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Storage Media for Dental Avulsion: A Literature Review

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ABSTRACT

Tooth avulsion is a critical dental emergency where immediate and appropriate management is essential for the successful reimplantation of the tooth. The choice of storage medium during the extra-alveolar period significantly impacts the viability of periodontal ligament (PDL) cells, crucial for the long-term retention of the replanted tooth. This review examines various storage media proposed for avulsed teeth, assessing their pH, osmolality, and overall efficacy in maintaining PDL cell viability. A comprehensive literature search was conducted across databases including PubMed, Scopus, and Google Scholar, identifying media such as Hank's Balanced Salt Solution (HBSS), milk, saline, propolis, coconut water, and more. Ideal storage media should exhibit antimicrobial properties, maintain physiological osmolality and pH, support PDL cell viability, and be readily available. HBSS is highlighted as the gold standard, with its ability to preserve PDL cells for up to 48 hours. Other effective media like milk, soy milk, and oral rehydration solutions are discussed for their accessibility and performance. Emerging media such as propolis and epigallocatechin-3-gallate show promise but require further research. This review emphasizes the importance of public awareness and accessibility to effective storage media to improve the prognosis of avulsed teeth.

Keywords: Storage Media, Dental Avulsion, PDL viability.

INTRODUCTION

There are many solutions that have been proposed and/or tested as storage media for avulsed teeth. The following were identified and reviewed as a result of the literature search: Hank's balanced salt solution (HBSS), Eagle's medium (EM), milk, ViaSpan, Gatorade, propolis, tooth rescue box (Dentosafe), conditioned medium, contact lens solution, tap water, egg white, saliva, normal saline, ORS, and coconut water.[1] An appropriate storage medium should maintain or improve the viability of the cells during the extra-alveolar period by preventing cell desiccation. The maintenance of periodontal ligament (PDL)

cellular viability is essential for long-term retention of replanted teeth. The American Association of Endodontists (AAE) recommend HBSS as a storage medium of choice for treatment of avulsed teeth due its ability to provide long-term preservation of PDL cell viability. It is well known that all cellular reactions are dependent upon the pH of the environment as alterations may influence biological processes. In addition to this, the osmolality of a medium affects the water absorption of the cells. Both the rise and the reduction of osmolality is critical for cell viability. The suitable pH and osmolality for cell growth are around 6.6-7.8 and 230-400 mosmol/kg, respectively. Media that have pH and osmolality values in the ideal range are milk, HBSS, DMEM, aloe vera (10%, 20%, 50%), and ORS (25%, 50%, 100%). Media that have pH out of range are egg white and aloe vera (100%). Media that have osmolality values out of suitable range are tap water and saliva. Media that have both pH and osmolality out of range are Gatorade green tea, and green tea extract. Thus, pH and osmolality were in the ideal range for 9 of the 16 tested storage media.[2]

IDEAL REQUIREMENTS OF A STORAGE MEDIUM FOR AVULSED TEETH:

Ideal storage medium should:

- Have antimicrobial characteristics.
- Be capable of preserving the feasibility of cellular PDL. Be able to maintain the viability of periodontal fibers for an acceptable period of time.
- Favor proliferative capacity of cells and should have the same osmolality as that of body fluids.
- Not react with body fluids.
- Not produce any antigen antibody reactions.
- Reduce the risk of post-reimplantation root resorption or ankylosis.
- Have a good shelf life.
- Effective in different climate and under different conditions.
- Wash off extraneous materials and toxic waste products.
- Aid in the reconstitution of depleted cellular metabolites.[3]

The storage media are listed according to their origin and composition.

1. Tap water:
   Use of tap water to store avulsed teeth is not recommended as it is not compatible with PDL cells because of its hypotonic osmolarity which causes cell lysis, and is reported to causes replacement resorption in avulsed teeth when they are place in it. It is considered the least desirable storage medium.

2. Saliva:
   Saliva is a readily available, natural storage medium. Despite this fact, due to the presence of substances like enzymes and bacteria and its non-physiologic osmolarity, which can exert harmful effects on the PDL cells, this can at best be used as an interim storage medium (no longer than 30 minutes). If stored for more than 60 minutes, a significant decrease in functional capacity of PDL cells occurs.[4]

3. Milk and Variants:
   The use of milk as storage media has gained much popularity due to its ease of availability, cost effectiveness and physiologic osmolarity. Studies in literature have investigated various forms of milk including whole milk, skimmed milk, low fat content milk, baby formula and long shelf-life milk. With a pH of 6.5-7.2 and the presence of essential nutrients important for maintaining the viability of PDL cells, milk can be considered as acceptable storage medium in most situations, increasing the life of the PDL cells on the root surface. Milk can maintain the viability of PDL cells from 2hrs to 6 hours. Milk that has been refrigerated or which has a lower fat content demonstrated better results as storage

media in various studies. Its clonogenic and mitogenic capacity for PDL cells is considered equivalent to Hank's Balanced Salt Solution [HBSS]. Some of the drawbacks of using milk as storage medium are the presence of antigens that could interfere with the process of reattachment of PDL cells when the tooth is re-implanted. Also the milk needs to be fresh and refrigerated. Sour milk should not be used as it is considered to be harmful. Milk also has no proven role in assisting cell mitosis in PDL cells.

4. Soy Milk:
   Soy milk, the water extract of soybean, contains no cholesterol or lactose and very small amounts of saturated fatty acid. It is considered an excellent culture medium forcell growth and biochemical activities. Recent studies have shown that soy milk in contact with periodontal ligament cells promoted good cell viability, comparable to HBSS and milk and hence is recommended as a storage medium.

5. Saline:
   Although isotonic saline has been used in various studies for its effect as a storage medium, it is unable to maintain the metabolism of PDL cells. It has comparable osmolality to the PDL cells, but lacks nutrients. It is considered acceptable to place an avulsed tooth in isotonic saline rather than storing it dry although in another study, no significant difference was found in the development of ankylosis between teeth kept dry or placed in normal saline. It is acceptable to place avulsed teeth in this storage medium for not more than 10 minutes.[5]

6. Oral Rehydration Solutions:
   Ricetral is a commercially available oral rehydration formulation, consisting of essential nutrients like glucose and vital salts which help in maintaining cell metabolism. They are marketed in sealed sterile pouches and easily available over the counter in addition to being economical. It does not promote cell mitosis and regenerative capacity of the PDL fibroblasts. Its ability to maintain PDL cell viability was demonstrated to be equal to HBSS in a study, both retaining PDL vitality better than milk.

7. Hank’s Balanced Salt Solution:
   HBSS is a salt-solution which is pH balanced and contains essential metabolites needed for viability of PDL cells. It is considered a gold standard for storage media used in transport of avulsed teeth and it is used to compare the efficacy of other storage media. Its ability to maintain the vitality, clonogenic and mitogenic capacity of PDL cells for up to 48 hours has been proven. It is considered superior to many other media in this regard and can be used to store the avulsed tooth for at least 24 hours. It has also shown to replenish metabolites which have been depleted from PDL cells. Hence, it has been recommended to place avulsed teeth in HBSS for 30 minutes before reimplantation in order to replenish the PDL cells, even if the avulsed teeth have been stored in an appropriate storage medium. HBSS is not readily available to public as its use is more in research laboratories, although in some countries it is available in emergency kits [Save-A-Tooth, PA, USA]. This kit comprises a small basket to hold the avulsed tooth while it is submerged in HBSS, until the tooth is re-implanted. Lack of availability and cost are considered the major draw-backs for this storage medium.[6]

8. Propolis:
   Propolis is a resin obtained from conifer trees. This sticky material is used by bees for constructing and maintaining their hives. It is a non-toxic biological material with anti-inflammatory, anti-

bacterial, antioxidant, anti-fungal and tissue regenerative properties. The main ingredient of propolis are flavonoids. Its chemical composition can be highly variable due to the different variety of plants the honey bees can visit while collecting this material. Recent studies have shown 10% propolis to be an effective storage media when compared with milk, HBSS, tap water and DMEM. Due to the fact that propolis is not readily available, its utility as a storage media is diminished when compared to other ready available materials.

9. Coconut Water:
   Coconut water is a biological liquid which is pure, sterile and rich in nutrients like amino acids, proteins, vitamins and minerals. Readily available in tropical countries, it is an isotonic solution which can be obtained fresh directly from coconuts or commercially in packages and bottles. When compared with other media like HBSS, propolis and milk, it was found that coconut water was the most effective in maintaining viability of PDL cells. The same study found the combination of coconut water with sodium bicarbonate to be more effective but some studies have also demonstrated contradicting results. Since the pH of coconut water is 4.1, it has harmful effects on cell metabolism until sufficiently neutralized. Further research in this regard needs to be undertaken before coconut water can be used effectively as a storage medium.

10. Egg white:
    This medium has not been found to be significantly different than HBSS in some studies in terms of cell viability and demonstrates greater PDL healing when compared with milk. It can be used to store avulsed teeth for up to 10 hours. One study found no difference between milk, egg white and artificial saliva. Although it is easily available, its major setback is impracticality of use.[7]

11. Salvia officinalis:
    Salvia officinalis is a perennial, evergreen shrub with blue to purplish flowers. It is a member of the family Lamiaceae and is native to the Mediterranean region; it has a long history of medicinal and culinary use. The extract from this plant has been used as spasmolytic, antiseptic and astringent. This extract has been proposed as a storage medium for avulsed teeth because of the anti-oxidants effects caused due to the presence of its phenolic components like rosmarinic acid, carnosic acid, salvianolic acid and derivatives. These antioxidants help to prevent root resorption by inhibiting the effect of osteoclastic cells. Studies have shown that Salvia extract at 2.5% helps maintain PDL cells viability over longer periods of time (3, 6, 12 or 24 hours) when compared with HBSS, phosphate buffered saline and tap water. It has also demonstrated anti-microbial and anti-inflammatory properties. Thus salviaofficinalis can be recommended as suitable storage media for avulsed teeth.

12. Morus rubra:
    Morusrubra [red mulberry] belongs to the Moraceae family and active components include flavonoids, alkaloids an polysaccharides. Morusrubra juice at 4% concentration was found to be superior to HBSS for maintaining PDL cell viability for upto 12 hours.

13. Epigallocatechin-3-gallate [EGCG]:
    Epigallocatechin-3-gallate [EGCG] is a major polyphenol of green tea, is known to have various biological effects such as anti-oxidative, anti-carcinogenic, anti-mutagenic, anti-inflammatory, anti-microbial, and anti-viral activities. Recently, research has been conducted

to determine its role as an adequate storage medium. Greater viability of PDL cells of guinea pig and Beagle dog has been maintained when placed in EGCG. A study on extracted human teeth showed that EGCG can be used adequately as a storage medium, with a higher potential than HBSS and milk to promote favorable reimplantation, with less risk of root resorption and ankylosis.

14. Caesin Phosphopeptide:
    Casein phosphopeptides [CPP] are derived from casein, which account for 80% of the total protein in bovine milk. They can form soluble organophosphate salts and may function as carriers for different minerals, especially calcium. Their role in preventing demineralization and aiding in remineralization has been demonstrated. In a study which investigated the use of different concentration of commercial CPP-amorphous calcium phosphate as storage media by observing morphological changes in fibroblast cells, it was found that cell apoptosis did not occur when very dilute concentrations of CPP-ACP was used as storage medium and further research was deemed necessary to determine the ideal concentration for preserving PDL cell viability.

15. Conditioned medium:
    This medium is derived from supernatant of human gingival fibroblasts grown in culture. Since this medium contains stimulatory growth factors produced by the gingival fibroblasts, it is believed to have a beneficial effect on the PDL cells and their proliferation. This medium is also not readily available for general use, which limits its practicality.

16. Culture media:
    Culture media can include Eagle's medium, alpha- Minimum Essential Media and alpha-MEM-S(with addition of foetal calf serum and antibiotics). Eagle's medium contains many nutrients like amino acids, vitamins and bicarbonates considered essential for maintaining the viability and proliferative capacity of PDL cells for longer time periods when compared with other storage media (48-53 hrs). The addition of growth factors like platelet derived growth factor, insulin like growth factor, epidermal growth factor and many others, helps to enhance the clonogenic and mitogenic capacity of PDL cells for longer periods of time. Recently, research has demonstrated that the use of special cell culture medium (SCCM), which has been formulated especially to be used as a storage medium for avulsed teeth, is better at maintaining PDL cell viability than HBSS for time period of longer than 24 hours. Another variation of Eagle's Modified Essential Medium (EMEM) is Dubelco's modified Eagle's Medium (DMEM) which contains a greater concentration of vitamins and amino acids as well as glucose compared to the EMEM. Despite their excellent properties, due to their need for refrigeration and lack of availability, all these culture media are not considered as practical for use as storage medium for avulsed teeth.[8]

17. Custodial:
    This medium is the registered trademark of Dr. Franz. It contains a histidine-tryptophan ketoglutarate solution containing high flow properties and low potassium content. It is basically an organ transport medium, also used for perfusing and flushing donar organs prior to their removal. In a study it was reported that custodial was comparable to HBSS in terms of cell viability. Similar to other organ storage medium, it is not available to public which limits its practicality as a storage medium for avulsed teeth.

18. Via Span:
    This is a medium which was formulated for use in transplant procedures. It is used for cold storage of organs when they are removed from a donor. It is clear to light yellow in color, sterile and non-pyrogenic, with a pH of 7.4. It has shown to maintain the viability of PDL cells effectively, while maintaining cell morphology. Drawbacks of using Via Span include the need for refrigeration, high cost and inaccessibility.[9]

19. Dentosafe:
    Dentosafe (Miradent, Germany) is the commercial name of a tooth rescue box containing Special Cell Culture Medium (SCCM) which is a combination of amino acids, vitamins and glucose. In the USA it is marketed as EMT Tooth Saver (Phoenix, USA). It has demonstrated the maintenance of vitality of PDL cells for 48 hours at room temperature. If unopened, this medium has a shelf-life of 3 years. A study by Pohl et al showed that avulsed teeth placed in Dentosafe solution showed functional healing and recommended that Dentosafe should be included in all first aid kits, especially in locations prone to tooth avulsion injuries like schools, sports ground and facilities, public pools as well as emergency units like hospitals and ambulances. The use of this system is self-explanatory and simple to understand for lay persons. Although effective, this medium is still not easily available in many countries.

20. Contact lens solutions: Since contact lens solutions are basically saline solutions, there use as storage media has been researched in some studies. However, when compared with other storage media, they were deemed to be harmful and thus are not recommended for storing and transporting avulsed teeth.

21. Gatorade:
    This drink was originally developed for athletes in order to replenish electrolytes lost during exercise and physical activity. Compared to tap water, the use of Gatorade as storage medium yielded better results for PDL cells survival. Although it is relatively easily available at sporting events, where avulsion injuries to teeth tend to occur, its osmolarity causes cells destruction and hence it is not recommended for long term storage of avulsed teeth.[10] Table 1 depicts the comparative analysis of various storage media used in dentistry for avulsion.

CONCLUSION

The choice of an appropriate storage medium for avulsed teeth is critical for maintaining PDL cell viability and ensuring the best possible outcome for reimplantation. Hank's Balanced Salt Solution (HBSS) remains the gold standard due to its ability to maintain cell viability for extended periods. However, other media such as milk, soy milk, and oral rehydration solutions are also effective and more readily available. Further research is needed to explore and validate the use of emerging media such as propolis, coconut water, and epigallocatechin-3-gallate. Public awareness and accessibility of effective storage media should be improved to enhance the prognosis of avulsed teeth.

TABLE 1: COMPARATIVE ANALYSIS OF VARIOUS STORAGE MEDIA USED IN DENTISTRY FOR AVULSION.

S.no	Author	Year	Place	Source	Storage medias compared	Parameters taken into consideration	Outcome	Additional findings
1	Hiltz J et al[11]	1991	USA	Human lip fibroblasts	1.Viaspan 2.Milk 3.HBSS	Viability of fibroblasts	Viaspan had better viability of cells comparatively	Time kept in storage media and viability checked after every 2,6,12,24,48,72,144,168 hrs
2	Patel S et al[12]	1994	Maryland, USA	32 orthodontically extracted sound teeth with healthy PDL extracted from age group of 13-25 years	1.Milk 2.Saline	Pdl cell viability	Both milk and saline showed similar number of viable cells close to positive control	Time kept in storage media and viability checked after 2hrs
3	Ashkenazi M et al[13]	1999	Israel	Data not available	1.Culture medium, 2. minimal essential medium 3.milk 4.HBSS 5.ViaSpan 6.conditioned medium (CM	Pdl viability	Milk>HBSS>Culture media>CM>viaspan>MEM HBSS and milk were the most effective media for preserving the viability, mitogenicity and clonogenic capacity after storage for up to 24 h at 4 C.	Time kept in storage media and viability checked after every 2,8,24 HRS
4	Ashkenazi M et al[14]	2000	Israel	Data not available	1.HBSS 2.Culture medium 3.Alpha minimal essential medium 4.ViaSpan	Pdl viability	Culture medium, followed by HBSS and Via Span, was the most effective media for preserving the viability, mitogenicity and clonogenic capacity of PDLF stored for up to 24 h at room temperature. The lowest functional abilities were found in PDLF stored in a-MEM.	Time kept in storage media and viability checked after every 2,8,24 HRS
5	Ashkenazi M et al[15]	2001	Israel	Data not available	1.ViaSpan, 2.Hanks' balanced salt solution 3.Minimal essential medium 4.Alpha MEM supplemented	Pdl viability	the mitogenic and clonogenic effects of GF were observed only after 24 h of storage at room temperature. HBSS and a MEM-S supplemented with GF were the most effective media for preserving the viability, mitogenicity and clonogenic capacity of PDLF stored for 24 h at room temperature. For short periods of storage (2 and 8 h), HBSS and a MEM-S without GF	Time kept in storage media and viability checked after every 2,8,24 HRS

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<tr><td></td><td></td><td></td><td></td><td></td><td>with FCS and antibiotic (a MEM-S).</td><td></td><td>were preferable.</td><td></td></tr>
<tr><td>6</td><td>Babaji P et al[16]</td><td>201</td><td>Kerala</td><td>Fifty orthodontically extracted sound teeth with healthy PDL</td><td>1.HBSS
2.Propolis
3.Aloe vera
4. Pomegranate juice</td><td>Periodontal ligament viability
Osmolarity
pH</td><td>1.Positive control - collagenase dispase 360,000
2.HBSS 262,000
3.Propolis 285,000
4.A. vera 226,000
5.Pomegranate juice 214,000
6.Negative control - bench drying 2000
Propolis showed more viable PDL cells followed by HBSS, A. vera, and PJ.
<table><tr><td>MEDIUM</td><td>OSMOLARITY</td><td>PH</td></tr><tr><td>HBSS</td><td>320</td><td>7.2</td></tr><tr><td>PROPOLIS</td><td>350</td><td>7.4</td></tr><tr><td>ALEO VERA</td><td>280-300</td><td>6.8</td></tr><tr><td>PJ</td><td>280</td><td>6.7</td></tr><tr><td>IDEAL</td><td>230-400</td><td>6.6-7.8</td></tr></table></td><td>-</td></tr>
<tr><td>7</td><td>B. D. M. Souza et al[17]</td><td>2011</td><td>Brazil</td><td>Data not available</td><td>1.Skimmed milk 2. whole milk 3. Natural coconut water 4. HBSS 5.Save-A-Tooth system’s HBSS 6.Industrialized coconut water 7.Tap water</td><td>Pdl viability</td><td>Cell viability maintenance level skimmed milk and whole milk > natural coconut water > HBSS > Save-A-Tooth system’s HBSS > industrialized coconut water > tap water</td><td>Time kept in storage media and viability checked for every After 3, 6,24, 48, 72, 96 and 120 h</td></tr>
<tr><td>8</td><td>N. K. Mahal et al[18]</td><td>2012</td><td>Punjab</td><td>Fifty-five sound human premolars with closed apices that were freshly extracted for orthodontic purposes were obtained</td><td>1.HBSS
2.Egg albumen
3.Propolis</td><td>PDL Viability</td><td>HBSS 86.2%
Egg albumen 88.0 %
Propolis 87.1 %
Positive control 98.25 %
Negative control 0.00%</td><td>Time kept in storage media and viability checked after 45mins</td></tr>
<tr><td>9</td><td>Meenakshi</td><td>2016</td><td>Rajasthan</td><td>45 non-carious</td><td>1.Rice water</td><td>Periodontal</td><td>Maximum percentage of viable cells was</td><td>Time kept in storage</td></tr>
</table>

	Sharma[19]			human mature premolars with apparently normal periodontium and closed apices, extracted for orthodontic therapeutic purposes, were included in this study.	2.Egg white 3.Milk	ligament viability	found in rice water (85.38%) followed by egg white (75.8%) and least in milk (74.07%).	media – 30 mins
10	Adeli F et al[20]	2016	Iran	surgically extracted unerupted third molar teeth, which were planned to be removed due to space management.	1.DMEM as positive Control 2.distilled water as negative control 3.HBSS, 4.Pasteurized long-life whole milk 5.Hypotonic sucrose solution 6. GTE 7.GTE + sucrose	Acidity and osmolarity Periodontal ligament viability	Acidity and osmolality of DMEM were estimated 7.3 and 305 mosmol, tap water: 4.8 and 15 mosmol; HBSS:7.2 and 280 mosmol; whole milk: 4.6 and 286 mosmol; HSS: 5.7 and 213 mosmol GTE: 5.4 and 87 mosmol; GTE + sucrose: 5.2 and 300 mosmol,	Time kept in storage media and viability checked for every 1hr,2hr,4hr,24hr
11	Divya Saini et al[21]	2016	Karnataka	Sixty-nine non-carious premolars that required extraction for orthodontic purposes were selected for the study.	1.Hbss as control 2. coconut milk 3.Probiotic milk	Periodontal ligament viability	The mean number of viable cells in the coconut milk, probiotic milk and HBSS groups were 8.75 3.16 cells /cu mm, 143 21.61 cells /cu mm and 144.79 14.40 cells /cu mm respectively.	Time kept in storage media – 30 mins
12	Awawdeh L[22] Et al	2018	Jordan	Extracted tooth under sterial condition from healthy individual aged between 9-21 years	1.Custodiol 2. immature CW 3.half mature CW 4.mature CW 5.Five serial dilutions of propolis in DMEM	Pdl viability	1.Custodial showed significant higher viability than the negative control 2.Half mature and mature coconut water had better results than immature coconut water 3. 6.25 mg/ml conc of propolis had better viability	Time kept in storage media and viability checked for every 1,2,6,24,48,72,168 hrs

					(12mg/mL, 6.25 mg/mL, 3.13 mg/mL, 1.56 mg/mL, and 0.78 mg/mL) 6.DMEM. 7.Distilled water - negative control 8. DMEM with 10% FBS - positive control			
13	Shah D, et al[23]	2018	Mumbai	A premolar tooth was extracted from a young healthy volunteer for orthodontic purposes after obtaining written informed consent of the guardians	1.DMEM 2.HBSS 3.Egg albumin	Clonogenicity	1.There was a significant reduction in the clonogenic capacity of the PDLF's when exposed to egg albumin as opposed to DMEM or HBSS for all three time points (i.e. 2, 4 and 6 hours) 2. DMEM was highest (~93%) 3. HBSS was 87%, 83.53% and 74.60%	Time kept in storage media and viability checked for every 2,4,6 hrs
14	Lee W et al[24]	2018	USA	Primary HPDLF were purchased from ScienCell Research Laboratories	1.HBSS 2.SAT 3.EMT 4. Deionized water	1.Osmolarity 2.pH 3.Cell viability	1.Osmolarity SAT-295 mOsmol/kg EMT-331 HBSS-279 mOsmol/kg, 2.The pH levels of EMT was the closet to the physiological level, followed by HBSS, SAT, and water. 3.Presto-blue assay at 3, 12 and 24 hours confirmed the superiority of EMT over SAT at all time points	Time kept in storage media and viability checked for every 0.5, 1, 3, 6, 12 and 24 hour
15	Sinpreechanon P et al[25]	2019	Thailand	premolars that had been atraumatically extracted for orthodontic purposes, with informed consent	1.HBSS (Invitrogen); 2.UHT whole cow's milk 3.UHT low-fat cow's milk 4.unsweetened UHT almond milk	1.Analysis of gene expression by quantitative real-time PCR 2. Collagen matrix analysis using Picrosirius Polarization	Cell viability 1.Whole cow milk-87.8% 2.Low fat milk-90.4% 3.Almond milk-74% Cell proliferation 1.almond milk - 165%, 2.HBSS (118%) 3.whole milk(107%) 4.low-fat milk (102%) 5.DMEM control (100%) Collagen matrix	Time kept in storage media – 1hr

						3. Cell viability and cell-proliferation assays	1.almond milk(103%) 2.ow-fat milk (113%), 3.whole-milk (90.8%) 4.HBSS (79.5%)	
16	Shruti Singh et al[26]	2020	Manipal	Maxillary and mandibular premolars indicated for orthodontic extraction, free from dental caries collected from 18-29 years old healthy individual	1.Cornisol 2.HBSS 3.Normal saline 4. dmem as control	Periodontal ligament cells viability	Cornisol showed the highest cell viability at 30 min (58.9%) followed by a gradual reduction in percent cell viability with time yet superior to other groups.	Time kept in storage media and viability checked for every 30 min, 1 h, 24 h, 48 h and 96 h.
17	Nam OH et al[27]	2020	Korea	Human PDL cells were obtained from the Cell Engineering for Origin	1.DMEM (control), 2.HBSS 3.Milk (Seoul Milk co, Gyeonggi-do)	Gene expression Pdl viability	1. The number of DEGs in human PDL cells varied in all groups. However, the groups shared several DEGs with each other. 2.no remarkable differences in the number of viable cells between HBSS and milk for both storage durations	Time kept in storage media and viability checked for every 3 and 6 hrs

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