ABSTRACT

Objectives: The aim of the present systematic review was to investigate the cryopreservation process of dental pulp mesenchymal stromal cells and whether cryopreservation is effective in promoting cell viability and recovery.

Materials and Methods: This systematic review was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the research question was determined using the population, exposure, comparison, and outcomes strategy. Electronic searches were conducted in the PubMed, Cochrane Library, Science Direct, LILACS, and SciELO databases and in the gray literature (dissertations and thesis databases and Google Scholar) for relevant articles published up to March 2019. Clinical trial studies performed with dental pulp of human permanent or primary teeth, containing concrete information regarding the cryopreservation stages, and cryopreservation performed for a period of at least 1 week were included in this study.

Results: The search strategy resulted in the retrieval of 185 publications. After the application of the eligibility criteria, 21 articles were selected for a qualitative analysis.

Conclusions: The cryopreservation process must be carried out in 6 stages: tooth disinfection, pulp extraction, cell isolation, cell proliferation, cryopreservation, and thawing. In addition, it can be inferred that the use of dimethyl sulfoxide, programmable freezing, and storage in liquid nitrogen are associated with a high rate of cell viability after thawing and a high rate of cell proliferation in both primary and permanent teeth.

Keywords: Cryopreservation; Dental pulp; Mesenchymal stromal cells; Stem cells

INTRODUCTION

Dental pulp, which is constituted by connective tissue, mesenchymal cells, neural fibers, and blood and lymphatic vessels, is located inside dental elements, circled by dentin and contained in a structure known as the pulp chamber [1,2]. The multiple functions of dental pulp include the production of dentin and its biological and physiological maintenance [2]. Dental pulp mesenchymal stem cells can be obtained from both permanent and primary teeth; however, in primary teeth, they are in a less mature stage, making the process of differentiation easier [2].
Dental pulp mesenchymal stem cells present positive expression for CD44, CD90, CD105, and CD146 surface markers and negative expression for CD34 and CD45 hematopoietic markers, a pattern that classifies them as mesenchymal stem cells or mesenchymal stromal cells \[3\]. Moreover, the cells that present mesenchymal surface markers have immunomodulatory properties and can differentiate into osteoblasts, chondrocytes, adipocytes and neural cells due to their origin from the same embryonic leaflet from which dental pulp originates \[3\]. Furthermore, the cells from dental pulp present a higher differentiation potential in odontogenic lineages and are better than bone marrow cells in terms of differentiation phenomena \[2,4,5\].

Dental pulp mesenchymal stem cells have shown multiple applications relevant to dentistry. These cells are able to form mineralized tissues involved in dentin-pulp complex constitution \textit{in vivo} with assistance from biomaterials, matrix, or carrier materials \[1,6\]. Moreover, some studies have demonstrated that these cells can regenerate dental pulp in the presence of an irreversible inflammatory process in rats and humans \[7-9\]. It was also found that dental pulp mesenchymal stem cells participated in bone neoformation during reconstruction surgery \[10\].

Because of the multiple possibilities of stem cell use, the necessity to store them emerged with the goal of maintenance for future applications. With time, these cells can present a reduction in their differentiation potential and genetic alterations due to multiple passages in culture and aging, which implies the need for a rigorous process of storage to prevent or postpone these alterations \[11,12\]. The most widely used method for conservation of these cells has been cryopreservation based on the use of extremely low temperatures with the aim of living tissue maintenance \[13\]. The freezing process should be carried out very carefully, following certain rules to avoid the formation of ice crystals within the cells, what could be responsible for cell lysis, culture contamination, and reduction of the cell viability rate \[14\]. One of the main stages involved in successful cryopreservation is previous immersion culture in a mixture of penicillin and streptomycin for disinfection, in laminar flow \[12\], as well as the use of a cryoprotectant, usually dimethyl sulfoxide (DMSO) \[15,16\], with the goal of reducing sample dissolution and thereby diminishing the probability of ice crystal formation \[11,15\]. These processes are applicable to primary and permanent teeth cells \[15,17\].

However, there currently is no standardized protocol for the cryopreservation of cells from dental pulp. Jesus \textit{et al.} \[18\] emphasized that the presence of dental pulp mesenchymal cells in primary teeth is a recent discovery and that, regarding the cryopreservation process, it is necessary to standardize the techniques and procedures employed in order to enhance the results. Thus, the aim of the present study was to perform a systematic review to investigate the process of cryopreservation of human dental pulp stromal mesenchymal cells and whether cryopreservation is effective in promoting cell viability and recovery.

**MATERIALS AND METHODS**

This systematic review was elaborated according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (www.prisma-statement.org).

**PECO question**

The research question was determined using the population, exposure, comparison, and outcome (PECO) strategy, as follows: population: dental pulp stromal cells; exposure and
comparison: materials and methods of cryopreservation; and outcome: cell recovery and viability. Based on this method, the following research question was established: “Which are the materials and methods of cryopreservation that promote cell recovery and viability of dental pulp mesenchymal stem cells?”

**Inclusion and exclusion criteria**

Clinical trials with dental pulp from primary and permanent teeth, containing information about extracted teeth disinfection, pulp extraction with mesenchymal stem cell separation, cell proliferation, and cryopreservation process and thawing, in which cryopreservation was performed for at least 1 week, were included in this review. Articles, dissertations, monographs, coursework, and theses published in English and Portuguese without restriction of the year of publication were considered eligible. Case reports, case series, letters to the editor, conference summaries, literature reviews, and animal studies were excluded.

**Search strategy**

The systematic review was started on December 2018, and the search were performed up to March 2019 at Universidade Federal do Paraná, Curitiba-PR, Brazil. The PubMed, Cochrane Library, Science Direct, LILACS and SciELO electronic databases were searched using the keywords “stem cells” or “mesenchymal cells,” “dental pulp,” “cryopreservation,” and “cell culture” in English and Portuguese. All search terms were indexed in MeSH and there was no individualized strategy for each database. A manual search in the reference lists of the articles included in the review and in the gray literature (Google Scholar and thesis/dissertation databases) was also performed to complement the initial search. Two researchers independently performed the searches and the references were organized using the EndNote X7 software. When additional data and figures for some studies were needed, we contacted the relevant authors.

**Article selection and data extraction**

Two independent researchers selected articles based on title and abstract analysis (pre-selection), followed by a full-text analysis of the pre-selected articles. The primary outcome sought were the materials and methods used for cryopreservation and the secondary outcome was viability and cellular recovery after the cryopreservation process. The data extraction form was created with the following variables: author/year/country in which the study was performed, study design, type of tooth analyzed (deciduous or permanent), sample size, type of sample storage, steps involved in the process (tooth disinfection, pulp extraction, cell isolation, cell proliferation, cryopreservation, time and thawing), results, and conclusions. Data extraction was also performed independently by two reviewers and divergences of opinion were resolved by consensus between them.

**Qualitative analysis**

For a methodological quality analysis of the articles, the Joanna Briggs Institute Critical Appraisal Checklist for Quasi-Experimental Studies was carefully adapted and applied [19]. The instrument consists of nine questions: (Q1) Is it clear in the study what is the ‘cause’ and what is the ‘effect’ (i.e., there is no confusion about which variable comes first)?; (Q2) Were the participants included in any comparisons similar?; (Q3) Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?; (Q4) Was there a control group?; (Q5) Were there multiple measurements of the outcome both pre and post the intervention/exposure?; (Q6) Was follow-up complete and if not, were differences between groups in terms of their follow-up adequately described and
analyzed? (Q7) Were the outcomes of participants included in any comparisons measured in the same way? (Q8) Were outcomes measured in a reliable way? and (Q9) Was appropriate statistical analysis used? The answers “yes,” “no,” “unclear,” or “not applicable” could be given to each question. The risk of bias was rated high when the study reached up to 49% of “yes” scores, moderate when the study had 50% to 69% of “yes” scores, and low when the study had more than 70% of “yes” scores [19,20].

RESULTS

Study selection
The initial search led to the retrieval of 185 articles: 17 from PubMed, none from Cochrane Library, 141 from Science Direct, 1 from LILACS, none from SciELO, and 26 from the gray literature. After the removal of 37 duplicate articles, the titles and abstracts of 148 articles were analyzed, which led to the exclusion of 123 articles, leaving 25 pre-selected articles for full-text analysis. From the manual search of the reference lists of the selected articles, 9 other papers were identified as eligible, however, they were excluded after full reading. After the full-text analysis, 21 articles were included in the review and qualitatively analyzed [14-17,21-37] (Figure 1).

Extraction of article data
Table 1 shows the characteristics of the data of the 21 articles included in the review. All studies were randomized controlled trials conducted in 12 different countries, and the publication date ranged from 2006 to 2018. Sixteen articles used permanent teeth

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**Figure 1.** Flowchart of the bibliographic search and selection process adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol.
| Authors          | Year | Country   | Study design | Sample                          | Disinfection | Pulp extraction | Cell isolation | Stages                                      | Cryopreservation | Time          | Thawing      |
|------------------|------|-----------|--------------|---------------------------------|--------------|----------------|---------------|--------------------------------------------|-----------------|---------------|--------------|
| Papaccio et al.  | 2006 | Italy     | RCT          | Teeth of healthy patients aged between 21 and 45 years (unspecified number of teeth) | Immediate disinfection | Gracey’s dentin digger and curette | Enzymatic digestion with collagenase I and dispase | Immersion in alpha-MEM medium supplemented with 20% FCS, 2-phosphate ascorbic acid, glutamine, penicillin, streptomycin. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Transfer of cells to medium containing 10% FCS-supplemented DMEM with immediate storage in liquid nitrogen | More than 2yr   | Water bath at 37°C |
| Zhang et al.     | 2006 | China     | RCT          | Third molar teeth of patients aged between 18 and 24 yr | Immersion in alpha-MEM containing 0.5 mg/mL gentamicin and 3 g/mL amphotericin B | High-speed drill for crown cutting and pulping | Explication technique associated with enzymatic digestion with collagenase type I for 1 hr | Immersion in alpha-MEM medium containing 20% FCS. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Liquid nitrogen storage immediately after handling | Less than 1 mon | Water bath at 37°C |
| Perry et al.     | 2008 | India     | RCT          | 31 third molars in patients aged between 18 and 30 yr | HypoThermosol, Mesencult basal medium, or PBS, the medium being chosen randomly | High-speed drill for crown and endodontic cutting | Enzyme digestion with dispase and collagenase type I | Cells were transferred to Mesencult medium supplemented with Pen-Strep and amphotericin B. | Cell transfer to Mesencult medium containing DMSO. Gradual freezing at −1°C/min to −85°C and subsequent transfer to liquid nitrogen | 1 mon          | Water bath at 37°C |
| Woods et al.     | 2009 | United States of America | RCT          | Teeth of patients aged between 18 and 30 yr | PBS solution | High-speed drill for crown cutting and pulping | Enzyme digestion with collagenase type I and II and thermolin | Immersion in Mesencult. Incubation at 37°C in a humid atmosphere with 5% CO₂ | This solution was supplemented with DMSO. Gradual freezing at −1°C/min to −85°C and subsequent transfer to liquid nitrogen | 1 mon          | Water bath at 37°C |
| Lee et al.       | 2010 | Japan     | RCT          | Premolar dental pulp of adults aged 18–30 yr | Immersion in PBS | High-speed drill for crown and endodontic cutting | Scalpel explantation technique and enzymatic digestion with collagenase type I and dispase | Immersion in Optimem I medium with Pen-Strep, gentamicin and FCS. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Medium cell transfer with FBS and DMSO. Gradual freezing at −1°C/min to −80°C with subsequent transfer to liquid nitrogen | 1 wk           | Water bath at 37°C |
| Temmerman et al. | 2010 | Belgium   | RCT          | Third molar of patients aged 15–25 yr | Immersion in medium containing DMEM, FCS, sodium thiosulfate (0.1%) | Carborundum disc for crown and endodontic pulp cutting | Explantation technique with scalpel | Immersion in Optimem I medium with Pen-Strep, gentamicin and FCS. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Medium cell transfer with FCS and DMSO. Gradual freezing at −1°C/min to −80°C with subsequent transfer to liquid nitrogen | 1 mon          | Water bath at 37°C |
| Abedini et al.   | 2011 | Japan     | RCT          | Third molar dental pulp of 10 patients aged 18–30 yr | Not specified | Vertical cut of tooth and pulp removal with curette | Explantation technique with scalpel | Immersion in alpha-MEM medium supplemented with FBS, penicillin, amphotericin B and kanamycin. Incubation at 37°C in a humid atmosphere with 5% and 10% CO₂ | Prior immersion in 10% DMSO associated with FBS. Freezing in a magnetic freezer with initial maintenance at −5°C for 15 min and subsequent cooling at a rate of −0.5°C/min to −30°C. Subsequent culture transfer at −150°C. | 3 mon           | Water bath at 37°C |
| Chen et al.      | 2011 | Taiwan    | RCT          | 50 teeth of patients with an average age of 56.5 yr | PBS immersion | High rotation drill for cutting the crown at the dentin junction and endodontic file | Enzymatic digestion with collagenase type I and dispase | Immersion in alpha-MEM medium containing FBS, Pen-Strep, and ascorbic acid. Incubation at 37°C in a humid atmosphere of 5% CO₂ up to 80% confluence | Transfer of cells to medium containing DMSO and FBS. Gradual freezing to 4°C for 2 hr, up to −80°C for 8 hr and transfer to liquid nitrogen. Cooling rate −0.05°C/min. | 1 mon          | Water bath at 37°C |

(continued to the next page)
| Authors                         | Year | Country | Study design | Tooth type | Sample | Disinfection | Pulp extraction | Cell isolation | Cell proliferation | Cryopreservation | Time | Thawing |
|--------------------------------|------|---------|--------------|------------|--------|--------------|----------------|---------------|-------------------|-----------------|------|---------|
| Gioventu et al. [23]           | 2012 | Italy   | RCT          | P          | 10 non-exfoliated teeth obtained from children aged 7-11 yr-old | Immersion in sterile RPMI 1640 medium | Making a cavity at the cementoenamel junction height with Nd:YAG laser | Enzymatic digestion with collagenase type A | Immersion in alpha-MEM Glutamax 1% medium supplemented with 20% FBS and 1% Pen-Strep. Incubation at 37°C in a humid atmosphere containing 5% CO₂ until 80% confluence. | Immersion in sterile RPMI 1640 medium containing 10% DMSO and 10% human albumin. −80°C culture storage in programmable freezer | 10 | days Water bath at 37°C |
| Lee et al. [28]                | 2012 | Taiwan  | CCT          | P          | Orthodontically exposed incisors of a 28-yr-old woman and a 25-yr-old man | Immersion in Dulbecco’s phosphate buffered saline solution | High speed drill for crown and endodontic file cutting | Scalpel explantation technique and enzymatic digestion with collagenase type I and dispase | Immersion in alpha-MEM medium supplemented with 15% FBS, 2-phosphate ascorbic acid, antibiotics and antimicrobials. Incubation at 37°C in a humid atmosphere with 9% CO₂ | Non-magnetic freezing group: freezes for 1 day at −80°C and −150°C for storage. Magnetic freezing: immersed in 10% DMSO, performed in a programmable freezer, cooling rate from −0.5°C to −32°C and storage at −150°C | 1wk | Water bath at 37°C |
| Antunes [17]                   | 2013 | Brazil  | RCT          | D          | 3 teeth of children aged 6 to 12 yr | Immersion in alpha-MEM medium and transport on ice | Diamond blade for crown cutting and pulp tissue curettage | Enzymatic digestion with collagenase type I and dispase | Immersion in solution with alpha-MEM and FBS. Incubation at 37°C in a humid atmosphere of 5% and CO₂ up to 70%–90% confluence | Transfer of cells to medium containing DMSO and FBS. Freeze gradually at 4°C for 2 hr, −20°C for 18 hr and up to −80°C with transfer to liquid nitrogen | 1mon | Water bath at 37°C |
| Ji et al. [16]                 | 2014 | South Korea | RCT         | D          | 122 teeth obtained from 105 healthy patients aged 3-16 yr | Immersion in medium containing alpha-MEM, penicillin, streptomycin, gentamicin, and amphotericin B | Made by a device called “Barbed Broach” (Mani, Utsunomiya Toshi-ken, Japan) | Scalpel explantation technique | Same composition of medium used for decontamination. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Transfer of cells to medium containing DMSO and FBS. Gradual freezing to 4°C for 1 hr and to −80°C with transfer to liquid nitrogen. Cooling rate −1°C/min | 1–9 | mon Water bath at 37°C |
| Lindemann et al. [29]          | 2014 | Brazil  | RCT          | D          | 26 teeth of children aged 9–11 yr | Direct immersion in disinfection medium | Endodontic file for pulp collection | Enzyme digestion with collagenase type I | FBS (20%) added to the enzyme digestion solution. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Immersion in 10% DMSO medium associated with FBS with initial temperature maintenance at 4°C for 1 hr. The temperature was gradually cooled at a rate of −1°C/min to −80°C and maintained for 24 hr, with subsequent transfer to liquid nitrogen at −196°C. | 1wk | Water bath at 37°C |
| Kumar et al. [25]              | 2015 | India   | RCT          | P          | Impacted teeth pulp of 16-yr-old patients | Hank’s balanced solution | High-rotation drill for crown cutting and curettage | Scalpel explantation technique | Immersion in alpha-MEM medium containing glutamine, FBS, and Pen-Strep incubation at 37°C in a humid atmosphere with 5% CO₂ | Transfer of cells to medium containing DMSO and FBS. The best protocol was gradual freezing at 0°C for 15 min, −20°C for 1 hr, and up to −80°C with transfer to liquid nitrogen. Freezing at −1°C/min | 1yr | Water bath at 37°C |

(continued to the next page)
Table 1. (Continued) Characteristics of studies included

| Authors            | Year  | Country | Study design | Tooth type | Size                        | Disinfection              | Pulp extraction          | Cell isolation                  | Stages                                      | Cryopreservation                  | Time | Thawing |
|--------------------|-------|---------|--------------|------------|-----------------------------|---------------------------|--------------------------|-----------------------------|-------------------------------------------|---------------------------------------|------|---------|
| Lee et al. [26]    | 2015  | Korea   | RCT          | D          | 20 teeth of children aged 5–14 yr | Immersion in PBS          | Endodontic pulp collection file | Scalpel explantation technique | Immersion in alpha-MEM medium supplemented with 10% FBS, ascorbic acid, glutamine, penicillin, and streptomyacin. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Pre-storage in FBS medium supplemented with 10% DMSO. Gradual freezing starting at 4°C with cooling rate at −1°C/min to −80°C and then storage in liquid nitrogen at −196°C | 1–8 mon | Water bath at 37°C |
| Munavar et al. [30]| 2015  | Colombia | RCT          | P          | Teeth of patients aged 18–31 yr | Immersion in PBS          | Immersion in 1% sodium hypochlorite and PBS baths | High-speed drill for crown and endodontic cutting | Enzyme digestion with dispase and collagenase type I | Immersion in DMEM medium supplemented with Pen-Strep and amphotericin B. Incubation at 37°C in a humidified atmosphere with 5% CO₂ | Transfer of cells to medium containing FCS and DMSO. The samples were stored in liquid nitrogen | 2 yr | Water bath at 37°C |
| Alsulaimani et al. [15] | 2016  | Saudi Arabia | RCT        | P          | 17 teeth of 30-yr-old | Chlorehxidine gluconate for 30 sec, immersion in saline and lysozyme | Diamond blade for crown and file cutting K-file | Explant and enzymatic digestion with collagenase type I and dispase | Immersion in solution with DMEM, FBS, penicillin, streptomyacin and alpha-MEM medium. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Transfer of cells to medium containing DMEM, FBS, Pen-Strep and DMSO. Gradual freezing to −20°C for 20 min and −80°C for 4 days and transfer to liquid nitrogen | 2 yr | D-MEM added to the environment and gentle aspiration |
| Malekfar et al. [30] | 2016  | India    | RCT          | P          | 20 teeth pulp samples from patients aged 15–30 yr | Pulp tissue was washed with Dulbecco and PBS solution | High-speed drill for crown and endodontic cutting | Scalpel explantation technique and type I collagenase enzymatic digestion | Immersion in DMEM medium supplemented with alpha-MEM, glutatione, FBS, and Pen-Strep. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Transfer of cells to medium containing FBS and DMSO maintained at 4°C for osmotic balance. Freezing gradually at −1°C/min to −80°C. Subsequent transfer to liquid nitrogen | 3 mon | Water bath at 37°C |
| Han et al. [24]    | 2017  | Korea    | RCT          | P          | 12 teeth of patients with an average age of 19 yr | Immersion in PBS medium containing Pen-Strep | High-rotation drill for crown cutting and curettage | Scalpel explantation technique, type I collagenase enzyme digestion | Immersion in Dulbecco medium supplemented with 10% FBS, Pen-Strep. Incubation at 37°C in a humid atmosphere with 5% CO₂ | Immersion in cryo-protective solution containing glucose, sucrose and ethylene glycol. Culture maintained at 11°C for 30 min, cooled to −2°C/min to −7°C, maintained for 5 min and cooled again to −0.3°C/min to −40°C and to −10°C/min to −140°C with liquid nitrogen storage | 1 yr | Water bath at 37°C |
| Huynh et al. [14]  | 2017  | Vietnam  | RCT          | P          | Third molars of patients aged 18–25 yr | Immersion in DMEM medium containing FBS and Pen-Strep | Immersion in DMEM medium containing glutamine and Pen-Strep with subsequent immersion in PBS | High-rotation drill for cutting the crown at the dentin-junction and endodontic file | Scalpel explantation technique | Immersion in DMEM medium with glutamine, FBS, and antibiotics. Incubation at 37°C in a humid atmosphere with 5% CO₂ to 80% confluence | Medium cell transfer with different percentages of DMSO and FBS. Dual freezing to −80°C and transfer to liquid nitrogen. Cooling rate −1°C/min | 6 mon | Water bath at 37°C |
| Mochizuki and Nakahara [31] | 2018  | Japan    | RCT          | P          | Dental pulp of 8 healthy young adults aged 20–37 yr | Immersion in DMEM/F12 medium supplemented with FBS-free, M-M1S, penicillin, streptomycin, and fungizone | High-rotation drill for crown cutting and curettage | Scalpel explantation technique and enzymatic digestion with collagenase type I and dispase | Immersion of cells in serum-free xenograft medium. Incubation at 37°C in a humid atmosphere with 4.7% CO₂ up to 80% confluence | Transfer of cells to medium containing DMSO-free medium and store at −80°C | 1–3 mon | Water bath at 37°C |

RCT, randomized clinical trial; CCT, controlled clinical trial; P, permanent; D, deciduous. MEM, modified Eagle’s essential medium; FCS, fetal calf serum; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; DMEM, Dulbecco’s modified Eagle’s essential medium.
Cryopreservation of dental pulp stem cells

Table 2 shows the 3 main cryopreservation methods observed in this study. Prior to the cryopreservation process, all samples in the eligible studies were immersed in DMSO for cryoprotection [14-17,21-37]. Regarding the cryopreservation process itself, the studies by Perry et al. [34], Woods et al. [36], Temmerman et al. [35], Gioventu et al. [23], Ji et al. [16], Lindemann et al. [29], Lee et al. [26], Malekfar et al. [30], Huynh et al. [14], and Mochizuki and Nakahara [31] employed programmable freezing with a cooling rate of −1°C/min to −80°C or −85°C and subsequent transfer to liquid nitrogen for storage. Eight other studies also employed programmable freezing, but with the addition of pauses at set temperatures to promote osmotic balance and reduce the risk of cell lysis. Of these studies, Lee et al. [27], Abedini et al. [21] and Lee et al. [26] employed a fixed cooling rate of −0.5°C/min starting at −5°C, maintained for 15 minutes, with subsequent cooling to −30°C or −35°C and transfer to medium storage at −150°C or −152°C. The work of Han et al. [24] had a greater variation in the cooling rate, as the samples were kept at 1°C for 30 minutes with cooling at −2°C/min to −9°C for 5 minutes, followed by subsequent cooling at −0.3°C/min to −40°C and at −10°C/min to −140°C with transfer to liquid nitrogen for storage. However, the studies by Papaccio et al. [33], Zhang et al. [37], and Munevar et al. [32] performed direct immersion in liquid nitrogen without programmable freezing.

The storage time of the samples ranged from 1 week to 2 years. Defrosting was performed in a 37°C water bath in all studies [15-17,21-37]. To assess the cryopreservation process, several parameters were measured to confirm stem cell characterization in the various studies. Flow cytometry or immunofluorescence were used to evaluate stem cell surface markers in all selected studies. Other parameters such as differentiation potential, cell proliferation, cell activity, and karyotype analysis were considered by the authors to verify the viability of the mesenchymal stem cells post-thawing (Figure 2).

Table 2. Cryopreservation methods

| Method 1                        | Method 2                                               | Method 3                                               |
|--------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| Direct immersion in liquid nitrogen | Programmable freezing with the cooling rate at −1°C/min to −80°C or −85°C and subsequent transfer to liquid nitrogen for storage | Programmable freezing with the addition of breaks at set temperatures. A fixed cooling rate of −0.5°C/min starting at −5°C, maintained for 15 minutes, further cooled to −30°C or −35°C and then transferred to storage medium at −150°C or −152°C |
| Papaccio et al. [33], Zhang et al. [37], and Munévar et al. [32] | Perry et al. [34], Woods et al. [36], Temmerman et al. [35], Gioventu et al. [23], Ji et al. [16], Lindemann et al. [29], Lee et al. [26], Malekfar et al. [30], Huynh et al. [14], and Mochizuki and Nakahara [31] | Lee et al. [27], Abedini et al. [21], Chen et al. [22], Lee et al. [28], Antunes [17], Kumar et al. [25], Alsulaimani et al. [15], and Han et al. [24] |
Figure 2. Parameters assessed post-thawing by the selected studies in this systematic review.

Regarding the results obtained after the cryopreservation process, the articles by Perry et al. [34], Lee et al. [27], Chen et al. [22], Lee et al. [28], Antunes [17], Munevar et al. [32], Alsulaimani et al. [15], Malekfar et al. [30], Han et al. [24], and Huynh et al. [14] showed cell viability rates ranging from 56.2% to 100% for cryopreserved cells and from 80 to 100% for fresh-cultured cells. The lowest viability rates were presented by Munevar et al. [32], who compared 2 cryopreservation methods used in 2006 and 2007 that are not as efficient as the current ones. However, all of the included articles showed that cryopreserved cells maintained their fibroblastic shape and their differentiation capacity similar to the control group of freshly maintained culture cells [14-17, 21-37].

**Risk of bias appraisal**

As seen in Table 3, 20 studies showed a low risk of bias [14,15,17,21-37] and 1 study showed a moderate risk of bias [16]. No studies were classified as having a high risk of bias. Five studies [16,25,32-34] did not present a negative control group and one study did not make this comparison clear [37]. The study by Ji et al. [16] did not show how multiple measurements of the outcome were made. Question 9 of the Joanna Briggs Institute Critical Appraisal Checklist for Quasi-Experimental Studies was considered not applicable for all studies due to the heterogeneity of the data presented.

**DISCUSSION**

To prolong the possibility of using dental pulp stromal mesenchymal cells for regenerative procedures, a well-established and standardized cryopreservation process is required to achieve a higher cell viability rate [38]. The process of cryopreservation of dental pulp mesenchymal cells includes some essential steps, such as dental element disinfection, dental pulp extraction, cell isolation, cell proliferation, cryopreservation, time setting, and thawing [38,39].

As proposed by Hilkens et al. [38], the first step in the cryopreservation process after obtaining a dental element is disinfection. Many studies presented a disinfection process involving the use of successive immersions of the dental element in phosphate-buffered saline (PBS) in combination with an antibiotic solution [14,16,24,29,31,33,35,37]. These...
Cryopreservation of dental pulp stem cells

Table 3. Risk of bias assessed through the Joanna Briggs Institute Critical Appraisal Checklist for Quasi-Experimental Studies

| Studies                          | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | % Yes | Risk  |
|----------------------------------|----|----|----|----|----|----|----|----|----|-------|-------|
| Mochizuki and Nakahara [31]      | Y  | Y  | Y  | Y  | Y  | Y  | Y  | NA | Y  | 88.88 | Low   |
| Huynh et al. [14]                | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Han et al. [24]                  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Malekfar et al. [30]             | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Alsulaimani et al. [15]          | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Minevar et al. [32]              | Y  | Y  | N  | Y  | Y  | Y  | Y  | Y  | Y  | 77.77 | Low   |
| Lee et al. [26]                  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Kumar et al. [25]                | Y  | Y  | Y  | N  | Y  | Y  | Y  | Y  | Y  | 77.77 | Low   |
| Lindemann et al. [29]            | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| JI et al. [16]                   | Y  | Y  | N  | UC | Y  | Y  | Y  | Y  | Y  | 66.66 | Moderate |
| Antunes et al. [17]              | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Lee et al. [28]                  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Gioventu et al. [23]             | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Chen et al. [22]                 | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Abedini et al. [21]              | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Temmerman et al. [35]            | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| LEE et al. [27]                  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Woods et al. [36]                | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 88.88 | Low   |
| Perry et al. [34]                | Y  | Y  | Y  | N  | Y  | Y  | Y  | Y  | Y  | 77.77 | Low   |
| Zhang et al. [37]                | Y  | Y  | UC | Y  | Y  | Y  | Y  | Y  | Y  | 77.77 | Low   |
| Papaccio et al. [33]             | Y  | Y  | Y  | N  | Y  | Y  | Y  | Y  | Y  | 77.77 | Low   |

Y, yes; N, no; UC, unclear; NA, not applicable.

Studies showed greater concern regarding the initial stage of access to the dental element, because the dental pulp framework may come into contact with it during cutting and, if not properly disinfected, it may contaminate the resident cells and induce complete loss of that material [13,38]. Papaccio et al. [33] and Alsulaimani et al. [15] corroborated the use of chlorhexidine gluconate gel in combination with PBS as a potent dental surface disinfectant. Perry et al. [34] and Woods et al. [36] pointed to the use of iodopovidone and sodium thiosulfate in combination with PBS as sufficient to obtain adequate disinfection. Thus, PBS seems to be an essential element for disinfection, but alone, it is insufficient for the full accomplishment of this task and therefore requires combination with another component. The study by Gioventu et al. [23] was unprecedented in using sterile RPMI 1640 for cleansing and disinfection of the dental element, which would be a new method for this step.

An important point to be considered is the period of time between the tooth extraction and cell isolation and/or cryopreservation. Some studies recommended the use of Hank's balanced salt solution or cell culture medium during transportation [34,40] or saline solution with the addition of antibiotics to prevent bacterial infections [14]. There was a time-dependent reduction in the number of pulp stem cells that could be isolated from extracted teeth as the length of time of storage increased [41], and they could remain viable for up to overnight or 12 hours [14].

The extraction of dental pulp is a critical moment because it can induce contamination if not performed properly, and the sterilization of the cutting materials helps to protect against this outcome [13]. Most of studies used a high-speed diamond drill or diamond disk for cutting the dental element at the cementoenamel junction. In general, after cutting, regardless of its shape, the pulp was excised by curettage with endodontic files or periodontal curettes. However, the details of this step at the end of the process do not seem to have an important impact. For instance, Temmerman et al. [35] compared 3 cuts at different heights of the roots of the teeth to verify whether there the region where the mesenchymal cells resided influenced their morphological characteristics and differentiation capacity beyond
alterations in the cryopreservation process. Regardless of the region of the cut for pulp removal, the morphological characteristics, differentiation capacity, proliferation rate, and cell viability remained the same, whether in fresh or cryopreserved cultures; only the quantity of cells was variable. Complementarily, Abedini et al. [21], with cuts made along the long axis of the dental element, showed no difference in morphology, differentiation capacity, cell viability, and proliferation rate after cryopreservation, and the results were similar to those of the other studies selected. In addition, Gioventù et al. [23] made only 1 cavity at the height of the cementoenamel junction with a Nd:YAG laser, which could be an alternative to cutting using high rotation instruments.

Cellular isolation is the stage in which mesenchymal cells are detached from each other after pulp tissue removal [13]. It was found that 38% (n = 8) of the selected studies used the enzyme digestion technique, 28% (n = 6) the explantation technique, and 34% (n = 7) used both techniques, showing a lack of consensus on which method is the most appropriate. Studies by Temmerman et al. [35], Abedini et al. [21], Li et al. [16], Kumar et al. [25], Lee et al. [28], and Huynh et al. [14] adopted only the explantation technique for cell isolation. This decision could be supported by the studies by Salehinejad et al. [42] and Hilkens et al. [38], who claimed that stromal mesenchymal cells obtained from explant-promoted cell isolation are purer, more heterogeneous, suffer less enzymatic damage, and have a higher proliferation rate than those obtained by enzymatic digestion. Other studies presented the use of enzymatic digestion containing collagenase type I and dispase in different concentrations, and Woods et al. [36] used type I and II collagenases associated with thermolysin bound to a neutral protein in place of dispase.

In order for dental pulp mesenchymal cells to be sufficient to carry out the experimental studies, cell proliferation in the culture medium is required [13]. Essentially, the selected studies pointed to the need to immerse these cells in a nutritive culture medium, either Dulbecco’s modified Eagle’s essential medium (DMEM), minimal essential alpha medium (alpha-MEM) or Mesencult, supplemented with a mixture of antibiotics and fetal bovine or calf serum (FBS or FCS, respectively). Studies prior to 2010 used FCS as a nutrient solution [33,35,37] and, in some cases, the Mesencult medium for culture [34,36]. Studies from 2010 onwards used FBS as a nutrient factor and DMEM medium for cell culture combined with a mixture of antibiotics, which is variable in types and quantities, although a combination of penicillin and streptomycin (Pen-Strep) was the most commonly used [15,16,22-24,28-30,32-35]. In general, there was a unanimous consensus in the selected papers that, after immersion in the growth solution, the cultures should be stored in a humid incubator at 37°C containing 5% CO₂ for a variable time.

The cryopreservation process itself involves freezing the cells for later use and should only occur after immersion in cryoprotectant to prevent the formation of ice crystals within the cell, which induce plasma membrane lysis and consequent reduction of viability [1,13]. Except for the study by Mochizuki and Nakahara [31], which explored the formulation of serum-free compounds for cryopreservation, agreement exists on the use of 5% or 10% DMSO as a cryoprotectant for mesenchymal cell cultures. In addition, gradual freezing of cultures with a fixed cooling rate, commonly −1°C/min, has been used in studies from 2008 onwards, and was associated with a noticeable increase in the cell viability rate [14,16,23,26,29-31,34-36]. Papaccio et al. [33], Zhang et al. [37], and Munear et al. [32] immersed the cells in liquid nitrogen immediately after cryoprotectant application and presented very low cell viability rates compared to the control group of fresh cells in culture,
which proves the need for gradual freezing. There is a consensus in the selected studies that cell cultures should be immersed in liquid nitrogen at −196°C after gradual freezing in a programmable freezer. The temperatures employed in the cryopreservation process are still debatable and explicit in the studies. There are methodologies in which the temperature has been gradually reduced and set for a few hours at some timepoints [15,17,21,22,24,25,27,28] and others that only used a fixed cooling rate until an ambient temperature of the culture at −80/−85°C, followed by storage in liquid nitrogen at −196°C [14,16,26,29-31,34-36].

Regarding the results of the selected studies, Papaccio et al. [33], Zhang et al. [37], and Munevar et al. [32] showed lower cell viability and recovery rates, possibly due to the direct storage of cell culture in liquid nitrogen without gradual freezing. Regarding the cell viability rate of the cryopreserved cell group after thawing, Perry et al. [34] reported a rate of 89.5%, Lee et al. [27] reported a rate of 73%, Chen et al. [22] reported a rate of 100%, Lee et al. [28] reported a rate of 73.2%, Antunes [17] reported a rate of 97.7%, Alsulaimani et al. [15] reported a rate of 85%, Malekfär et al. [30] reported a rate of 60%, Han et al. [24] reported a rate of 81.2% and Huynh et al. [14] reported a rate of 79.7%. These cell viability rates are considered high relative to those obtained in studies in which freezing was not gradual [32,33,37]. All studies analyzed herein found that the morphophysiological characteristics, cell proliferation rate, and differentiation capacity of dental pulp mesenchymal cells were not altered after cryopreservation compared to the control group. Thus, they were positive for CD73, CD90 and CD105 surface markers, negative for CD45 and CD34, and had the ability to differentiate into chondrocytes, osteocytes, adipocytes, and fibroblast-like cells [14-17,21-37].

CONCLUSIONS

According to this systematic review, the cryopreservation process involves 6 steps: dental element disinfection, pulp extraction, cell isolation, cell proliferation, cryopreservation itself, and thawing. Most studies showed a high rate of cell viability after thawing and a high rate of cell proliferation in both primary and permanent teeth using DMSO, programmable freezing, and storage in liquid nitrogen. In addition, none of the methods employed in the selected studies affected cell differentiation capacity or the fibroblastic morphology of the mesenchymal cells.

REFERENCES

1. Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Xu GT, Liang A, Liu S. Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells 2015;33:627-638. PUBMED | CROSSREF
2. Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med 2015;9:1205-1216. PUBMED | CROSSREF
3. Kawashima N, Noda S, Yamamoto M, Okiji T. Properties of dental pulp-derived mesenchymal stem cells and the effects of culture conditions. J Endod 2017;43:531-534. PUBMED | CROSSREF
4. Mayo V, Sawatari Y, Huang CY, Garcia-Godoy F. Neural crest-derived dental stem cells--where we are and where we are going. J Dent 2014;42:1043-1051. PUBMED | CROSSREF
5. Yamamoto A, Sakai K, Matsubara K, Kano F, Ueda M. Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury. Neurosci Res 2014;78:16-20.

6. Wang J, Ma H, Jin X, Hu J, Liu X, Ni L, Ma PX. The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells. Biomaterials 2011;32:7822-7830.

7. Nakashima M, Iohara K. Mobilized dental pulp stem cells for pulp regeneration: initiation of clinical trial. J Endod 2014;40:526-532.

8. Rosa V, Zhang Z, Grande RH, Nör JE. Dental pulp tissue engineering in full-length human root canals. J Dent Res 2013;92:970-975.

9. Saghiri MA, Asatourian A, Sorenson CM, Sheibani N. Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. J Endod 2015;41:797-803.

10. Chamieh F, Collignon AM, Coyac BR, Lesieur J, Ribes S, Sadoine J, Llorens A, Nicoletti A, Letourneur D, Colombier ML, Nazhat SN, Bouchard P, Chassagne C, Rochefort GY. Accelerated craniofacial bone regeneration through dense collagen gel scaffolds seeded with dental pulp stem cells. Sci Rep 2016;6:38814.

11. Estrela C, Alencar AH, Kitten GT, Vencio EF, Gava E. Mesenchymal stem cells in the dental tissues: perspectives for tissue regeneration. Braz Dent J 2011;22:91-98.

12. Martin-Piedra MA, Garzon I, Oliveira AC, Alfonso-Rodriguez CA, Carriel V, Sciorti G, Alaminos M. Cell viability and proliferation capability of long-term human dental pulp stem cell cultures. Cytotherapy 2014;16:266-277.

13. Pegg DE. Principles of cryopreservation. Methods Mol Biol 2007;368:39-57.

14. Huynh NC, Le SH, Doan VN, Ngo LT, Tran HL. Simplified conditions for storing and cryopreservation of dental pulp stem cells. Arch Oral Biol 2017;84:74-81.

15. Alsulaimani RS, Ajlan SA, Aldahmash AM, Alnabaheen MS, Ashri NY. Isolation of dental pulp stem cells from a single donor and characterization of their ability to differentiate after 2 years of cryopreservation. Saudi Med J 2016;37:551-560.

16. Ji EH, Song JS, Kim SO, Jeon M, Choi BJ, Lee JH. Viability of pulp stromal cells in cryopreserved deciduous teeth. Cell Tissue Bank 2014;15:67-74.

17. Antunes FG. Atividade biológica de células-tronco da polpa de dentes deciduos humanos submetidas à criopreservação. Natal: Universidade Federal do Rio Grande do Norte; 2013.

18. Jesus AA, Soares MB, Soares AP, Nogueira RC, Guimarães ET, Araújo TM, Santos RR. Coleta e cultura de células-tronco obtidas da polpa de dentes deciduos: técnica e relato de caso clínico. Dental Press J Orthod 2011;16:111-118.

19. Tufanaru C, Munn Z, Aromataris E, Campbell J, Hopp L. Chapter 3: Systematic reviews of effectiveness. In: Aromataris E, Munn Z, editors. Joanna Briggs Institute reviewer’s manual. Adelaide: JBI; 2017.

20. Lima IF, de Andrade Vieira W, de Macedo Bernardino I, Costa PA, Lima AP, Pithon MM, Paranhos LR. Influence of reminder therapy for controlling bacterial plaque in patients undergoing orthodontic treatment: A systematic review and meta-analysis. Angle Orthod 2018;88:483-493.

21. Abedini S, Kaku M, Kawata T, Koseki H, Kojima S, Sumi H, Motokawa M, Fujita T, Ohtani J, Owada N, Tanne K. Effects of cryopreservation with a newly-developed magnetic field programmed freezer on periodontal ligament cells and pulp tissues. Cryobiology 2011;62:181-187.

22. Chen YK, Huang AH, Chan AW, Shieh TY, Lin LM. Human dental pulp stem cells derived from different cryopreservation methods of human dental pulp tissues of diseased teeth. J Oral Pathol Med 2011;40:793-800.
23. Gioventù S, Andriolo G, Bonino F, Frasca S, Lazzari L, Montelatici E, Santoro F, Rebulla P. A novel method for banking dental pulp stem cells. Transfus Apheresis Sci 2012;47:199-206.

24. Han YJ, Kang YH, Shivakumar SB, Bhatari D, Son YB, Choi YH, Park WJ, Byun IH, Rho GJ, Park BW. Stem cells from cryopreserved human dental pulp tissues sequentially differentiate into definitive endoderm and hepatocyte-like cells in vitro. Int J Med Sci 2017;14:1418-1429.

25. Kumar A, Bhattacharyya S, Rattan V. Effect of uncontrolled freezing on biological characteristics of human dental pulp stem cells. Cell Tissue Bank 2015;16:513-522.

26. Lee HS, Jeon M, Kim SO, Kim SH, Lee JH, Ahn SJ, Shin Y, Song JS. Characteristics of stem cells from human exfoliated deciduous teeth (SHED) from intact cryopreserved deciduous teeth. Cryobiology 2015;71:374-383.

27. Lee SY, Chiang PC, Tsai YH, Tsai SY, Jeng JH, Kawata T, Huang HM. Effects of cryopreservation of intact teeth on the isolated dental pulp stem cells. J Endod 2010;36:1336-1340.

28. Lee SY, Huang GW, Shiang JN, Huang YH, Jeng JH, Kuo TF, Yang JC, Yang WC. Magnetic cryopreservation for dental pulp stem cells. Cells Tissues Organs 2012;196:23-33.

29. Lindemann D, Werle SB, Steffens D, García-Godoy F, Franke P, Casagrande L. Effects of cryopreservation on the characteristics of dental pulp stem cells of intact deciduous teeth. Arch Oral Biol 2014;59:970-976.

30. Malekfar A, Vali KS, Kanafi MM, Bhonde RR. Isolation and characterization of human dental pulp stem cells from cryopreserved pulp tissues obtained from teeth with irreversible pulpitis. J Endod 2016;42:76-81.

31. Mochizuki M, Nakahara T. Establishment of xenogeneic serum-free culture methods for handling human dental pulp stem cells using clinically oriented in-vitro and in-vivo conditions. Stem Cell Res Ther 2018;9:25.

32. Munévar JC, Gutiérrez N, Jiménez NT, Lafaurie GI. Evaluation of two human dental pulp stem cell cryopreservation methods. Acta Odontol Latinoam 2015;28:114-121.

33. Papaccio G, Graziano A, d’Aquino R, Graziano MF, Pirozzi G, Menditti D, De Rosa A, Carinci F, Laino G. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair. J Cell Physiol 2006;208:319-325.

34. Perry BC, Zhou D, Wu X, Yang FC, Byers MA, Chu TM, Hockema JJ, Woods El, Goebel WS. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. Tissue Eng Part C Methods 2008;14:149-156.

35. Temmerman L, Beele H, Dermaut LR, Van Maele G, De Pauw GA. Influence of cryopreservation on the pulp tissue of immature third molars in vitro. Cell Tissue Bank 2010;11:281-289.

36. Woods El, Perry BC, Hockema JJ, Larson L, Zhou D, Goebel WS. Optimized cryopreservation method for human dental pulp-derived stem cells and their tissues of origin for banking and clinical use. Cryobiology 2009;59:150-157.

37. Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Eng 2006;12:2813-2823.

38. Hilkens P, Driessen RB, Wolfs E, Gervois P, Vanganswinkel T, Ratajczak J, Dillen Y, Bronckaers A, Lambrichts I. Cryopreservation and Banking of Dental Stem Cells. Adv Exp Med Biol 2016;951:199-235.

39. Oh YH, Che ZM, Hong JC, Lee El, Lee SI, Kim J. Cryopreservation of human teeth for future organization of a tooth bank—a preliminary study. Cryobiology 2005;51:322-329.

40. Khademi AA, Saei S, Mohajeri MR, Mirkheshti N, Ghassami F, Torabi nia N, Alavi SA. A new storage medium for an avulsed tooth. J Contemp Dent Pract 2008;9:25-32.
41. Eubanks EJ, Tarle SA, Kaigler D. Tooth storage, dental pulp stem cell isolation, and clinical scale expansion without animal serum. J Endod 2014;40:652-657.
   PUBMED | CROSSREF

42. Salehinejad P, Alietheen NB, Ali AM, Omar AR, Mohit M, Janzamin E, Samani FS, Torshizi Z, Nematollahi-Mahani SN. Comparison of different methods for the isolation of mesenchymal stem cells from human umbilical cord Wharton's jelly. In Vitro Cell Dev Biol Anim 2012;48:75-83.
   PUBMED | CROSSREF