Stem Cells: An Arrow In The Quiver… Does It Lead To Bull's Eye????!!!!

Review Article

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ABSTRACT

The periodontium is an unusually complex tissue comprised of two hard (cementum and bone) and two soft (gingiva and periodontal ligament) tissues. To date, restoration of damaged or diseased periodontal tissues has relied almost entirely on the use of implantation of structural substitutes, often with little or no reparative potential. More recently, biological approaches based on the principles of tissue-engineering have emerged as prospective alternatives to conventional treatments. These new approaches based on an understanding of the cell and molecular biology of the developing and regenerating periodontium, offer interesting alternatives to existing therapies for the repair and regeneration of the periodontium. Stem cells prove to be a promising as well as an effective novel approach in the regeneration of the periodontal apparatus.

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INTRODUCTION:

The periodontium is an unusually complex tissue comprised of two hard (cementum and bone) and two soft (gingiva and periodontal ligament) tissues. Once damaged, the periodontium has a limited capacity for regeneration. Although new cementum formation, remodelling of the periodontal ligament and new bone formation can be observed during orthodontic tooth movement, this may be classified more as a physiological response rather than as true repair or regeneration of pathologically damaged tissue. During the early phases of periodontal disease some minor regeneration of the periodontium may be seen. However, once periodontitis becomes established, only therapeutic intervention has the potential to induce regeneration.[1] The complex series of events associated with periodontal regeneration involves recruitment of locally derived progenitor cells to the site which can subsequently differentiate into periodontal ligament-forming cells, mineral-forming cementoblasts, or bone-forming osteoblasts.[2]

Periodontal regeneration is the reproduction or reconstitution of a lost or injured part of the periodontium so that the form and function of lost structures is restored. The strategy of periodontal tissue regeneration therapies has been to control inflammation and stimulate stem progenitors to regenerate new periodontal tissues. Recent advances in stem cell biology and regenerative medicine have presented opportunities for tissue engineering as well as gene-based approaches in periodontal therapy.[3]

Definition and types of stem cells

The term "stem cell" first appeared in the literature during the 19th century. A "stem cell" refers to a clonogenic, undifferentiated cell that is capable of self-renewal and multi-lineage differentiation. In other words, a stem cell is capable of propagating and generating additional stem cells, while some of its progeny can differentiate and commit to maturation along multiple lineages giving rise to a range of specialized cell types. Depending on intrinsic signals modulated by extrinsic factors in the stem cell niche, these cells may either undergo prolonged self-renewal or differentiation.[4] A pluripotent stem cell can give rise to cell types from all three germ layers of the body (i.e., ectoderm, mesoderm and endoderm) whereas a multipotent stem cell can produce cell types from more than one (but not all) lineages. Descriptive and experimental studies support the notion that stem cells exist in both embryonic and adult tissues.[5] To date, six types of stem cells have been isolated in humans and these are depicted in the figure-1.

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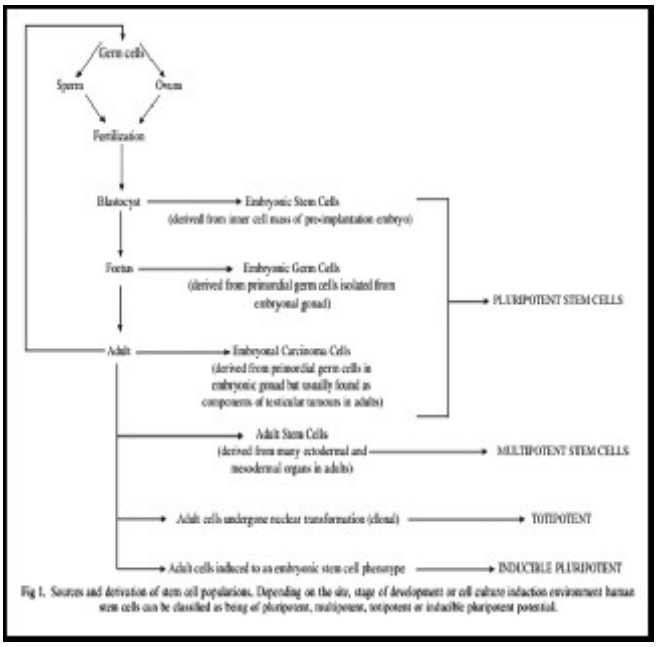

Fig 1. Sources and derivation of stem cell populations. Depending on the site, stage of development or cell culture induction environment human stem cells can be classified as being of pluripotent, multipotent, totipotent or inducible pluripotent potential.

Embryonic stem cells

In 1998, Thomson and co-workers derived the first human embryonic stem (ES) cell line from the inner cell mass of 4 to 7 days old blastocyst-stage embryos donated by couples under going infertility treatment.[6] The capacity of human ES cells to form teratomas containing derivatives of all three germ layers highlights their potential to differentiate into a range of cell types. To date, human ES cells have not been tested for their ability to participate in human embryonic development in vivo or to contribute to germ lines because of ethical concerns. The use of embryonic stem cells for clinical therapies is a relatively new endeavor and currently this development has been hampered by ethical concerns.

Defining properties of embryonic stem cells

1. Derived from the inner cell mass/epiblast of the blastocyst of pre-implantation or peri-implantation embryo.

2. Capable of undergoing unlimited proliferation in an undifferentiated state.

3. Exhibit and maintain a stable, diploid normal complement of chromosomes.

4. Can give rise to differentiated cell types that are derivatives of all three embryonic germ layers (ectoderm, mesoderm and endoderm) even after prolonged culture.

5. Capable of integrating into all foetal tissues during development.

6. Capable of colonizing the germ line and giving rise to egg or sperm cells.

7. Clonogenic, i.e. a single ES cell can give rise to a colony of genetically identical cells or clones, which have the same properties as the original cell.

8. Expresses the transcription factor Oct-4, which then activates or inhibits a host of target genes and maintains ES cells in a proliferative, non-differentiating state.

9. Can be induced to continue proliferating or to differentiate.

10. Lacks the G1 checkpoint in the cell cycle. ES cells spend most of their time in the S phase of the cell cycle, during which they synthesize DNA. Unlike differentiated somatic cells, ES cells do not require any external stimulus to initiate DNA replication.

11. Do not show X inactivation. In every somatic cell of a female mammal, one of the two X chromosomes becomes permanently inactivated but this does not occur in undifferentiated ES cells

Adult stem cells and mesenchymal stem cells

Adult stem cells, also known as somatic stem cells, are undifferentiated cells found in specialized tissues and organs of adults. It appears that all specialized tissues with renewal capacity throughout life probably contain adult stem cells in very small numbers that probably help replenish cell loss during normal senescence or tissue injury.[7] Hematopoietic stem cells from bone marrow were the first type of adult stem cells to be identified. Another population of adult non-hematopoietic

stem cells also resides in the bone marrow microenvironment.[8] These are termed bone marrow stromal stem cells (BMSSCs) or mesenchymal stem cells (MSCs) and their biological properties are less understood.

The primary source of MSCs is the bone marrow where they exist at a low frequency (one per 34 000 nucleated cells), which declines with age.[9] MSC-like cell populations have also been identified in other tissues, including adipose tissue, muscle, peripheral blood, foetal pancreas and liver.[10] Because of their widespread distribution, it has been proposed that MSCs arise from a perivascular stem cell niche[11] where it has been suggested that MSC exhibit a phenotype characteristic of pericytes. They are identified by the expression of a number of phenotypic characteristics of osteoblasts, endothelial, perivascular cells, neural or muscle cells and a range of surface markers.[12] A particularly distinguishing feature of human MSCs is their ability to form colonies (i.e., they are clonogenic).

PERIODONTAL TISSUE ENGINEERING:

Tissue engineering is a contemporary area of science based on the principles of cell biology, bioengineering, biomaterials, biochemistry, and biophysics to solve clinical and surgical problems related to tissue loss and organs' functional failures.[13] These include high - quality regeneration of damaged tissues without forming fibrous tissue,[14] minimum donor-site morbidity compared to autografts, and low risk of autoimmune rejection and disease transmission.[15] Therefore, as undifferentiated cells capable of self-renewing at a high rate of proliferation, and differentiating into multiple cell lineages including mesodermal, endodermal, and ectodermal cells, MSCs represent a valuable resource for tissue engineering. MSCs were first identified by Friedenstein et al.[16] in 1966 from bone marrow. They defined them as a population of postnatal stem cell shierarchically organized with the capacity to differentiate into specialized cells of at least one mesenchymal lineage, such as bone, cartilage, fat, muscle or neuronal cells. Adult mesenchymal stem cells and progenitor cells differentiated to PDL cells. (see figure-2)

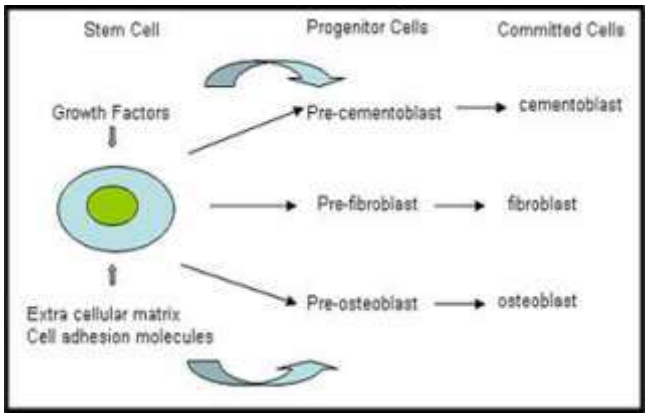

Figure-2 Differentiation of Adult Mesenchymal stem cells and progenitor cell in to PDL cells

The tissue-recombination technique aims to replicate key reciprocal interactions between the dental epithelium and the ectomesenchyme during odontogenesis to regenerate the periodontium. Furthermore, the combination of oral epithelium with nondental-derived mesenchyme (e.g. embryonic stem cells, neural stem cells and adult bone-marrow derived cells), results in the formation of both tooth crown and bone in vivo. Several studies have reported that engineered tooth primordia give rise to proper tooth development after being transferred into the adult mandible,[17] indicating that cultured stem cells can replace lost or damaged dental structures following transplantation into the adult oral cavity. However, the periodontal structures regenerated using tissue-recombination techniques are not formed in isolation from other dental tissues and this may pose problems for implantation into periodontal defects. In addition, there is currently no suitable substitute for the embryonic epithelial compartment of the engineered tooth germ, and the use of human embryonic tissues for periodontal engineering may limit the practical application of this approach.

Stem cells in human periodontal ligament

The presence of multiple cell types (fibroblasts,

cementoblasts and osteoblasts) within the postnatal periodontal ligament has led researchers to speculate that these cells may share common ancestors. The possibility that progenitor cells might exist in the postnatal periodontal ligament has been recognized for some time but until recently had never been formally proven.[18] These cells are believed to provide a renewable cell source for normal tissue homeostasis and periodontal wound healing. Recently, multipotent stem cell populations, termed periodontal ligament stem cells (PDLSCs), have been isolated from the periodontal ligament of extracted human third molar teeth. These PDLSCs give rise to adherent clonogenic clusters that resemble fibroblasts and are capable of developing into adipocytes, osteoblast- and cementoblast-like cells in vitro, and demonstrate the capacity to produce cementum and periodontal ligament-like tissues in vivo.[18]

PDLSCs express an array of cementoblast and osteoblast markers as well as the BMSSC associated markers, STRO-1 and CD146 antigens, which are also present on dental, pulp stem cells.[19] The similarity betweenPDLSCs, dental pulp stem cells and BMSSCs suggests that PDLSCs represent another MSC-like population. Further work is now focusing on identifying markers uniquely expressed by PDLSCs to discriminate these cells from other types of MSC-like cells identified in dental tissues. However, this is likely to be a complex task as earlier studies have indicated that there is considerable heterogeneity amongst cells of the periodontal tissues with regenerative capacity.[20] The first reported isolation and identification of mesenchymal stem cells in human periodontal ligamentwas in 2004.

All of these studies have confirmed the multipotent nature of periodontal ligament stem cells, and while the initial studies indicated this to include an ability to differentiate into osteoblast, cementoblast or lipidogenic phenotypes at least one recent study has indicated an ability of these cells to differentiate into neuronal precursors.[21] "Banking" of these cells become a clinical necessity

Potential applications of stem cells in periodontal therapy

Identification of stem cells in post natal dental tissues has presented exciting possibilities for the application of tissue engineering as well as gene and cell-based therapies in reconstructive dentistry. The use of stem cells with these technologies may constitute novel strategies for regenerative periodontal therapy. After stem cell isolation from donor tissue, it is transplanted to recipient site. (see figure 3)

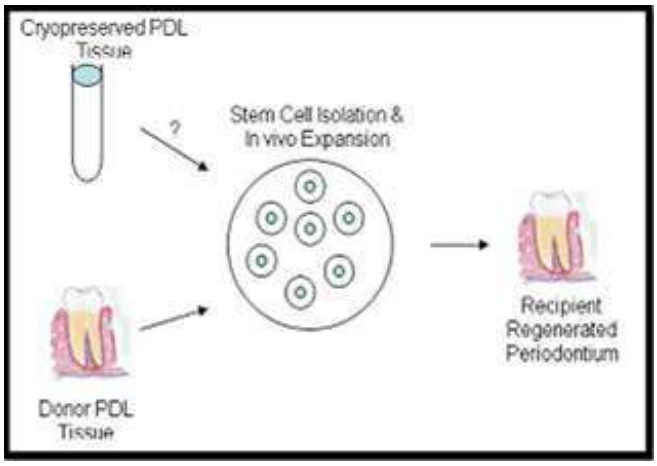

Figure-3 Cell transplantation from donor to recipient site

Tissue engineering

Tissue engineering is a specialized field of science based on principles of cell biology, developmental biology and biomaterials science to fabricate new tissues to replace lost or damaged tissues.[22] Successful tissue engineering requires an appropriate extracellular matrix or carrier construct which contains regulatory signals and responsive progenitor cells. A potential tissue engineering approach to periodontal regeneration involves incorporation of progenitor cells and instructive messages in a prefabricated three-dimensional construct, which is subsequently implanted into the defect site.

This strategy eliminates some of the limitations associated with conventional regenerative

procedures because of direct placement of growth factors and progenitor cells into the defect site overcomes the normal lag phase of progenitor cell recruitment to the site. The technical requirements for successful cell-based tissue engineering can be divided into two main categories: Engineering issues related to maintenance of an in vivo cell culture in the defect (e.g., biomechanical properties of the scaffold) and biological functions of the engineered matrix (including cell recruitment, neovascularization and bioavailability of growth factors).[23] With respect to the biochemical features of the matrix scaffold, these compounds should act in a manner consistent with the principles of membrane based guided tissue regeneration and have similar design feature. In particular, these properties should include: ease of handling, rigidity to withstand soft tissue collapses into the defect, and ability to maximize cell colonization and tissue in growth of desired type.[24] It is also important that unwanted epithelium is not totally excluded, but rather encouraged to form a biological seal over the scaffold and onto the tooth in the vicinity of the cemento-enamel junction, protecting the regenerating events occurring beneath.[25]

The concept of cell transplantation into periodontal defects was first described over 15 years ago.[26] Since then, other studies have attempted to induce periodontal regeneration using implantation of cultures of periodontal ligament fibroblasts and alveolar bone cells. More recently, the use of purified stem cells for tissue engineering approaches to facilitate periodontal regeneration has been investigated. Transplantation of autologous bone marrow MSCs in combination with atello collagen into class III defects in dogs has been shown to regenerate cementum, periodontal ligament and alveolar bone.[27] Ex vivo expanded PDLSCs co-transplanted with hydroxyapatite / tricalcium phosphate ceramic (HA/TCP) particles into nude rats are capable of forming cementum / periodontal ligament - like structures.

One novel report has shown that stem cells isolated from the root apical papilla of human teeth and PDLSCs can be combined to regenerate the root /periodontal structure respectively. After a three month healing phase this biologically created "root" was restored with a porcelain crown. Collectively, these findings demonstrate the feasibility (and potential) of using a combination of MSC-like cell populations for functional tooth regeneration.

Gene and cell-based therapy

The inherent proliferative and pluripotent capabilities of stem cells may offer lifelong opportunities for treatment of some important human diseases, including periodontitis, by repairing, replacing or regenerating damaged tissues. Stem cells may act as suitable vehicles for the delivery of therapeutic genes in gene therapy, and as therapeutic agents per se in cell-based therapy. It relies on genetic engineering, which involves molecular techniques to introduce, suppress or manipulate specific genes, thereby directing an individual's own cells to produce a therapeutic agent. In the context of periodontal regeneration, gene therapy seeks to optimize the delivery of agents such as growth factors to periodontal defects so that the limitations associated with topical application (e.g., short duration of action) can be overcome.[28]

Two major strategies for delivering therapeutic transgenes into human recipients are: (1) direct infusion of the gene of interest using viral or non-viral vectors in vivo; and (2) introduction of gene into delivery cells (often a stem cell) outside the body ex vivo followed by transfer of the delivery cells back into the body. The use of both in vivo and ex vivo gene delivery strategies via adenoviral (Ad) vectors encoding growth promoting molecules such as platelet-derived growth factor (PDGF) and bone morphogenetic protein-7 (BMP-7) has been investigated for its potential in periodontal regeneration by Giannobile and colleagues.[29] The introduction of transgenes into dental stem cells may offer an alternative to conventional methods because stem cells have the potential to provide a sustained source of growth factors for regeneration

Further research is also needed to address potential risks of viral recombination and immune responses towards viral antigens which could potentially hinder the progress of gene therapy in treating periodontal diseases.

CONCLUSION:

Regeneration of tissues destroyed by periodontitis has long been an altruistic goal of periodontal therapy. Periodontal regeneration requires consideration of many features that parallel periodontal development, including the appropriate progenitor cells, signaling molecules and matrix scaffold in an orderly temporal and spatial sequence. It is clear that current regenerative procedures are less than ideal but the identification of stem cells in human dental tissues in recent years holds promise to the development of novel, more effective approaches to periodontal regeneration and reconstructive therapy. However, before this is feasible, many biological, technical and clinical hurdles need to be overcome and a thorough understanding of underlying healing processes in periodontal regeneration is required

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List of abbreviations:

- ES: embryonic stem
- BMSSC: bone marrow stromal stem cells
- MSC: mesenchymal stem cells
- PDLSCs: periodontal ligament stem cells
- HA: hydroxyapatite
- TCP: tricalcium phosphate
- Ad: adenoviral
- PDGF: platelet derived growth factor
- BMP: bone morphogenetic protein