the first 20 minutes after avulsion. Results of milk probably occur due to the presence of nutritional substances such as amino acids, carbohydrates and vitamins⁽¹⁰⁾.

Being a gland secretion, milk contains epithelial growth factor (EGF), which stimulates the proliferation and regeneration of epithelial cell rests of Malassez and activates the alveolar bone resorption⁽¹²⁾. This will ultimately contribute to isolate the bone tissue from the tooth and decrease the likely hood of ankylosis. In spite of offering no conditions for the restoration of cell morphology nor does cell differentiation or mitosis, milk prevents cell death⁽¹²⁾. The pasteurization of milk is responsible for diminishing the number of bacteria and bacteriostatic substances, also for the inactive presence of enzymes, which could be potentially harmful to the fibroblasts of the periodontal ligament.

Milk has a pH of 6.5 to 7.2 and osmolality of 270 mOsm/kg, which is similar to extracellular fluid. Milk can potentially maintain PDL cell viability for up to 2 h. The vitality; clonogenic and mitogenic capacity of PDL cells in milk are similar to the values for HBSS. The colonogenic capacity can be maintained at the same level for an additional 45 min. by keeping the milk chilled with an ice pack or in the refrigerator.⁽¹¹⁾ Cooler temperatures reduce cell swelling, increase cell viability and improve PDL cells recovery.⁽³⁾

Blomlof⁽²⁾ showed that 50% of the cells were viable after 12 h storage in milk and no cells were viable after 3 h storage in saliva. Brief storage in saliva followed by storage in milk was better than storage in saliva only. Milk maintains the viability of PDL cells at a clinically significant level for up to 1 h of extra-oral time Milk can usually be obtained on short notice, but even 10 min of desiccation can affect the outcome of replantation. The drawbacks are that milk needs to be fresh and kept refrigerated, it does not replace depleted cell metabolites, and it does not facilitate cell mitosis. However, milk has a compatible osmolality with PDL cells and is readily available to the public in most situations. It prevents cell death, but does not restore the cells normal morphology and ability to differentiate and undergo mitosis. It is reported that sour milk is harmful.

Gamsen et al.⁽¹³⁾ showed that milk is able to maintain the osmotic pressure for periodontal ligament cells but it does not have the ability to reconstitute depleted cell metabolites and restore viability. **Harkacz et al.**⁽¹⁴⁾ showed that milk with lower fat content may be more appropriate at maintaining cell viability than milk with higher fat content.

Marino et al.⁽¹⁵⁾ reported that there was no significant difference between regular pasteurized milk and long shelf-life ultra high temperature (UTH) pasteurized whole milk at any time period. Regular pasteurized milk has a short shelf life and requires refrigeration, which makes it less readily available at the trauma site. Thus a long shelf-life milk having identical composition, pH, and osmolality to regular milk with a storage capability of 6 months without the need for refrigeration has gained more acceptance.

Pearson et al.⁽¹⁶⁾ evaluated the efficacy of several milk substitutes, including reconstituted powdered milk, evaporated milk and two baby formulas (Similac and Enfamil) compared with regular pasteurized whole milk and reported that Enfamil, which is supplied in powder form, does not require special storage and has a shelf life of 18 months, is a more effective storage medium for avulsed teeth than the pasteurized whole milk for at least 4 hour. Some authors reported superior results with the green tea, Eurocollins, Coconut water, Propolis, Egg and Ricetral media in relation to milk. In spite of this, milk has been widely recommended to dentists and general population for keeping avulsed teeth to be replanted, being the second or third best transportation media for avulsed teeth include (in order of preference), after Viaspan and or Hank's Balanced Salt Solution, according to the International Association of

Dental Traumatology, and the American academy of Pediatric Dentistry due to its beneficial effects and characteristics, and its ease of access at the moment of trauma.

EGG WHITE - has a pH of 8. Difference between egg white and HBSS at storage times of 1, 2, 4, 8 and 12 hour has been established and egg white was more suitable than water and milk.⁽¹¹⁾ Egg albumin is a good choice because of its high protein content, vitamins, water, lack of microbial contamination and easy accessibility.

Khademi et al.⁽¹⁰⁸⁾ have compared milk and egg white as solutions for storing avulsed teeth, and the results have shown that teeth stored in egg white for 6 to 10 hours had a better incidence of repair and lower surface resorption than those stored in milk for the same amount of time and in the dextran controls.

Sousa et al.⁽⁴⁾ found no difference between milk, egg white and artificial saliva. Some experiments indicate that egg white is a very good medium to maintain cell viability, but others show a small loss of efficacy overtime, possibly due to egg's high pH and also because the PDL cells could target the several egg proteins as strange bodies. Further studies are required to confirm these adverse effects, as there are wide variations in egg composition and quality.

HANKS BALANCED SALT SOLUTION-It was introduced by John H Hanks in 1975 as a solution for preservation of tissue culture. Among all the storage medium HBSS is considered as the gold standard and is often used as a comparison reference medium to deduce the clinical efficacy of other media. The American Academy of Endodontics has accepted HBSS as an acceptable medium for avulsed teeth because of its capability to maintain vitality and proliferative capacity of PDL for an extended period of time (up to 48 hours)⁽¹¹⁾.

It contains sodium chloride, D-glucose, potassium chloride, sodium bicarbonate, potassium phosphate (monobasic), calcium chloride and magnesium sulphate anhydrous. It can preserve cells and tissues for 24 h and both the pH (7.4) and the osmolality (280 mOsmol kg-1) are ideal.⁽¹¹⁾ It can maintain the viability of PDL cells for several hours with a success rate of 90% reported when degenerated PDL cells were stored in HBSS for less than 30 min.

An added advantage of the system is, inner suspension netting and removable basket, which permits gentle washing and removal of the tooth without crushing of the periodontal ligament by the operator. The vitality, clonogenic and mitogenic capacity of PDL cells using this medium are excellent. HBSS is the only medium that can replenish metabolites in depleted PDL cells. Commercially available form of Hanks' Balanced Salt Solution (HBSS) is marketed as Save-A-Tooth (Save-A-Tooth; Phoenix Lazerus Inc., Pottstown, PA, USA), to maintain periodontal ligament cell viability.⁽¹¹⁾

According to **Ashkenazi et al.**⁽¹⁸⁾ HBSS was the most effective medium for preserving viability, mitogenicity, and clonogenic capacities of PDL cells for up to 24 h at 4°C when compared with other solutions.

Table No. 1:Composition Of Hanks Balanced Salt Solution

COMPONENT	CONCENTRATION (g/L)
Inorganic Salts	
Potassium Chloride	0.4
Potassium Phosphate Monobasic (anhydrous)	0.06

Sodium Chloride	8.0
Sodium Phosphate Dibasic.7 H ₂ O	0.09
Others	
D-Glucose	1.0
PhenolRed	0.011
Sodium Bicarbonate	0.35

The method of preparation of the reagent is as follows:

1. Measure the quantity of Hanks' solution needed into a container that will contain 8X the volume dispensed.
2. Measure 7X the volume dispensed of distilled or high purity water into the container and mix.
3. Reagent is ready for use. The pH of the diluted Hanks' Balanced Salt Solution is 6.7 plus or minus 0.2. Sodium Bicarbonate can be added to the solution (0.35 g/L). The pH of the solution can be adjusted with 1N HCl or 1N NaOH.
4. When reagent bottle is empty, dispose of it responsibly by recycling.

A special kit available in some countries, which has been designed for the public to use for the emergency management of avulsed teeth. This kit contains a small basket in which the avulsed tooth is suspended and submerged in HBSS⁽¹¹⁾.

Successful reimplantation of avulsed teeth can be achieved if the teeth are stored in an optimal storage environment (OSE) such as the Save-A-Tooth system. Save-A-Tooth is a 6-part system that was designed to prevent damage to the PDL cells of avulsed teeth. Each part of the system addresses a potentially damaging situation that can occur between the time of avulsion and reimplantation.

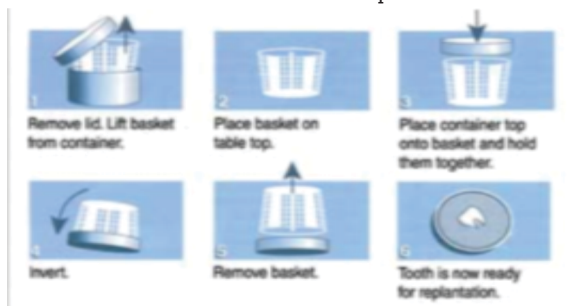


Fig. No.1 Save-A-Tooth System

Among these situations

- (1) Removal of debris from the root surface.
- (2) Spillage of storage medium.
- (3) Removal of the avulsed tooth from the storage container.

The 6 parts of the Save -A-Tooth system and their functions are the following:

1. Shatterproof container to prevent leakage of HBSS.
2. Tightly fitting top to prevent spillage during transport.
3. Removable basket that permits atraumatic removal of teeth.
4. Suspension net with divider fins that permits atraumatic washing of debris from the tooth surface and prevents bumping of the avulsed tooth against the walls of the container or against another tooth.
5. Sponge on underside of lid that allows for atraumatic removal of the avulsed tooth.
6. HBSS, which provides optimum osmotic pressure and replacement of PDL cell metabolites.

There is space on the Save-A-Tooth container to write the patient's name. One suggested use of this system is in ambulances. If the accident victim has severe injuries and several teeth avulsed, emergency personnel are often reluctant to take the time to find the avulsed teeth and

reimplant them. If the Save-A-Tooth system is utilized, a friend or family member can gather the teeth and place them in the container and bring it to the hospital, where the teeth can be reimplanted when the patient is stabilized.

It has been reported that when an avulsed tooth that has been kept dry for 15–60 min is replanted after being soaked in HBSS for 30 min, less root resorption occurred. It is recommended that avulsed teeth should be soaked in HBSS for 30 min before replantation even if they have been stored in a suitable physiologic medium because of the replenishing ability of HBSS.⁽¹¹⁾

Hwang et al.⁽¹⁹⁾ reported 94% cell viability after storage of cultured human PDL cells for 24 h in this medium, which is considered an excellent result.

GATORADE - was originally formulated as a drink for sports people to replenish electrolytes during training and sports events. This substance has pH 3 and osmolality between 280 and 360 mOsm, which may cause damage to the cells due to the low pH and hypertonicity. Sigalas et al.⁽²⁰⁾ evaluated Gatorade efficacy in maintaining the viability of PDL cell culture by the Tripzan blue exclusion method and the results showed that at 37°C it was toxic to the cells; but this may be an alternate medium to HBSS and milk when used ice cold and for a short time. Gatorade preserves more viable cells than tap water but fewer than all other media. It can only serve as a short time storage medium if other more acceptable media are not available⁽⁹⁾.

CONTACT LENS SOLUTION– It is a sterile, buffered and isotonic saline. These properties led to its investigation for use as a storage medium. These solutions are fatty acid monoester composites with an antimicrobial cationic component.⁽¹⁾

Sigalas et al.⁽²⁰⁾ studied the efficacy of different contact lens solutions in maintaining the viability of cultured PDL cells and the results showed that the preservatives in the formula damaged the cells. Nonetheless, in the absence of another storage medium, they may be used instead of water or saline for short periods of time. The presence of preservatives in its formula was harmful to the cells of the PDL and they are not commonly recommended. Gatorade® and contact lens solutions do not present quite positive results, probably because their characteristics are not very favorable to the cells; however, they may replace water and saliva if required.⁽¹⁾

EURO-COLLINS – COLLINS - solution as a storage medium for avulsed teeth is due to the fact that it is a hypothermal medium developed for preserving organs to be transplanted. Its characteristics include a pH of 7.4, electrolytes and phosphate buffer to control cell acidosis, a high concentration of potassium to decrease the intracellular cation loss, a low concentration of sodium and chlorine, a osmolality of 420 mOsm/kg, maintained by the addition of glucose, which avoids cell edema.⁽²¹⁾

EAGLE'S MEDIUM- contains amino acids, vitamins and bicarbonates. This solution must be refrigerated and is not readily available except in research laboratories. Although EM is an excellent storage medium. It is not practical to recommend it for general use because of these limiting factors. One study has shown that human PDL cells proliferated in EM's storage medium. Soaking teeth in EM for up to 60 min after they had been stored dry for 5–14 days resulted in better PDL healing and reduces the level of inflammatory resorption.⁽³⁾

MEM (Minimum Essential Medium) cell culture medium

contains L-glutamin, penicillin, streptomycin, nistatin, bovine serum and nutrients for cell growth and proliferation and several authors have reported its efficacy in preserving the viability of PDL cells and have indicated it as a storage medium before tooth replantation.⁽¹²⁾ Teeth preserved in the culture for 5 to 7 days showed significantly reduced levels of inflammatory resorption. **Pohl et al.**⁽²²⁾ investigated the suitability of specially composed cell culture media for storage of extracted teeth for up to 48 hours. Auto radiographic investigations revealed that the proliferative activity of periodontal ligament (PDL) cells of teeth stored in cell culture medium for up to 48 hours increased with storage time.⁽³⁾ Several authors have reported its efficacy in preserving the viability of PDL cells and have indicated it as a storage medium before tooth replantation.

COCONUT WATER - Coconut (*Cocosnucifera* L) is popularly known as the "Tree of life" and the coconut water is a natural sterile product biologically produced and hermetically sealed inside the coconut. Coconut water is biologically pure and sterile, with a rich presence of amino acids, proteins, vitamins, and minerals. This natural isotonic fluid is available in its natural form directly from the coconut or in long shelf life packages and plastic bottles, mainly in tropical countries.⁽³⁾ It aids in replenishing the fluids, electrolytes and sugars lost from the body during heavy physical work, has been suggested as a promising storage medium for avulsed teeth. It was observed that coconut water was superior to HBSS, milk or propolis in maintaining the viability of PDL cells.

Coconut water was found to have a pH of 4.1 which is deleterious to cell metabolism.⁽¹¹⁾ The high osmolality of coconut water helps in maintaining viability of periodontal ligament cells.⁽¹⁰⁾

Gopikrishna et al.⁽²³⁾ observed that coconut water was superior to HBSS, milk or propolis in maintaining the viability of PDL cells. **Thomas et al.** found that 15 to 120 min storage in coconut water is as efficient as storage in HBSS. On the other hand, **Pearson et al.**⁽¹⁶⁾ and **Thomas et al.**⁽²⁴⁾ observed that inflammatory resorption was more frequent when the tooth was maintained in coconut water compared with milk. **Moreira-Neto et al.**⁽⁶⁾ observed that coconut water has an acidic pH of 4.1, which is deleterious to cell metabolism and concluded that the capacity of the storage media in maintaining human fibroblast cell viability in a decreasing order was milk > saline and coconut water with sodium bicarbonate > coconut water > mineral water. It is therefore difficult to consider coconut water as an adequate storage medium for avulsed teeth because of the difficulty of neutralizing the coconut water to obtain a pH of 7.0 under clinical condition. Standardized studies with similar methods are required to avoid diverging results and eliminate doubts over its use, as this is a medium with easy access and good biological characteristics that could be promising for its indication.

EMDOGAIN - Emdogain (Biora, Malmo, Sweden) is a commercial Enamel Matrix Derivative (EMD) extracted from developing embryonic enamel of porcine origin and contains several matrix proteins. It can influence the migration, attachment, proliferative capacity and biosynthetic activity of PDL cells.⁽¹¹⁾ It has also been used in anti-resorptive-regenerative therapy along with topical glucocorticoids and systemic doxycycline. Use of emdogain has been shown to increase the incidence of healed PDL when this gel was applied to root surface of the avulsed tooth and /or inserted directly into alveolar socket before implantation. It appeared to aid in preventing or retarding root resorption and ankylosis. Thus, it is a recommended therapeutic agent for the management of avulsed

permanent teeth.

PROPOLIS - Propolis is a natural product used by bees in the construction and maintenance of their hives. It has anti-inflammatory, antibacterial, anti-oxidant, anti-fungal and has tissue regenerative actions. It can inhibit the late stages of osteoclast maturation so it may be useful as an intracanal medicament to reduce resorption of traumatized teeth. A recent study showed that propolis could be used for avulsed teeth and that a 6-hour period of storage was more appropriate than 60 min of storage.⁽¹¹⁾

Propolis has a complex chemical composition and is mainly composed of resin (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds (5%). The application of propolis as a storage media has been studied by many authors.⁽²⁵⁾

Ahangari et al.⁽²⁶⁾ compared the number of viable cells at 1 hour and 3 hours after storage in 10% propolis, 50% propolis, milk, egg white and HBSS. A significantly more number of viable PDL cells were found in propolis as compared to the other experimental groups. No significant difference was found between the performance of 10% and 50% concentration of Propolis.

Therefore, propolis can be a more beneficial storage medium for avulsed teeth. Propolis has synergistic effect with Dubelcco's modified eagle's medium (DMEM) for the maintenance of cell viability and preservative potential. In combination, 10% propolis + DMEM was found to be better than 20% propolis + DMEM, which shows that 20% concentration of propolis might have some cytostatic/cytotoxic effect on the cell physiology.

Propolis is a promising medium for the maintenance of PDL cell viability. The major disadvantage of propolis is that it is not readily available to the public and therefore of little value as a storage medium for avulsed teeth. Further research is required to improve its applicability and widen its indication of use.

GREEN TEA EXTRACT - has strong antioxidant, anti-inflammatory and antibacterial properties, good ability to extend the survival of grafts and capacity to protect the periodontal tissues against the resorption of alveolar bone as a result of infectious processes caused by pathogenic microorganisms.

Hwang et al.⁽¹⁹⁾ and **Jung et al.**⁽²⁷⁾ in the search for a medium capable of minimizing the infections after tooth replantation, maintaining PDL cell viability and reducing root resorption and ankylosis, reported enthusiastic results with green tea, with the maintenance of 90% of cell viability for up to 24 h, similar to the HBSS control. **Jung et al.**⁽²⁷⁾ also observed that the higher the extract concentration the more efficient the medium. In view of this, the use of green tea extract and its compounds may be an alternative for the conservation of avulsed teeth and its beneficial effect is enhanced by higher extract concentrations.

In view of their study, the use of green tea extract and its compounds may be a suitable, alternative storage medium for the conservation of avulsed teeth, but further in vivo research is necessary before its use can be recommended.

MORUS RUBRA (red mulberry) - *Morusrubra* (red mulberry) is a natural product available in different climates, which contains flavonoids, alkaloids and polysaccharides, all of them are very important for cell preservation.⁽¹²⁾

Ozan et al.⁽⁸⁾ reported when teeth were stored in red

mulberry for up to 12 h, its capacity to maintain the viability of PDL cells was better than that of HBSS; however, if a longer storage time is required, it is advisable to employ higher concentrations of the fruit juice. There are very few studies evaluating the use of red mulberry juice as a

transport medium for avulsed teeth and its biological properties have not been established yet. Further research is necessary before its use can be recommended.

Table No.2 Main Characteristics, Efficacy And Accessibility Of Each Storage Medium For Avulsed Teeth.⁽¹²⁾

Storage medium	Characteristics	Efficacy	Accessibility
Hank's Balanced Salt Solution (HBSS)	Physiological pH, osmolality and nutrients	Excellent	--
Euro-Collins®	Physiological pH and hypothermal capacity	Excellent	--
Minimum Essential Medium (MEM)	Nutrients, antimicrobial property and growth factors	Excellent	--
Saline	Physiological pH and osmolality	Poor	+
Water	Microbial contamination, hypotonic, nonphysiological pH and osmolality	Very poor	++
Saliva	Microbial contamination, hypotonic, nonphysiological pH and osmolality	Very poor	++
Milk	Small bacterial contents, isotonic, physiological pH, osmolality, growth factors and nutrients	Excellent	+
Propolis	Anti-inflammatory, antibacterial and antioxidant properties	Excellent	-
Green Tea	Anti-inflammatory, antibacterial and antioxidant properties	Excellent	-
Red mulberry	Not established	Good	-
Coconut water	Sterile, natural product and contains nutrients	Good	+
Gatorade	Low pH and hypertonic	Poor	+
Contact lens solution	Antimicrobial property, preservatives	Poor	+

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