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DENTAL STEM CELL BANKING: A PROMISING FUTURE FOR REGENERATIVE MEDICINE APPLICATIONS

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ABSTRACT Dental stem cells originating from different oral tissues in and around dental structures have recently gained attention as a potential alternative for regenerative medicine applications. To date, many dental stem cells are identified specific to the tissue from which they originate. They exhibit many valuable advantages including high proliferation ability, self-renewal capacity, and multiple differentiation potentials that make them an important candidate for clinical applications, especially in treating degenerative and inflammatory diseases. The fact that they can be easily obtained from an individual's waste tooth without any ethical concern provides them an excellent opportunity for autologous treatment with a low risk of immune rejection. Nowadays, the storage of autologous dental stem cells isolated from wisdom teeth or healthy extracted teeth in biobanks without ethical concerns has become a very important approach for the regeneration of damaged and diseased tissue and for the treatment of life-threatening diseases that may be encountered in the future life of the donor. This study provides a comprehensive overview of dental stem cells, recent advances in their clinical use, long-term preservation processes, and the latest advances in Dental Stem Cell Banking.

Keywords Dentistry, biobanks, stem cells, regeneration

1. INTRODUCTION

Stem cells are cell populations comprised of unspecialized cells that have a remarkable capacity for proliferation, clonality, and differentiation into various cell types [1]. Considering their regenerative capacity, stem cells are classified as totipotent, pluripotent, and multipotent. Cells that can differentiate into any cell type and possess the potential to form an entire organism are defined as totipotent. Although pluripotent stem cells display differentiation ability into any cell type, they differ from totipotent stem cells in that they cannot form an entire living organism on their own. On the other hand, multipotent stem cells only exhibit the potential to differentiate into a limited number of specific cells in the body [2, 3].

Regarding their origin, stem cells are classified as embryonic stem cells which are isolated from the inner cell mass of the blastocyst of the embryo. Besides,

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adult stem cells can be isolated from diversified tissues. While embryonic stem cells are distinguished by their pluripotency, adult stem cells possess limited differentiation capacity which is the general barrier to their regenerative medicine applications. Nevertheless, the use of embryonic stem cells has societal limitations and ethical issues due to the derivation of cells from early human embryos that inhibits embryonic development. Scientists aiming not only to leave ethical concerns behind but also to obtain cells with high differentiation capacity discovered the induced pluripotent stem cells which exhibit simulant proliferation capacity and gene expression characteristics compared to embryonic stem cells. Unlike embryonic stem cells, these cells were developed from individual somatic cells with certain gene modifications but no ethical limitations. With this scientific discovery, Shinya Yamanaka and John Gurdon earned the Nobel Prize in Physiology or Medicine in 2012 [4, 5].

In addition to different types of stem cells, dental stem cells are also considered excellent candidates for stem cells that can be easily obtained from wisdom teeth or healthy extracted teeth and used with less ethical concerns [6]. To date, several types of dental stem cells have been isolated from different parts of tooth and tooth-related tissues, which possess remarkable regenerative potential. Therefore, they have recently gained great attention as a potent platform for developing regenerative medicine applications, in the future [7].

Personalized stem cell-based therapy is going to be the face of the future of medicine which holds hope for many previously incurable cases such as type 1 spinal cord injuries, neurodegenerative diseases, diabetes, and many others. As a source of stem cell therapy, cord blood banking which involves invasive and expensive procedures, is now being widespread all over the world through different government agencies and private companies [8]. If patient lost the opportunity to store their stem cells from the umbilical cord at the time of birth, they have now another opportunity to preserve their stem cells originating from their extracted teeth which are generally thought to be medical waste. Therefore, instead of discarding teeth, storing their cells in a professional dental stem cell bank will be a better option for their future life. Preserving stem cells derived from tooth or tooth-related tissues which includes the same procedures as storing the umbilical cord stem cells in biobanks, is called “Tooth Banking” or “Dental Stem Cell Banking”. For this process, baby teeth, wisdom teeth, or healthy extracted teeth are considered a precious source of highly potent stem cells. When a child’s or an adult’s tooth is extracted by dental professionals, stem cells can be isolated from different tissues, cryogenically frozen following their characterization, and then preserved. Banking these stem cells provides a reliable source for medical treatments in the future [3].

In the last decade, the number of stem cell banks that store the stem cells obtained from bone marrow and placenta cord blood has been increasing rapidly all over the world. Despite this trend, the number of banks specializing in dental stem cells is still very small worldwide. Moreover, in many countries, there is no awareness of this issue yet. This review focuses on the recent advances in dental

stem cells, and procedures related to dental stem cells and serves as an introduction to their “banking”.

2. DENTAL STEM CELLS

Dental stem cells are defined as multipotential mesenchymal stem cells that can be isolated from oral tissues [9]. Similar to mesenchymal stem cells, dental stem cells can undergo self-renewal and have multipotent differentiation ability. In recent years, they have been considered a relatively non-invasive source of autologous stem cell therapy. Besides, they have many remarkable advantages such as being nonimmunogenic, easily accessible, having a good match for the entire family, displaying a higher capacity for proliferation and differentiation, and having the potential to remedy organ shortage which is an expected future necessity [10]. Additionally, they do not pose any ethical problems compared to other stem cell sources.

To date, dental stem cells have been identified from different parts of oral tissues in and around the tooth, and named according to their origin such as dental pulp stem cells from dental pulp (DPSCs), stem cells from human primary exfoliated deciduous (SHEDs) teeth (primary teeth of children), periodontal ligament stem cells (PDLSCs) from periodontium, dental follicle stem cells (DFSCs) from human third molars, gingival mesenchymal stem cells (GMSCs) from gingiva, stem cells from alveolar bone (ABSCs), and stem cells from the apical papilla (SCAPs) (Figure 1).

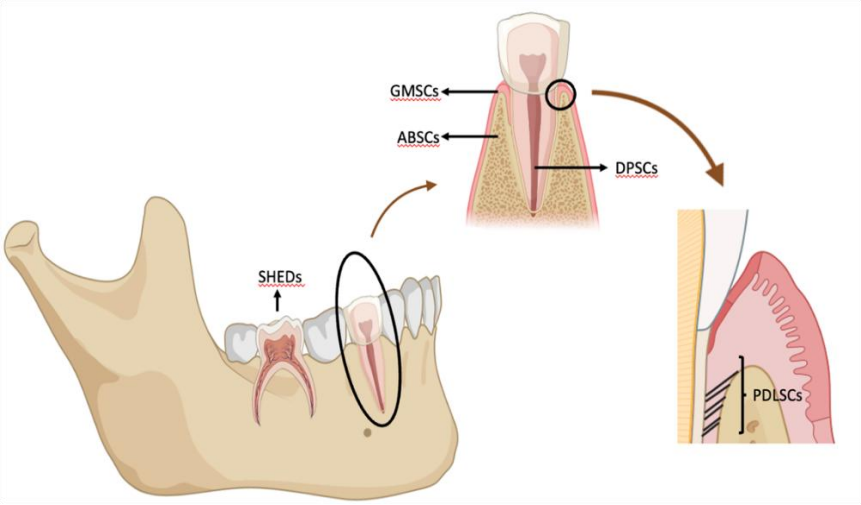

FIGURE 1. Dental tissue sources for therapeutically relevant stem cells. SHEDs, Stem Cells from Human Deciduous Teeth; DPSCs, Dental Pulp Stem Cells; PDLSCs, Periodontal Ligament Stem Cells; ABSCs, Alveolar Bone Stem Cells; GMSCs; Gingival Mesenchymal Stem Cells. Figure created with BioRender.com (accessed on 24 January 2024).

2.1 Dental pulp stem cells (DPSCs): Dental pulp stem cells (DPSCs) were first isolated and identified from the dental pulp of adult 3rd molar (19-29 years old) teeth without caries or infections by Gronthos et al. in 2000 [11]. Since their identification, DPSCs have been the subject of an increasing number of scientific studies in the field of tissue engineering, especially in dental tissue regeneration. In addition to their easy accessibility, these cells exhibit mesenchymal stem cell-like properties with their self-renewal potential and multilineage differentiation capacity. Besides, they have positive surface markers such as CD105, CD90, and CD70, similar to mesenchymal stem cells. Recent studies have shown that DPSCs have a higher proliferation capacity than bone marrow mesenchymal stem cells. Besides, the ability of these cells to differentiate into neuronal and adipose cells, odontoblast and osteoblast cells, and chondrocytes, has been shown by different studies [12, 13]. Therefore, DPSCs are now considered a potential candidate for autologous regenerative therapy.

2.2 Human primary exfoliated deciduous stem cells (SHEDs): In 2003, Miura et al. [14] identified the stem cells from the pulp tissue of human exfoliated deciduous teeth and named the cells as human primary exfoliated deciduous stem cells (SHEDs). Similar to DPSCs, SHEDs express the surface markers including CD13, CD44, CD73, CD90, CD146, and CD166. As a novel and non-invasive source of mesenchymal stem cells, SHEDs exhibit higher proliferation capacity than DPSCs and can differentiate into neurons, adipocytes, odontoblast, and hormone-secreting cells. Other advantages exhibited by SHEDs include painless cell collection without major ethical concerns and minimal risk of invasion. All these valuable properties make them important candidates to be used in tissue engineering-based treatments [15].

2.3 Periodontal ligament stem cells (PDLSCs): Periodontal ligament stem cells (PDLSCs) were first identified from the periodontal ligament of third molars by Seo et al. (2004) [16]. With the exhibition of important characteristics similar to mesenchymal stem cells, they include positivity for mesenchymal stem cell surface markers such as CD90, CD73, and CD105. Recent studies have also proven that PDLSCs have a significant capability of differentiating into cementoblast-like cells, adipocytes, glial and neuron-like cells, and collagen-forming cells when stimulated with appropriate growth factors. They are considered a source of multipotent stem cells that play important roles, especially not only in periodontal tissue regeneration but also in other dental and non-dental tissues [16].

2.4 Dental follicle stem cells (DFSCs): Stem cells isolated from the dental follicle tissue of third molars are defined as dental follicle stem cells (DFSCs). Within a group of dental mesenchymal stem cells, they display multipotential differentiation ability into periodontium, alveolar osteoblast, periodontal ligament, fibroblast, neuronal, adipogenic, chondrogenic, and cementoblast cells in specifically induced culture media. They express a series of classic cell surface

markers specific to mesenchymal stem cells, including CD44, CD73, CD90, and CD105 [17].

2.5 Alveolar bone stem cells (ABSCs): Alveolar bone stem cells (ABSCs) were first isolated from the alveolar bone of the jaw by Matsubara et al. (2005). With their multipotential ability, they exhibit chondroblast, osteoblasts, and adipocyte differentiation. Therefore, ABSCs are considered a potential candidate for the regeneration of cranial bones especially the alveolar bone [18].

2.6 Apical papilla stem cells (SCAPs): Apical papilla stem cells (SCAPs) were initially isolated from the apical papilla of immature permanent teeth of swine by Sonoyama et al. (2006). SCAPs express the cell surface markers including CD24, CD29, CD73, CD90, CD105, CD106, CD146, and CD166. They can differentiate into osteoblasts, adipocytes, and odontoblasts. Besides, SCAPs have higher proliferation and mineralization capacity than DPSCs [19].

2.7 Gingival mesenchymal stem cells (GMSCs): In 2009, Zhang et al. [20] defined the human gingival tissue as a source of mesenchymal stem cells (GMSCs). They are characterized by the markers of Stro-1, Oct-4, and SSEA-4. Like mesenchymal stem cells isolated from human bone marrow or umbilical cord, GMSCs have shown excellent self-renewal capacity, multipotent differentiation ability and exhibit successful differentiation into neural cell lineages [21].

Especially their differentiation properties and being easy to obtain without ethical concerns paved the way for dental stem cells to be used in regenerative and therapeutic applications for human disorders.

3. REGENERATIVE MEDICINE APPLICATIONS

Regenerative medicine, based on the principles of stem cell and tissue engineering, is an important and rapidly developing field of application that has attracted attention among scientific innovations in recent years. The main aim of regenerative medicine is to repair, replace, or renew the injured, diseased, or dysfunctional tissues or organs to restore their normal functionalities [22]. Remarkable progress in regenerative medicine applications has recently been made using stem cells derived from dental tissues due to their glorious properties such as easy accessibility and high proliferative ability. Recent clinical experiments and trials have shown their potential in regeneration and various therapeutic applications, particularly in inflammatory, metabolic, or neurodegenerative diseases. Besides, dental stem cells can serve as a valuable platform for oral and maxillofacial tissue homeostasis, regeneration, and repair.

3.1 Regenerative treatments of dental stem cells in dentistry: Generally, dental stem cells such as DPSCs, SHEDs, and PDLSCs are considered as trustworthy and easily accessible cell sources for the regeneration of oral tissues [23]. In dentistry, they are commonly used for the regeneration of periodontal

tissue, pulp-dental complex, entire-tooth, and salivary gland with or without scaffolds. PDLSCs are frequently applied in periodontal tissue (periodontal ligament, cementum, and alveolar bone) regeneration procedures, especially in the case of periodontitis which is characterized by the loss of the alveolar bone that supports teeth [23]. The first application of human PDLSCs resulted in the successful reconstitution of the cementum and periodontal ligament in an animal model and opened up new scientific research including different experimental models and scaffold-cell combinations [16]. Current studies have shown promising results that induce periodontal regeneration using allogeneic or autologous grafts seeded with PDLSCs [24]. In a clinical trial, 3 patients with periodontitis were treated with autologous PDLSCs on a membrane. After using autologous PDLSCs cell membrane, the cementum and periodontal ligament formation was improved [25]. For long-term treatment of autologous PDLSCs cell membranes also confirmed their safety and efficiency [26]. In addition to PDLSCs, GMSCs are commonly used for repairing jaw-bone defects and regenerating the periodontal ligament as well as dentin. Besides, SHEDs have potential roles in promoting the repair of periodontium including cementum, alveolar bone, dentin, and the periodontal ligament [27].

Dental pulp tissue is located in the center of the tooth and has important functions in maintaining vitality and the neural network of teeth. Therefore, neurogenic and angiogenic potential is essential for the dental pulp regeneration. DPSCs with their neurovascular differentiation characteristics are the best candidates for the regeneration of dental pulp tissue. Numerous preclinical and clinical studies have shown their critical roles in dentin-pulp tissue repair via stimulating the proliferation and migration of progenitor cells, inhibiting apoptosis and inflammation, and enhancing angiogenesis [11, 12]. These important properties make DPSCs a potential alternative to traditional endodontic treatments for some cases. One of the clinical studies carried out by Nakashima et al. (2017) declared that the transplantation of DPSCs to patients with irreversible pulpitis resulted in positive responses for the neurologic reactions following the 24-week application [28]. Likewise, Xuan et al. (2019) proved that autologous implantation of SHEDs highly regenerated and vascularized the dental pulp with tooth root development [29].

3.2 Application of dental stem cells for the regeneration of non-dental tissues: Apart from oral practices, dental stem cells are valuable candidates for use in other tissue engineering applications due to their multipotent differentiation capacity to non-dental tissues [30]. With their versatility, they can be applied in neuronal [31, 32], bone [33, 34], muscle [35], corneal [35], renal [36], and hepatic [37] regeneration.

Owing to the neurodifferentiation potential, DPSCs are considered attractive candidates for the therapy of various systemic diseases. Recent studies have shown their neurotrophic effects on neurodegenerative diseases including Alzheimer's and Parkinson's [23]. The potential effects of DPSCs for neural regeneration have been also shown under *in vitro* [38], and *in vivo* [39]

conditions for spinal cord injury. Besides, the angiogenic potential of DPSCs has been defined in muscular dystrophy. The results exhibited their integration in muscle fibers and the improvement in angiogenesis [40]. The implementation of DPSCs in a rat myocardial infarction model increased the number of vessels and decreased the size of the infarction [41]. Recent studies have shown that the main effects of DPSCs on cardiac repair effect after myocardial infarction can be through the growth factors and cytokines they secret [42]. Studies providing strong evidence about the differentiation capacity of the DPSCs into hepatocyte-like cells indicate that these cells may be potential candidates for the treatment of liver diseases [43]. Besides, DPSCs exhibited a suppressive effect on the colon cancer cells via mitogen-activated protein kinase pathways [44]. Recent studies also suggest that DPSCs and SHEDs with their differentiation ability into pancreatic cell lineages could be effective in the treatment of diabetes [45, 46, 47]. A clinical report demonstrated that intravenous application of SHEDs decreased the rate of unified Huntington's disease [48]. It has also been reported that SHEDs display chondrogenic differentiation ability which makes them potential candidates for cartilage regeneration [49]. Besides, Nishino et al. (2011) have shown that SHEDs promote re-epithelialization and extracellular matrix formation that enhances wound healing [50]. In another study, SHEDs were used for limbal stem cell deficiency. Following the transplantation into the rabbit eye, corneal regeneration was observed [51]. When the DPSCs were transferred to the damaged cornea of the human eye by the contact lens, they inhibited the conjunctival diseases [52].

Nowadays, immunomodulatory and anti-inflammatory characteristics of dental stem cells have gained attention for treating diseases related to the immune system or inflammation. Many preclinical and clinical studies in the literature confirm that SHEDs exert immunomodulatory effects by regulating the proliferation of immune cells, suppressing the inflammatory response, and adjusting immune-related mediators [53]. DPSCs have also been demonstrated for cell-based therapy of immune and inflammation-related diseases [54]. Similar to other dental stem cells GMSCs also display immune-modulating and anti-inflammatory properties. They modulate macrophage immunity, significantly reduce the degranulation of mast cells, and control macrophage polarization [55].

Consequently, dental stem cells hold great potential in the field of tissue engineering and regenerative medicine applications. However, further clinical studies are needed to fully understand their regenerative potential and their safety and efficacy.

4. DENTAL STEM CELL BANKING

Generally, human biobanks are defined as organizations that store biological materials with personal information, and perform operations when necessary [56]. Among them, stem cell banks have gained attention as a promising approach for research and clinical applications, worldwide [57]. Recently, dental

stem cells represent a new and potential source for regeneration remarkably. Their collection and storage for therapeutic usage is a new service called “Dental Stem Cell Banking”. Long-term preservation of dental stem cells for the treatment of different diseases such as cancer, autism, and neural degeneration has led to the establishment of dental stem cell banks. After the first establishment of a dental stem cell bank by Hiroshima University (Japan) in 2004 [58], many private Dental Stem Cell Banks have been opened in more than 20 countries, particularly in Norway, the United Kingdom, Germany, India, Singapore, Mexico, and the United States. In our country, dental stem cell storage started in 2016. However, there is still no licensed dental stem cell bank, yet. Therefore, dental stem cells can be stored in special cord blood banks. However, health insurance does not currently cover the long-term preservation of stem cells [3]. The processing fee including laboratory analysis can cost in the range of $500-$2000 and annual maintenance from $100-$250. Some private companies offer a 20-year plan with no annual maintenance costs of $2000 - $3000.

To translate dental stem cells into clinical applications, numerous standards and regulations must be followed. In the United States, the Food and Drug Administration (FDA) is responsible for the regulations and procedures. For Europe, the European Medicines Agency (EMA) is the main unit for the regulatory mechanisms [57]. Although each country has its additional regulatory system, bank facilities must fulfill certain requirements according to ISO 9000 which includes quality management and assurance standards [59]. Besides, guidelines on European Good Manufacturing Practices (GMP) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) are considered other official international regulatory ways for quality [60, 61]. Additionally, the TRS 878 document provided by the World Health Organization (WHO) and the documents related to ICH, ICHQ7, GMP, ICHQ9, ICHQ10, Quality Risk Management and Pharmaceutical Quality Systems, are recommended as the other relevant guidelines for scientific and technical aspects [61].

Banking of dental stem cells is a critical step for the efficient advancement of clinical translation [57]. A licensed dental stem cell bank usually consists of four main departments including laboratory services, medical services, cryogenic services, and sale services with the purpose of collection, isolation, characterization, preservation, and marketing of the dental stem cells (Figures 2a and 2b).

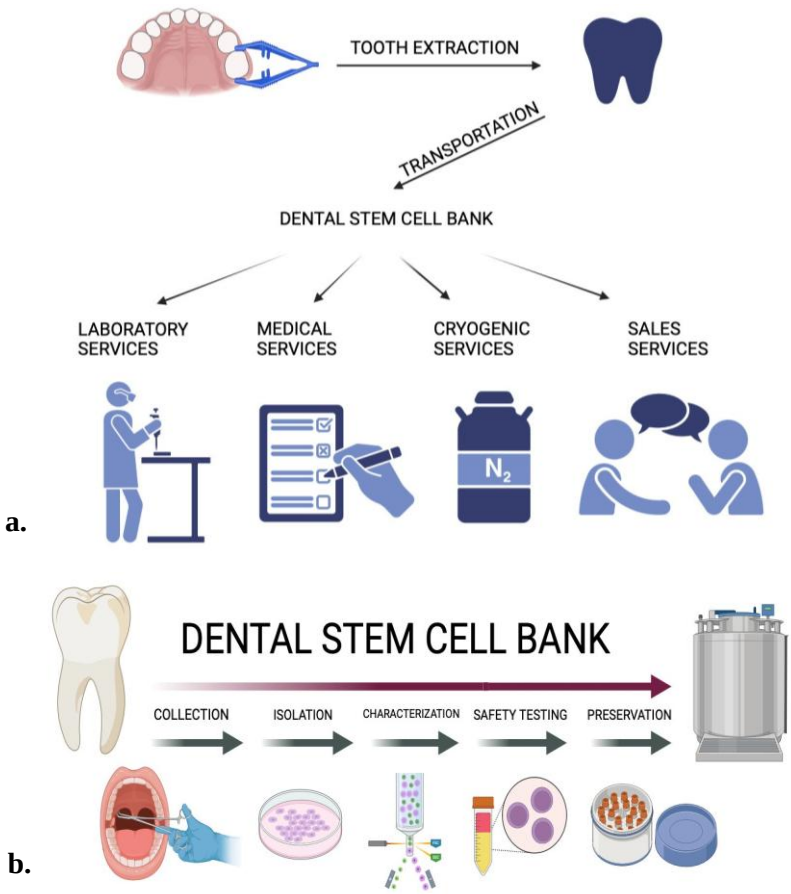

FIGURE 2. Schematic representation of main services (a) and general banking procedures (b) of dental stem cell banks. Figure created with BioRender.com (accessed on 24 January 2024).

4.1 Harvesting and transport: Since the tooth has to meet some requirements, the tooth selection process for dental stem cells is considered as a critical stage in dental stem cell banking. Extracted deciduous teeth with two-thirds of the root with a pulp are preferred over exfoliated teeth. For the adult teeth, only vital teeth without any infection and pathology and with a sufficient amount of pulp have to be harvested. The tooth that will be used as a source for dental stem cell isolation has to be first examined by a dental surgeon to rule out any infection in the targeted area. Extraction and the transfer protocol have to be carried out under aseptic conditions [10]. Transportation is carried out using special collection kits provided by the banking company.

4.2 Stem cell isolation, quality control, and characterization: One of the main critical issues for applying dental stem cells in clinical approaches is collecting a significantly high number of desired cells from different parts of the dental tissues. According to their location on tooth or oral tissue, dental stem cells can be harvested and then isolated using various techniques in the laboratory. Mechanical and enzymatic methods can be used for cell isolation. Following the isolation, the isolated stem cells have to be tested according to the quality and control assessments of the cell bank as well as within the framework of regulation rules of the country [10, 62-65]. According to international guidelines; the characteristics, viability, and purity of the isolated cells have to be checked. Besides, their genetic stability and identity have to be confirmed, and osteogenic, adipogenic, and chondrogenic differentiation capabilities have to be tested. The cells that do not meet these requirements have to be excluded from the cell banks [61].

4.3 Storage (Cryopreservation): Once cells are isolated and characterized, they have to be quickly suspended in a preservation medium containing cryoprotectants and dimethyl sulfoxide (DMSO). Following the transfer of cell suspensions into specialized cryo-vials, they have to be immediately frozen and stored in low-temperature storage containers including liquid nitrogen that maintains the cells below -195,8 °C / -320.5 °F. Considering the entire process, the preservation of the stem cells is still considered the most crucial step of the banks. Therefore, new technological improvements are being developed to optimize a more efficient storage protocol. It has been shown that using magnetic fields in the freezing process increases the viability of frozen cells and reduces DMSO usage in the cryopreservation medium [3]. However, appropriate preservation procedures for dental stem cells are still needed.

4.4 Post-thaw quality control: Post-thaw quality control is another critical step for dental stem cell banks. Optimal past-thaw cell recovery has to be analyzed according to the total cell count, cell viability which is expected to be more than 80%, membrane integrity, metabolic activity, etc. [66, 67].

In particular, the storage of dental stem cells which offer potential applications for the donor and other family members in the future, has gained increasing attention. However, this application still has significant disadvantages. For instance, every extracted tooth cannot be equally suitable for stem cell harvesting. Stem cells should preferably be obtained from extracted teeth with a bleeding pulp and uninfected teeth [68]. In addition, ethical regulations on the use of biospecimens and the slow engraftment rates are also considered other important disadvantages [69]. Recently, dental practitioners reported that cost might be the biggest barrier to hindering a prospective dental stem cell bank [70]. Importantly, the sector still lacking in terms of education and raising awareness about the effects of dental stem cells on different health issues. Patients, dentists, and the government must be aware of the presence, source, and therapeutic effects of dental stem cells. Therefore, educational health campaigns can also provide the public with a great amount of information about the therapeutic benefits of dental stem cell banking for their health in the future [71]. Internet

and social media which are the best sources of information can be utilized to increase public awareness about dental stem cell banking. Furthermore, the shortage of medically trained personnel is considered an important problem, worldwide [69]. There are few adequately trained personnel for sample collection, storage, and processing of dental stem cells to provide banking services and stem cell transplantation. The lack of trained medical laboratory scientists, doctors, nurses, medical laboratory scientists, and other related personnel is one of the major disadvantages in this field. Low-trained personnel to the world population density pose a major challenge to implementing stem cell banking. Therefore, medical personnel qualified to collect stem cells and provide banking services should be trained appropriately. Education of stem cell banking personnel has to be applied through media, workshops, symposiums, etc. Adequate funding should be allocated to this sector and medical research in particular by the federal government, non-governmental organizations, and international agencies [72]. To overcome these disadvantages, finding resources, training the employees, and providing easy access for the citizens to such services have to be the main focus of this sector. Overall, additional efforts are still needed for dental stem cell banking to become a more accessible application for patients in the future.

5. CONCLUSION

Over the last few decades, several preclinical and clinical studies have been performed, and their results make stem cells an attractive candidate in the field of tissue engineering and regenerative medicine applications. As reviewed in this article, dental stem cells have recently received the greatest attention due to their superiorities such as easy accessibility, self-renewal ability, and higher capacity to differentiate into several cell types. Therefore, dental stem cell banking is rapidly finding its place in health strategies similar to umbilical cord blood banking. However, it is necessary to conduct further studies into their cryopreservation. In addition, studies to raise awareness of individuals and dentists about dental stem cell banking need to increase rapidly.

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Author Contribution Statements HBE and FK: designed the study, wrote and reviewed the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest.

REFERENCES

[1] Fortier, L.A., Stem cells: Classifications, controversies, and clinical applications. *Veterinary Surgery*, 34 (2015), 415-423. https://doi.org/10.1111/j.1532-950X.2005.00063.x

[2] Ratajczak M.Z., Zuba-Surma E.K., Wysoczynski M., Wan W., Ratajczak J., Wojakowski W., Hunt for pluripotent stem cell - regenerative medicine search for almighty cell. *Journal of Autoimmunology*, 30 (2008), 151-162. https://doi.org/ 10.1016/j.jaut.2007.12.003

[3] Zeitlin, B.D., Banking on teeth - Stem cells and the dental office. *Biomedical Journal*, 43 (2) (2020), 124-133. https://doi.org/10.1016/j.bj.2020.02.003

[4] Yamanaka, S., Induced pluripotent stem cells: Past, present, and future. *Cell Stem Cell*, 10 (2012), 678-684. https://doi.org/10.1016/j.stem.2012.05.005

[5] Gurdon, J.B., The egg and the nucleus: A battle for supremacy. *Development*, 140 (12) (2013), 2449-2456. https://doi.org/10.1242/dev.097170

[6] Bojic, S., Volarevic, V., Ljujic, B., Stojkovic, M., Dental stem cells - characteristics and potential. *Histology and Histopathology*, 29 (2014), 699-706. https://doi.org/10.14670/HH-29.699

[7] Li, Y., Duan, X., Chen, Y., Dental stem cell-derived extracellular vesicles as promising therapeutic agents in the treatment of diseases. *International Journal of Oral Science*, 14 (2) (2022). https://doi.org/10.1038/s41368-021-00152-2

[8] Dhot, P.S., Nair, V., Swarup, D., Sirohi, D., Ganguli, P., Cord blood stem cell banking and transplantation. *Indian Journal of Pediatrics*, 70 (12) (2003), 989-992. https://doi.org/10.1007/BF02723826.

[9] Lymperi, S., Taraslia, V., Tsatsoulis, I.N., Samara, A., Velentzas, A.D., Agrafioti, A., Anastasiadou, E., Kontakiotis, E., Dental stem cell migration on pulp ceiling cavities filled with MTA, dentin chips, or bio-oss. *Biomed Research International*, (2015), 189872. https://doi.org/10.1155/2015/189872

[10] Sunil, P.M., Manikandan, R., Yoithapprabhunath, M.T.R., Sivakumar, M., Harvesting dental stem cells – Overview. *Journal of Pharmacy and Bioallied Sciences*, 7 (2) (2015), 384-386. https://doi.org/10.4103/0975-7406.16346

[11] Gronthos, S., Mankani, M., Brahim, J., Robey, P. G., Shi, S., Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences*, 97 (2000), 136213630. https://doi.org/10.1073/pnas.240309797

[12] Gronthos, S., Brahim, J., Li, W., Fisher, L. W., Cherman, N., Boyde, A., Stem cell properties of human dental pulp stem cells. *Journal of Dental Research*, 81 (2002), 531-535. https://doi.org/10.1177/154405910208100806

[13] Ulmer, F.L., Winkel, A., Kohorst, P., Stiesch, M., Stem cells-prospects in dentistry. *Schweiz Monatsschr Zahnmed*, 120 (10) (2010), 860-883.

[14] Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L.W., Robey, P.G., Shi, S., SHED: stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences USA*, 100 (10) (2003), 5807-5812. https://doi.org/ 10.1073/pnas.0937635100

[15] Huang, Y.H., Yang, J.C., Wang, C.W., Lee, S.Y., Dental Stem Cells and Tooth Banking for Regenerative Medicine. *Journal of Experimental and Clinical Medicine*, 2 (3) (2010), 111-117. https://doi.org/10.1016/s1878-3317(10)60018-6

[16] Seo, B.M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., Young, M., Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet*, 364 (2004), 149-155. https://doi.org/10.1016/S0140-6736(04)16627-0

[17] Sowmya, S., Chennazhi, K.P., Arzate, H., Jayachandran, P., Nair, S.V., Jayakumar, R., Periodontal specific differentiation of dental follicle stem cells into osteoblast, fibroblast, and cementoblast. *Tissue Engineering Part C Methods*, 21 (10) (2015), 1044-1058. https://doi.org/10.1089/ten.TEC.2014.0603

[18] Matsubara, T., Suardita, K., Ishii, M., Sugiyama, M., Igarashi, A., Alveolar bone marrow as a cell source for regenerative medicine: differences between alveolar and iliac bone marrow stromal cells. *Journal of Bone and Mineral Research*, 20 (3) (2005), 399-409. https://doi.org/10.1359/JBMR.041117

[19] Sonoyama, W., Liu, Y., Fang, D., Yamaza, T., Seo, B.M., Zhang, C., Liu, H., Gronthos, S., Wang, C.Y., Wang, S., Shi, S., Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PLoS One*, 20 (1) (2006), e79. https://doi.org/ 10.1371/journal.pone.0000079

[20] Zhang, Q., Shi, S., Liu, Y., Uyanne, J., Shi, Y., Shi, S., Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *Journal of Immunology*, 183 (12) (2009), 7787-7798. https://doi.org/10.4049/jimmunol.0902318

[21] Kim, D., Lee, A.E., Xu, Q., Zhang, Q., Le, A.D., Gingiva-derived mesenchymal stem cells: Potential application in tissue engineering and regenerative medicine - A Comprehensive review. *Frontiers in Immunology*, 16 (12) (2021), 667221. https://doi.org/10.3389/fimmu.2021.667221

[22] Sampogna, G., Guraya, S.Y., Forgione, A., Regenerative medicine: Historical roots and potential strategies in modern medicine. *Journal of Microscopy and Ultrastructure*, 3 (3) (2015), 101-107. https://doi.org/10.1016/j.jmau.2015.05.002

[23] Zhang, W., Yelick, P.C., Tooth repair and regeneration: Potential of dental stem cells. *Trends in Molecular Medicine*, 27 (5) (2021), 501-511. https://doi.org/ 10.1016/j.molmed.2021.02.005

[24] Soudi, A., Yazdanian, M., Ranjbar, R., Tebyanian, H., Yazdanian, A., Tahmasebi, E., Keshvad, A., Seifalian, A., Role and application of stem cells in dental regeneration: A comprehensive overview. *EXCLI Journal*, 20 (2021), 454-489. https://doi: 10.17179/excli2021-3335

[25] Feng, F., Akiyama, K., Liu, Y., Yamaza, T., Wang, T.M., Chen, J.H., Utility of PDL progenitors for in vivo tissue regeneration: A report of 3 cases. *Oral Diseases*, 16 (1) (2010), 20-28. https://doi: 10.1111/j.1601-0825.2009.01593.x

[26] Iwata, T., Yamato, M., Washio, K., Yoshida, T., Tsumanuma, Y., Yamada, A., Periodontal regeneration with autologous periodontal ligament-derived cell sheets - a safety and efficacy study in ten patients. *Regenerative Therapies*, 9 (2018), 38-44. https://doi: 10.1016/j.reth.2018.07.002

[27] Zhao, J., Zhou, YH., Zhao, Y.Q., Oral cavity-derived stem cells and preclinical models of jaw-bone defects for bone tissue engineering. *Stem Cell Research and Theraphy*, 14 (1) (2023), 39-59. https://doi.org/10.1186/s13287-023-03265-z

[28] Nakashima, M., Iohara, K., Murakami M., Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. *Stem Cell Research and Theraphy*, 8 (2017), 61-74. https://doi.org/10.1186/s13287-017-0506-5

[29] Xuan, K., Li, B., Guo, H., Sun, W., Kou, X., He, X., Zhang, Y., Sun, J., Liu, A., Liao, L., Liu, S., Liu, W., Hu, C., Shi, S., Jin, Y., Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. *Science Translational Medicine*, 10 (455) (2018), eaaf3227. https://doi.org/10.1126/scitranslmed.aaf3227

[30] Song, W.P., Jin, L.Y., Zhu, M.D., Wang, H., Xia, D.S., Clinical trials using dental stem cells: 2022 update. *World Journal of Stem Cells*, 15 (3) (2023) 31-51. https://doi.org/10.4252/wjsc.v15.i3.31

[31] Shi, X., Mao, J., Liu, Y., Pulp stem cells derived from human permanent and deciduous teeth: Biological characteristics and therapeutic applications. *Stem Cells Translational Medicine*, 9 (4) 2020, 445-464. https://doi.org/10.1002/sctm.19-0398.

[32] Sramkó, B., Földes, A., Kádár, K., Varga, G., Zsembery, Á., Pircs, K., The wisdom in teeth: Neuronal differentiation of dental pulp cells. *Cell Reprogram*, 25 (1) (2023), 32-44. https://doi.org/10.1089/cell.2022.0102

[33] Liu, P., Zhang, Y., Ma, Y., Tan, S., Ren, B., Liu, S., Application of dental pulp stem cells in oral maxillofacial tissue engineering. *International Journal of Medical Science*, 19 (2) (2022), 310-320. https://doi.org/10.7150/ijms.68494

[34] Awais, S., Balouch, S.S., Riaz, N., Choudhery, M.S., Human dental pulp stem cells exhibit osteogenic differentiation potential. *Open Life Science*, 15 (2020), 229-236. https://doi.org/10.1515/biol-2020-0023

[35] Armiñán, A., Gandía, C., Bartual, M., García-Verdugo, .M., Lledó, E., Mirabet, V., Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. *Stem Cells and Development*, 8 (6) (2009), 907-918. https://doi.org/10.1089/scd.2008.0292

[36] Bosch, B.M., Salero, E., Núñez-Toldrà, R., Sabater, A.L., Gil, F.J., Perez, R.A., Discovering the potential of dental pulp stem cells for corneal endothelial cell production: A proof of concept. *Frontiers in Bioengineering and Biotechnology*, 9 (2021), 617724. https://doi.org/10.3389/fbioe.2021.617724

[37] Hirata, M., Ishigami, M., Matsushita, Y., Ito, T., Hattori, H., Hibi, H., Multifaceted therapeutic benefits of factors derived from dental pulp stem cells for mouse liver fibrosis. *Stem Cells Translational Medicine*, 5 (10) (2016), 1416-1424. https://doi.org/10.5966%2Fsctm.2015-0353

[38] Király, M., Porcsalmy, B., Pataki, A., Kádár, K., Jelitai, M., Molnár, B., Hermann, P., Gera, I., Grimm, W.D., Ganss, B., Zsembery, A., Varga, G., Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. *Neurochemistry International*, 55 (2009), 323-332. https://doi.org/10.1016/ j.neuint.2009.03.017

[39] Young, F., Sloan, A., Song, B., Dental pulp stem cells and their potential roles in central nervous system regeneration and repair. *Journal of Neuroscience Research*, 91 (2013), 1383-1393. https://doi.org/10.1002/jnr.23250

[40] Martínez-Sarrà, E., Montori, S., Gil-Recio, C., Núñez-Toldrà, R., Costamagna, D., Rotini, A., Atari, M., Luttun, A., Sampaolesi, M., Human dental pulp pluripotent-like stem cells promote wound healing and muscle regeneration. *Stem Cell Research and Therapies*, 278 (1) (2017), 175-195. https://doi:10.1186/s13287-017-0621-3

[41] Gandia, C., Armiñan, A., García-Verdugo, J.M., Lledó, E., Ruiz, A., Miñana, M.D., Sanchez-Torrijos, J., Payá, R., Mirabet, V., Carbonell-Uberos, F., Llop, M., Montero, J.A., Sepúlveda, P., Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. *Stem Cells*, 26 (3) (2008), 638-645. https://doi:10.1634/stemcells.2007-0484.

[42] Bluteau, G., Luder, H.U., De Bari, C., Mitsiadis, T.A., Stem cells for tooth engineering. *European Cells and Materials*, 6 (2008), 1-9. https://doi: 10.22203/ecm.v016a01

[43] Ishkitiev, N., Yaegaki, K., Imai, T., Tanaka, T., Fushimi, N., Mitev, V., Okada, M., Tominaga, N., Ono, S., Ishikawa, H., Novel management of acute or secondary biliary liver conditions using hepatically differentiated human dental pulp cells. *Tissue Engineering Part A*, 21 (3-4) (2015), 586-593. https://doi: 10.1089/ten.TEA.2014.0162

[44] Nikkhah, E., Kalalinia, F., Asgharian Rezaee, M., Tayarani-Najaran, Z., Suppressive effects of dental pulp stem cells and its conditioned medium on development and migration of colorectal cancer cells through MAPKinase pathways. *Iran Journal of Basic Medicinal Science*, 24 (9) (2021), 1292-1300. https://doi.org/ 10.22038/ijbms.2021.58273.12946

[45] Kanafi, M.M., Rajeshwari, Y.B., Gupta, S., Dadheech, N., Nair, P.D., Gupta, P.K., Transplantation of islet-like cell clusters derived from human dental pulp stem cells restores normoglycemia in diabetic mice. *Cytotherapy*, 15 (10) (2013), 1228-1236. https://doi.org/10.1016/j.jcyt.2013.05.008

[46] Tonsekar, P., Tonsekar, V., Jiang, S., Yue, G., Dental stem cell-based therapy for glycemic control and the scope of clinical rtanslation: A systematic review and meta-analysis. *International Journal of Translational Medicine*, 4 (2024), 87-125. https://doi.org/10.3390/ijtm4010005

[47] Xu, Y., Chen, J., Zhou, H., Wang, J., Song, J., Xie, J., Effects and mechanism of stem cells from human exfoliated deciduous teeth combined with hyperbaric oxygen therapy in type 2 diabetic rats. *Clinics*, 75 (2020), e1656. https://doi.org/10.6061/clinics/2020/e1656

[48] Huntington Study Group, Unified Huntington's disease rating scale: Reliability and consistency. *Movement Disorders*, 11 (1996), 136-142. https://doi.org/10.1002/mds.870110204

[49] Chen, K., Xiong, H., Xu, N., Shen, Y., Huang, Y., and Liu, C., Chondrogenic potential of stem cells from human exfoliated deciduous teeth in vitro and in vivo. *Acta Odontologica Scandinavica*, 72 (8) (2014), 664-672. https://doi:10.3109/00016357.2014.888756

[50] Nishino, Y., Yamada Y., Ebisawa, K., Nakamura, S., Okabe, K., Umemura, E., Hara, K., Ueda, M., Stem cells from human exfoliated deciduous teeth (SHED) enhance wound healing and the possibility of novel cell therapy. *Cytotherapy*, 13 (5) (2011), 598-605. https://doi: 10.3109/14653249.2010.542462

[51] Gomes, J.Á.P., Monteiro, B.G., Melo, G.B., Smith, R.L., da Silva, M.C.P., Lizier, N.F., Corneal reconstruction with tissue-engineered cell sheets composed of human immature dental pulp stem cells. *Investigative Ophthalmology and Visual Science*, 51 (3) (2010), 1408-1414. https://doi: 10.1167/iovs.09-4029

[52] Kushnerev, E., Shawcross, S.G., Sothirachagan, S., Carley, F., Brahma, A., Yates, J.M., Regeneration of corneal epithelium with dental pulp stem cells using a contact lens delivery system. *Investigative Ophthalmology and Visual Science*, 57 (13) (2016), 5192-5199. https://doi: 10.1167/iovs.15-17953

[53] Gao, X., Shen, Z., Guan, M., Huang, Q., Chen, L., Qin, W., Immunomodulatory role of stem cells from human exfoliated deciduous teeth on periodontal regeneration. *Tissue Engineering Part A*, 24 (17-18) (2018), 1341-1353. https://doi:10.1089/ten.tea.2018.0016

[54] Djouad, F., Plence, P., Bony, C., Tropel, P., Apparailly, F., Sany, J., Noël, D., Jorgensen, C., Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood*, 102 (2003), 3837-3844. https://doi.org/10.1182/blood-2003-04-1193

[55] Tolouei, A.E., Oruji, F., Tehrani, S., Gingival mesenchymal stem cell therapy, immune cells, and immunoinflammatory application. *Molecular Biology Reports*, 50 (2023), 10461-10469. https://doi.org/10.1007/s11033-023-08826-2

[56] Hewitt, R., Watson, P. Defining biobank. *Biopreservation and Biobanking*, 11 (5) (2013), 309-315. https://doi.org/10.1089/bio.2013.0042

[57] Collart-Dutilleul, P., Chaubron, F., de Vos, J., Cuisinier, F., Allogenic banking of dental pulp stem cells for innovative therapeutics. *World Journal of Stem Cells*, 7 (7) (2015), 1010-1021. https://doi.org/10.4252/wjsc.v7.i7.1010.hal-03147103

[58] Kaku, M., Kamada, H., Kawata, T., Koseki, H., Abedini, S., Kojima, S., Cryopreservation of periodontal ligament cells with magnetic field for tooth banking. *Cryobiology*, 61 (2010), 73-78. https://doi.org/10.1016/j.cryobiol.2010.05.003

[59] Sirchia, G., Rebulla, P., Lecchi, L., Mozzi, F., Crepaldi, R., Parravicini, A., Implementation of a quality system (ISO 9000 series) for placental blood banking. *Journal of Hematotherapy*, 7 (1) (1998), 19-35. https://doi.org/10.1089/scd.1.1998.7.19

[60] Liikanen, E., Laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. *Official Journal of the European Union*, (2003).

[61] Khaseb, S., Orooji, M., Pour, M. G., Safavi, S. M., Eghbal, M. J., Rezai Rad, M., Dental stem cell banking: Techniques and protocols. *Cell Biology International*, 45 (9) (2021), 1851-1865. https://doi.org/10.1002/cbin.11626

[62] Cobo, F., Stacey, G.N., Hunt, C., Cabrera, C., Nieto, A., Montes, R., Cortés, J.L., Catalina, P., Barnie, A., Concha, A., Microbiological control in stem cell banks: approaches to standardization. *Applied Microbiology and Biotechnology*, 68 (4) (2005), 456-466. https://doi.org/10.1007/s00253-005-0062-2

[63] Pakzad, M., Hassani, S.N., Abbasi, F., A Roadmap for the production of a GMP-compatible cell bank of allogeneic bone marrow-derived clonal mesenchymal stromal cells for cell therapy applications. *Stem Cell Reviews and Reports*, 18 (2022), 2279-2295. https://doi.org/10.1007/s12015-022-10351-x

[64] World Health Organization, Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (Report No. 878), (2010).

[65] European Medicines Agency, ICH Topic Q5D quality of biotechnological products: Derivation and characterisation of cell substrates used for production of biotechnological/biological products, (2007), 1-3.

[66] Raik, S., Kumar, A., Rattan, V., Seth, S., Kaur, A., Bhatta Charyya, S., Assessment of post-thaw quality of dental mesenchymal stromal cells after long-term cryopreservation by uncontrolled freezing. *Applied Biochemistry and Biotechnology*, 191 (2) (2020), 728-743. https://doi.org/10.1007/s12010-019-03216-6

[67] Xie, J., Ekpo, M.D., Xiao, J., Zhao, H., Bai, X., Liang, Y., Zhao, G., Liu, D., Tan, S., Principles and protocols for post-cryopreservation quality evaluation of stem cells in novel biomedicine. *Frontiers in Pharmacology*, 13 (2022), 907943. https://doi.org/10.3389/fphar.2022.907943

[68] Çevik, Z.M., Erkmen, E., Kahraman, Ş., Significance of dental stem cells in dentistry and stem cell banking. *Journal of Clinical Sciences*, 13 (2) (2024), 402-408. https://doi.org/10.54617/adoklinikbilimler.1431309

[69] Narayanan, D., Phadke, S.R., Concepts, utility and limitations of cord blood banking: What clinicians need to know. *Indian Journal of Pediatrics*, 86 (2019), 44-48. https://doi.org/10.1007/s12098-018-2651-y

[70] Alomar, R.K., Aladhyani, S.M., Aldossary, M.N., Almohaimel, S.A., Salam, M., Almutairi, A.F., A prospective Saudi dental stem-cell bank from the perspective of the public and dental practitioners: A cross sectional survey. *Journal of Family Medicine and Primary Care*, 9 (2) (2020), 864-870. https://doi.org/10.4103/jfmpc.jfmpc_978_19

[71] Olayanju, A.O., Nkanga, A.E., Olayanju, A.J., Oluwatayo, B.O., Adesina, O., Enitan, S.S., Oladele, A.A. A Cord blood banking: the prospects and challenges of implementation in Nigeria. *Hematology and Transfusion International Journal*, 5 (4) (2017), 273-278. https:// doi.org/10.15406/htij.2017.05.00126