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SYSTEMATIC REVIEW

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In vitro evaluation of plant-based storage media compared to Hanks Balanced Salt Solution for avulsed teeth: a systematic review

Avaliação *in vitro* de meios de armazenamento fitoterápicos comparados à Solução Salina Balanceada de Hanks para dentes avulsionados: uma revisão sistemática

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ABSTRACT

Background: Tooth avulsion, complete loss of tooth from trauma, affects 1-11% of permanent teeth injuries, often in children aged 7-10. Diagnosis involves examining severed vascular and nerve supply, leading to pulp death and periodontal ligament (PDL) damage. Successful replantation depends on viable PDL cells. Immediate replantation is preferred to minimize PDL compromise. Alternatively, the tooth can be taken to a dental professional, requiring careful storage to maintain PDL cell viability. Natural products are explored for storage due to potential benefits. This study reviews plant effects on avulsed tooth tissue restoration, assessing if plant-based preservation aids PDL re-establishment post-replantation. **Objective:** The aim is to systematically review literature on herbal storage mediums compared to Hanks Balanced Salt Solution (HBSS) for avulsed permanent teeth using in-vitro studies. **Design:** Relevant literature from PubMed, Google Scholar, Web of Science, Cochrane, and Scopus was reviewed. Only laboratory-based studies on PDL cells from adult permanent teeth were included. From 692 initial articles, 19 were selected. RoB 2 was used for quality assessment. **Results:** Of 692 articles, 19 were selected. Green tea extract was most recommended, followed by Morinda citrifolia and HBSS. Propolis and coconut water were also frequently recommended. Recommendations were based on PDL cell viability, availability, cost, and shelf life. **Conclusion:** Plant-based preservation has positive effects on PDL cell viability in avulsed teeth. Further research is needed to validate clinical impacts and explore regenerative treatments.

KEYWORDS

Dental avulsion; HBSS; PDL cell viability; Plant derivatives; Transport media.

RESUMO

Introdução: A avulsão dentária, caracterizada pela perda completa de um dente devido a trauma, representa de 1 a 11% das lesões dentárias permanentes, principalmente em crianças entre 7 e 10 anos. O rompimento do suprimento vascular e nervoso leva à necrose pulpar e danos ao ligamento periodontal (LP), cuja viabilidade é essencial para o sucesso da reimplantação. Embora a reimplantação imediata seja o ideal para minimizar o comprometimento do LP, caso não seja possível, o dente deve ser transportado a um profissional em condições de armazenamento que preservem a viabilidade celular do LP. Este estudo revisa os efeitos desses meios na viabilidade do LP de dentes avulsionados, comparando-os à Solução Salina Balanceada de Hanks (HBSS). **Objetivo:** Revisar sistematicamente estudos in vitro que avaliaram meios de armazenamento fitoterápicos em comparação à HBSS para dentes permanentes avulsionados. Métodos: Foram analisados artigos das bases PubMed, Google Scholar, Web of Science, Cochrane e Scopus. Apenas estudos laboratoriais em células do LP de dentes permanentes adultos foram incluídos. De 692 artigos encontrados, 19 foram selecionados. O risco de viés foi avaliado pelo RoB 2. **Resultados:** O extrato de chá verde foi o meio mais indicado, seguido por Morinda citrifolia, HBSS. Própolis e água de coco também foram frequentemente recomendados. As recomendações

consideraram viabilidade celular, disponibilidade, custo e vida útil. **Conclusão:** Meios de armazenamento à base de fitoterápicos mostraram benefícios na preservação do LP de dentes avulsionados. Mais estudos são necessários para confirmar os impactos clínicos e explorar tratamentos regenerativos.

PALAVRAS-CHAVE

Avulsão dentária; HBSS; Viabilidade celular; Medicamento fitoterápico; Meio de transporte.

INTRODUCTION

Tooth avulsion is the complete loss of a tooth from its alveolar socket, typically occurring due to trauma such as a fall, road traffic accident, assault, sports injuries, or occupational incidents [1]. This type of dental trauma constitutes 1-11% of all traumatic injuries to permanent teeth, with maxillary central incisors being the most commonly affected, especially among children aged 7-10 years [2]. Children most commonly suffered trauma between the ages of 8 and 11 years, with boys having a greater incidence [3-5]. Clinical and radiographic examination, revealing the absence of the tooth from its socket, is used as diagnostic criteria [6]. When a tooth is avulsed, it presents with severed vascular and nerve supply, leading to pulp death, particularly in mature permanent teeth with a closed apex. Avulsion also results in the tearing of the periodontal ligament (PDL), causing damage to PDL cells that play a crucial role in tooth attachment [7]. It causes functional, psychological, and aesthetic challenges [6]. The success of replanting an avulsed tooth into its socket largely depends on the presence of viable PDL cells on the root surface [7].

After retrieving an avulsed tooth from the incident site, two management approaches become prominent. The preferred method involves careful handling of the avulsed tooth, either by the patient or an attendant (in the case of a minor patient), leading to immediate replantation into the alveolar socket. This careful approach aims to minimize further compromise to the integrity of periodontal ligament (PDL) cells, facilitating the prompt initiation of tissue repair. In a more commonly encountered situation, the avulsed tooth is not immediately replanted but is instead brought to a dental professional or medical professional for subsequent replantation [8,9]. In such cases, it is crucial to prevent desiccation of the avulsed tooth and promptly immerse it in an appropriate storage or transport medium until the optimal moment for replantation arises [6,8]. The effectiveness of this interim storage step significantly influences the course of the subsequent replantation outcome, as a carefully chosen storage medium ensures the viability of PDL cells on the root surface of avulsed teeth.

An optimal storage medium should be able to maintain the viability of periodontal ligament (PDL) cells, a crucial factor for fibroblast repopulation on the root surface to prevent the adherence of osteoclasts in that region [10]. The exploration of natural products as alternative sources of medications has gained attention in the field of complementary and alternative medicine, leading to numerous studies on their use in tissue repair [11]. The bioactive compounds within medicinal plants contribute to their pharmacological effects, and various phytochemicals have been scrutinized to understand the therapeutic impact of natural products. In comparison to synthetic products, natural products may offer greater efficacy in preserving the viability of PDL cells [12].

Several phytotherapies have been explored as potential plant-based storage mediums for avulsed teeth due to their bioactive properties that promote tissue repair and maintain cell viability. Natural products like green tea, aloe vera, propolis, coconut water, honey, neem, turmeric, Morinda citrifolia (noni), pomegranate, and ginger exhibit anti-inflammatory, antioxidant, and antimicrobial effects. Compounds such as catechins, curcumin, flavonoids, and phenolic acids in these plants enhance periodontal ligament (PDL) cell viability, support fibroblast repopulation, and prevent osteoclast activity. These phytotherapies provide a promising alternative to synthetic storage mediums, warranting further investigation for their clinical applicability in preserving and restoring avulsed tooth tissues. Given this existing research, there is a requirement for a systematic review to ascertain which plants yield the most favorable effects. The objective of this study was to delineate the effects of plants on the restoration of tissues in avulsed teeth and to ascertain whether a

plant-based preservation medium enables the re-establishment of the periodontal ligament (PDL) following tooth avulsion and replantation.

MATERIALS AND METHODS

Protocol

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2020 Statement and the Cochrane Handbook were followed in the preparation of the review's protocol - CRD42024520907 (PROSPERO Registration number). This review's main question was: What are the effects of various plants or plant-based materials on the tissue repair of avulsed teeth after replantation when compared to using Hanks Balanced Salt Solution (HBSS)?

Eligibility criteria

A PICOS criterion was developed to help with the search strategy and ascertain which studies should be included in the present systematic review [13]. The following is the PICOS formula:

- Population (P) - Human PDL cells (isolated from avulsed permanent teeth)
- Intervention (I) - Plant-based/ derived, herbs or medicinal plant compounds used as storage mediums
- Comparison (C) - HBSS
- Outcome (O) - PDL cell viability
- Study Design (S): Original research investigating the effects of different storage mediums on Human PDL cells in *In-vitro* studies was included; overviews, narrative reviews, letters to the editor, brief communications, case reports, and case series were not.

For this review, only articles that were published were reviewed. Only articles published in the English language were included in the review process.

INFORMATION SOURCES AND SEARCH STRATEGY

To find all peer-reviewed studies relevant to the review's question, a comprehensive search of PubMed, Cochrane CENTRAL, Scopus and Web of Science, was conducted for articles published between January 2014 and January 2024 (10 years). When developing thorough search strategies, each database's unique vocabulary and syntactical restrictions were considered.

The following MeSH terms were used in search strategy **(**((avulsed permanent tooth) OR (dental avulsion)) OR (dental trauma)) OR (tooth injury) AND ((((((((((((((((((herbal derivatives) OR (coconut water)) OR (pomegranate juice)) OR (green tea extract)) OR (plants)) OR (herb)) OR (Plant derivatives)) OR (plants)) OR (Green tea extract)) OR (Aloe Vera)) OR (transport media)) OR (biological transport)) OR (Nigella sativa)) OR (Cocos nucifera)) OR (castor oil)) OR (Red mulberry)) OR (Marmosa Rubra)) OR (Salvia officinalis)) OR (Punica granatum)) OR (Dragon's blood sap)) OR (soy milk) AND ((Hanks balanced salt solution) OR (HBSS)) OR (storage medium) AND (pdl cell viability) OR (periodontal ligament viability).

When more information on a particular article was required, attempts were made to contact the respective authors.

Study screening and selection

One author carried out the search strategy from the individual databases. The overall titles obtained were scanned and evaluated independently by two authors, to select the most relevant articles. The research work that were duplicated in multiple databases were eliminated. In case of any disagreement between the authors the selection was made after a debate between the authors. When the information provided in the title was insufficient, assessment of abstracts were done. Based on the inclusion and exclusion criteria the research articles were chosen. When the information provided by the abstract proved to be unsatisfactory, the full text was evaluated. The references of all the selected articles were checked as they might have been missed out in the databases due to various reasons. A manual search was conducted, and the reference lists of all papers were reviewed to identify any additional studies that were not found in the computerized search. A screening of the electronic databases identified 692 records, of which 24 were excluded after the removal of duplicates and 633 were excluded after title screening. 35 articles were screened, out of which 14 were excluded as they did not match the inclusion criteria. 19 full-text articles satisfied the eligibility criteria

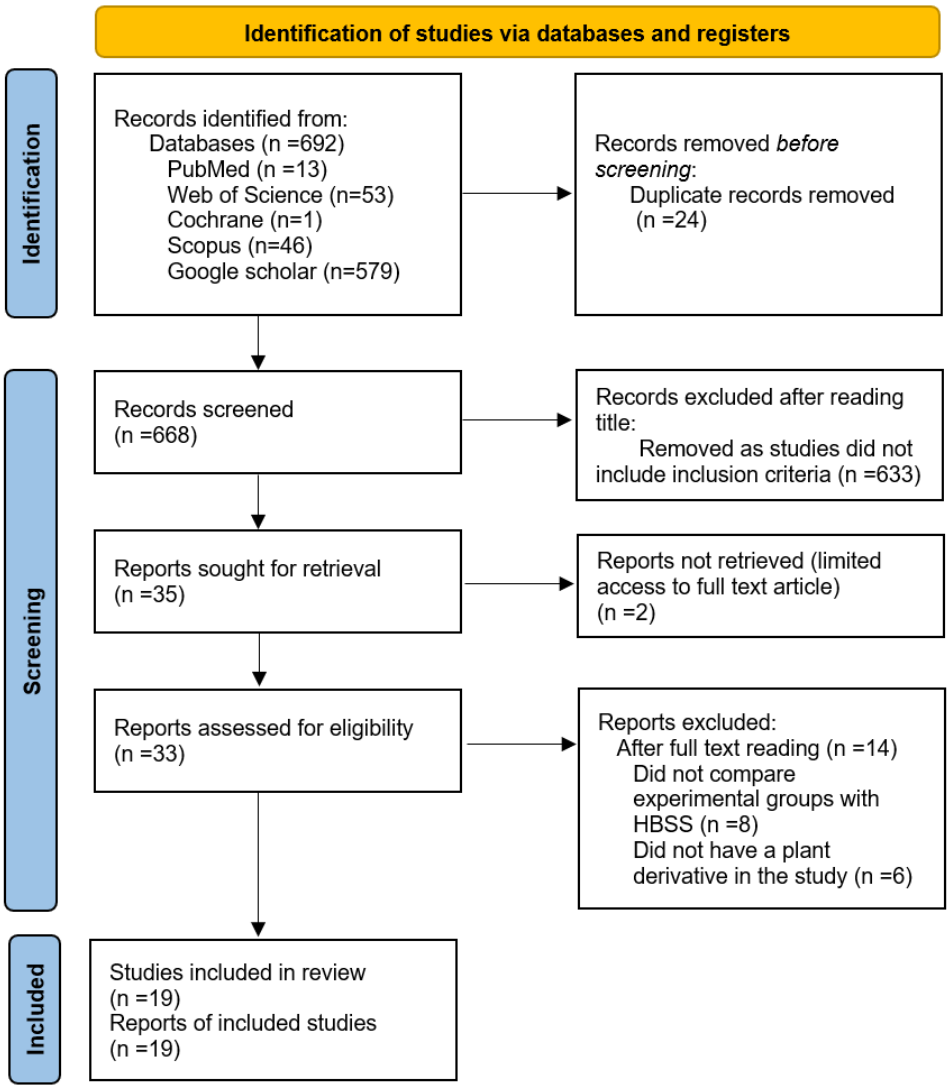

Figure 1 - Flowchart of the retrieved studies through the selection process.

of the targeted research and were covered in this systematic review. Figure 1 gives the PRISMA flow diagram. Desired information such as the design of the study, bibliographic data, characteristics of the participants, intervention and outcome were recorded.

Data extraction

Two reviewers (AC) and (VR) carried out the data extraction. Information like year of publication, study design, tooth origins, storage media, viability evaluation, period of storage, and results were taken from the studies that satisfied the requirements for inclusion and eligibility and entered into a Microsoft Excel (Microsoft Corp., Redmond, WA, USA) spreadsheet.

Assessment of risk of bias

The Risk of Bias tool (RoB 2) for randomized trials provided by the Cochrane Handbook for Systematic Reviews was used to assess the quality of the included studies. The included studies were evaluated using the RevMan 5.4.1 software for the following domains: random sequence generation and allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), absence of incomplete outcome data assessment (attrition bias), bereft from baseline imbalance (reporting bias) and adequate reliability. The risk of bias evaluation was carried out independently by both authors, who resolved any disagreement through discussions.

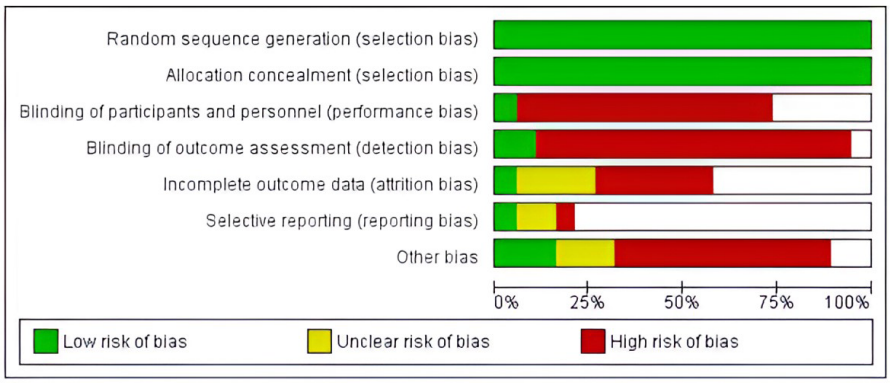

Figure 2 - Risk of bias reported as percentage across all included studies.

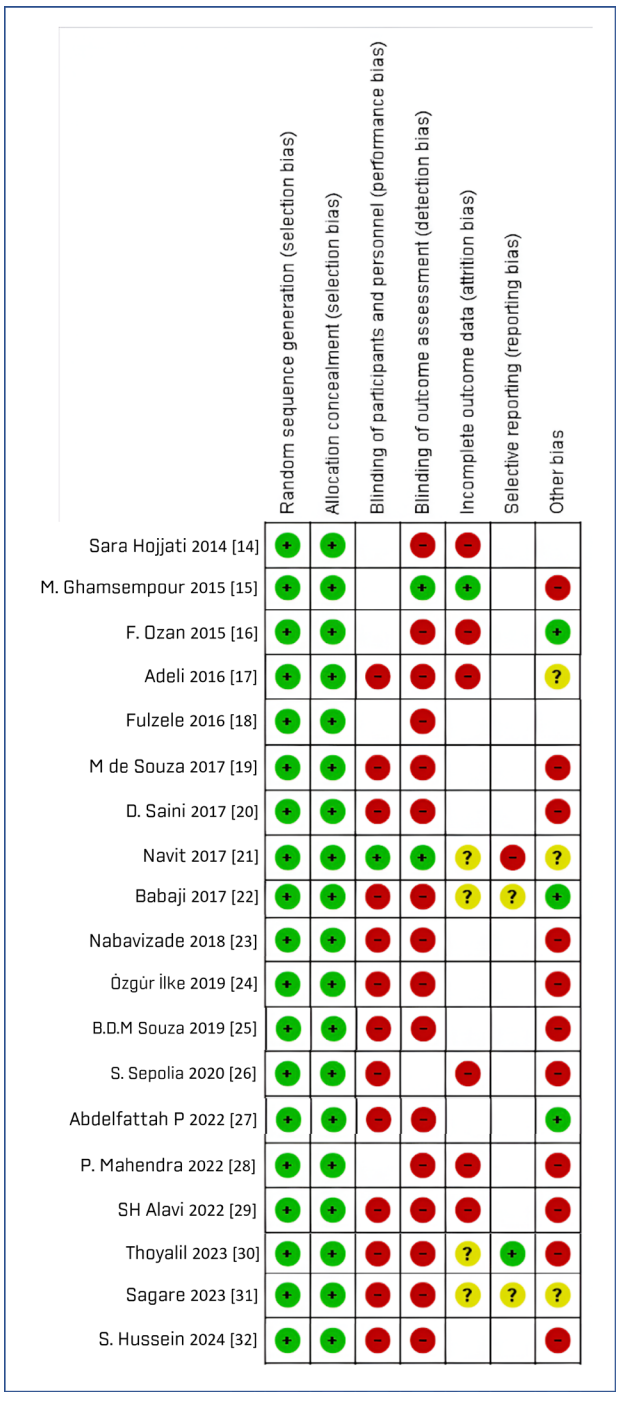

Figure 3 - Risk of bias summary represented in traffic light plot.

RESULTS

A screening of the electronic databases identified 692 records, of which 24 were excluded after the removal of duplicates and 633 were excluded after title screening. 35 articles were screened, out of which 14 were excluded as they did not match the inclusion criteria. 19 full-text articles satisfied the eligibility criteria of the targeted research and were covered in this systematic review.

The characteristics of the included studies were determined. The articles included in vitro studies into the effects of plant use on avulsed periodontal ligament tissues in humans. However, each study presented a different type of evaluation on the effects in the tissues, with the methods being described in Table I.

The systematic review encompasses studies employing various storage mediums for preserving periodontal ligament (PDL) cells post-tooth avulsion. Pomegranate juice (PJ) was investigated in three studies [14,22,30], in which the study by Sara Hojjati et al. [14] revealed promising results, with a 7.5% concentration exhibiting the highest viability of PDL cells. Green Tea Extract (GTE), was explored in five studies [15,17,27-29], demonstrated varied outcomes, emphasizing the significant effects of GTE on cell viability, Adeli et al. [17] showed that GTE was better than HBSS, Alavi et al. [21] showed that higher concentrations of GTE and the combination of GTE with aloe vera (AV) showed increased cell viability, both Ghamsempour et al. [15] and Abdelfattah et al. [27] studies showed no significant difference between HBSS and GTE, P. Mahendra et al. [28] was the only study where HBSS showed more cell viability. AV was studied in six articles [18,21,22,26,28,29] Fulzele et al. [18] showed that both HBSS

and AV were effective in maintaining PDL cell viability, S. Sepolia et al. [26] showed most viability with AV. Navit et al. [21], Babaji et al. [22] and P. Mahendra et al. [28] were the studies where HBSS showed more cell viability. Milk, including Skimmed Milk (SMilk), Whole Milk (WMilk), Coconut Milk (CMilk), Probiotic Milk (PMilk) and Almond Milk (AMilk), featured prominently in 5 studies [17,20,23,25,32]. Two studies were plant-based milk extract studies. The study by D. Saini et al. [20] showed HBBS was better than CM in maintaining cell viability, its efficacy in maintaining PDL cell viability across different temperatures. Combination of AV and propolis emerged as potential mediums in various studies [18,20,26,31]. Additionally, HBSS featured prominently in several studies [14-21,23,24,26,30], highlighting its prevalence as a standard medium. Other notable studies explored DMEM [16,17,27], Save-A-Tooth® system's HBSS (SAVE) [19,25] and AMilk [32], contributing valuable insights into alternative storage solutions. This categorization provides a comprehensive overview of the diverse storage mediums investigated in the included studies.

Among the 19 studies evaluating the effect of plants on avulsed teeth, 15 showed positive effects of plant treatment on the cells of the PDL. In addition, all studies evaluated the primary cultures of cells. No studies were found that used cells of other dental tissues, such as cementum, gingiva or alveolar bone.

About 14 studies evaluated cell viability using different means of verification. Trypan blue was used 11 times. The MTT assay was used in eight studies, the neutral red assay was used in three studies.

About 4 studies evaluated the temperature of the storage medium. The temperatures recorded in these studies were 4°C, 5°C, 20°C,

Table I - Study characteristics of included studies

Sl. No.	Studies	Teeth type/ number	Storage media	Methodology	Cell viability evaluation method	Storage time	Main results
1.	Sara Hojjati et al. 2014 [14]	PDL cells from freshly extracted orthodontic premolars.	HBSS, pomegranate juice (PJ), tap water.	Isolation of PDL cells after placement in storage solution.	Neutral Red assay.	1,3,6 and 24 hours storage time in all mediums.	7.5% concentration of PJ showed most viability, 1% of PJ was as effective as HBBS.
2.	M. Ghasempour et al. 2015 [15]	44 freshly extracted teeth.	Green tea extract (GTE), HBSS (positive control), water (negative control).	Isolation of PDL cells after placement in storage solution.	Tryptan blue exclusion technique.	Specimens immersed for 1,3 and 15 hours at 4°C.	No significant difference was found between HBSS and GTE at all time intervals.
3	F. Ozan et al. 2015 [16]	PDL cells obtained from extracted permanent teeth.	HBSS, Dulbecco's Modified Eagles Medium (DMEM) (control), C.Spinosa, light milk.	Isolation of PDL cells prior to incubation.	Tryptan blue exclusion technique.	Monitored every 5 minutes for 26 hours.	DMEM and C.Spinosa had higher cell value index than HBSS and light milk. C.Spinosa had better results than DMEM but was not statistically signifcant
4.	Adeli et al. 2016 [17]	PDL cells from freshly extracted third molars.	Dulbecco's Modified Eagles Medium (DMEM), HBSS, tap water, whole milk, hypotonic sucrose solution, Green Tea Extract (GTE), GTE + sucrose.	Isolation of PDL cells prior to incubation.	MTT assay.	1, 2, 4 and 24 hours at 37°C.	GTE was better than HBSS at 2, 4 and 24 hours, DME at 2 hours, and milk at 4 hours.
5.	Fuzele et al. 2016 [18]	PDL cells from freshly extracted third molars.	Hanks Balanced Salt Solution (HBSS), Aleo Vera gel (AV), packaged drinking water.	Isolation of PDL cells prior to incubation.	Tryptan blue dye exclusion test with haemocytometer.	15,30,60,90 and 120 minutes.	Both HBSS and AV are effective in maintaining PDL cells.

Table I - Continued...

Sl. No.	Studies	Teeth type/ number	Storage media	Methodology	Cell viability evaluation method	Storage time	Main results
6.	M de Souza et al. 2017 [19]	PDL cells from freshly extracted third molars.	5°C and 20°C, in skimmed Milk (SMilk), whole milk (WMilk), HBSS, Save-A-Tooth® system's HBSS (Save), natural coconut water, propolis,and egg white (Egg).	Isolation of PDL cells prior to incubation.	MTT assay.	3, 6, 24, 48, 72, 96, and 120 hours, 14 plates at 5°C and 7 at 20°C.	At 5°C, SMilk and WMilk were better than HBSS in maintaining cell viability, from 24 hours onward. At 20°C, HBSS was the best storage medium at 96 and 120 hours. At both temperatures, from 6 hours onward, Coconut, Propolis and Egg were less effective than SMilk, WMilk, and HBSS, the lowest temperature undermined the effectiveness of HBSS from 24 hours and favored SMilk and WMilk, from 96 and 48 hours onward, respectively.
7.	D. Saini et al. 2017 [20]	69 freshly extracted premolars.	HBSS (control), coconut milk, probiotic milk (PM).	Isolation of PDL cells after placement in storage solution.	0.5% tryptan blue staining.	Stored dry- 20 minutes and then 30 minutes in storage media.	PM and HBSS are better than coconut milk. No significant difference between PM and HBSS.
8.	Navit et al. 2017 [21]	58 freshly extracted premolars.	HBSS, coconut water, aloe vera (AV), saline.	Isolation of PDL cells after placement in storage solution.	Haemocytometer.	Air dried for 30 minutes, stored in different mediums for 45 minutes followed by 30 minutes with collagenase and dispase II.	HBSS most effective then coconut water and then AV.
9.	Babaji et al. 2017 [22]	50 orthodontically extracted teeth.	HBSS, propolis, aloe vera (AV), pomegranate juice (PJ).	Isolation of PDL cells after placement in storage solution.	Tryptan blue test.	Immediately placed in various storage medium for 45 minutes, controls were bench dries for 8 hours then placed in dipase and collagenase.	Propolis showed more viable cells than HBSS, then AV and last PJ.
10.	Nabavizadeh et al. 2018 [23]	40 freshly extracted teeth.	Two control groups, HBSS, milk, castor oil.	Isolation of PDL cells after placement in storage solution.	Tryptan blue assay.	2 hours dry time for control groups, experimental groups had 30 minutes of dry time and then immersed in mediums for 30 minutes.	Castor oil showed significantly lower viability.
11.	Özgür İlke et al. 2019 [24]	40 freshly extracted teeth.	HBSS, virgin olive oil (VOO), soyabean (SO), one positive control, one negative control.	Isolation of PDL cells after placement in storage solution.	0.4% Tryptan blue assay.	Air dried for 30 minutes and then soaked in mediums.	SO and VOO had more viable cells than HBBS. No significant difference between SO and VOO.
12.	B.D.M Souza et al. 2019 [25]	Human PDL cells extracted from permanent teeth.	Skimmed and whole milk, HBSS, Save-A-Tooth system's, coconut water, propylene glycol with 20% propolis, egg white, tap water.	Isolation of PDL cells prior to incubation.	MTT 24, 48, 72, 96, and 120 hours at 20°C and MEM 24, 48, 72, 96, and 120 hours at 37 °C.	Incubated till 120 hours at different temps (5 5 °C, milk (5°C, 20°C and 37°C) tap water (negative control) at 5 °C and 20 °C, for 24 hours.	5 °C, milk maintained more viable cells immediately after exposure, 20 °C, milk and HBSS were similar till 24 and 48h, HBSS superior at 72 hours.

Table I - Continued...

Sl. No.	Studies	Teeth type/ number	Storage media	Methodology	Cell viability evaluation method	Storage time	Main results
13.	S. Sepolia et al 2020 [26]	90 freshly extracted teeth.	HBSS, propolis, aloe vera (AV), one control.	Isolation of PDL cells after placement in storage solution.	0.4% Tryptan blue assay.	Soaked in mediums for 30 minutes.	AV showed (82%) most viability followed by propolis (68%) and HBSS (66%).
14.	Abelfattah et al. 2022 [27]	30 freshly extracted premolars.	HBSS, Dulbecco's Modified Eagles Medium (DMEM) (control group), Green tea extract (GTE).	Isolation of PDL cells after placement in storage solution.	MTT assay.	1,3,6,12,24,48, hours viability seen.	No significant difference at 1 and 3 hour between HBBS and GTE. GTE was superior in 6, 12, 24 and 48 hour.
15.	P. Mahendra et al. 2022 [28]	55 freshly extracted permanent teeth.	HBSS, green tea extract (GTE), aloe vera (AV), 2 control groups (one negative, one positive).	Isolation of PDL cells after placement in storage solution.	Tryptan blue dye test.	Air dried for 30 minutes, then 45 minutes in storage mediums. Negative control bench dried for 8 hours, positive group immediately treated with collagenase).	HBSS had most viable cells out of all experimental groups. No significant difference between AV and GT. all groups were significantly higher than negative control and significantly lower than positive control.
16.	SH Alavi et al. 2022 [29]	Human PDL cells extracted from permanent teeth.	Aloe vera (AV), green tea extract (GTE), combination of GTE and AV,HBSS (positive control), culture medium (negative control).	Isolation of PDL cells prior to incubation.	MTT Assay.	4 hours of incubation.	Higher concentrations of green tea and the combination of the two extracts significantly increased cell viability. Higher concentrations of Aloe vera had the least positive effect on maintaining the viability of these cells.
17.	Thoyalil et al. 2023 [30]	65 freshly extracted premolars.	Placentrex, propolis 10%, pomegranate juice (PJ) 5%, HBSS.	Isolation of PDL cells after placement in storage solution.	Tryptan blue test with haemocy-tometer.	Placed in storage medium for 45 minutes each, then placed in incubator for 30 minutes.	HBSS has most viability, Placentrex is a better alternative to PJ and propolis.
18.	Sagare et al. 2023 [31]	65 freshly extracted premolars.	HBSS, Morinda citrifolia juice, ocimum sanctum.	Isolation of PDL cells after placement in storage solution.	MTT assay, Trypsin dye exclusion technique.	Air dried for 30 minutes, stored in different mediums for 45 minutes followed by 30 minutes with collagenase and dispase II.	PDL cells viability was most in Morinda citrifolia juice followed by HBSS followed by Ocimum sanctum extract.
19.	S.Hussein Adam et al. 2024 [32]	66 wells with extracted human PDL cells.	HBSS, almond milk, control.	Isolation of PDL cells prior to incubation.	Annexin V-FITC fluorescent cytometry test, IC50 cytotoxic assay multiple range test.	Incubation for 1 hour.	In necrotic, early apoptosis phase and late apoptosis phase HBBS was highly significant to control and almond milk, while in normal intact cells phase, almond milk showed highest significant Q3 apoptotic cells amongst the other groups.

37°C and room temperature. The detailed data are described in Table I.

Another drawback identified in the assessment of the employed tests is the inadequacy of data, posing a notable risk of bias. Unlike the majority of studies that focused solely on evaluating cell viability, only three studies conducted multiple tests. Overall, the findings lack consistency in suggesting that the studied

plants could be recommended for the clinical management of avulsed teeth.

DISCUSSION

Natural products have proven to be more effective than synthetic storage mediums for avulsed teeth, although alternative options exist. Studies indicate that plants may contribute to the treatment of periodontal diseases by facilitating tissue regeneration, osteogenic differentiation, and mineralisation. However, identifying suitable plant storage media for tooth preservation is presently unviable due to the variability of existing data and several methodological deficiencies. Consequently, the level of evidence identified in contemporary research is conveyed through a qualitative analysis, which is the sole methodology employed.

This analysis revealed multiple plant species that have been studied for their ability to maintain PDL cell viability. None of the trials included, however, offered compelling evidence that the effect approached the expected degree of confidence. The heterogeneity of data rendered another systematic review's assessment of in vivo experiments insufficient for determining the optimal storage medium.

It was determined that propolis was the most extensively studied botanical extract. The anti-inflammatory, anti-cancer, antioxidant, and regenerative properties of propolis have been evidenced in numerous studies [29]. The majority of the studies referenced in this review focused on Brazilian propolis. Nonetheless, the study exclusively employed propolis sourced from the Apis mellifera L. species [30]. This review suggests that propolis may be an effective component for maintaining the pulp-density liposome (PDL) of avulsed teeth due to its impact on cell viability, anti-inflammatory properties, and osteogenic differentiation. A recent study examined the effect of polyphenols on the reduction of bacterial proliferation utilising Chilean propolis. Because avulsed teeth are susceptible to contamination once they are out of the alveolus and exposed to the environment, it is crucial to evaluate their antibacterial potential when treating them. The pharmacological effects of propolis in a 0.4% ethanolic solution may have affected the outcomes of that investigation. The antimicrobial properties of the plant media were not, however, tested in any of the planned research.

The antibacterial actions, inclusion of vital amino acids, vitamins, and electrolytes of coconut water prompted its investigation as another plant medium. Though it is recognised that coconut water comes from the species Cocos nucifera L., as cited in some papers of this study, four articles [22,25,31,32] reported that the coconut studied was from Thailand. Based on the data we have, coconut water may have a protective effect on the survival and health of PDL cells from avulsed teeth.

The inexpensive and widely-available aloe vera plant has more than 75 nutrients and a transparent gel within its leaves. Aloe vera's phytotherapeutic characteristics, including its antibacterial, antioxidant, and anti-inflammatory effects, have been detailed in scientific studies [33]. According to all research, this plant keeps PDL cells alive, making it an attractive option for tooth storage. Seven of the research [16,20,22,24] made use of gel-form Aloe vera. Information regarding the plant shape was lacking in the other research.

As a potential antibacterial agent, green tea has been investigated in the field of dentistry. In periodontal diseases, the catechins in green tea extracts have shown that they prevent the production of osteoclasts and macrophages by reducing the expression of matrix metalloproteinase-9 (MMP-9). Avulsed teeth have also been tested in green tea, which is a physiological medium and widely available across the world. When it comes to preserving PDL cells, green tea outperformed the more typical options of water and milk in cases of tooth trauma.

Pomegranate, which is abundant in polyphenolic flavonoids, has been previously studied for their potential effects on tissues in the mouth. Besides being an antioxidant, pomegranate also has anti-inflammatory and antibacterial properties. Research evaluating PDL cells following avulsed tooth storage in pomegranate juice is cited in this review [14-16]. According to two papers that were included, pomegranate juice could be suggested as a good medium for avulsed teeth.

Cell proliferation and survival are influenced by the physiological osmolality, pH, and temperature. One recent study recommended looking at a medium's pH and osmolality before doing anything else. Reducing the osmolality of the medium is crucial for cell survival since it impacts the water uptake of the cells. Biological

processes are affected by changes in pH, which affect all cellular responses. Three of the experiments maintained their storage media at 4°C, which is about the same as the temperature in a standard household fridge. On the other hand [25], was the only study to compare two temperatures (5°C and 20°C) in this review, and it demonstrated that keeping the cells at 20°C generated better results.

As a last stage, we looked at how different plants affected the periodontal ligament (PDL) cell survival in avulsed teeth, and we found that they helped maintain the cells intact. There is a shortage of evidence in the existing literature to determine which plants can be employed as appropriate storage mediums to alter the results of replantation. To further understand how maintaining avulsed teeth in plant-based medium affects distinct cells involved in tooth reintegration, larger-scale clinical trials are urgently needed. Improving the prognosis for avulsed teeth and finding practical clinical applications are the goals of this approach. Additionally, it is highly desirable to identify plants and their derivatives that are both economical and conveniently available for persons who have endured trauma and lost a tooth. It is crucial to have an easily available and user-friendly tooth preservation medium on hand in case of accidents. This will allow the traumatised person to seek replantation and tissue repair therapies from a dentist without delay.

The methodological differences across the publications were a major shortcoming of this comprehensive study. One difference was that different investigations employed different plants to treat periodontal ligament cells. Different tests were utilised to examine the same outcome, which further prohibited direct comparison between the researches. There was a deficiency of information regarding the age and number of cell culture passages, even though all the articles focused on human PDL cells in primary culture. Lack of validated methodologies for analysing in vitro study evidence levels and bias issues is another key constraint. Due to their failure to fully address the research themes provided in this systematic review, no publications were assessed to be of high quality. Additional non-biased research is needed to test the validity of systematic review instruments in order to remove these constraints.

CONCLUSION

This systematic review revealed a diversity of studies exploring the utilization of plants for preserving avulsed teeth. Despite the varied approaches among the reviewed studies, the overall results indicate encouraging impacts of plants on the viability of periodontal ligament (PDL) cells. The findings from this comprehensive analysis offer insights for prospective research, proposing the need for additional studies to validate the clinical effects of these plants on the PDL. Moreover, the review suggests investigating regenerative treatments involving cells from other tissues within the periodontal complex.

Author's Contributions

AC: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft Preparation, Writing – Review & Editing, Visualization, Supervision, Project Administration and Funding Acquisition. VR: Conceptualization, Validation, Formal Analysis, Writing – Review & Editing, Visualization, Supervision, Project Administration. GJ: Writing – Review & Editing, Visualization, Supervision.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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