

Stem cells – The powerhouse of tissue engineering

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Abstract

Periodontal tissue rebuilding is one of the major processes, involving the regeneration of the periodontal ligament, cementum, and bone, the periodontium's three principal structures. This approach to bone and periodontal regeneration combines three key elements to enhance regeneration: Cells, Scaffolds, and Signaling molecules. When applying tissue engineering strategies the source of the cell is a crucial factor to consider to restore lost functions and tissues. Through a process of asymptomatic mitosis, differentiation and self-renewal are both possible to result from stem cells. That lead to two daughter cells and are capable of differentiation into more mature cells. In this review, the classification of cells, that is, totipotent, pluripotent, multipotent, adult stem cells and embryonic stem cells, and the various dental stem cells that have the capacity for self-renewal and multilineage differentiation potential has been discussed. The different cell storage methodologies to maintain the viability of these cells such as cryopreservation, magnetic freezing, and tooth stem cell banking as well as the recent advances in stem cell engineering such as induced pluripotent cells, cell sheet engineering, bio tooth, and hybrid tooth have been discussed. The biological, technical, and clinical challenges associated with cell based approach and future perspectives have been considered in the review.

Introduction

Tissue engineering has evolved as a promising field that draws on polymer chemistry, surgery, molecular biology, physiology, advances in medicine, and cellular biology.

The aim of using tissue engineering has been to extract primed and selected cells together with an appropriate mixture of regulatory factors, to allow specialization and growth of matrix and cells. In 1993, Langer *et al.* offered tissue engineering as a method for repairing lost periodontal tissue which combines three key elements to enhance regeneration: Cells, Scaffolds, and Signaling molecules to bone and periodontal regeneration to restore lost tissues and functions, when applying tissue engineering strategies source of the cell is a crucial factor to consider.^[1]

What are Stem Cells?

Self-renewing cells that differentiate into multi-lineage cells through an asymptomatic mitotic process that produces two daughter cells, one of which can differentiate into more mature cells (progenitor cells) and the other of which is similar to the stem cell (daughter stem cell).^[2]

Classification of Stem Cells

Stem cells may be:^[3]

1. Totipotent, embryonic cells that can give rise to all embryonic organs including the placenta (1–3 days after oocyte fertilization)
2. Pluripotent, that is, blastocystis embryonic cells (4–14 days after oocyte fertilization) that can only differentiate into embryonic tissues of the inner cell mass (ectoderm, mesoderm, and endoderm)
3. Multipotent, that is, embryonic cells that can give rise to tissues belonging to only one embryonic germ layer from the 14th day onwards (ectoderm or mesoderm or endoderm).

Stem cells can be split into two groups based on the stage of development of the tissue from which they are extracted.^{.[4]}

- Adult stem cells and
- Embryonic stem cells.

Blastocysts are 2–11-day-old embryos that are derivatives of embryonic stem cells. They are totipotent cells, which means they have unlimited potential. Its application has been limited to the field of study due to ethical issues and the possibility of tumorigenicity and teratoma development.

Adult stem cells are multipotent stem cells that can be divided into hematopoietic stem cells and mesenchymal stem cells (MSCs) depending on their origin. Friedenstein (1976) were the first to discover MSCs in adult bone marrow aspirates. Adult stem cells derived from bone marrow, also known as MSCs are adherent, growing cells that can differentiate into a variety of cell types. Allowing them to differentiate into a variety of tissue types such as bone, cartilage, muscle, and tendon, and have a lot of potential for autologous cell-based therapy. In the field of periodontal tissue engineering, mesenchymal derived cells have been used to regenerate the attachment apparatus components at the same time.^[5]

Sources of Stem Cells

Several oral tissues such as periostium, oral mucosa, gingival craniofacial bone, dental follicle, dental pulp, tooth germ PDL, and apical papilla can be used isolate stem cells.^[6]

Dental Stem Cells (DSC)

The DSCs are capable of self-renewal and differentiate to multi-lineage cell populations. These cells are distinct from MSCs generated from bone marrow (BMMSCs), which are derived from mesoderm and the neural crest. Because of their easy accessibility, less invasive collection, and clinical potential, oral tissue-derived stem cells are among the most popular, human dental pulp stem cells have been widely studied. These cells were found to differentiate into osteoblast-like cells that formed bone *in vitro* and form dentin-like tissue. In the presence of particular stimuli, DPSCs developed into chondrocytes, adipocytes, and neurons, among other cell types. Vascular endothelial cells and DPSCs have been reported to develop into osteoblasts and endothelial cells in a synergistic manner.^[7]

DSC

The dental pulp is derived from the dental papilla and is ectomesenchymal and mesenchymal in nature. They are derived from various developmental phases of dental tissue-derived MSCs, and there are around eight distinct populations that have been identified and described. The first human dental MSCs discovered in pulp tissue were post-natal DPSCs. Other dental MSC-like populations include stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), dental follicular progenitor cells (DFPCs), and alveolar bone derived MSCs.^[8]

The potential to regenerate into multiple tissues is demonstrated by DPSC and SHED. It has recently been demonstrated that implanting DPSCs or SHED enhances functional recovery following spinal cord damage. In neonatal mice, DPSC also protects against ischemic brain injury.^[9]

SHED

SHED showed a high degree of flexibility, able to differentiate into neurons, osteoblasts, adipocytes, and odontoblasts.

These cells, which were isolated from the pulp tissue of exfoliated deciduous teeth' crowns, did not grow as individual cells, but rather in clusters, creating colonies that, following separation, expanded as individual fibroblast-like cells. SHED have a higher rate of proliferation as well as a great potential for odontogenic and osteogenic differentiation.^[9]

DPSC

The first type of DSC derived from dental pulp was DPSCs. These multi potential cells had a fibroblast-like shape to them.^[10]

Chondrogenic, osteogenic, and adipogenic cell type can all be formed from these cells, exhibit epithelial markers, and share characteristics with neural stem cells; *in vitro*, these cells can also develop into vascular endothelial or neural cells. They exhibit multiple markers, including STRO-1 and CD146, which are indicators for mesenchymal and bone marrow stem cells, as well as OCT4, which is a marker for embryonic stem cells. STRO-1, CD29, CD44, CD73, CD90, CD105, CD146, CD166, and CD271 are some of the possible DPSC markers.^[11]

PDLSC

PDL is made up of many cell types that can be divided into cementoblasts and osteoblasts. These cells proliferated at a faster rate than DPSCs and expressed CD146, a tendon-specific transcription factor and STRO-1. When cultivated with the appropriate inductive conditions, PDLSCs can differentiate into osteogenic, adipogenic, and chondrogenic cells.^[12]

DFPC

The dental follicle is made up of ectomesenchymal cells that form a protective sac around the unerupted tooth. During tooth eruption, it regulates the processes of osteoclastogenesis and osteogenesis and develops into the periodontium. When induced with BMP-2 and BMP-7, as well as enamel matrix derivatives, the ability to develop and express cementoblast markers (EMDs).^[13]

ABMS

These cells have a fibroblast-like spindle-shaped morphology, colony formation, and plastic adhesion. The markers CD73, CD90, CD105, and STRO-1 are expressed on these cells, while the hematopoietic markers CD14, CD34, and CD45 are not. They can differentiate into chondrogenic and adipogenic tissues in the same way as other stem cell types can.^[9]

SCAP

When compared to PDLSCs, SCAP possesses the ability to develop into odontoblasts. Early mesenchymal surface markers, particularly CD24, are present in SCAP, which may be a population-specific marker. When cultivated in the appropriate inductive media, these cells showed their potential to differentiate into neurogenic, chondrogenic, adipogenic, and osteogenic cells.^[14]

TGPC

TGPCs are a type of stem cell that was discovered in the late bell stage of the third molar tooth germ, dental mesenchyme, and can be maintained and expanded for sixty population doublings while keeping their spindle-shaped form and strong proliferation rate.^[15]

In vitro, TGPCs can develop into cells with morphological, phenotypic, and functional features similar to hepatocytes. TGPC has the ability to differentiate into chondrocytes, adipocytes, osteoblasts, odontoblasts, and neurons, which is similar to that of other dental MSCs.^[16]

GMSC

Multipotent differentiation, self-renewal, and clonogenicity are all capabilities of GMSC, which also have stem cell-like and immunomodulatory features. In long-term cultures, GMSCs have exhibited the ability to self-renew and produce connective tissue-like structures *in vivo*, as well as the ability to create mineral, fat, and cartilage-like matrix *in vitro*, demonstrating their multilineage differentiation potential. GMSCs had osteogenic capacity *in vivo* after being incubated in an osteoinductive media *in vitro*. These characteristics suggest that clinical use of GMSCs for tissue regeneration and repair is a promising therapeutic strategy.^[17]

BMSSCs

For therapeutic purposes, BMSSCs have the ability to produce bone. In athymic mice, *ex vivo* expanded human BMSSCs were observed to improve bone growth and bone strength. The effectiveness of employing transplanted BMSSC produced from autologous or HLA-compatible bone marrow to promote bone formation in patients with a variety of illnesses including osteogenesis imperfecta, Hurler syndrome, and metachromatic leukodystrophy.^[18]

Storage of Stem Cells^[19]

The success of stem cells banking over the long term is critically dependent on the methodology.

Cryopreservation

Cells or entire tissues are frozen to sub-zero temperatures in order to preserve them. Cells taken at the completion of the log phase of development (about 80–90% confluent) are excellent candidates for cryopreservation. Liquid nitrogen vapor is employed to keep cells at a temperature of 150°C. 1.5 ml of freezing media per vial is ideal for 1–2 10⁶ cells.

Magnetic freezing

The cells alive system (CAS) is a device that operates on the premise of introducing water or cell tissue in the presence of a tiny magnetic field, lowering the freezing point of that body by up to 6–7°C. Hiroshima University believes that with CAS, the rate of cell survival in teeth may be increased to 83 percent.

Tooth stem cell banking

Although tooth banking is not widely practiced, the trend is gaining traction, particularly in industrialized countries. BioEden (Austin, Texas, United States) has international laboratories in the United Kingdom (serving Europe) and Thailand (serving Southeast Asia), with aspirations to expand globally. Store –A- Tooth (Provia Laboratories, Littleton, Massachusetts, USA) and StemSave (Stemsave Inc, New York, USA) are two stem cell banking organizations that are expanding abroad. The first tooth bank in Japan was created at Hiroshima University in 2005, and the company was titled “Three Brackets” (Suri Buraketto). Last but not least, Stemade recently launched operations in Mumbai and Delhi, bringing the notion of DSC banking to India.

Recent Advances in Stem Cells and Tissue Engineering

Induced pluripotent stem cells

Experiments have been conducted to see if somatic cells, in addition to MSCs, may be induced into pluripotent stem cells. Takahashi *et al.*, 2007^[20] showed that inducing the expression of specific transcription factors can turn adult somatic cells into pluripotent stem cells. Thus, utilizing the essential transcription factors, cells derived from the oral epithelium or other dental sources can be easily converted into the desired cell type. These stem cells have gotten a lot of interest since they are quite similar to embryonic stem cells, which means they have a lot of potential for periodontal regeneration.^[21]

Cell sheet engineering

Rationale - demonstrating non-enzymatic cell harvesting is noninvasive, gentle, and safe for cells while preserving the extracellular matrix cells were cultivated on a temperature-responsive intelligent cell culture surface. Cultured cells can be harvested as an unbroken contiguous cell sheet and transferred to host tissue by lowering the temperature from 37°C to 20°C. This technique, known as “cell sheet engineering,” has been successfully utilized to manufacture tissue-like grafts utilizing a variety of cells in multiple clinical trials.^[22]

Bio-tooth

A bio-tooth is a type of biological tooth that may be re-integrated into the jaw and perform the same tasks as a natural tooth, including the ability to regenerate after an injury. In dental tissue engineering, using the notion of epithelial-mesenchymal interactions to direct tooth regeneration has become a prevalent method. Many research have shown that dental cells recombined with or without scaffolds, pre/post-natal dental cells, and even non-dental cells can restore the bio-tooth.^[23]

Hybrid tooth

The scaffolds were made by mixing tooth bud cell-seeded scaffolds were combined with autologous iliac crest bone marrow derived stem cell-seeded scaffolds and then implanted into surgically

created mandibular deficiencies in the same minipig. After 12 and 20 weeks of growth, the constructions were harvested. X-ray, ultra-high-resolution volume computed tomography, histological, and immunohistochemical studies. The creation of tiny tooth-such as structures with structured dentin, enamel, pulp, cementum, periodontal ligament, and surrounded by regenerated alveolar bone shows that teeth and related alveolar bone can be regenerated in a single surgery. This model presented a method that could be used in human clinical trials in the future.^[24]

Challenges in Stem Cell Based Research^[25]

Biological challenge

Although biological evidence demonstrates that regeneration can occur in humans, complete, and consistent regeneration remains an elusive clinical aim (particularly in severe periodontal abnormalities). Understanding the role of progenitor cells in periodontal healing has benefited from the characterization and isolation of stem cells from periodontal tissues. Most basic discoveries on periodontal ligament cells are based on cell culture and animal models. The pathways that underlie stem cell self-renewal and differentiation are also largely unknown.

Technical challenge

Cell manipulations, scaffold materials, and delivery systems all provide technical challenges. Culture conditions are not yet advanced enough to replicate the cell microenvironment *in vivo*. Infections are a common occurrence. Second, there is an inherent limit in terms of timing. *Ex vivo* processing can take weeks or months in some cases. The next step is to find the most biocompatible scaffolding materials and delivery system.

Clinical challenge

Oncogenic features of stem cells, functional integration, and immunological rejection after delivery of transplanted tissues into the host are all factors in stem cell periodontal therapy.

Future Perspective

- The current laboratory techniques need to be refined to facilitate translation and in order to advance the field, it will be equally important to handle stem cells in a therapeutic context
- Studies on both and adult embryonic stem cells should continue to be studied as part of a larger effort to learn more about what goes wrong in the illness process. and how cells function
- This knowledge base will help to decide future treatment options, making gene therapy and stem cell-based tissue engineering a viable option for periodontal regeneration.

Conclusion

The regeneration of periodontal tissues that have been destroyed owing to trauma or illness is a substantial challenge.

Many elements that parallel periodontal growth must be considered during periodontal regeneration, including the use of an optimum progenitor cell population, signaling molecules, and matrix scaffold in an orderly temporal and geographic sequence.

Adult stem cells are adherent, proliferative, and capable of differentiating between many lineages, allowing them to divide into different types of tissue. They show significant potential for autologous cell-based therapy. MSCs have been used for simultaneous regeneration in the field of periodontal tissue engineering.

The periodontal ligament is now recognized as a rich source of stem cells, and though this tissue looks to have significant regenerative potential, harnessing and utilizing this capability for clinical use is problematic. While efforts to improve our understanding of periodontal regeneration biology will continue, advances in biologic and materials sciences are also expected.

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