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Review Article

Banking on teeth – Stem cells and the dental office

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ABSTRACT

Science and commerce advance together and the stem cell field is no exception. With the promise of cures for conditions as diverse as cancer, autism, neural degeneration, organ replacement and addiction, long-term preservation of dental stem cells is a growth market. The discovery nearly twenty years ago, of viable, multipotent, stem cells in dental pulp from both baby and adult teeth initiated, and drives, this market.The dental stem cell preservation services, "tooth banks", focus on the collection of a child's baby teeth, as they are shed naturally, and storage of the stem cells from within the pulp for therapeutic use in later years should the child require them. This review focuses on the procedures related to these stem cell storage services and may serve as an introduction for many to the practice of "tooth banking".

Dentists have been changing lives for hundreds of years - removing pain, enabling people to eat normally, returning faces to their original splendor - but modern dentists are not only changing lives, they are saving them.

Over the last decade a service once solely the purview of hospital clinics, is becoming more and more popular in the dental office. The collection of stem cells for long-term storage for therapeutic use is a service now provided by dentists. More accurately, the dentist collects teeth and "tooth banking" services extract and preserve the stem cells within the pulp for the future benefit of the patient (Fig. 1).

Stem cell collection from bone marrow, blood, fetal material and umbilical cords present unique practical and conflicting ethical challenges [1,2]. However, the discovery of postnatal stem cell populations in the tooth pulp by Gronthos and Shi [3,4], around two decades ago, opened up new horizons to stem cell research and propelled the dental profession further into the exciting field of regenerative medicine. Post-natal stem cells are present in pulp from deciduous teeth (baby or milk teeth) lost - or exfoliated - by all children during the first 6–12 years of development and are also commonly available from orthodontic extraction of third molars (wisdom teeth) in adults.

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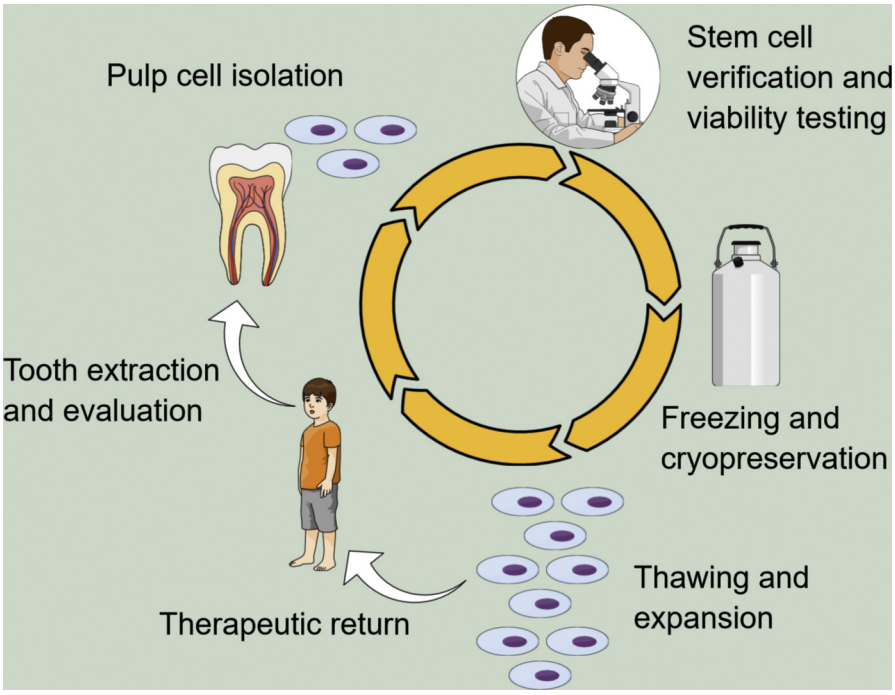

Fig. 1. Schematic representation of dental stem cell banking process. Teeth lost by exfoliation, avulsion or extraction are preserved in transport medium, to tooth banking facility. Dental pulp is removed and stem cells are isolated, tested for various parameters including viability then frozen. Frozen stem cells are finally stored under liquid nitrogen until required by the donor. At that time, they can be thawed and grown to a therapeutically appropriate number of cells before return to the donor.

This review will primarily focus on the practical aspects of "tooth bank" services for storage of dental stem cells.

Therapeutic stem cells

Stem cell populations are comprised of unspecialized cells with the ability to keep dividing, proliferating and thus surviving until they receive specific signals. The signals take the form of molecules secreted by cells at a site of growth, development, tissue damage or repair, into the blood stream or surrounding tissue. These chemoattractant messages cause the stem cells to home in to their source entering the tissue at that site where further chemical signals direct them to specialize. During growth or tissue renewal, they add to the overall tissue development and in tissue damage, they replace the damaged cells aiding the reparative process.

Not all stem cells are equal however, designations based on regenerative capacity define each class into totipotent, pluripotent and multipotent [5]. Totipotent cells have the potential to recapitulate an entire organism *in utero* and thus can differentiate into any cell type. Other stem cells, including early human embryonic stem cells, are termed "pluripotent", meaning they can differentiate into any cell type but cannot recapitulate an entire living organism by themselves. Multipotent stem cells are able to differentiate into more than one type of cell in the body, for example nerve, muscle, bone, blood cells, but without the full regenerative capacity of pluripotent stem cells [6,7]. Generally they specialize within one family of cells, for example either mesenchymal, neural or hematopoietic [5]. With such remarkable capacity for growth and repair, it is little wonder that both medical and commercial interests have long-standing interest in the potential of stem cells.

The use of bone marrow, containing hematopoietic (blood) stem cells, is established in cancer treatment and other therapies [8–10]. However, compatible bone marrow is not always available. Embryonic stem cell therapy, another well studied source of these cells, has societal limitations due to ethical objections to the use of embryonic stem cells and has resulted in a divide that in the USA alone runs from allowing active research through to outright bans, depending on the state [11]. This picture is broadly reflected worldwide; from controlled access to complete prohibition.

One of the most exciting developments in recent stem cell science, following decades of embryonic stem cell research has been the demonstration of fully differentiated cells induced to de-differentiate then re-differentiate along a new lineage. These cells are termed induced Pluripotent Stem Cells (iPSC) and were the subject of the 2012 Nobel Prize for Physiology or Medicine awarded to Shinya Yamanaka and John Gurdon who showed that iPSC regain many aspects of stemness [12,13]. This opened the door wide to future stem cell therapy however at this time inducible stem cells are still in clinical development and while clinical trials are underway in Japan it may be far longer before wider international iPSC treatments are available [14].

It is little wonder then that a comparatively new source of stem cells, the dental pulp - a readily available, relatively non-

invasive source of autologous (an individual's own) stem cells - has created such interest. Since their initial identification nineteen years ago, there remains much to learn about dental stem cell biology and the regenerative capacity of these cells. Many excellent reviews exist describing the multi-faceted biology of dental stem cells in tissue engineering [15,16]. However little has been published recently about the process of dental stem cell banking, the remainder of this review examines some of the practical aspects of dental stem cell banking.

What do the public know about dental stem cells?

A broad search using the terms "tooth" and "stem cell" returns results dominated by companies and dental offices offering to collect extracted teeth and preserve the dental pulp stem cells within. Many of these web sites list as possibly benefiting from dental stem cell therapy pathologies as sweeping as diabetes, heart attack, cancer, autism, drug addictions and aging. Yet the research quoted in relation to these lists invariably cite either clinical trials for non-dental mesenchymal stem cells or pre-clinical studies for dental-derived stem cells. It is unclear whether this important difference is plain to even informed - but non-specialist - members of the public. In fact, there is abundant *in vitro* and *in vivo* evidence that dental pulp cells do have a high *potential* for therapeutic benefit but the clinical evidence, critical to the benefits implied by tooth banks, is sparse [17]. And while it is beyond the scope of this review to recount the large body of pre-clinical work relating to dental stem cell biology a brief description of the sources and function of dental stem cells will help to explain the rise of commercial tooth banking or more accurately, long term storage of stem cells from baby and adult teeth.

Oral tissue sources of stem cells

A number of oral tissues have yielded discrete populations of stem cells (Fig. 2). The dental pulp of both adult dental pulp stem cells (DPSC) and Stem cells from human exfoliated deciduous (SHED) teeth (baby teeth) comprise the most studied populations and with periodontal ligament stem cells (PDLSC), alveolar bone stem cells (ABSC), stem cells from the apical papilla (SCAP) derive from tissue in and around the tooth itself (Fig. 2). Tooth germ progenitor cells (TGPC), and dental follicle stem cells (DFSC) present in the tooth bud are stem cells that contribute to the developing tooth and so are not readily accessible for therapeutic use. Associated oral tissue such as epithelium, gingiva (gums) and salivary gland also house unique populations of stem cells. Clearly, however, the least invasive source of dental stem cells are those released naturally as part of development - SHED which are contained in the pulp of deciduous baby teeth.

SHED and DPSC

Tooth banking services focus on SHED and DPSC but are they appropriately functional stem cells? Gene expression and proteomic analyses show that DPSC broadly express much the same genes as the bone marrow stem cells [4,18]. Both cell types reside in perviscular niches, ready to migrate to the site of need within the local tissue [19]. One of the functional differences they display is an increased proliferative capacity for DPSC compared to bone marrow stem cells [20,21]. Indeed, repeated studies have noted that SHED have even greater proliferative properties than DPSC and may have a greater propensity for survival than their adult counterparts [20,22,23]. This property is significant when considering the small numbers of stem cells available within the confines of a tooth pulp. One of the primary services provided by dental stem cell banking companies is the expansion of the few cells isolated from the pulp to a number that could be therapeutically useful.

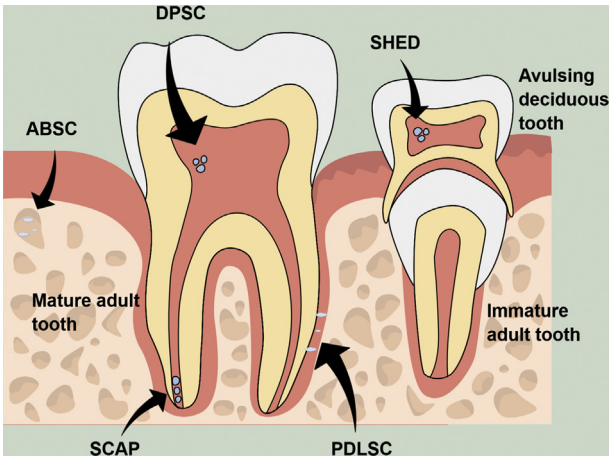

Fig. 2 Dental tissue sources for therapeutically relevant stem cells. Cells are found primarily in the dental pulp of deciduous or adult teeth, the surrounding connective tissue and the alveolar bone although other oral sites also contain stem cell populations. SHED, Stem Cells from Human Deciduous Teeth; DPSC, Dental Pulp Stem Cells; SCAP, Stem Cells from the Apical Papilla; PDLSC, Periodontal Ligament Stem Cells; ABSC, Alveolar Bone Stem Cells; TGPC, Tooth Germ Progenitor Cells; DFSC, Dental Follicle Stem Cells.

However, it seems that other than a higher rate of proliferation and survival there is little functional difference between DPSC and SHED. Although protein analyses have shown some very specific differences in protein expression the capacity for either DPSC, SHED or SCAP - also pulp-derived cells - their ability to differentiate into specialized tissue seems quite similar [24–27]. They have the capacity to become cells of both soft and hard tooth tissue recapitulating dental pulp including endothelial cells and dentin secreting odontoblasts both critical to regenerating the integrity of a damaged tooth [27–29].

SHED and DPSC appear to have a greater propensity to differentiate into neural cells compared to bone marrow cells [21,26]. Unlike other mesenchymal stem cells, it appears clear now that dental pulp stem cells are derived from neural-crest tissue, and that neural cells such as Schwann cells and their precursors, have the capacity to migrate into the pulp chamber and become pulp stem cells able to produce dentin matrix material [30]. In turn, isolated dental pulp cells - and other

Table 1 Tooth banking services examined in this review.

Dental Stem Cell Banking Service	Primary Company	Collection by (per web site)	Web Page
BioEden	USA	patient or dentist	https://www.bioeden.com/us/
Dentcell	Mexico	patient or dentist	https://dentcell.com.mx/
Future Health Biobank	UK	patient	https://futurehealthbiobank.com/
Mothercell	India	dentist	https://www.mothercell.com
National Dental Pulp Laboratory	USA	dentist	https://ndpl.net/
Oothy	USA	patient or dentist	https://www.oothy.com/
ReeLabs	India	unspecified	https://reelabs.com
Stemade	India	dentist	http://www.stemade.com
Stemodontics	USA	dentist	https://stemodontics.com/
Stem Protect	UK	patient	https://www.stemprotect.co.uk/
Stem Save	USA	dentist	https://www.stemsave.com
Store-A-Tooth/Provia Labs	USA	dentist	http://www.store-a-tooth.com/
Store Your Cells	India	dentist	https://www.storeyourcells.com
Tooth Bank/Cryopoint	USA	patient or dentist	https://www.toothbank.com/

neural crest-derived dental stem cells such as PDLSCs, DFSCs and gingiva-derived stem cells - can become functional neurons with SHED again having a greater capacity to do so than DPSC [23,31–33]. *In vivo* studies have demonstrated that implanted SHED can regenerate functional neurons, which is an exciting clinical prospect if successfully translated [34–36].

Therefore, under experimental conditions SHED and DPSC are able to reconstitute human tissue in general and more complex dental tissues - such as pulp - specifically. Dental stem cells *in vivo* seem to repair damaged tissue in a variety of ways that include direct cell replacement and also release of cytokines and chemokines mediating immunomodulation, vascular remodeling by angiogenesis, synaptogenesis and apoptosis in target tissue [37]. In addition to tissue repair, these secreted mediators may allow local stem cells to exert an immunomodulatory influence on tissue response to oral pathogens in the progression of oral diseases such as periodontitis [38,39]. Recently demonstrated, dental stem cells also secrete nanoscale extracellular vesicles that may contribute to their therapeutic functions [39,40]. These vesicles carry specifically packaged proteins, lipids and small RNA species and are taken up by, and capable of reprogramming, target cells. Yet, while stem cells from early tooth buds can generate entirely new teeth, and while dental stem cells may remediate specific lesions, growing any other replacement organs from these accessible dental pulp stem cells may yet be just out of reach [41,42].

Carious teeth as a source for banked stem cells

A proportion of deciduous teeth extracted are carious and so are bacterially infected; tooth-banking companies are divided over acceptance of diseased teeth. The scientific literature is similarly split. Werle et al. demonstrated that stem cells extracted from both carious and healthy deciduous teeth demonstrated equivalent capacity for tissue differentiation [43]. In similar manner, Tsai et al. compared the efficiency of stem cell recovery from deciduous teeth in the absence or presence of caries of increasing levels of disease severity [44]. They also concluded that stem cells could be isolated from carious teeth but with numbers in inverse correlation to the clinical severity of the caries. A greater than four-fold difference in successful stem cell isolation from healthy versus carious teeth was demonstrated. In contrast, Werle et al. noted only a 10% difference in successful stem cell isolation from carious versus healthy teeth. Studies of adult stem cells from healthy teeth and post-caries teeth with inflamed pulps confirmed that cell recovery was lower in the diseased teeth but that both sources of stem cells retained similar differentiation capacities [45,46]. Notably, Tsai et al. reported an increase in both inflammatory mediator expression and of innate immune system molecules in stem cells from the diseased teeth compared to healthy. It is unclear how those differences may influence the long-term use of SHED or DPSC banked for regenerative purposes but it is reasonable to be cautious at the very least. The questions surrounding carious teeth clearly highlight the paucity of information regarding teeth as a source of banked stem cells.

Tooth banking: the process

Although stem cell banks collecting bone marrow and placental cord blood have been in operation for decades, banks specializing in stem cells isolated from teeth are relatively new in comparison. In North America, India and the United Kingdom in particular, dental stem cell banks are expanding in number. An English language internet search of the first 100 hits for "tooth + stem + cell + bank" recovered 15 separate tooth-banking services (Table 1). While this is not an exhaustive search, and other tooth banks function locally, many of these companies, the USA-based ones in particular, extend their banking facilities continentally and globally. It is worth noting that one of the first tooth banks looking at stem cell recovery, not listed in the search, was started in Hiroshima University, Japan as early as 2004 [47]. Cell banks accepting placental cord blood and other sources of stem cells existed well before then and some of these accept teeth for recovery of dental pulp stem cells.

The following discussion will examine the procedures used by some specific tooth banking services however; no specific positive or negative opinion towards any individual dental stem cell banking service is intended or implied in this review.

Collection

Many collection protocols for dental stem cell banks are similar yet there are some notable and important differences in both sample requirements and sample processing. The majority of services recommend that a dentist extract the deciduous tooth as soon as it is loose. In contrast, BioEden (www.Bioeden.com) claim to use a process for collection and transport optimized for naturally exfoliated teeth (Table 1). Indeed their transport medium for the tooth is pasteurized cow milk, an interesting choice discussed further below. For the other services accepting teeth from home extractions the requirement that the tooth have an existing blood supply - evidenced by post removal bleeding - is common to most. For all the Indian tooth banks and five of the US tooth banks extraction by a dental professional is required.

Professional extraction shortens the time the tooth is out of physiological conditions likely to preserve the pulp tissue. While there is modest literature on the preservation of whole teeth specifically for banking stem cells, there is a long history of avulsed tooth preservation, that is, preservation of teeth lost to physical trauma for re-implantation [48]. The requirement for overall survival of healthy tissue makes avulsed tooth data a useful guide for survival of the entire tooth tissue, including pulp. Long-term success rates for re-implantation of avulsed teeth range from around 20% to above 90% depending on how the tooth was handled and how the patient was treated post implant [49,50]. However, the readiness of an otherwise healthy tooth for extraction and the manner in which it is extracted are not the primary factors concerning tooth-banking services. Once a tooth has been extracted or exfoliated, storage for transport has the biggest impact on living pulp, and therefore stem cell survival.

Transport

A number of studies have evaluated different media for successful preservation of live teeth although many are *ex vivo* models and have looked at the specific survival of periodontal ligament cells after tooth storage in the media. Of the tooth banking services that describe the transport medium, balanced salt solutions such as phosphate buffered saline (PBS) or hanks buffered saline solution (HBSS) predominate with mention of undefined nutrients. As mentioned above, BioEden calls for the use of bovine milk as the transport medium. Avulsed teeth are often recommended to be brought to the dentist in milk or even held in the mouth with saliva acting as the carrier [51]. In the short term, tissue drying is the greatest enemy to successful tooth recovery and the same is likely true for stem cell recovery from banked teeth. Times varying from overnight up to 48 h are presented as maximum transport times from tooth extraction to receipt by the banking facility. Yet simply keeping the tooth hydrated, in water for example, is insufficient to that purpose instead an isotonic fluid mimicking physiological conditions is required [52,53].

Bovine milk meets a number of practical standards that are absent from many other media [54]. It is biocompatible, has neutral pH and is naturally buffered but most critically, dental tissue demonstrably survives in it and teeth can be successfully re-implanted if milk is used as a carrier [52,55,56]. Importantly, it is commonly available. This is more important for the unplanned loss of teeth by avulsion but still also, perhaps to a lesser extent, for exfoliated teeth where the tooth is shed at home, school or anywhere other than the dental clinic. Bovine milk is still the most widely recommended preservation media for avulsed tooth storage during transport to the dentist [57]. Other biological media investigated for tooth preservation include propolis (a honeybee natural product), soymilk, almond milk, pomegranate and other fruit juices, chicken egg white, coconut water, green tea extract, Gatorade sports drink and the Brazilian plant extract Dragon Blood *Croton Lechleri* sap [58–63]. For the most part and with the possible exception of Gatorade, these non-defined media have proven viable as tooth tissue preservation vehicles yet none standing out as consistently better than milk.

Closely following bovine milk in frequency of recommendation is HBSS, which is commonly used for tissue and cell preservation and maintenance under *in vitro* experimental conditions [57]. Notably, HBSS is the medium contained in the tooth preservation kit, Save-a-Tooth™ the kit officially used by the tooth bank Store-A-Tooth [57,64]. The main apparent benefit of HBSS, and similar saline solutions is that they are of a defined and consistent formulation, unlike milk [56]. For tooth banks requiring the use of their proprietary collection media kits and preferred or required extraction by a dentist it is this matter of consistency that drives the tooth preservation process.

Stem cell isolation and preparation

Successfully obtaining viable cells is the critical step in this process although most tooth banks do not publicize their proprietary isolation processes. Stem cells are generally extracted from the pulp by mechanical and enzymatic preparation of single cell populations or alternatively by tissue outgrowth - where cells are allowed to naturally migrate from the extracted pulp onto plastic culture surfaces (Fig. 3). In early studies on the isolation and characterization of dental pulp stem cells, Songtao Shi and Stan Gronthos enzymatically digested the pulp tissue with collagenase type 1 and dispase - to free cells from the extracellular matrix - then they passed them through a 70 μm sieve. Single cells were able to grow in culture thereafter [3]. This recovery protocol is still used essentially unchanged two decades later [65,66]. Given the rapidity of this process, 24–48 h from start to finish, this protocol of is likely employed with minor variation by most of the tooth banks. The primary alternative method reported commonly in the literature, tissue outgrowth, is a simpler yet lengthier protocol where the pulp tissue is macerated and then placed in a culture vessel in balanced salt solution with appropriate nutrients to maintain stemness. The cells migrate out from the tissue and establish colonies on the plastic culture surface [44,67]. In both cases, the population of cells surviving in culture is greatly enriched in stem cells following two or three rounds of subculture.

However, the presence of stem cells needs to be tested and the tooth banks detailing their processes mention both viability testing - testing the cell population for number of

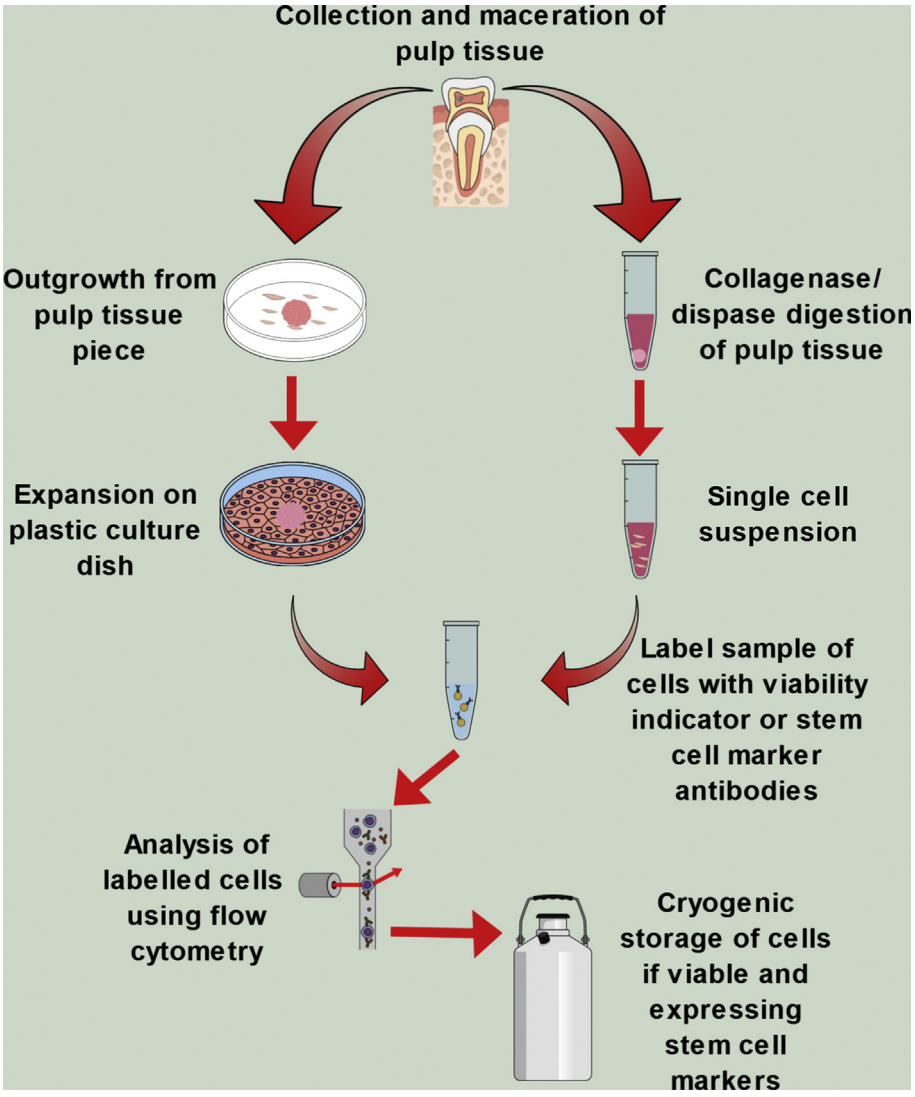

Fig. 3. Summary of dental pulp stem cell isolation processes. Stem cells are routinely collected from pulp tissue, by tissue outgrowth (left) or enzymatic digestion (right). In both cases, the resulting populations are tested for viability, contamination and often the presence of stem cell markers using flow cytometry. Viable cells are then cryopreserved.

living versus dead cells - and flow cytometry (Store-a-Tooth). Flow cytometry is a common analytical technique requiring only a few thousand cells out of the entire sample [68]. This is critical as pulp tissue volumes vary between incisors and molars but are consistently small in deciduous teeth. Cells can be simultaneously labelled by fluorescent molecular probes or antibodies, which could include markers for viability and also stem cell-specific markers. All tooth-banking services test cell viability but stem cell marker testing may not be performed unless explicitly noted. The reason for this is possibly that excessive handling of the pulp-derived cells in the early stages of isolation could result in loss of stemness or viability [69]. At least one company, The National Dental Pulp Laboratory tooth bank, state that they adhere to the US Food and Drug Administration (FDA) non-binding regulatory guidance on minimal handling of cell and tissue based products for homologous use. Homologous use refers to use of collected tissue for the same purpose that tissue would have served in the system from which it was extracted. For extracted cells this means, in the words of the FDA, "… processing that does not alter the relevant biological characteristics of cells or tissues.".

Cryopreservation

Once viable cells or pulp tissue have been isolated they must be successfully frozen and stored. Cells are suspended in a preservation medium, possibly containing growth factors and a cryoprotectant, commonly dimethyl sulfoxide (DMSO) which inhibits the growth of ice crystals that may disrupt the cell membrane and so reduce overall viability. They are transferred to specialized cryo-vials generally constructed from high-density polypropylene then the samples are frozen and placed in low temperature storage containers filled with liquid nitrogen.

The freezing process and storage, when reported by the banking service, is similar to standard procedures common to

research laboratories. It comprises a slow or staged freezing process followed by long term storage in the presence of liquid nitrogen which maintains the samples below −195.8 °C/-320.5 °F. There is a significant body of literature looking at the cryogenic preservation of dental tissue from initial studies simply demonstrating that DPSC can be frozen and successfully recovered, to comparison of cryogenically frozen whole pulp tissue and isolated DPSC/SHED, and to technological improvements for the freezing process [47,70–73].

The majority of tooth banks store isolated, viable stem cells but at least one, Store-A-Tooth, mentions the preservation of at least some of the original pulp material along with the isolated cells. Although whole tooth storage for future recovery of stem cells has proven technically possible, the efficiency of stem cell recovery is highly variable and it is not in general use by commercial banking services [73–76]. Preservation of extracted, intact pulp tissue likewise is not offered by banking services but it is comparable, post-cryopreservation, to fresh pulp for recovery of stem cells [69,76,77]. Indeed both *in vitro* and *in vivo* functions are maintained if stem cells are recovered from frozen pulp tissue [69,73,77]. However, from a commercial perspective it seems desirable to be able to reassure a paying client that viable stem cells are definitely being preserved. This is likely the reason that most banking services isolate cells and, at a minimum, verify cell viability and lack of contamination or test for the presence of stem cell markers. Interestingly, Lizier et al. developed a protocol for greatly scaling up the numbers of extracted stem cells from pulp tissue prior to freezing in which the same piece of pulp tissue is transferred from culture plate to culture plate seeding each of them in turn with stem cells over a number of days [78]. Under these conditions, cells maintained stemness throughout the process suggesting that dental stem cells and pulp tissue are relatively resistant to handling and manipulation if done carefully. Notably, this group demonstrated that these Immature Dental Pulp Stem Cells (IDPSC) are a stem population distinct from SHED or DPSC gathered from equivalent pulps [79].

Gradual, staged freezing at -1 °C per minute is a standard both in experimental cell culture and also for cell banks. Digital freezers allow the gradual and controlled reduction in temperature and once frozen the cells are transferred to a dewar containing liquid nitrogen for long-term storage. That said, both Woods et al. and Kumar et al. demonstrated that both gradual yet uncontrolled freezing and long term storage at higher temperatures of −80 °C had no detrimental effect on DPSCs compared to controlled freezing and storage under liquid nitrogen suggesting again that dental stem cells are remarkably robust [69,80]. At the other extreme vitrification, instant freezing, results in optimal recovery of embryonic stem cells although some evidence suggests that rapid freezing is not optimal for dental stem cells [75,81]. In addition, the application of magnetic fields during the freezing has been shown to increase the viability of frozen cells. Freezing causes ice-crystal formation and a combination of rapid dehydration, excessive osmotic and physical disruption of the plasma membrane by the crystals can occur [82]. Importantly, application of magnetic fields allow reduction in the concentration of cryoprotective - yet cytotoxic - DMSO in the cryopreservative medium towards a higher cell recovery on thawing [75,83]. A fuller discussion of the technical aspects of stem cell cryopreservation and the selection of cryoprotective agent is available in two excellent reviews [84,85].

The cost of banking

Although the technical aspects of the banking process must concern a potential patient or their parent, there is generally insufficient, information available from the public face of the tooth banks to allow members of the public to differentiate between services. Their decision will likely be strongly influenced by both local availability and the commercial or rather, financial differences between banking facilities. Notably, health insurance plans in the USA do not currently cover long-term stem cell preservation and it is not a standard service in most countries with national health services. As prices are subject to change and as there are multiple types of plan available, a direct cost comparison may be meaningless, however as a guide the initial processing can cost in the range of $500 - $2000 (US dollars) and annual maintenance from $99 - $264. Some services offer a flat rate of around $2000 - $3000 for a 20-year plan with no annual maintenance costs. Indeed this is the sole offering from Oothy, with the idea that stem cell preservation from deciduous teeth is intended as a long-term therapeutic investment. So basing a choice of service on the overt cost is clearly a complicated and daunting prospect and it still does not take into account the exact services offered by each tooth bank. For example, NDPL, Oothy, and Stem-Save will accept multiple teeth at the same time whereas BioEden and Tooth Bank have no extra charge for processing teeth until successful stem cell isolation is achieved. Some services offer a refund if the entire process is unsuccessful and some offer credit for future teeth. Accreditation and security are other incalculable factors and while each facility assures security for the cryopreservation facilities, liquid nitrogen storage and patient confidentiality it would be impractical for an individual consumer to make a comparison based on those parameters. Ultimately, it is a reasonable likelihood that dentists or other parents will introduce new consumers to the concept of dental stem cell banking and will likely recommend their own affiliated banking services, and that this will be the strongest influence on any such decision.

Conclusion

Given the rate of technical innovation over the last century, it is impossible to predict the medical advances over the life span of a child with dental stem cells stored right now. So, are the costs of banking stem cells warranted and reasonable, based on current knowledge? If money is no object then the answer is likely yes. However, it is quite possible that medical genomics, biotechnology, nanotechnology, DNA editing and even traditional pharmaceutical sciences will successfully answer therapeutic questions that we might think to address with stem cells at this time. It is clear that hematopoietic, bone marrow and cord blood stem cells already provide viable solutions to some medical challenges. Moreover, dental stem

cells, a source more readily available than those others also demonstrate exciting functionality *in vivo* and *in vitro*. The *potential* of dental stem cells is unquestionable but much more needs to be understood about these cells towards their practical, therapeutic use. Ultimately, it will be the patient or parent who will decide the fate of stem cell banking as an enterprise; yet cost and the Tooth Fairy aside there can be little reason not to bank dental stem cells.

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Conflicts of Interest

The author declares that they have no conflicts of interest or link of any sort to any commercial entity mentioned in this document.

REFERENCES

[1] Rosemann A, Luo HY. Attitudes towards the donation of human embryos for stem cell research among Chinese IVF patients and students. J Bioethic Inq 2018;15:441–57.
[2] Sivaraman MAF. Using surplus embryos and research embryos in stem cell research: ethical viewpoints of buddhist, hindu and catholic leaders in Malaysia on the permissibility of research. Sci Eng Ethics 2018;24:129–49.
[3] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 2000;97:13625–30.
[4] Shi S, Robey PG, Gronthos S. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone 2001;29:532–9.
[5] Ratajczak MZ, Zuba-Surma EK, Wysoczynski M, Wan W, Ratajczak J, Wojakowski W, et al. Hunt for pluripotent stem cell - regenerative medicine search for almighty cell. J Autoimmun 2008;30:151–62.
[6] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–7.
[7] Viswanathan S, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, et al. Mesenchymal stem versus stromal cells: international society for cellular therapy mesenchymal stromal cell committee position statement on nomenclature. Cytotherapy 2019;21:1019–24.
[8] Gorin NC, Douay L, Laporte JP, Lopez M, Mary JY, Najman A, et al. Autologous bone marrow transplantation using marrow incubated with Asta Z 7557 in adult acute leukemia. Blood 1986;67:1367–76.
[9] Lamo-Espinosa JM, Mora G, Blanco JF, Granero-Molto F, Nunez-Cordoba JM, Sanchez-Echenique C, et al. Intra-articular injection of two different doses of autologous bone marrow mesenchymal stem cells versus hyaluronic acid in the treatment of knee osteoarthritis: multicenter randomized controlled clinical trial (phase I/II). J Transl Med 2016;14:246.
[10] Ghoryani M, Shariati-Sarabi Z, Tavakkol-Afshari J, Ghasemi A, Poursamimi J, Mohammadi M. Amelioration of clinical symptoms of patients with refractory rheumatoid arthritis following treatment with autologous bone marrow-derived mesenchymal stem cells: a successful clinical trial in Iran. Biomed Pharmacother 2019;109:1834–40.
[11] Legislatures NCoS. Embryonic and Fetal Research Laws, http://www.ncsl.org/research/health/embryonic-and-fetal-research-laws.aspx. [accessed 23/8/19.2019].
[12] Yamanaka S. Induced pluripotent stem cells: past, present, and future. Cell Stem Cell 2012;10:678–84.
[13] Gurdon JB. The egg and the nucleus: a battle for supremacy. Development 2013;140:2449–56.
[14] Kobayashi Y, Okada Y, Itakura G, Iwai H, Nishimura S, Yasuda A, et al. Pre-evaluated safe human iPSC-derived neural stem cells promote functional recovery after spinal cord injury in common marmoset without tumorigenicity. PLoS One 2012;7:e52787.
[15] Yang XR, Li L, Xiao L, Zhang DH. Recycle the dental fairy's package: overview of dental pulp stem cells. Stem Cell Res Ther 2018;9:347.
[16] Anitua E, Troya M, Zalduendo M. Progress in the use of dental pulp stem cells in regenerative medicine. Cytotherapy 2018;20:479–98.
[17] Yamada Y, Nakamura-Yamada S, Kusano K, Baba S. Clinical potential and current progress of dental pulp stem cells for various systemic diseases in regenerative medicine: a concise review. Int J Mol Sci 2019;20:E1132.
[18] Tamaki Y, Nakahara T, Ishikawa H, Sato S. In vitro analysis of mesenchymal stem cells derived from human teeth and bone marrow. Odontology 2013;101:121–32.
[19] Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 2003;18:696–704.
[20] Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod 2009;35:1536–42.
[21] Karaoz E, Demircan PC, Saglam O, Aksoy A, Kaymaz F, Duruksu G. Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. Histochem Cell Biol 2011;136:455–73.
[22] Kaukua N, Chen M, Guarnieri P, Dahl M, Lim ML, Yucel-Lindberg T, et al. Molecular differences between stromal cell populations from deciduous and permanent human teeth. Stem Cell Res Ther 2015;6:59.
[23] Majumdar D, Kanafi M, Bhonde R, Gupta P, Datta I. Differential neuronal plasticity of dental pulp stem cells from exfoliated deciduous and permanent teeth towards dopaminergic neurons. J Cell Physiol 2016;231:2048–63.
[24] Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531–5.
[25] Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cell 2008;26:1787–95.
[26] Isobe Y, Koyama N, Nakao K, Osawa K, Ikeno M, Yamanaka S, et al. Comparison of human mesenchymal stem cells derived from bone marrow, synovial fluid, adult dental pulp, and exfoliated deciduous tooth pulp. Int J Oral Maxillofac Surg 2016;45:124–31.
[27] Sakai VT, Cordeiro MM, Dong Z, Zhang Z, Zeitlin BD, Nor JE. Tooth slice/scaffold model of dental pulp tissue engineering. Adv Dent Res 2011;23:325–32.

[28] Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod Dent Pulp Tissue Eng Stem Cells Exfoliated Deciduous Teeth 2008;34:962–9.
[29] Zhang Z, Nor F, Oh M, Cucco C, Shi S, Nor JE. Wnt/beta-Catenin signaling determines the vasculogenic fate of postnatal mesenchymal stem cells. Stem Cell 2016;34:1576–87.
[30] Kaukua N, Shahidi MK, Konstantinidou C, Dyachuk V, Kaucka M, Furlan A, et al. Glial origin of mesenchymal stem cells in a tooth model system. Nature 2014;513:551–4.
[31] Tomokiyo A, Hynes K, Ng J, Menicanin D, Camp E, Arthur A, et al. Generation of neural crest-like cells from human periodontal ligament cell-derived induced pluripotent stem cells. J Cell Physiol 2017;232:402–16.
[32] Isaac J, Nassif A, Asselin A, Taihi I, Fohrer-Ting H, Klein C, et al. Involvement of neural crest and paraxial mesoderm in oral mucosal development and healing. Biomaterials 2018;172:41–53.
[33] Chai Y, Jiang X, Ito Y, Bringas Jr P, Han J, Rowitch DH, et al. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 2000;127:1671–9.
[34] Pereira LV, Bento RF, Cruz DB, Marchi C, Salomone R, Oiticicca J, et al. Stem cells from human exfoliated deciduous teeth (SHED) differentiate in vivo and promote facial nerve regeneration. Cell Transplant 2019;28:55–64.
[35] Wang J, Wang X, Sun Z, Wang X, Yang H, Shi S, et al. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. Stem Cell Dev 2010;19:1375–83.
[36] Zhang N, Lu X, Wu S, Li X, Duan J, Chen C, et al. Intrastriatal transplantation of stem cells from human exfoliated deciduous teeth reduces motor defects in Parkinsonian rats. Cytotherapy 2018;20:670–86.
[37] Raza SS, Wagner AP, Hussain YS, Khan MA. Mechanisms underlying dental-derived stem cell-mediated neurorestoration in neurodegenerative disorders. Stem Cell Res Ther 2018;9:245.
[38] Andrukhov O, Behm C, Blufstein A, Rausch-Fan X. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: implication in disease and tissue regeneration. World J Stem Cell 2019;11:604–17.
[39] Kang H, Lee MJ, Park SJ, Lee MS. Lipopolysaccharide-preconditioned periodontal ligament stem cells induce M1 polarization of macrophages through extracellular vesicles. Int J Mol Sci 2018;19:3843.
[40] Wu JY, Chen LL, Wang RF, Song Z, Shen ZS, Zhao YM, et al. Exosomes secreted by stem cells from human exfoliated deciduous teeth promote alveolar bone defect repair through the regulation of angiogenesis and osteogenesis. ACS Biomater Sci Eng 2019;5:3561–71.
[41] Oshima M, Mizuno M, Imamura A, Ogawa M, Yasukawa M, Yamazaki H, et al. Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. PLoS One 2011;6:e21531.
[42] Ono M, Oshima M, Ogawa M, Sonoyama W, Hara ES, Oida Y, et al. Practical whole-tooth restoration utilizing autologous bioengineered tooth germ transplantation in a postnatal canine model. Sci Rep 2017;7:44522.
[43] Werle SB, Lindemann D, Steffens D, Demarco FF, de Araujo FB, Pranke P, et al. Carious deciduous teeth are a potential source for dental pulp stem cells. Clin Oral Invest 2016;20:75–81.
[44] Tsai AI, Hong HH, Lin WR, Fu JF, Chang CC, Wang IK, et al. Isolation of mesenchymal stem cells from human deciduous teeth pulp. BioMed Res Int 2017:2851906.
[45] Pereira LO, Rubini MR, Silva JR, Oliveira DM, Silva IC, Pocas-Fonseca MJ, et al. Comparison of stem cell properties of cells isolated from normal and inflamed dental pulps. Int Endod J 2012;45:1080–90.
[46] Malekfar A, Valli 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.
[47] Kaku M, Kamada H, Kawata T, Koseki H, Abedini S, Kojima S, et al. Cryopreservation of periodontal ligament cells with magnetic field for tooth banking. Cryobiology 2010;61:73–8.
[48] Pasqualini U, Pasqualini ME. Treatise of implant dentistry: the Italian tribute to modern implantology. Carimate (IT); 2009.
[49] Wang G, Wang C, Qin M. A retrospective study of survival of 196 replanted permanent teeth in children. Dent Traumatol 2019;35:251–8.
[50] Krasner P. Treatment of avulsed teeth by oral and maxillofacial surgeons. J Oral Maxillofac Surg 2010;68:2888–92.
[51] Andersson L, Andreasen JO, Day P, Heithersay G, Trope M, Diangelis AJ, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent Traumatol 2012;28:88–96.
[52] Chen FB, Qi SC, Yang QX, Zhang X, Xu YZ, Wang RR. Effect of temperature and six storage media on human dental pulp cells. Acta Med Mediterr 2019;35:461–6.
[53] Moazami F, Mirhadi H, Geramizadeh B, Sahebi S. Comparison of soymilk, powdered milk, Hank's balanced salt solution and tap water on periodontal ligament cell survival. Dent Traumatol 2012;28:132–5.
[54] Courts FJ, Mueller WA, Tabeling HJ. Milk as an interim storage medium for avulsed teeth. Pediatr Dent 1983;5:183–6.
[55] Sottovia AD, Sottovia D, Poi WR, Panzarini SR, Luize DS, Sonoda CK. Tooth replantation after use of euro-coffins solution or bovine milk as storage medium: a histomorphometric analysis in dogs. J Oral Maxillofac Surg 2010;68:111–9.
[56] Hasan MR, Takebe H, Shalehin N, Obara N, Saito T, Irie K. Effects of tooth storage media on periodontal ligament preservation. Dent Traumatol 2017;33:383–92.
[57] Adnan S, Lone MM, Khan FR, Hussain SM, Nagi SE. Which is the most recommended medium for the storage and transport of avulsed teeth? A systematic review. Dent Traumatol 2018;34:59–70.
[58] Moura CCG, Soares PBF, Reis MVD, Neto AJF, Barbosa DZ, Soares CJ. Potential of coconut water and soy milk for use as storage media to preserve the viability of periodontal ligament cells: an in vitro study. Dent Traumatol 2014;30:22–6.
[59] Hwang JY, Choi SC, Park JH, Kang SW. The use of green tea extract as a storage medium for the avulsed tooth. J Endod 2011;37:962–7.
[60] Martins CM, Hamanaka EF, Hoshida TY, Sell AM, Hidalgo MM, Silveira CS, et al. Dragon's blood sap (Croton Lechleri) as storage medium for avulsed teeth: in vitro study of cell viability. Braz Dent J 2016;27:751–6.
[61] Ozan F, Polat ZA, Er K, Ozan U, Deger O. Effect of propolis on survival of periodontal ligament cells: new storage media for avulsed teeth. J Endod 2007;33:570–3.
[62] Sinpreechanon P, Boonzong U, Sricholpech M. Comparative evaluation of periodontal ligament fibroblasts stored in different types of milk: effects on viability and biosynthesis of collagen. Eur J Oral Sci 2019;127:323–32.
[63] Babaji P, Melkundi M, Devanna R, Suresh SB, Chaurasia VR, Gopinath PV. In vitro comparative evaluation of different storage media (hank's balanced salt solution, propolis, Aloe vera, and pomegranate juice) for preservation of avulsed tooth. Eur J Dermatol 2017;11:71–5.

[64] Souza BD, Luckemeyer DD, Felippe WT, Simoes CM, Felippe MC. Effect of temperature and storage media on human periodontal ligament fibroblast viability. Dent Traumatol 2010;26:271–5.
[65] Piva E, Susan AT, Jacques EN, Zou D, Hatfield E, Guinn T, et al. Dental pulp tissue regeneration using dental pulp stem cells isolated and expanded in human serum. J Endod Dent Pulp Tiss Regen Using Dental Pulp Stem Cells Isolated Expand Hum Serum 2017;43:568–74.
[66] Suchanek J, Kleplova TS, Rehacek V, Browne KZ, Soukup T. Proliferative capacity and phenotypical alteration of multipotent ecto-mesenchymal stem cells from human exfoliated deciduous teeth cultured in xenogeneic and allogeneic media. Folia Biol-Prague 2016;62:1–14.
[67] Nowwarote N, Pavasant P, Osathanon T. Role of endogenous basic fibroblast growth factor in stem cells isolated from human exfoliated deciduous teeth. Archives Oral Biol Role Endogenous Basic Fibroblast Growth factor Stem cells Isolated Hum Exfoliated Deciduous Teeth 2015;60:408–15.
[68] Adan A, Alizada G, Kiraz Y, Baran Y, Nalbant A. Flow cytometry: basic principles and applications. Crit Rev Biotechnol 2017;37:163–76.
[69] Woods EJ, 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;59:150–7.
[70] Zhang W, X FW, Shi S, Fan M, John AJ. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Engin Multilineage Differ Potential Stem Cells Derived Hum Dent Pulp After Cryopreservation 2006;12:2813–23.
[71] Perry BC, Zhou D, Wu X, Yang FC, Byers MA, Chu TM, et al. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. Tissue Eng C Methods 2008;14:149–56.
[72] Papaccio G, Graziano A, d'Aquino R, Graziano MF, Pirozzi G, Menditti D, et al. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair. J Cell Physiol 2006;208:319–25.
[73] Lee HS, Jeon M, Kim SO, Kim SH, Lee JH, Ahn SJ, et al. Characteristics of stem cells from human exfoliated deciduous teeth (SHED) from intact cryopreserved deciduous teeth. Cryobiology 2015;71:374–83.
[74] Gioventu S, Andriolo G, Bonino F, Frasca S, Lazzari L, Montelatici E, et al. A novel method for banking dental pulp stem cells. Transfus Apher Sci 2012;47:199–206.
[75] Huynh NCN, Le SH, Doan VN, Ngo LTQ, Tran HLB. Simplified conditions for storing and cryopreservation of dental pulp stem cells. Arch Oral Biol 2017;84:74–81.
[76] 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.
[77] Ma L, Makino Y, Yamaza H, Akiyama K, Hoshino Y, Song G, et al. Cryopreserved dental pulp tissues of exfoliated deciduous teeth is a feasible stem cell resource for regenerative medicine. PLoS One 2012;7:e51777.
[78] Lizier NF, Kerkis A, Gomes CM, Hebling J, Oliveira CF, Caplan AI, et al. Scaling-up of dental pulp stem cells isolated from multiple niches. PLoS One 2012;7:e39885.
[79] Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Massironi SMG, Pereira LV, et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 2006;184:105–16.
[80] Kumar A, Bhattacharyya S, Rattan V. Effect of uncontrolled freezing on biological characteristics of human dental pulp stem cells. Cell Tissue Bank 2015;16:513–22.
[81] Li Y, Tan JC, Li LS. Comparison of three methods for cryopreservation of human embryonic stem cells. Fertil Steril 2010;93:999–1005.
[82] Pilbauerova N, Suchanek J. Cryopreservation of dental stem cells. Acta Med 2018;61:1–7.
[83] Lin SL, Chang WJ, Lin CY, Hsieh SC, Lee SY, Fan KH, et al. Static magnetic field increases survival rate of dental pulp stem cells during DMSO-free cryopreservation. Electromagn Biol Med 2015;34:302–8.
[84] Elliott GD, Wang S, Fuller BJ. Cryoprotectants: a review of the actions and applications of cryoprotective solutes that modulate cell recovery from ultra-low temperatures. Cryobiology 2017;76:74–91.
[85] Hunt CJ. Technical considerations in the freezing, low-temperature storage and thawing of stem cells for cellular therapies. Transfus Med Hemotherapy 2019;46:134–50.