Review

Storage Media to Preserve Avulsioned Teeth Maintaining the Viability of Periodontal Cells: A Review

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Abstract: Background: Dental avulsion is the most serious dentoalveolar traumas because its management requires immediate reimplantation to maintain the periodontal ligament. The success of the treatment depends largely on the appropriate storage medium that ensures the viability of the periodontal cells. The purpose of this review was to identify the most efficient media to store and transport avulsed permanent teeth, according to the viability of the periodontal ligament cells. Methods: The Scopus, Web of Science and PubMed electronically searched using the keywords "Storage media"; "Avulsed teeth"; and "Periodontal ligament". The inclusion criteria were in vitro studies and articles published in English. The exclusion criteria were literature reviews, studies in primary teeth, studies in animals, and cell culture studies. Results: Hank's saline solution and honey were equally effective, maintaining a cell viability rate of 98.89% and 96.43%, respectively, over a period of 3 hours. Ringer's lactate solution maintained the viability of 906.40 viable cells/mm3 at 1 hour. Propolis maintained the viability of 285,000 cells/mm3 at 45 minutes, and the Neem extract maintained a cell viability rate of 88% at 30 minutes. Conclusions: Hank's saline solution is the most effective storage medium for maintaining the viability of periodontal cells in avulsed teeth.

Keywords: storage media; avulsed teeth; periodontal ligament

1. Introduction

Dental avulsion is one of the most dentoalveolar traumas, and it has a poor prognosis, such that 1 to 16% of all cases result in permanent dentition. (1-4) This trauma is very common among children aged 7-9 years old, and the maxillary central incisors are the most commonly affected teeth; additionally, dental avulsion causes functional, psychological and aesthetic problems. (5-8)

The ideal management of dental avulsion consists of immediate reimplantation within the socket in a time interval of 5 minutes, which improves the repair of the periodontal ligament (LPD) and significantly reduces root resorption and ankylosis. (2-6,9) The success of reimplantation of a dental organ depends largely on the presence of viable cells (fibroblasts) on the surface of the root, and an appropriate storage medium ensures the viability of these cells, thus improving the prognosis of the affected piece. (6.10)

There is little knowledge regarding how to treat dental avulsions. The lack of access to storage media in the place where the trauma occurs leads to failures in dental reimplantation due to the lack of conservation of the fibroblasts from the periodontal ligament between the time of injury and reimplantation. (1,9)

It has been shown that the capacity of the storage medium to maintain the viability of the LPD cells is more important than the duration of time that the piece is outside the alveolus. (2,6,11) The use of an inadequate transport medium can cause necrosis of the periodontal ligament, root resorption and tooth loss. (1,9,12) It has been reported that the periodontal ligament has a constant blood supply, a pH of 7.2 and an osmolarity of 280 to 300 mOsm/L. (4)

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An ideal storage and transport medium must meet the following characteristics: neutral pH between 7.2 and 7.4, osmolality between 230 and 400 mOsm/kg, temperature of 9.5 °C, sterile, contain nutrients, available at the time of injury, long useful life and low cost. (1-7,11-14) In the search for an ideal storage medium, these characteristics of a wide variety of products have been studied as possible media of transport for avulsed teeth that are divided into three groups: milk and its derivatives; natural products; and rehydration solutions. (5-8,15,16) The purpose of this research is to identify the most effective media for the storage and transport of avulsed permanent teeth, according to the viability of the periodontal ligament cells.

2. Materials and Methods

The present review of the literature was conducted in accordance with the PRISMA 2019 guidelines.

Search strategy

The population, intervention, outcomes (PIO) framework was used for the search strategy: P (avulsed teeth), I (storage media), C (milk) and O (Viability of periodontal cells), and the following question was posed: What is the ideal storage medium to preserve avulsed teeth while maintaining the viability of periodontal cells?

Inclusion criteria

In vitro studies, articles in published English and articles published from 2017 to January 2, 2022.

Exclusion criteria

Literature reviews, studies performed on primary teeth, animal studies, and cell culture studies.

Selection criteria

Studies were selected if the variables included storage media, viable periodontal cells, storage time, and cell count.

Information sources

The Scopus, Web of Science and PubMed electronic databases were searched by two researches.

Search and selection of studies.

A search of articles in English was performed using the following search query ("storage" [All Fields] OR "" storages "[All Fields]) AND (" culture media "[Pharmacological Action] OR" culture media. "[MeSH Terms] OR (" culture "[All Fields] AND" media" [All Fields]) OR "" culture media "" [All Fields] OR "" media "" [All Fields] OR "" media s "" [All Fields] OR "" media "" [All Fields]) AND ("avulse" [All Fields] OR "" avulsed "" [All Fields] OR "" avulsing "" [All Fields] OR "" avulsive "" [All Fields] OR "" fractures, avulsion "" [MeSH Terms. "] OR (“fractures” [All Fields] AND “avulsion” [All Fields]) OR “avulsion fractures” [All Fields] OR “avulsion” [All Fields] OR “avulsions” [All Fields]) AND (“teeth s "[All Fields] OR" teeths "[All Fields] OR" tooth "[MeSH Terms] OR" tooth "[All Fields] OR" teeth "[All Fields] OR" tooth s "[All Fields] OR" tooths " [All Fields]) AND (“periodontal ligament” [MeSH Terms] OR (“periodontal” [All Fields] AND “ligament” [All Fields]) OR “periodontal ligament” [All Fields]).

The retrieved articles were then screened bases on the titles, and duplicate studies were excluded.

Then, the abstracts were screened, and those that mentioned two of the four key selection points in this review (storage medium, viable periodontal cells, storage time and cell count) were chosen to be evaluated in the next stage. Animal studies were excluded. Then, the full texts were screened, and articles that examined three of the key points were included. Additionally, a manual search was performed using Google Scholar to find relevant information. The study selection process is shown in the PRISMA flow chart (Fig. 1).

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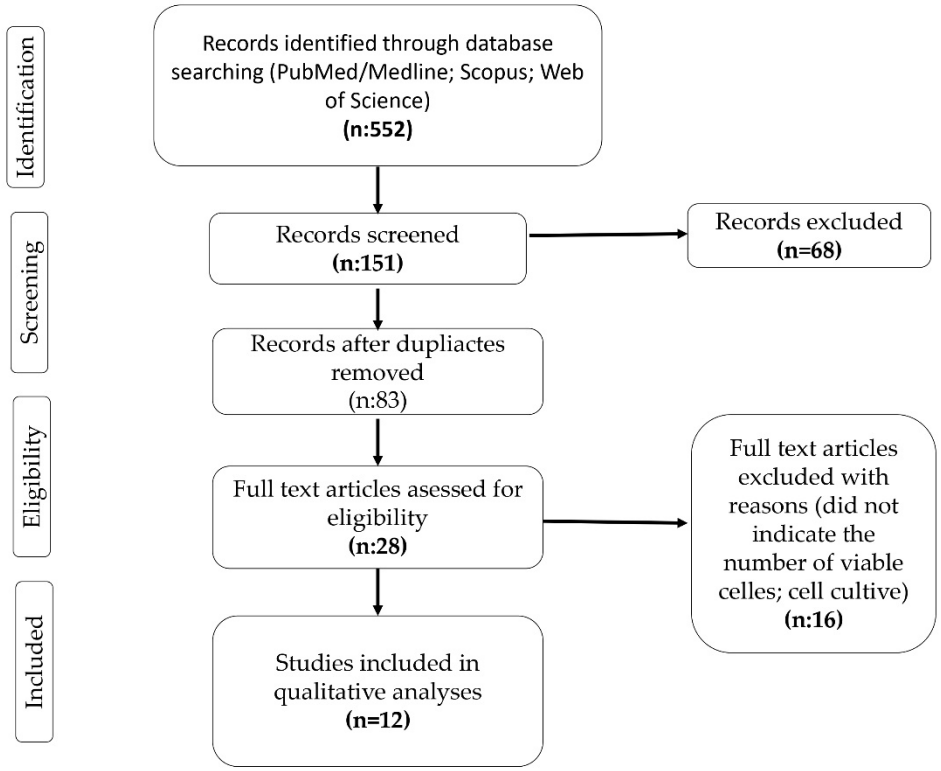

Figure 1. Flowchart of the search and selection process of articles, according to PRISMA guidelines (2019).

Quality analysis

Two investigators evaluated the methodological quality of the twelve in vitro studies included using the bias analyses based on the "Modified Consort" scale (CONSOLIDATED STANDARDS OF REPORTING TRIALS) (17). The studies were classified as follows: a score from 1-4 indicated a high risk of bias; a score of 5-8 indicate a medium risk of bias; a score of 9-12 indicated a low risk of bias.

Data extraction

The following data were extracted from the included articles using a summary table: title, year and author, number of teeth used in the study, storage media, time, viable periodontal cells, and p value.

3. Results

Regarding the risk of bias of the articles included in the present review, it was determined that 3 of the 12 studies had a low risk of bias and 9 had a medium risk of bias. It should be noted that with respect to the mechanism used to implement the random assignment sequence and who generated it, none of the articles included met these 2 criteria.

To facilitate the analysis and interpretation of the results, the data of each study were captured in two different tables. Table 1 shows the cell viability as a percentage, and Table 2 indicates the viability of the cells per mm3.

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Table 1. Analysis of the variables according to the percentage of viable periodontal cells.

Title and year	n	Storage media	Time	Periodontal viable cells (%)	p value	Level of BIAS
An In vitro comparison of coconut water, milk, and saline in maintaining periodontal ligament cell viability. (Vivian Flourish, et al. 2017)	40	Coconut Water	45 min	82.00*	0.0000	MEDIUM
		Milk		59.00		
		Saline Solution		15.00		
Evaluation of Periodontal Ligament Cell Viability in Honey as a Storage Media at Different Time Intervals: An In Vitro Study (Sheth P, et al. 2020)	50	Honey - Immediately	3 hours	96.43 ± 3.83*	a-b: p=<0.001 a-c: p=0.339 b-c: p=<0.001	MEDIUM
		Honey - Extraoral drying [b]		84.76 ± 2.00		
		Hank's balanced salt solution (HBSS) [c]		98.89 ± 1.07*		
Nature's Benefaction as a Life Saver for an Avulsed Tooth: An In vitro Study (Saumya Navit et al. 2017)	58	Hank's balanced salt solution (HBSS)	45 min	87.33*	<0.001	LOW
		Coconut water		79.87		
		Aloe Vera		70.59		
		Salina		50.56		
Evaluation of the efficacy of neem (Azadirachta indica) and turmeric (Curcuma longa) as storage media in maintaining periodontal ligament cell viability: An in vitro study. (Dhimole P et al. 2019)	90	Neem extract	30 min	88,00 ± 5.85 *	<0.001	MEDIUM
		Turmeric extract		81,63 ±7.12		
Efficiency of Castor oil as a Storage medium for avulsed teeth in maintaining the viability of periodontal ligament cells (Navavizadeh M et al. 2018)	40	Castor oil [a]	30 min	46,93 ±3,24	a-b: p=<0.05 a-c: p=<0.05 b-c: p=>0.05	MEDIUM
		Hank's balanced salt solution (HBSS) [b]		52,85±4,04*		
		Milk with 2.5% fat [c]		61,02±2,55*		
Viability and reproducibility of periodontal ligament cells on avulsed teeth stored in ham's f-10 solution (Talebi M et al. 2018)	60	Ham's F-10 solution	1 hour	91.27 ± 4.75*	<0.001	MEDIUM
		Pasteurized skim milk		68.33 ± 8.47		

* Most efficient storage media according to the percentage of viable cells.
(<0.001- <0.05): Statistically significant. (> 0.001-> 0.05): Not statistically significant.

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Table 2. Analysis of the variables according to the number of viable cells.

Title and year	n	Storage media	Time	Viable periodontal cells (cells/mm³)	p value	Level of BIAS
In vitro comparative evaluation of different storage media (Hank's balanced salt solution, propolis, Aloe vera, and pomegranate juice) for preservation of avulsed tooth. (Babaji P et al. 2017)	50	Hank's balanced salt solution (HBSS)	45 min	282,000	> 0.05	MEDIUM
		Aloe vera gel	45 min	226,000		
		Propolis	45 min	285,000*		
		Pomegranate juice	45 min	214,000		
Comparative Evaluation of Efficacy of Platelet-Rich Fibrin and Hank's Balanced Salt Solution as a Storage Medium for Avulsed Teeth: An In Vitro Study (Shetty et al. 2019)	30	Hank's balanced salt solution (HBSS)	45 min	76,800	0.001	MEDIUM
		Platelet Rich Fibrin		79,072*		
Evaluating the effectiveness of rehydrating solutions in preserving periodontal ligament cells vitality: An in vitro study. (Mahesh C, et al. 2018)	60	Distilled water + Electrolyte powder	1 hour	496.00 ± 31.62	<0,001	LOW
		Ringer's Lactate Solution		906.40 ± 60.57*		
		Oral rehydration saline solution		385.60 ± 31.32		
		Tender Coconut Water		555.20 ± 36.49		
Coconut milk and probiotic milk as storage media to maintain periodontal ligament cell viability: an in vitro study. (Saini D et al. 2017)	69	Coconut milk	1 hour	8.75 ± 3.166	000	MEDIUM
		Probiotic milk		143.25 ±1.616*		
Comparative evaluation of posttraumatic periodontal ligament cell viability using three storage media (Kokkali V et al. 2017)	55	Cow's milk	45 min	23,213.33 ± 2664.56*	<0,001	LOW
		Tender coconut water		13,920.00± 2094.61		
		Whey		10,566.67 ± 1415.05		
Comparative evaluation of the efficacy of aloe vera gel with milk and hank's balanced salt solution in maintaining the viability of PDL cells in avulsed teeth. (Baren A et al.2019)	40	Hank's balanced salt solution (HBSS).	45 min	921.40 ± 608.438*	<0,001	LOW
		Low-fat pasteurized cow's milk		812.70 ± 449.170		
		Aloe vera gel		241.00 ± 194.572		

(*): Most efficient storage media according to the number of viable cells.
(<0.001- <0.05): Statistically significant. (> 0.001-> 0.05): Not statistically significant.

The results of Table 1 show that the Neem extract is the best storage medium in an interval of 30 minutes, with a total of 88% viable cells, while at 3 hours, Hank's saline solution and honey showed a viability rate of 98.89% and 96.43% of cells, respectively (p> 0.001-0.005).

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The results of Table 2 show that Propolis is the best storage medium to maintain cell viability at 45 minutes, with a total of 285,000 viable cells/mm3. At 1 hour, the best medium was Ringer's lactate solution, with a total of 906.40 viable cells/mm3.

4. Discussion

The present review used the modified CONSORT scale to assess the risk of bias of the 12 included in vitro studies. The scale has 15 items that assess how a study was developed. In this review, 3 items of the scale were not included because they were not relevant to the experimental designs of the included studies. (17) No article complied with all 12 items; that is, no article provided a detailed report about the randomization process, and only 2 articles indicated how the sample size and the blinding of the evaluators were determined, thereby decreasing the reliability of the reported estimates.

Within the search strategy, the exclusion of articles published in Spanish may have helped to improve the scientific evidence. The articles included in this review were in vitro studies, which attempt to approximate the natural conditions of a phenomenon; however, the findings of these studies are not conclusive since they only partially elucidate the underlying mechanism and do not example the toxicokinetic and toxicodynamic process in the organism. (18) The selected studies only evaluated the number of viable cells after using a storage medium but did not evaluate the reaction of the organism when reimplanting the avulsed piece.

There was heterogeneity in the storage time among the studies, which allowed us to establish which storage medium was more effective and for how long. Only articles that examined cell count were included because studies that use cell culture tend to excessively manipulate the samples, thus causing cell death and decreasing the reliability of the results. The studies evaluated experimental groups and control groups (positive and negative); however, for the results of this review, only the experimental groups were analyzed because the sample sizes and storage times differed between the two groups. The experimental groups simulated the reality of a dental avulsion since the tooth was extracted by allowing it to air dry for a certain time and finally placed in a storage medium. Teeth in the positive control groups were immediately immersed in the postextraction storage medium. Teeth in the negative control groups were dried on a bench and, in some cases, were not immersed in any medium.

It was determined that the most effective storage medium at 30 minutes is the Neem extract, with a total of 88% viable cells. Neem is considered an ideal storage medium due to its active components that give it its antimicrobial action, its pH of 7 to 7.5 and its osmolality of 270 mosmol/kg, which is similar to that of extracellular fluid. (8,19) This medium was found to be more effective than milk and Hank's saline solution, which are considered standard storage media.

Hank's saline solution was more effective at 45 minutes, with a total of 87.33% viable cells, due to the large amount of metabolites and glucose it contains, which are essential for cell viability. (1) It has an osmolarity between 270 and 320 mOsm/kg and a physiological pH of 7.2, which is considered ideal for preserving fibroblast cell viability for up to 24 hours. Sheth P. et al. compared honey and Hank's saline solution over a 3-hour storage interval, where Hank's solution maintained 98.89% of viable cells, showing a longer storage time in this medium led to greater viability. (6)

At 1 hour of storage, Ham's F-10 solution had a cell viability rate of 91.27% due to its growth factor vitamin B7 (18) at pH 7.2 to 7.4 and its osmolarity of 280-300 mOsm/kg. (21) Talebi et al. demonstrated in an in vitro study that Ham's F-10 solution is as effective as the HBSS solution for the storage and transport of avulsed teeth because it maintained a significant percentage of viability for up to 24 hours. (22)

Based on the number of viable cells per mm3, propolis is the best storage medium to maintain cell viability at 45 minutes, with a total of 285,000 cells/mm3, due to its anti-inflammatory, antifungal and antibacterial properties (which enable the prevention of osteoclastic reabsorption), its pH of 7.4 and its osmolality of 350 mosmol/kg (23-26). Babaji et al. found that propolis is more effective than HBSS in maintaining the cell viability of avulsed teeth. (9)

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At a time interval of 1 hour, the best storage medium was Lactate Ringer's solution, with a total of 906.40 viable cells/mm3. Ringer's lactate has been used as a rehydrator in burns and trauma due to its tissue-friendly properties, such as pH ranging from 6.2 to 7.5 and an osmolality of 274 mOsm/kg. It also has the ability to preserve cell viability thanks to sodium glucose, potassium, chloride and a series of components that keep the PDL fibroblasts viable. (27.28)

Although all these media had excellent characteristics, it is difficult to access them at the time of avulsion, and most of the population does not have knowledge about the advantages of their use.

Milk is considered one of the most effective media of transporting avulsed teeth according to the American Association of Endodontists (7) because it is available in most homes, and the population is aware of its benefits. Its success rate ranges between 70-90%; it has a pH of 6.5-7.2; it has an osmolality of 275 mosmol/kg; it has low bacterial content; it contains nutritional substances (amino acids, carbohydrates and vitamins); and it contains ideal growth factors to prevent osteoclasia and decrease ankylosis and reabsorption. (29-32) Despite having all these characteristics, milk did not have promising results in the present study. Therefore, more studies should be conducted to compare milk with the storage media described above.

The difference in values between studies that analyzed the efficiency of the storage media to maintain the viability of fibroblasts in the same time interval (45 minutes) is due to the manipulation and storage technique of the extracted teeth. In the study by Babaji P et al. (9), the extracted teeth were immediately immersed in the storage medium, producing greater viability of the periodontal ligament cells and therefore a greater count of them. In the study by Kokkali V et al. and Baren et al. after extraction, the teeth underwent an air-drying period of 30 minutes and were finally placed in the storage medium, causing cell death and thereby significantly reducing the number of viable cells. (7,13)

5. Conclusions

According to the studies analyzed herein, it can be concluded that Hank's saline solution is the most effective storage medium to maintain the viability of periodontal cells of avulsed teeth, since it has been shown that the longer storage time leads to greater cell viability. However, honey has been observed to maintain the viability of fibroblasts for a long period of time; of these two, honey is easier to access. Given that the studies analyzed in this review were in vitro studies, it is recommended to analyze periodontal viability in clinical studies.

Supplementary Materials: Figure S1: title; Table S1: title; Video S1: title.

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