



Dental Pulp Stem Cell: A Promising Tool in Forensic Odontology

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ABSTRACT-

Forensic odontology, also known as forensic dentistry, is a specialized field within dentistry that deals with the application of dental science in medico-legal investigations, identifying accidental human remains during mass disasters, identifying victims from the site of crime scene. Most of human body parts perish after death, but tooth is one such organ which is incredibly resilient, preserving DNA well even in extreme conditions that may destroy other human body parts. Therefore, they serve as an excellent source of genetic material for identifying individuals in forensic cases. Stem cells, with their ability to develop into different cell types, have various applications in forensic investigations, including identification, reconstruction, and age estimation. Dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) are particularly valuable in forensic identification. Amongst other methods used in Forensic odontology the use of DPSCs gives us significant results and aid in an accurate finding. The article intends to discuss the basis of DPSCs, its sources and methods of isolation, applications, accuracy of DPSCs through case study and its limitations.

Keywords: Forensic Odontology; Forensic Dentistry; Stem Cells; Dental Pulp Stem Cells

Introduction

Ambiguity and uncertainty pertaining to several tools of forensic odontology such as cheiloscopy, rugae patterns, bite mark analysis, dental records, photographs, radiographs etc. can lead to difficulties owing to their lack of confirmatory nature. There is a need for more certain and confirmed results and hence Dental Pulp Stem Cells (DPSC) have emerged as a promising tool in forensic odontology. The application of stem cells in forensic dentistry offers several benefits for both identification as well as for investigative purposes. This article elucidates the role of stem cells in advancing forensic dentistry thus showcasing the impact of stem cell technology in this field.

What is stem cell?

Stem cells are primitive cells found in all multi-cellular organisms that are characterized by self-renewal and the capacity to differentiate into any mature cell type.¹

Based on the origin adult stem cells can be classified in to two types: germline and somatic stem cells. Variety of somatic stem cells have been discovered in different parts in the human body like mesenchymal stem cells (MSCs) are found in the stroma of adult bone marrow which can differentiate in to osteoblast, adipocytes in response to appropriate signal.

Noteworthiness of the dental pulp and dental pulp stem cells

Human tooth consists of hard and soft tissues. Enamel, dentin and cementum are the hard tissues. Dental pulp has rich innervation, vascular connective tissue and periodontal ligament (PDL) which attaches the tooth to the bony socket are the soft tissues present in the tooth. Dental pulp is a soft tissue that is encased within a hard mineralized tissue that protects and preserves it. Human teeth can withstand the temperature of 1600o C and extreme environmental conditions.² This persistence of dental tissue makes it useful in various applications in forensic dentistry. Also, the rich cellularity and vascularity makes the dental pulp a reservoir of Dental Pulp Stem Cells (DPSC) which has the lifelong ability to regenerate and differentiate into odontoblasts,

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neural progenitors, osteoblasts, chondrocytes, and adipocytes.³

Different sources of stem cells in orofacial region:

Apart from Dental Pulp Stem Cells (DPSC), we have other sources of stem cells in the orofacial region such as Stem Cells From Human Exfoliated Deciduous Teeth (SHED), Periodontal Ligament Stem Cells (PDLSC), Dental Follicle Stem Cells (DFSC), Stem Cells from Apical Papilla (SCAP), Epithelial Stem Cells (EpSC), Induced Pluripotent Stem Cells (iPSC), Oral Epithelial Stem Cells (OESCs), Immature Dental Pulp Stem Cells (IDPS), Gingiva derived MSCs (GMSCs), Tooth Germ Progenitor Cells (TGPCs), Salivary gland stem cells (SGSCs) and Periosteum derived Stem Cells (PSCs), Bone Marrow Stem Cells (BMSC).

Methods of isolation and storage of dental pulp stem cells:

Methods of isolation: Table no. 1 and 2 shows various methods of isolation and approaches used for stem cell storage respectively.⁴

Methods of isolation (a) Size-sieved isolation Enzymatic digestion of whole dental pulp tissue in solution of 3% collagenase Type I for 1 h at 37°C is done followed by the process of filtering, seeding, culture and amplification. (b) Stem cell colony cultivation Enzymatic digestion of the dental pulp tissue is done to prepare single cell suspension cells followed by colony formation and amplification. (c) Magnetic activated cell sorting (MACS) Immune-magnetic method used for separation of stem cell populations based on their surface antigens (CD271, STRO-1, CD34, CD45, and c-Kit). (d) Fluorescence activated cell sorting (FACS) Isolation of stem cells from cell suspension based on cell size and fluorescence. Table no. 1. Name of various methods of isolation of DPSCs and its procedure.

Approaches used for stem cell storage (a) Cryopreservation Liquid nitrogen vapour is used to preserve cells at a temperature of <-150°C. (b) Magnetic freezing. It works on principle of applying a weak magnetic field to water or cell tissue which will lower the freezing point of that body by up to 6-7°C. Table no. 2. Methods of stem cell storage and its procedure.

DPSCs maintain their stem cell properties even after cryopreservation as reported by Zhang et al.⁴

Applications of stem cell in forensic odontology:

1. Regeneration and Repair of Dental Tissues

Stem cells have the capacity to regenerate and repair dental tissues, which is useful in reconstructive forensic

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(b) Stem cell colony cultivation	Enzymatic digestion of the dental pulp tissue is done to prepare single cell suspension cells followed by colony formation and amplification.
(c) Magnetic activated cell sorting (MACS)	Immune-magnetic method used for separation of stem cell populations based on their surface antigens (CD271, STRO-1, CD34, CD45, and c-Kit)
(d) Fluorescence activated cell sorting (FACS)	Isolation of stem cells from cell suspension based on cell size and fluorescence.

Table 2: Methods of stem cell storage and its procedure

Approaches used for stem cell storage	
(a) Cryopreservation	Liquid nitrogen vapour is used to preserve cells at a temperature of <-150°C.
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cases where teeth have been damaged or are missing. They can aid in the identification process by reconstructing dental features which are unique to individuals.⁵ Methods such as cell homing, transplantation, explant outgrowth and enzymatic digestion can be used to regenerate tissues using DPSC.⁶

2. Age Estimation

DPSCs are valuable in forensic investigations due to their regenerative properties and ability to provide high-quality DNA. Changes in the cellular and molecular composition of DPSCs can be indicative of age, making them useful for age estimation in forensic cases.⁷ Age estimation has applications both in postmortem identification as well as in living individuals where chronological age is under dispute. The dental pulp can be considered a tissue, in which biological age approximates chronological age.⁸ As age progresses cell density of dental pulp cells of crown and



root region changes, fibroblasts of the older age group appeared to be flattened and spindle shaped when compared with the fibroblasts of the younger age group.

3. Sex Determination

Dental stem cells can be used to identify the sex of an individual. Amelogenin gene, located on the X and Y chromosomes, is used in forensic science for determination of sex. Differences in the length of the amelogenin gene on the X and Y chromosomes helps in this through polymerase chain reaction analysis.⁹ DPSC is a good source for extraction of DNA. Restriction fragment length polymorphism, polymerase chain reaction (PCR), and microarrays are various methods to analyze DNA.¹⁰ Barr bodies are strongly stained chromatin material present in nucleus of female somatic cells and F bodies are available in Y chromosome which can be used in sex determination. The accuracy of DPSCs in sex determination is as high as 99%.¹¹

4. Disaster Victim Identification

In mass disasters, dental pulp stem cells can be used to rapidly extract DNA which will help in victim identification and even in cases where other tissues are compromised.¹²

DNA in dental pulp remains stable for long periods, even in harsh conditions like extreme temperatures or post-mortem changes. This stability is significant in disaster victim identification where body remains may be exposed to severe environmental conditions.¹³

5. Criminal Identification

The dental pulp is a good source of genomic DNA. Extraction of genomic DNA and amplification of dental pulp stem cells through Polymerase chain reaction technique leads to a positive identification of the human in question.¹⁴

6. Blood Group Determination

Blood group antigens are preserved for over 2 years after the death of an individual and can be retrieved from the dental pulp. ABO genotyping using dental pulp can be used to identify blood group of an individual by 'absorption elution technique'.¹⁵

7. Post Mortem Interval Estimation

The correct estimation of post mortem interval (PMI) can be crucial aspect on the success of a forensic investigation. Researchers used diverse method to

estimate PMI, considering physical changes that occur after death using DPSCs.¹⁶

Discussion

In 2000, Gronthos et al. isolated the first MSC like cells from the human dental pulp.¹⁷ Studies in the stem cells of dental pulp showed that it has a high potential for proliferation in turn promotes auto renewal.¹⁸

The pilot study conducted by Ilenia Bianchi et al "Dental DNA as an Indicator of Post-Mortem Interval (PMI): A Pilot Research" explores the feasibility of using Next-Generation Sequencing (NGS) to analyze genetic mutations in dental pulp DNA post-mortem samples. By examining mutations in a 56-gene panel, they aimed to correlate these changes with the time since death. The findings suggest that there are specific genetic mutations in dental pulp DNA can indicate PMI, thus providing a potential tool for forensic investigations.¹⁹

A study conducted in the year 2010 by Nayar et al determined gender by pulp tissue using fluorescent Y body test and reported 100% specificity.²⁰ A study conducted by Saxena et al. proved the sensitivity of blood group determination using dental pulp by the absorption-elution technique to be 80% accurate.²¹

Gawande et al in a study showed that postmortem DP shows well-preserved histological appearance for up to 72 hours in contrast with destruction and degeneration seen in other body tissues as early as 24 hours, making it a viable candidate for soft-tissue age estimation. Vavpotic et al have demonstrated that odontoblast numbers can be used to estimate time of death for about 5 days postmortem, further signifying the role of DP in forensic odontology. Another application of DP in forensics has been described by Sandoval et al who validated that the Barr body test can be applied to DP fibroblasts of human remains exposed to extreme conditions of pH, humidity and salinity for gender identification with 98.9% accuracy.²² Daud et al. in their study used the histological method for age determination and found that cell density of dental pulp cells of crown and root region changes with age.²³ Sweet and Sweet in 1995 presented a case of human remains identification using a preserved unerupted third molar which enabled 1.35 µg DNA extraction from the dental pulp.²⁴

A study conducted in the year 2011 by Nalini A to emphasize the sensitivity and specificity of dental pulp in identifying the ABO Blood group, Rhesus factor and also to emphasize the role of dental pulp in forensic odontology to identify the



deceased individual. 60 individuals were studied out of which 57 showed positive results wherein blood group identification from the capillary blood and the dental pulp extract was compared. The study concluded and highlighted the high potential of dental pulp in the identification of blood group and hence serve a great tool of identification in forensic identification.²⁵

Limitations:

Despite the potential, stem cells in the field of forensic odontology also face some limitations. Usage of stem cells in the field of forensic can also raise legal as well as ethical concerns regarding the consent and privacy. There is a need to adapt to the ever-advancing techniques for the evaluation of these stem cells without which the results will fall short of its accuracies.

Conclusion:

The potential of dental stem cells in forensic odontology is significant although it is accompanied by few limitations. Their applications in forensic dentistry include aiding in the regeneration and repair of dental tissues, age estimation, sex determination, and disaster victim identification through DNA analysis and criminal identification. But, challenges such as ethical and legal concerns, the need for advanced technology limits their use. Despite these limitations, ongoing research, like studies on the expression of mesenchymal stem cell markers and genetic mutations in dental pulp DNA, all these endeavors have the potential to advance the field, thus pointing towards more accurate and reliable forensic tools in the future.

Conflicts of interest:

None.

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