**UNLOCKING THE POTENTIAL: DENTAL PULP STEM CELLS IN REGENERATIVE MEDICINE**

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**INTRODUCTION**

 Stem cells are undifferentiated primitive cells with the remarkable capacity to divide and differentiate into specialized cells, making them of paramount importance in the field of medicine. Stem cells can be sourced from various origins, including embryos, fetuses, and adult tissues. In adults, major sources include bone marrow, adipose (fat) tissue, brain tissue, human exfoliated deciduous teeth (SHED), dental pulp (DPSCs), and the periodontal ligament (PDLSCs). Researchers firmly believe that stem cells hold the potential to offer treatment options for a diverse array of diseases.

The recent discovery of dental stem cells, coupled with advances in cellular and molecular biology, has opened up exciting possibilities for the regeneration of oral tissues damaged by disease or trauma. These breakthroughs have paved the way for novel therapeutic approaches, promising improved outcomes in reparative dentistry.

The rapid expansion of knowledge in stem cell technology across all medical disciplines underscores the necessity for innovative strategies in various fields, including reparative dentistry. With the integration of tissue engineering, the long-cherished goal of repairing and regenerating defective tissues and organs is on the brink of becoming a reality.

**HISTORY**

The term "stem cell" was introduced by Alexander Maksimov, a Russian histologist, during the 1908 Congress of the Hematologic Society in Berlin. It wasn't until the early 1960s that Canadian scientists began to yield promising results in stem cell research. A significant milestone came in 1998 when the first human embryonic stem cell line was derived at the University of Wisconsin-Madison.

Stem cells are a type of biological cell found in all multicellular organisms. They possess the unique ability to divide and differentiate into various specialized cell types and can also self-renew, generating more cells. Stem cells hold great promise for tissue repair and regeneration. There are two primary categories of stem cells: embryonic stem cells and adult stem cells.

Embryonic stem cells originate from the inner cell mass of the blastocyst, a hollow, thin-walled structure formed during early embryonic development. The inner cell mass contains a cluster of cells from which the entire embryo develops. In contrast, the outer layer of cells gives rise to the placenta and other supportive tissues necessary for fetal development within the uterus. Embryonic stem cells have the remarkable capacity to differentiate into cells from all three germ layers and possess the ability to develop into a wide range of cell types.

On the other hand, adult stem cells are found in various tissues, including the umbilical cord blood and bone marrow. While pluripotent stem cells in umbilical cord blood are relatively scarce, they have still proven valuable in certain medical treatments. Adult stem cells have been successfully used for years in the treatment of conditions like leukemia and bone/blood cancers through bone marrow transplantation, as well as some hematopoietic diseases.

**STEM CELL BANKING**

Stem cell banks are increasingly recognized as vital resources for both basic and translational research. These banks and registries play a crucial role in maintaining international access to high-quality and ethically sourced stem cell lines originating from diverse sources and of varying grades, such as research-grade versus clinical-grade cells. Additionally, they serve as repositories for "biological standards," which are indispensable for standardizing research and medical applications.

Global efforts are underway to address synchronization and standardization processes within the field of stem cell research and banking. Notable initiatives include the International Society for Stem Cell Research and the International Stem Cell Banking Initiative. Stem cell banks are also at the forefront of ensuring domestic policy consistency concerning the ethical and practical aspects of conducting stem cell research.

The term "stem cell bank" can encompass a variety of operations and associations, spanning different levels and types of activities.

In India, Life Cell International introduced the country's first stem cell banking services in Bangalore in 2009. The cost for collecting and storing stem cells with them typically amounts to around 3000 USD, with separate annual storage fees. However, a notable challenge with stem cell banks is that their charges may not be affordable for individuals from lower economic groups.

**THE DENTAL PULP STEM CELLS**

In 2003, a Pedodontist named Dr. Songtao Shi made a groundbreaking discovery while examining the deciduous teeth of his six-year-old daughter. He identified and named these remarkable cells "stem cells from the human exfoliated deciduous teeth" (SHED). This discovery marked a significant milestone in dental science.

Dental Pulp Stem Cells (DPSCs) are found within the "cell-rich zone" of the dental pulp. Their origin from the embryonic neural crest explains their remarkable multipotency. Under specific stimuli, these versatile stem cells can differentiate into various cell types, including adipocytes, neurons, chondrocytes, and mesenchymal stem cells. DPSCs are among the most promising stem cells, with a wide range of therapeutic applications.

It's noteworthy that dental pulp stem cells can be found in both adults and children. Stem cells of dental origin hold the remarkable ability to generate dental tissues. SHED and DPSCs, in particular, exhibit the capacity to generate tissue with morphological and functional characteristics closely resembling those of human dental pulp.

These discoveries have opened up exciting possibilities in the field of dentistry and regenerative medicine, offering potential solutions for various dental and oral health issues.

**ADVANTAGES OF DENTAL PULP STEM CELLS**

Unlike umbilical cord blood cells, which must be collected immediately at birth, dental stem cells can be derived from both deciduous (baby) and permanent teeth. With a total of 20 viable deciduous teeth and 32 permanent teeth available for collection, the process is non-controversial and devoid of ethical concerns. Furthermore, obtaining dental stem cells is a straightforward procedure, posing no risk of mortality or morbidity.

**Sample selection criteria**

Deciduous teeth

(1) Pulp should be vital.

(2) Deciduous teeth with two third of root is preferred.

(3) Extracted teeth are preferred than loose teeth.

 **Adult teeth**

 (1) Only vital teeth should be harvested.

 (2) Teeth with infection and any pathology are avoided.

 (3) Mobile teeth with lack of blood supply can’t be harvested.

(4) Teeth should have sufficient amount of pulp.

**Steps in the dental clinic**

• Examine the tooth and rule out any infection. The tooth has to be removed

 • Rinse the tooth.

 • Transfer to transportation tube.

• Add saline solution. • Wait for five minutes.

• Seal the tube.

• Transport under room temperature before 48 hrs.

**Steps in the laboratory**

• Identification of stem cells with markers.

• Separation of viable cells by centrifuge.

• Cryopreservation.

• Retrieval.

**THE ISOLATION OF THE DENTAL PULP STEM CELLS**

Stem cells are identified through various techniques, including flow cytometer, fluorescence-activated cell sorting (FACS), and magnetic-activated cell sorting (MACS), as well as the use of biomarkers such as surface markers and side populations.

Magnetic-Activated Cell Sorting (MACS) is a method employed to separate different cell populations based on their surface antigens. This technique involves incubating cells with magnetic nanoparticles coated with antibodies specific to particular surface antigens. Cells expressing the targeted antigen then bind to the magnetic nanoparticles. Subsequently, a strong magnetic field is applied, causing the nanoparticle-bound cells to remain in the column while allowing other cells to flow through. MACS enables the separation of cells based on specific antigens of interest.

Fluorescence-Activated Cell Sorting (FACS) is a specialized form of flow cytometry that allows for the sorting of a heterogeneous mixture of cells into two or more containers, one cell at a time. This sorting is based on the unique light scattering and fluorescent characteristics exhibited by each cell. FACS provides a rapid, objective, and quantitative assessment of the fluorescent signals emitted by individual cells, and it allows for the physical separation of cells that are of particular interest.

Cell surface markers play a vital role in classifying and isolating stem cells and monitoring their differentiation states. These markers are valuable because they can be directly visualized on intact cells, aiding in the precise identification and isolation of specific cell populations.

**CRYOPRESERVATION**

 The haematopoietic stem cells have been cryopreserved and successfully utilized for transplantation. The dental pulp can be easily crayo stored for long periods and it can be used to form a crayo bank for adult tissue regeneration. The dental pulp stem cells retain their potential after cryopreservation. In a study which was performed on the cryopreserved tissue samples of periodontal ligaments. The cryopreservation of the whole dental pulp leads to a safe recovery. Different cryopreservation techniques are required for the whole pulp. These features make these cells for a therapeutic three-dimensional tissue reconstruction, with the potential of storage and recovery as per the needs of the patient. Dental pulp stem cells can also be obtained from the patient’s vital pulp, since we have 20 deciduous and 32 permanent teeth. This can be done with the help of stem cell markers, which help in the identification of stem cells.

**REGENERATION OF THE TOOTH TISSUE AND BLOOD VESSELS**

It has been observed that Stem Cells from Human Exfoliated Deciduous Teeth (SHED) have the potential to differentiate into functional vascular endothelial cells through a process that resembles vasculogenesis. These findings raise hope that dental pulp-derived stem cells may hold promise in treating severe ischemic conditions of the heart, brain, or limbs. One specific challenge in dental pulp tissue engineering is the creation of a functional vascular network, especially given the requirement that all vascularizations must access the root canal through the apical foramen. Therefore, additional research is necessary to induce vasculogenesis as part of the efforts in dental pulp tissue engineering.

**WHOLE TOOTH REGENERATION**

By placing stem cells on biodegradable scaffolds, tooth-like tissues have been successfully generated. Ikeda et al. reported the successful development of a fully functioning tooth replacement in an adult mouse through the transplantation of a bioengineered tooth germ into the alveolar bone at the site of the lost tooth.

Xu et al. seeded a tooth bud from a rat onto scaffolds made from silk fibroin, which had two pore sizes. These scaffolds were either used in their fabricated state or treated with the Arg-Gly-Asp attachment site binding peptide. While these studies have demonstrated the regeneration of dental tissues, the success rate for achieving the correct arrangement of a natural tooth is currently only 15-20%. Therefore, further research is necessary to achieve structurally sound teeth."

**BONE TISSUE REGENERATION**

DPSCs, when they undergo differentiation into pre-osteoblasts, form an extracellular matrix that becomes a calcified woven bone tissue. Other than this, it has been demonstrated that such tissues undergo remodelling, when they were transplanted in immunocompromised rats, and that they form a lamellar bone with entrapped osteocytes.

**IN TREATING VARIOUS DISEASES**

Stem cells play a vital role in treating various lives threatening diseases. Other than forming the bone, the blood vessels, the whole tooth and the dental tissues, the dental pulp stem cells can also be used to treat myocardial infarction, Parkinson’s disease, Diabetes mellitus and certain forms of cancer.

**CONCLUSION**

The identification of various types of epithelial and mesenchymal stem cells in teeth represents a significant achievement. However, controlling morphogenesis and cytodifferentiation remains a challenge, necessitating a thorough understanding of the cellular and molecular events involved in tooth development, repair, and regeneration.

Current stem cell research also contributes to our understanding of cancer stem cells, aiding the development of novel therapies to combat cancer. Nevertheless, there is an ongoing need to discover new, easily accessible sources of both epithelial and mesenchymal stem cells that can be reprogrammed for odontogenic potential and subsequently combined to create a fully functional tooth.

To address these challenges, we must harness technology, determination, and dedication. Achieving this will require sustained collaboration across various disciplines, including medicine, biotechnology, bioengineering, and biomaterials. It will also depend on significant support from governments and international agencies, as well as the understanding and endorsement of the public.

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