

Dental pulp stem cells and banking of teeth as a lifesaving therapeutic vista

SUKUMARAN ANIL^{1,2,*}; RAMYA RAMADOSS³; NEBU G. THOMAS⁴; JASMIN M. GEORGE⁴; VISHNUPRIYA K. SWEETY⁴

¹ Oral Health Institute, Department of Dentistry, Hamad Medical Corporation, Doha, Qatar

² College of Dental Medicine, Qatar University, Doha, Qatar

³ Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India

⁴ Pushpagiri College of Dental Sciences, Medicity, Perumthuruthy, India

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Abstract: Exfoliated deciduous or an extracted healthy adult tooth can be used to harvest, process, and cryogenically preserve dental pulp stem cells. Future stem cell-based regenerative medicine methods could benefit significantly from these mesenchymal stem cells. Teeth serve as a substantial source of mesenchymal stem cells, otherwise disposed of as medical waste. Care should be taken to store this treasure trove of stem cells. Collective responsibility of patients, dentists, and physicians is necessary to ensure that this valuable resource is not wasted and that every possible dental pulp stem cell is available for use in the future. The dental pulp stem cells (DPSC) inside teeth represent a significant future source of stem cells for regenerative medicine procedures. This review describes the ontogeny, the laboratory processing and collection, and isolation methods of DPSC. This review also discusses currently available stem cell banking facilities and their potential use in regenerative medicine procedures in dental and general medical applications in the future.

Introduction

Stem cell therapy and research have grown leaps and bounds in the last few decades. The exceptional scope of stem cells often necessitates and enhances the sources that generate them. Amidst diverse established stem cell sources, dental stem cells have been more compelling due to the ease of availability and manifest high plasticity and potentiality (Ferro *et al.*, 2012; Potdar and Jethmalani, 2015). Because of their regenerative and multilineage differentiation potential, it is possible to isolate stem cells from many oral tissues, including the periodontal ligament, dental pulp, dental follicle, and oral mucosa. Dental stem cells (DSC) resemble mesenchymal stem cells (MSC). Most stem cells that are widely used in regenerative medicine can be effectively derived from DSC due to their heterogeneity and mesenchymal properties (Chalisserry *et al.*, 2017).

Human dental pulp stem cells have been extensively explored for their multitudinous therapeutic possibilities (Ledesma-Martinez *et al.*, 2016). Dental pulp stem cells originate from the neural crest cells, which makes them

highly plastic than mesodermal derived bone marrow stem cells. In addition, neural crest cells have an inherent pluripotentiality to differentiate into species-specific tissue developmental patterns (Zhai *et al.*, 2019). DSCs thus are highly proliferative and can differentiate into various cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes (Galler *et al.*, 2014).

Stem cell banking refers to the process of collecting, isolating, and preserving stem cells for use in regenerative medicine and therapy. Cryopreservation is a process of sustaining the viability of cells and tissues by freezing and storing them at sub-zero temperatures when biochemical reactions do not occur. This concise review highlights the main strategies related to the use of dental pulp stem cells, their characterization, storage, and stem cell and tooth banking.

Dental pulp stem cells (DPSCs)

DPSCs can be harvested from either extracted teeth or naturally exfoliated deciduous teeth. Exfoliated anterior deciduous teeth with root resorption of less than two-thirds are preferred for DPSC extraction. There are several sources of DSCs, namely the third molar, premolars, supernumerary, permanent, and deciduous teeth (Miura *et al.*, 2003; Huang *et al.*, 2008; Wang *et al.*, 2012). DPSCs have the advantage of involving a

*Address correspondence to: Sukumaran Anil, drsanil@gmail.com

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minimally invasive process for retrieval and a capacity for long-term cryopreservation. DPSCs can be differentiated by modulating growth factors, transcriptional factors, extracellular matrix proteins, and receptor molecules into different cell types such as odontoblast, osteoblast, chondrocyte, cardiomyocytes, neuron cells, adipocyte, corneal epithelial cell, melanoma cell, and insulin-secreting Beta cells (Fig. 1) (Gronthos *et al.*, 2000; Yu *et al.*, 2010).

Therapeutic applications of dental pulp stem cells

DPSCs were found to be promising in many regenerative therapies such as: dental pulp regeneration (Iohara *et al.*, 2013), bone tissue engineering (Akkouch *et al.*, 2014), neurology (Ahmed Nel *et al.*, 2016), angiogenesis/vasculogenesis (Gandia *et al.*, 2008), endocrinology (Carnevale *et al.*, 2013), and healing (Hirose *et al.*, 2016) (Fig. 1). DPSCs are a unique type of mesenchymal stem cells of neural crest origin and express pluripotent stem cell markers such as Oct4, Nanog, Sox, and Klf4 with potent neurogenic and more immunosuppressive activities (Kerkis *et al.*, 2006; Mead *et al.*, 2014). By modulating DPSCs with growth factors, transcriptional factors, extracellular matrix proteins, and receptor molecules, they can be differentiated into numerous cell types, such as odontoblast, osteoblast, chondrocyte, cardiomyocytes, neuron cells, adipocyte, corneal epithelial cell, melanoma cell, and insulin-secreting beta cells (Potdar and Jethmalani, 2015).

Dental pulp stem cells (DPSCs) secrete a dentin matrix and differentiate into odontoblasts by stimulating specific cytokines or growth factors (Couble *et al.*, 2000). Stem cell-based dental pulp regeneration can maintain tooth vitality and regenerate a complete dental pulp containing blood vessels, nerves, and newly formed dentin in both ectopic

and *in situ* regeneration (Xuan *et al.*, 2018; Meng *et al.*, 2020). Furthermore, the possibility of regenerating whole teeth with functional properties by using scaffold and DPSCs cells is being carried out successfully (Huang *et al.*, 2021). Hence, stem cell-mediated root regeneration may be an alternative to replacing teeth (Meng *et al.*, 2020).

Dental stem cells are a potential source for the engineering of bone tissue, and the osteogenic differentiation capacity of DPSCs has been demonstrated both *in vitro* and *in vivo* (Akkouch *et al.*, 2014; Yasui *et al.*, 2017). Dexamethasone, L-ascorbic acid, and β -glycerol phosphate supplementation have been shown to extensively induce osteogenic differentiation. Due to their osteogenic potential, DPSCs have been combined with 3D polymeric scaffolds to generate bone-like hard tissues (Hao *et al.*, 2022; Xie *et al.*, 2022).

DPSC can stimulate long-term regenerations of nerves in the damaged spinal cord and can be effectively used to manage neurodegenerative diseases, including Alzheimer's (Ahmed Nel *et al.*, 2016). Compared to MSCs, DPSCs exhibit a higher proliferation rate, higher expression of trophic factors, potent neuroprotective effects, and neuro-supportive properties *in vitro* and *in vivo*, demonstrating their potential for use in stroke treatment (Nito *et al.*, 2022) and the treatment of pathologies caused by insufficient angiogenesis. The potential of DPSCs to treat myocardial infarction and ischemia has been confirmed (Loo *et al.*, 2014; Augustine *et al.*, 2021). DPSC is an excellent source of stem cells for repairing infarcted myocardium and regenerative therapy for ischemic heart disease (Gandia *et al.*, 2008).

DPSCs have been differentiated to generate hepatocyte-like cells with acquired hepatocyte functions, such as glycogen storage and urea production (Ishkitiev *et al.*, 2012). Chen *et al.* (2016) performed hepatic differentiation of

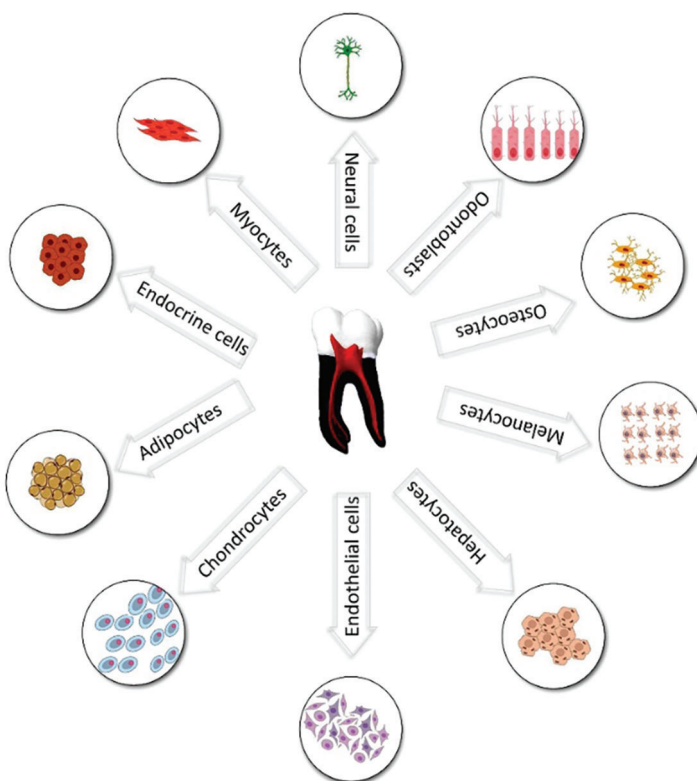


FIGURE 1. Multiple differentiation potential of dental pulp stem cells.

DPSCs using cryopreserved dental pulp tissue. DPSCs have the potential to differentiate into pancreatic cell lineage resembling islet-like cell aggregates. Human DPSCs express genes related to pancreatic β -cell development and function under appropriate stimuli, including insulin, pancreatic, and duodenal homeobox-1 (Carnevale *et al.*, 2013). Transplantation of islet-like cell clusters derived from human DPSC in diabetic mice showed reversal of hyperglycemia (Kanafi *et al.*, 2013). The current research is promising, and DPSCs could be used as an autologous stem cell therapy for diabetes (Hata *et al.*, 2020).

Tooth collection and storage

Vital deciduous and permanent teeth without any infections serve as a viable source of dental pulp stem cells (Perry *et al.*, 2008; Temmerman *et al.*, 2010). Chen *et al.* (2011) showed that the DPSCs could be retrieved from infected vital human teeth encompassing periodontitis, supernumerary teeth, inflamed malpositioned teeth, root resorption, and pericoronitis, as well as fractured teeth. Several other studies also confirmed that infected vital teeth could be a source of DPSCs (Pereira *et al.*, 2012; Malekfar *et al.*, 2016; Werle *et al.*, 2016; Tsai *et al.*, 2017). Greater root resorption might have a negative impact on the viability of the stem cells (Bernardi *et al.*, 2011). Challenge lies in the preservation of the cells from degradation, which begins immediately after extraction or exfoliation. In addition, teeth with diseases such as periapical abscesses, tumors, or cysts must not be considered for stem cell recovery (Cordeiro *et al.*, 2008). Most tooth banking services recommend and prefer professionally extracted deciduous teeth when they become mobile over naturally exfoliated ones. This is because deciduous teeth that become very mobile naturally through trauma, or disease, often have a compromised blood supply and may not be suitable for DPSC recovery. Harvested teeth should be washed with bactericidal solutions to minimize contamination. The tooth is stored at 4°C in phosphate buffer saline (PBS) during laboratory transfer to avoid dehydration and necrosis. The stem cell isolation should be performed within 120 h after extraction. Perry *et al.* (2008) demonstrated that DPSC cultures were established from 80% of extracted human third molars processed within 24 h of extraction. DPSC cultures can be initiated for at least 120 h post-extraction if teeth are stored at 48°C in a variety of collection or transport media. A longer time frame may lower post-thawing cell recovery efficiency. Exfoliated anterior deciduous teeth with less than two-thirds root resorption are preferred for DSC extraction. Increased root resorption reduces the stem cell viability from human exfoliated deciduous teeth (SHED). Dental pulp tissues from wisdom teeth and first premolars extracted for orthodontic purposes are the common sources of DPSC in adults. DPSC harvesting and isolation efficiency is better from an immature permanent tooth with incomplete root formation (Bernardi *et al.*, 2011).

The exfoliated or extracted tooth shows no signs of post-removal bleeding, and the pulp appears greyish. It indicates a necrotic pulp tissue and hence is not ideal for DPSC retrieval. However, some services accept naturally

exfoliated or home-extracted deciduous teeth. The requirement for acceptance is the presence of bleeding after extraction/exfoliation as evidence of a vital blood supply (Zeitlin, 2020). BioEden® (Austin, TX) provides a safe home collection kit to store and transport the tooth to the tooth banking facility.

Once the tooth is extracted or exfoliated, it must be preserved from the naturally occurring degradation process. Keeping the tooth hydrated and preventing necrosis of the pulp tissue are essential concerns. The tooth must be immediately placed in a vial with a transport medium. The choice of the transport medium is a matter of preference of the banking facility. However, immersing it in water is not recommended. An isotonic solution mimicking physiological conditions would be ideal (Moazami *et al.*, 2012). PBS and Hanks buffered saline solution (HBSS) supplemented with nutrients are widely used solutions for transport. Save-a-Tooth™ kit contains HBSS as storage media (Zeitlin, 2020). BioEden® has pasteurized bovine milk as its transport medium in its home collection kit. It has been a transport medium for avulsed teeth over the years because of its biocompatibility and neutral pH (Andersson *et al.*, 2012). The maximum permissible time from harvesting to processing at the storage facility should not exceed 40 h, beyond which the tooth may lose its viability of the pulp tissue.

Once the vital tooth is transported to the laboratory, it is cleaned and disinfected, and the crown of the tooth is separated to reach the pulp chamber and pulp tissue. The separation of the crown from the root(s) must be done carefully to avoid damage to the soft tissue within. Diamond burs with coolants or special forceps can be used in the cemento-enamel junction at the cervical third of the tooth to open the pulp chamber. Alternately, the teeth could be mechanically fractured and the dental pulp gently isolated with forceps, without cutting or drilling. This could prevent any damaging effects on the viability of DPSCs (Hilkens *et al.*, 2013). Next, the dental pulp tissue is separated from the root canal walls and the pulp chamber. It is easier to isolate the dental pulp tissue from the pulp chamber in teeth with a large opening at the apical foramen region, like resorbed deciduous teeth or incompletely formed roots of permanent teeth. These large open areas pave a pathway through which the pulp tissue can be retrieved without splitting the tooth. This is a much safer technique to obtain the pulp tissue without damaging it. After the pulp tissue is accessed and retrieved, it is cut into numerous small pieces for further expansion (Pilbauerova and Suchanek, 2018).

Isolation of dental pulp stem cells

Stem cells account for 1% of the total pulp cell population; hence efficient isolation and expansion are imperative (Smith, 2005). Dental pulp retrieval is done by gently separating the root region to reach the pulp chamber. The pulp tissue can be retrieved either through the root apex of the resorbed roots of deciduous teeth or through the immature open apex of the permanent teeth. The pulp tissue is harvested with a sterile barbed broach or by slicing the dentinal wall with the diamond disc in the presence of a coolant (Fig. 2). The other approach is opening the pulp chamber by splitting the tooth at the cemento-enamel



FIGURE 2. A schematic of the dental pulp stem cell isolation and storage processes.

junction. The retrieved pulp tissue is minced into small pieces and stored (Spath *et al.*, 2010; Ferro *et al.*, 2012).

The DPSCs can be isolated either by the enzymatic digestion (ED) technique or the outgrowth from tissue explants (OG) technique, also known as the spontaneous growth technique (Gronthos *et al.*, 2000; Spath *et al.*, 2010). Researchers have tested both techniques to evaluate the efficiency of cell proliferation rate or unchanged morphological and phenotypic properties of isolated stem cells. Some researchers have reported that the stem cells isolated by the OG technique have a lower proliferation rate and weaker stem cell marker expression (Karamzadeh *et al.*, 2012). Others found that DPSCs isolated by the ED technique displayed a higher proliferation rate, differentiation, and expressed other surface markers when compared with the cells isolated by the OG technique. In contrast, dental pulp immature stem cells (IDPSCs) from deciduous teeth isolated using the OG method showed that the cell culture promoted the selective proliferation of IDPSCs, preventing premature differentiation (Kerkis *et al.*, 2006).

Enzymatic digestion technique

In this technique, the minced pulp tissue pieces are submerged in a cocktail of two or more enzymes. ED allows segregation of single-cell suspensions by enzymatic digestion of minced primary pulp tissues. Single cells in suspension are obtained by physically straining the digested tissue, and the cells are then allowed to grow in culture media. There are several digestion protocols developed for pulp tissue isolation. However, the combination of 3 mg/mL collagenase type I

and 4 mg/mL dispase has been used widely (Rodas-Junco and Villicana, 2017). Other enzyme combinations and mechanical isolation of DPSCs could be assessed for the increase in survival of the cells during the isolation process. Successful isolation with a blend was achieved with 0.1% collagenase IV in 0.25% trypsin EDTA and 5 mg/mL collagenase type II in 2.5 mg/mL dispase (Lucaciu *et al.*, 2015). A mechanical device, MACSTM dissociator (Miltenyi, Biotec), could be used to isolate the DPSCs mechanically.

Outgrowth from tissue explants technique

The OG technique is simple and fast and involves the direct placement of the minced pulp tissues, of about 1 to 2 mm³ size, into culture plates with a proliferation medium. This allows them to grow from the pulp tissue explants (Hilkens *et al.*, 2013). The culture plates are incubated at 37°C in 5% CO₂. The proliferation medium changes after outgrowths are observed, and the cells can be sub-cultured further (Karamzadeh *et al.*, 2012). Stem cells obtained at the end of the subculture from both techniques are assessed for their survival rates and expression of cell markers by running them through flow cytometric analysis. When the cells are deemed sterile and viable with the desired surface markers, they are ready for cryogenic preservation.

Purification of dental pulp stem cells

Purification of DPSC is pivotal for isolating highly regenerative cells. This requires the utility of specific cell markers. Markers LNGFR and THY-1 are routinely used to label MSCs as they can validate the phenotypic characters

(Yasui *et al.*, 2017). Cell selection is performed either with fluorescence or magnetic-activated cell sorting methods. In addition to the markers mentioned above, CD73, CD90, CD105, CD29, CD44, CD166, STRO-1, CD13, CD146, CD117, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, NANOG, Oct4, Sox2, and HLA-DR surface antigens are also used (Rodas-Junco and Villicana, 2017). The need for multiple markers for selection is due to the heterogeneous nature of the DSCs. To date, there is no single marker that can delineate the subpopulations. Hoechst dye has exhibited reasonable success in delineating DSCs with angiogenic potential; however, it is not advocated in therapeutic cell isolation due to its carcinogenic and growth-inhibiting properties.

Cryopreservation of dental pulp stem cells

Once stem cells have been extracted via enzyme digestion or tissue outgrowth, they are ready for cryopreservation to maintain their vitality, phenotypic stability, and differentiation capacity over the long term. This procedure is crucial as the differentiation capacity changes with cell passaging (Yu *et al.*, 2010). Cryopreservation is the application of low temperatures to preserve the structural and functional integrity of cells and tissues. During the process, the aqueous phase changes to a solid form of ice. The stability of the cells and tissue is maintained by storage at an ultra-low temperature of -196°C (Hunt, 2019). While most banking facilities prefer to store only the isolated cells, there are facilities, like a store-a-tooth, which freezes a part of the original pulp tissue along with the isolated cells (Zeitlin, 2020). The ideal cell concentration to be cryopreserved is reported to be $1.0\text{--}1.5 \times 10^6$ cell concentration to reap a maximum number of viable cells after thawing to produce optimal results (Woods *et al.*, 2009).

While considering cryopreservation, the rate at which the cells are cooled must be addressed with caution. This is because of the water content within the cells; freezing the cells rapidly causes the formation of ice crystals because of insufficient time for the water to leave the cells, which can cause rupture of the cell membranes and eventual structural and mechanical damage to the cells. On the other hand, freezing them slowly can cause dehydration of the cells, which increases the cytosol concentration of solutes leading to alteration in the osmotic gradient, thereby damaging the cell. (Zhurova *et al.*, 2010). To overcome these temperature-related cellular damages, cryoprotectants (CPAs) are added to the cryopreservative medium, which prevents the formation of the ice crystals due to optimal freezing rate and reduces freeze-thaw points (Stolzing *et al.*, 2012). CPAs can be grouped under two categories based on their molecular weights and mode of action. The low molecular weight CPAs exert their action intracellularly by penetration through the cell membrane and preventing the initiation of ice crystal nucleation and growth (Zambelli *et al.*, 1998). The high molecular weight CPAs do not cross the cell membrane but facilitate dehydration of the cell, reduce intracellular ice crystal formation, and maintain the integrity of the cell membrane (Stolzing *et al.*, 2012). The most widely used CPA is dimethyl sulfoxide (DMSO), a low molecular weight agent, documented for its efficacy in cellular protection and maintenance of cellular viability

(Naaldijk *et al.*, 2012). Once the cells are suspended in the appropriate cocktail of the cryopreservation medium and CPA, they are transferred into high-density polypropylene cryovials and stored in liquid nitrogen (LN_2) canisters.

The process of cryopreservation

The isolated cells are suspended in a preservation medium consisting of growth factors and a cryoprotective agent capable of inhibiting crystal growth and dehydration. Then, the cells are transferred to specialized high-density polypropylene cryovials and frozen containing LN_2 . For safe storage of the stem cells and their future clinical use, the choice of the cryopreservation method is crucial. Therefore, various freezing methods are used, such as rapid freezing (vitrification), controlled-rate freezing (slow freezing), uncontrolled freezing, and magnetic freezing.

Rapid freezing method (vitrification)

Vitrification is a simple process of cooling cells at ultrahigh speeds of freezing. This eliminates the formation of ice crystals and creates a glass-like (vitreous) state of the freezing solution. It involves direct contact between the vitrification solution containing the cryoprotectant agents and LN_2 . The vitrification process allows cells to be placed directly into the cryoprotectant, stored within small carriers, and then plunged into LN_2 (Liebermann *et al.*, 2002). Although the process uses a high concentration of cryoprotectant, rapid cooling reduces the toxicity induced by the CPA. The cooling rate is dependent on the structure of vitrification carriers and the manipulation procedures. Generally, an increased cooling rate is achieved by plunging small-volume samples inside small vitrification carriers, such as miniStraw, cryoleaf, and cryoloop, directly into LN_2 (Li *et al.*, 2010). Therefore, only small volumes of samples and a relatively high volume of LN_2 for each vitrification cycle are needed, limiting the potential use of this technique.

While the viability and growth rate of post-vitrification thawing of stem cells have been studied, only a few studies have reported on the viability of dental stem cells following the vitrification method. One study compared the cell survival, and proliferation of DPSCs cryopreserved by vitrification and controlled-rate freezing method in 5% and 10% DMSO for six months. The dental stem cells cryopreserved by the controlled rate cryopreservation showed significant improvement in cell survival and proliferation rate. However, the vitrified stem cells did not regrow after thawing. Additionally, irrespective of the six-month DMSO concentration, cryopreserved DPSCs could not grow after thawing (Huynh *et al.*, 2017).

Controlled-rate cryopreservation

In this cryopreservation method, the freezing process and storage comprise a gradual freezing process and storage in LN_2 (-196°C). The temperature at which the cells are cooled per minute can be controlled. The two widely used controlled-rate freezing methods are the slow freezing rate and the ultra-slow freezing rate cryopreservation. The rationale behind these methods is the elimination or reduction of cellular damage caused due to ice crystal formation or dehydration that can be encountered while

freezing cells. The optimal rate of slow freezing at which the viability and growth potential of the cells can be maintained and with minimal or no damage is 1°C to 2°C per min, and that of ultra-slow freezing is 0.3°C to 0.6°C per min (Pilbauerova *et al.*, 2021). Some studies reported that these methods show better results in post-thaw cell viability and growth rate than the vitrification technique (Huynh *et al.*, 2017). However, other studies claim no differences in cell viability and growth potential of the post-thaw cells obtained from either the controlled rate freezing or vitrification method (Naaldijk *et al.*, 2012). The uncontrolled-rate cryopreservation is a more straightforward and cost-effective method when compared to controlled-rate freezing. The cells are suspended in the cryopreserved medium with CPA and precooled to 4°C. This is followed by freezing the cells at -80°C or storing them directly into LN₂ (Davies *et al.*, 2014). Studies have shown that DPSCs cryopreserved by this method have shown good viability and growth rate of the cells (Kumar *et al.*, 2015).

Uncontrolled-rate freezing

Cryopreservation can also be done using an uncontrolled rate freezing protocol. In this method, CPA and stem cell samples are cooled to 4°C and placed directly into a freezer at 80°C or LN₂ (Davies *et al.*, 2014). Uncontrolled rate freezing is an efficient method for long-term cryopreservation of DPSCs without any post-thaw effect on cell viability, multipotency, proliferation, and differentiation (Kumar *et al.*, 2015).

Magnetic cryopreservation

Magnetic cryopreservation involves subjecting cells and tissues to a low-intensity magnetic field which reduces the freezing point by up to 6°C to 7°C. The concept applies the utilization of a slow controlled-rate magnetic field of 0.01 mT in a programmed freezer. The magnetic field lowers water accumulation during freezing, thereby eliminating the chances of ice formation and undesirable electric current generation (Lee *et al.*, 2012). Freezing occurs at a slower pace of 0.5 °C/min up to -32°C, after which the frozen cells are stored in a -150°C freezer (Lee *et al.*, 2012). The advantage of magnetic cryopreservation over other methods is that thawed cells present superior biological activity due to a low concentration of DMSO (3%) and minimal pre-equilibration time. Magnetic cryopreserved DPSCs have improved cell survival and more expansion and differentiation than other methods. Additionally, they also exhibit better cell surface markers (Lee *et al.*, 2012).

Cryopreservation of whole teeth

Isolation of DPSC from freshly extracted or exfoliated teeth is possible, although contamination and damage during cryopreservation might affect the viability of dental pulp stem cells. Also, the isolation and expansion of DPSC in a laboratory environment is expensive, time-consuming, and poses a risk of contamination and spontaneous differentiation (Lindemann *et al.*, 2014). Therefore, preservation as a whole tooth and deferring isolation and expansion when required have been proven to be more viable methods.

Cryopreservation maintains the viability of cells by freezing and storing them at sub-zero temperatures, thereby preventing biochemical reactions (Mullen and Critser, 2007).

A tissue that undergoes cryopreservation could sustain freezing injuries during the freezing or thawing process. Most likely, the injuries are due to ice crystals formed either within the cells (intracellularly) or outside the cells (extracellularly). Injuries to the cells can also happen due to the release of reactive oxygen species (ROS), which can trigger apoptosis and cause cell death (Mazzilli *et al.*, 1995). A cryoprotective agent (CPA) is added to the freezing medium to protect the cells from sustaining these injuries. CPAs slow the growth and formation of ice crystals, thereby inhibiting the binding of intracellular nuclei (Stolzing *et al.*, 2012).

The idea of preserving whole teeth cryogenically rather than the DPSCs has been explored. The rationale behind the concept is to isolate and expand the DPSCs when the future demands it. Both deciduous and permanent teeth have been attempted to be preserved cryogenically, and the process has been met with limitations. Isolation and expansion of DPSCs can be costly and time-consuming with risks of contamination and spontaneous differentiation (Lindemann *et al.*, 2014). When the whole tooth is cryopreserved, it has to undergo a “thawing” process when retrieving the DPSCs from it. A low number of viable cells, morphological changes, and low rate of isolation are some of the drawbacks faced while dealing with DPSCs obtained from cryopreserved whole teeth. The morphology of the roots of cryopreserved teeth might play a role in these defects. When the roots are fully formed, like in a permanent tooth or deciduous tooth with no root resorption, the extent of penetration of the CPA through the tiny apical foramen into the pulp tissue is less. This could reduce the protective effect of the CPA on the pulp during the freezing and thawing processes. Hence one would expect a better proliferation rate of the DPSCs in teeth with open root apices of permanent teeth or significantly resorbed deciduous teeth because of the ease of penetration of the CPA into the pulpal area and the protective effect it could render. However, this was not the case, and the proliferation rate of the DPSCs from such teeth remained low compared to non-cryopreserved teeth (Pilbauerova and Suchanek, 2018). One study reported that the percentage of viable stem cells retrieved from cryopreserved and thawed teeth is as low as 30% compared to that from non-cryopreserved teeth (61%) (Lindemann *et al.*, 2014). Morphological change in a more rounded cytoplasm was found in DPSCs of cryopreserved whole teeth compared to the spindle-shaped ones of the non-cryopreserved teeth. A novel technique of laser piercing on the tooth to create micro-holes was attempted to enhance the CPA’s penetration. However, the DPSCs showed a similar isolation rate as those of the non-cryopreserved teeth (Gioventu *et al.*, 2012). Based on the data published regarding cryopreservation of whole teeth, it can be concluded that there are no significant benefits of cryopreserving whole teeth. There are, however, several disadvantages of this process.

Cell thawing

A water bath maintained at 37°C is the most standard method, which is excellent at removing the ice crystals without any cell damage. The only drawback is that the contact with water may lead to a high bacterial or fungicidal contamination risk. The application of dry heat is another technique comparable to

those thawed using the standard water procedure. The cryoprotective agent must be washed out of the cryovials after thawing to avoid any cytotoxic effect (Triana *et al.*, 2013).

Dental stem cell banking

DSCs have a wide range of therapeutic benefits and applications in regenerative medicine. The preservation of DSCs necessitated a “tooth bank” for long-term preservation and retrieval for future regenerative therapies. This facility is essential for storing varied cells obtained from dental pulp, tooth apex, periodontal ligament, follicle, and gingiva. Cryopreservation or magnetic freezing are used to preserve dental stem cells (Oh *et al.*, 2005; Tamaoki *et al.*, 2010; Gioventu *et al.*, 2012).

Despite tooth bank’s enormous sources and prospects, the concept is yet to gain complete global acceptance. Developed countries have established tooth banks with extended centers in other countries. BioEden® (Austin, Texas, USA), Store-A-Tooth® (Provia Laboratories, Littleton, Massachusetts, USA), and StemSave® (Stemsave Inc., New York, USA) are a few of the prominent tooth banking facilities which also have their branches in the United Kingdom and Thailand.

Commercial tooth banks

Tooth banking is a process that entails tooth collection, stem cell isolation, and storage. Banking dental pulp stem cells is a relatively new concept that is rapidly gaining traction in several countries. The establishment of the world’s first tooth bank is credited to Hiroshima University in Japan in 2004, and the company was renamed “Three Brackets” in 2005. In 2008 (Kaku *et al.*, 2007), Hiroshima University and Taipei Medical University established Taipei’s first tooth bank as a source for natural implants. BioEden®, National Dental Pulp Laboratory, Oothy, Stemodontics, Stem Save, Store-A-Tooth/Provia Labs, and Tooth bank/Cryopoint are just a few of the companies located in the United States of America that offer dental stem cell storage services, with several of them expanding internationally (Zeitlin, 2020).

BioEden® has laboratories in the United Kingdom and Thailand and is expanding globally. The Norwegian Tooth Bank, established in 2008 in collaboration with the Norwegian Institute of Public Health’s Department of Clinical Dentistry & University of Bergen, has a collection of 100,000 children as part of “The Norwegian Mother and Child Cohort Study.” In this cohort study, Tooth Bank is a sub-project. This is intended to grow into the world’s largest tooth bank for research purposes. Cryocord is Southeast Asia’s premier stem cell bank, established in Malaysia in 2003. Cryocord operates nationwide with regional offices in the Philippines, Brunei, Indonesia, and Thailand. Cryocord offers tooth banking services in addition to stem cell storage. DentCell, based in Mexico, Future Cell Biobank, based in the United Kingdom, Mothercell, Reelabs, Stemade, and Store Your Cells, based in India, are a few other companies that provide dental pulp stem cell banking (Zeitlin, 2020).

Conclusion

The dental pulp stem cells are capable of extensive proliferation and differentiation, which make them an essential resource of stem cells for regeneration and repair of

diseased and injured organs and tissues. DPSC can be obtained from extracted vital teeth or deciduous teeth after shedding. Combining growth factors, small molecules, scaffold materials, and optimal culture conditions are essential for the proper differentiation of DPSCs and the release of their regenerative potential. The comprehensive knowledge regarding the biological characteristics and origin of DPSCs, their identification and harvesting, critical aspects related to their characterization, their multilineage differentiation potential, and current clinical applications would contribute to their potential use in regenerative medicine. Regarding cell banking processes, cryopreservation media and methods must be developed to retain the characteristics and viability of these cells for future application. Both cryopreserved intact tooth and dental stem cells can be used at a later stage in individual regenerative cell and tissue therapy. Even though significant progress has been made in the isolation, expansion, and preservation of DPSCs, further optimization is still required, particularly to follow more homogenous rules to limit phenotypic variance and address safety concerns to generate DPSCs for cellular treatment. Further research and clinical trials should focus on developing novel techniques to advance the use of DPSCs in stem cell-based therapy.

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