NARRATIVE REVIEW

Biobanking of human teeth for stem cell storage: Preserving stem cells for future needs

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Abstract

Human teeth serves as a potential reservoir for post-embryonic mesenchymal dental stem cells. Researchers have identified and isolated seven types of dental stem cells from pulp and periodontal ligament tissues. These cells have a wide range of clinical applications across the fields of medicine and dentistry due to their increased proliferative nature. Biobanking is a concept for storing human biological tissues to preserve and isolate stem cells. Until now, haematopoietic tissues have been the area of focus for biobanking facilities. Extracted human teeth may serve as a valuable resource for the isolation and preservation of dental stem cells for future therapeutic benefits. The current narrative review was planned to focus on the workflow of the teeth biobanking process, its isolation, cryopreservation, and potential therapeutic uses in medical and dental specialtiesxx

Keywords: Stem cells, Biobank, Dental stem cells.

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Introduction

Over the last decade, advancements in genetics, cell biology and regenerative medicine have made it possible to cure several human diseases using the body's own stem cells.[1] Historically, the concept of human stem cells was first reported in the 19th century.[2] Later, multiple colonies of stem cells within the human tissues were identified, including dental stem cells (DSCs).[3] Among the DSCs, dental pulp stem cells (DPSCs) were first discovered in 2006 and labelled as a pluripotent stem cell population.[4] Furthermore, researchers have identified and isolated seven types of DSCs from human teeth. These DSCs can be broadly classified as cells derived from the periodontal ligament and dental pulp.[5] Thus, human teeth serve as a potential niche for stem cells besides other tissues, such as bone marrow and skin. The therapeutic use of stem cells obtained from different human tissues, such as bone marrow, is well established for treating several blood disorders, like bone marrow transplant for the treatment of thalassemia.[6] Like other stem cells, DSCs also have a wide range of clinical applications in dentistry, ranging from regenerative endodontic procedures to tissue reconstruction of large bony defects in the oro-facial region.[7-10] Keeping in mind the vast clinical applications of DSCs, it is imperative to preserve the DSCs from extracted human teeth for future clinical use, thus providing an autologous source for the treatment of various dental and medical conditions.[11, 12]

Biobanking of human tissues is a concept based on the storage and preservation of human biological substances, from which stem cells can be isolated for future therapeutic benefits to patients throughout their life.[13] These preserved stem cells have the ability to be differentiated into various types of cells, like adipocytes, chondrocytes, osteocytes, odontoblasts, etc, depending on whether they are pluripotent or totipotent stem cells.[14] The application of stem cells is not only confined to the oro-facial region, but extends to the treatment of neurodegenerative conditions, like Parkinsonism, Alzheimer's disease, and spinal cord injuries.[15, 16] Until now, most biobanks have focussed on collecting and preserving biological tissues, such as bone marrow and foeto-maternal placental cord tissues.[17, 18] Clinicians often extract healthy and non-carious teeth due to orthodontic reasons, impacted third molars, and mobile deciduous teeth. These teeth are mostly extracted at a younger age, when the individual does not need any stem cell therapy. Thus, preserving healthy extracted teeth in a biobank is imperative for future therapeutic use.[19] However, human teeth are not extensively focussed by biobanks for the preservation and therapeutic use of DSCs.[1] The current narrative review was planned to focus on the process of collecting and preserving human teeth for biobanking, and their potential benefits across medical and dental fields.

Human tooth banking

The workflow of tooth banking starts with collecting and transporting human teeth specimens to the appropriate laboratory, where stem cell isolation and preservation is performed (Figure 1). There are 15 tooth banking facilities functioning globally (Table).[1]

The process of tooth banking consists of several steps.

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Table: List of teeth banking services available worldwide.[1]

Dental stem cell banking service	Year of establishment	Location	Collection by	Webpage
BioEden	2006	England	Patient or Dentist	https://www.bioeden.com/us/
Dentcell	Not mentioned	Mexico	Patient or Dentist	https://dentcell.com.mx/
Future Health Biobank	2002	England	Patient	https://dentcell.com.mx/
Mothercell	Not mentioned	India	Dentist	https://www.mothercell.com/
National Dental Pulp Laboratory	2007	USA	Dentist	https://ndpl.net/
Oothy	Not mentioned	USA	Patient or dentist	https://www.oothy.com/
ReeLabs	2010	India	Unspecified	https://reelabs.com/
Stemade	2009	India	Dentist	http://www.stemade.com/
Stemodontics	Not mentioned	USA	Dentist	https://stemodontics.com/
Stem Protect	2017	UK	Patient	https://www.stemprotect.co.uk/
Stem Save	Not mentioned	USA	Dentist	https://www.stemsave.com/
Store- A-Tooth	2006	USA	Dentist	http://www.store-a-tooth.com/
Store Your Cells	Not mentioned	India	Dentist	https://www.storeyourcells.com/
Tooth Bank/Cryopoint	2014	USA	Patient or Dentist	https://www.toothbank.com/

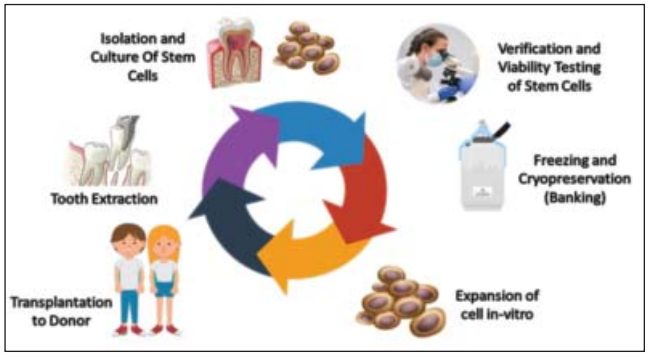

Figure: 1 Schematic representation of dental stem cell (DSC) banking process.

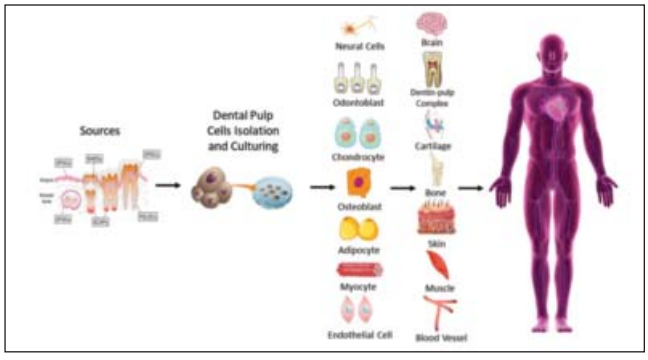

Figure: 2 Therapeutic uses of dental stem cell (DSCs).

Tooth collection and transport

The first step in collecting a tooth for a tooth bank is obtaining informed consent from the patient and the parents.[20,21] The protocols for collection by tooth bank vary according to the guidelines of laboratory processing.[1,22] Most of the tooth banking facilities recommend professional tooth extraction by a dentist having a valid practice license issued by state authority.[1] Teeth having active infection/pus, periodontal disease, cysts/tumours, or teeth that sustained a trauma mostly have compromised blood supply and are not suitable candidates for stem cell preservation. [23] Soon after the extraction of a healthy tooth, providing an adequate physiological medium and temperature is of paramount importance for maintaining the vitality of pulpal tissues.[24]

Several physiological and biological mediums for the transportation of teeth have been studied and recommended.[1] These include saliva, bovine milk, hanks balanced salt solution (HBSS), phosphate-buffered saline (PBS), propolis, and coconut water.[25] PBS and HBSS are most commonly used at tooth banking facilities.[25] Some companies have introduced their proprietary kits for storing avulsed teeth to maintain the pulp and periodontal ligament vitality.[1] After extraction, the tooth is immediately transferred to a physiologic medium and carefully sealed. To maintain an adequate temperature, it is transferred to a temperature-controlled vessel in a hypothermic state in the laboratory, and this process is called sustentation.[25] The time taken for the whole process till laboratory arrival should not exceed 40 hours.[25]

Stem cell isolation

Isolation of vital stem cells from human tissue is a critical step in laboratory procedures.[26] Most of the proprietary tooth banks do not disclose their techniques explicitly for the isolation of stem cells. As such, the current narrative review was limited to discussing the most common method performed for stem cell isolation.

When a tooth bank receives an extracted tooth, the specimen is cleaned with PBS.[27] Following this, the tooth is sectioned to expose the pulp, and, with the help of a sterile endodontic file or barbed broach, the pulp tissue is collected and placed in a sterile petri dish. The enzymatic dissolution of the pulpal tissue is carried out with collagenase and dispase enzymes for 1 hour at 37^{0}C. After this, isolated cells are passed through a 70µm filter to obtain single-cell suspensions. The cells are then cultured in a specialised medium, called the mesenchymal stem cell (MSC) medium, which contains glutamine, foetal bovine serum, L-ascorbic acid phosphate, penicillin and streptomycin. After 24 hours of culturing in MSC medium, individual colonies of cells are visible. The ingredients of the MSC medium can be tailored according to the growth of different cell lineages; adipocytes, neural cells,

chondrocytes, osteocytes and odontoblasts.[28, 29]

Storage of stem cells

Currently, there are two approaches for stem cell storage: cryopreservation and magnetic freezing.[30]

Cryopreservation is the science of storing biological tissues and cells at freezing temperature where biochemical reaction does not occur.[31] Once the stem cells are isolated, they are suspended in a preservation medium containing growth factors and dimethyl sulfoxide (DMSO), a cryoprotectant.[32] DSCs can be cryopreserved for longer and may remain viable for therapeutic use.[33] It is noteworthy that cells cultured near the log phase of the growth cycle are the most suitable candidates for cryopreservation.[34] The colonies of stem cells are preserved in vials containing liquid nitrogen at a freezing temperature. Several freezing protocols have been mentioned in the literature, depending upon the availability of resources and expertise of the scientists and laboratory technicians.[35] Controlled rate freezing is the technique in which 1-2^{0}C temperature is decreased per minute. This technique aims at maintaining stem cell viability by minimising the cells' dehydration.[36]

The ultra-slow freezing rate is a highly controlled way of decreasing the temperature by 0.3^{0}C to 0.6^{0}C.[37] Current scientific studies have shown increased cell viability using a controlled rate and ultra-slow freezing rate compared to rapid freezing methods.[38] A study observed a significantly higher number of viable cells by controlled rate freezing in 5% or 10% DMSO compared to rapid freezing methods.[39]

DSCs can be damaged during the cryopreservation process, thus leading to a decrease in cellular viability.[40] During cryopreservation, the cellular water content may crystallise and form ice crystals, leading to cell death.[41, 42] Secondly, the formation of reactive oxygen species (ROS) during cryopreservation is thought to be a culprit in the cellular death of stem cells.[43] A systematic review[44] analysed the effect of cryopreservation on the biological properties of DSCs. The review analysed 21 studies and concluded that DSCs could be safely preserved with DMSO for 2 years, maintaining their high proliferation, multipotency and stem cell markers.[44]

Magnetic freezing

The concept of magnetic freezing was first utilised at Hiroshima University, Japan, for the preservation of stem cells.[45] This technology is based on the concept that applying a weak electromagnetic field across tissue will decrease the temperature of the medium.[46] This technique has shown to be effective in terms of both whole teeth preservation and isolated DSCs. A study on the effect of magnetic freezing on intact rat teeth and pulpal tissues in the presence of DMSO as a cryoprotectant concluded that magnetic freezing was an effective method for intact tooth and pulp tissue banking.[47] Studies have reported 73% viability of cells following magnetic freezing.[48] The Hiroshima University Company is the first entity to use this novel technology, and claims an 83% survival rate of cells compared to a 63% survival rate of liquid nitrogen preservation. In terms of cost-effectiveness, magnetic freezing costs less than other cryogenics techniques.[49]

Therapeutic uses of DSCs

DSCs, due to their high proliferative and multilineage differentiation abilities, have wide applications across the fields of tissue engineering and regenerative medicine (Figure 2).[50] DSCs have the ability to reconstruct normal physiological function and repair of the injured cells.[51, 52]

DSCs have been studied for treating various kinds of neuronal tissue injuries. In animal-based studies, DSCs have improved limb functions in rats after sustaining cerebral ischaemia. DSCs could also be used in treating traumatic brain injuries replacing the neural tissue that was devitalised after traumatic injury.[53, 54] Two systematic reviews focussed on the use of DSCs to repair or regenerate dental and non-dental tissues.[55, 56] Among the included studies, most of the investigators concluded successful formation or repair of human tissues, but the clinical use of stem cells is still a subject of debate among the experts. An in vivo study observed that DSCs demonstrated the successful formation of pulp-like tissue in three-dimensional (3D) printed scaffolds, which holds a promising future for tissue engineering.[57]

DSCs can also be used to regenerate and repair bony defects. Researchers have shown that DSCs, both in-vitro and in-vivo, have the potential to re-form the tooth roots in the presence of appropriate growth factors and scaffolds.[58] Bone grafts manufactured by tissue engineering processes may be used in the future in various oral and maxillofacial surgeries. A study evaluated the role of DSCs in repairing large-size defects in mice, and found that DSCs isolated from primary teeth had significantly repaired the bony defects.[59] In addition, several anatomical structures, such as condyles, calvarium and adipose tissue, have been successfully engineered from DSCs. Thus, DSCs hold a promising position in the near future for oro-facial regenerative medicine.[60]

Endodontic treatment is the first-line treatment by clinicians for treating and preventing pulpal infections. Stem cell therapy has shown the ability to regenerate the lost pulpal and dentinal tissue in the presence of appropriate conditions. Studies have demonstrated the

formation of pulp-like tissue in artificial teeth having vascularity like pulp and dentine like hard tissue.[61, 62]

Conclusions

DSCs might be a helpful tool for numerous regenerative medicine applications. With advancements in research and technologies, DSCs have become one of the target adult stem cells that need further exploration and development. Although remarkable achievements have been made regarding the regenerative potential of DSCs in various medical and dental fields, several challenges yet remain. The existing scientific evidence is mostly based on animal and in-vitro studies. Thus, the clinical translation of these benefits has yet to be established. Biobanking in dentistry is an efficient tool for advancing research and clinical translation on oral and systemic diseases. It should also help generate therapeutic benefits and be a fundamental step towards personalised medicine.

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SH: Concept, writing and original draft.
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AA: Resources and visualisation.
FRK: Writing, reviewing, editing and supervision.