Review Article

Therapeutics of stem cells in periodontal regeneration

Rajiv Saini, Santosh Saini[1], Sugandha Sharma[2]

Departments of Periodontology and Oral Implantology, [1]Microbiology and [2]Prosthodontics, Rural Dental College - Loni, Maharashtra, India.

Address for correspondence:
Dr. Rajiv Saini, Department of Periodontology and Oral Implantology, Rural Dental College - Loni, Tehsil - Rahata, District - Ahmednagar, Maharashtra - 413 736, India.
E-mail: drperiodontist@yahoo.co.in

Abstract

The structure and composition of the periodontium are affected in many acquired and heritable diseases, and the most significant among these is periodontal disease. Periodontal regeneration is considered to be organically promising but clinically capricious. The principal requirements for tissue engineering are the incorporation of appropriate numbers of responsive progenitor cells and the presence of bioactive levels of regulatory signals within an appropriate extracellular matrix or carrier construct. Stem cell therapy is a treatment that uses stem cells, or cells that come from stem cells, to replace or to repair a patient's cells or tissues that are damaged. And, recent progress in stem cell research and in tissue engineering promises novel prospects for tissue regeneration in dental practice in the future, with regeneration of a functional and living tooth as one of the most promising therapeutic strategies for the replacement of a diseased or damaged tooth.

Key words: Dental, periodontal, regeneration, stem cells

INTRODUCTION

"Periodontium" refers to the tissues that collectively invest and support the teeth and consists of the gingiva, periodontal ligament, alveolar bone, and cementum. The structure and composition of the periodontium are affected in many acquired and heritable diseases, and the most significant among these is periodontal disease. The hallmarks of periodontal disease are destruction of soft connective tissues, bone loss, and loss of connective tissue attachment to the cementum. These alterations, if left untreated, lead to tooth loss.[1] Periodontal disease is a complex infectious disease resulting from the interplay of bacterial infection and host response to bacterial challenge, and the disease is modified by environmental, acquired risk factors and genetic susceptibility and is defined as an inflammatory disease of supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, the key organisms including *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Peptostreptococcus micros*, and *Campylobacter rectus*.[2] The aim of periodontal therapy is to regenerate and restore the various periodontal components affected by disease to their original form, function, and consistency.

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.82316

LOOM TO REGENERATE PERIODONTAL TISSUE

For decades, periodontists have sought ways to repair the damage that occurs during periodontitis. This has included the use of a range of surgical procedures, the use of a variety of grafting materials (autologous bone and bone marrow, allograft, xenografts, and various manmade bone substitutes) and growth factors, and the use of barrier membranes. Although these techniques had limited success, the need for a more effective regenerative approach resulted in the development of procedures that use biological mediators and tissue-engineering techniques.[3] Periodontal regeneration is considered to be organically promising but clinically capricious. The principal requirements for

tissue engineering are the incorporation of appropriate numbers of responsive progenitor cells and the presence of bioactive levels of regulatory signals within an appropriate extracellular matrix or carrier construct. Recent advances in mesenchymal stem cell isolation, growth factor biology, and biodegradable polymer constructs have set the stage for successful tissue engineering of many tissues, of which the periodontium could be considered a prime candidate for such procedures.[4]

Stem cells: Prologue and assortment in human tissue regeneration

Stem cells are unspecialized cells that develop into the specialized cells that make up different types of tissue in the human body. They are vital to the development, growth, maintenance, and repair of our brain, bones, muscles, nerves, blood, skin, hair, and other organs. The isolation, culture, and partial characterization of stem cells isolated from human embryos were reported in the November of 1998.[5] Stem cell therapy is a treatment that uses stem cells, or cells that come from stem cells, to replace or to repair a patient's cells or tissues that are damaged and stem cell technologies have, or are anticipated to have, applications for basic science (study of complex processes), medical research (to produce large numbers of genetically uniform cultures of organ tissues; e.g., liver, muscle, or neural), and therapies (repair or replace damaged or diseased tissues).[6] All stem cells, no matter what their source, are unspecialized cells that give rise to more specialized cells. Stem cells can become one of more than 200 specialized cells in the body. They serve as the body's repair system by renewing themselves and replenishing more specialized cells in the body, and the easiest way to categorize stem cells is by dividing them into two types: mature and early. Mature stem cells are found in specific mature body tissues as well as the umbilical cord and placenta after birth. Early stem cells, often called embryonic stem cells, are found in the inner cell mass of a blastocyst after approximately 5 days of development. Stem cells can now be grown and transformed into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies. Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies. Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell, such as totipotent, pluripotent, multipotent, oligopotent, and unipotent.

Periodontal regeneration: The eventual approach

In order for successful periodontal regeneration to occur, it will be necessary to use and recruit progenitor cells that can differentiate into specialized cells with a regenerative capacity, followed by proliferation of these cells and synthesis of the specialized connective tissues that they are attempting to repair. Clearly, a tissue-engineering approach for periodontal regeneration will need to utilize the regenerative capacity of cells residing within the periodontium, and would involve the isolation of such cells and their subsequent proliferation within a three-dimensional (3D) framework with implantation into the defect.[4] In wound healing, the natural healing process usually results in tissue scarring or repair. Using tissue engineering, the wound-healing process is manipulated such that tissue regeneration occurs. This manipulation usually involves one or more of the following three key elements: the signaling molecules, scaffold or supporting matrices, and cells;[3] the idiosyncratic feature and characteristic of the three key elements are illustrated in Tables 1–3.

Challenges in periodontal regeneration

The various challenges[7] that exist in periodontal regeneration are illustrated below:

1. A major unmet challenge is the modulation of the exuberant host response to microbial contamination that plagues the periodontal wound. Dual delivery of host modifiers and anti-infective agents is probably necessary to optimize periodontal regeneration.
2. The necessary interactions of multiple cell lineages should be clarified. These include cementogenic cells, fibroblasts, and osteogenic cells.
3. Despite the important role of exogenously delivered cells in the regeneration of severe periodontal defects, it is advantageous to attract endogenous periodontal tissue-forming cells by growth and/or trophic factors.

Clinical trials in periodontal regeneration

As human stem cell research is a relatively new area, companies developing cell therapies face several types of risk, and some are not able to manage them thus becoming highly speculative enterprises. Present clinical trials are being performed on recombinant human fibroblast growth factor-2, human platelet-derived growth factor, and tricalcium phosphate (GEM-21). Looking at the ongoing clinical trials, it is too early to tell whether all therapies based on stem cells will prove to be clinically effective. Thus, despite extensive research, there still remain problems with stem cell therapy because, in many cases, deep and exhaustive studies to determine the exact biology of stem cells are omitted and there are increasing pressures to start with insufficiently controlled clinical trials. It is very important to address all these issues.

Cell surface markings in stem cells

In recent years, scientists have discovered a wide array of

Table 1: Characteristics of signaling molecules in periodontal tissue engineering

Signaling molecule	Characteristics
Platelet-rich plasma concentration	• Use of platelet-rich plasma as a source of growth factors in bone and periodontal regeneration. • Autologous blood is drawn and separated into three fractions: platelet-poor plasma (fibrin glue or adhesive), platelet- rich plasma, and red blood cells. • Presence of platelet-derived growth factor, insulin-like growth factor, and transforming growth factor-beta in the cytoplasmic granules of platelet-rich plasma. • Mixture of growth factors in platelet-rich plasma putatively stimulates the proliferation of fibroblasts and periodontal ligament cells, extracellular matrix formation, and neovascularization and suppresses cytokine release and limits inflammation, thereby promoting tissue regeneration. • Platelet-rich plasma also contains a high concentration of fibrinogen. • Ineffective for complete periodontal regeneration.
Enamel matrix derivatives	• Harvested from developing porcine teeth has recently been reported to induce periodontal regeneration. • Contains a mixture of low-molecular weight proteins that stimulate cell growth and the differentiation of mesenchymal cells, including osteoblasts. • Emdogain is the only enamel matrix protein derivative approved by FDA and used clinically with promising results. • They promote bone cell attachment and cell spreading and enhance the proliferation of more immature bone cells while stimulating the differentiation of more mature bone cells.
Growth factors	• They are naturally occurring proteins that regulate various aspects of cell growth and development. • In periodontal regeneration, much of the focus has been on platelet-derived growth factor and basic fibroblast growth factor-2. • These biological mediators have been used to stimulate periodontal wound healing or to promote the differentiation of cells to become osteoblasts, thereby favoring bone formation.
Bone morphogenic proteins	• Group of regulatory glycoproteins that are members of the transforming growth factor-beta superfamily and stimulate differentiation of mesenchymal stem cells into chondroblasts and osteoblasts. • In the field of periodontal regeneration, much of the research interest has focused on bone morphogenetic protein-2 (OP-2), bone morphogenetic protein-3 (osteogenin), and bone morphogenetic protein-7 (OP-1).
Gene therapy	• Gene therapy can be used for minimizing major limitations associated with the use of growth and differentiation factors, which are their short biological half-lives. • Gene therapy approaches have also been used to study the regulation of bone morphogenetic protein, and gene regulation can be manipulated to result in a healing response that mimics regeneration.

Table 2: Characteristics of scaffold/supporting matrices in periodontal tissue engineering

Scaffold/supporting matrices	Characteristics

Membrane	• Guided tissue regeneration is a concept based on the principle that periodontal ligament cells have the potential to regenerate the supporting tissue of the teeth and, by using the membrane, it serves two basic goals:
	  • Isolates the cells of the periodontal ligament and bone from the gingival epithelium that leads to repopulation of cells with isolation and
	  • prevents epithelial migration in regeneration or merging cementum and periodontal ligament attachment thus preventing epithelial migration in wound.
	• Available in both a non-absorbable (expanded polytetrafluoroethylene membrane) and an absorbable form (collagen membrane, oxidizing cellulose membrane) but, used more extensively, is the absorbable form.
	• Use of membrane is usually combined with autogenous bone from adjacent areas or other graft materials and root biomodifiers.

Graft materials	• Evaluated on the basis of their osteogenic, osteoinductive, or osteoconductive potentials.
	• Orofacial regeneration in the past 30 years has been the use of bone grafts or substitutes to repair periodontal and maxillofacial defects.
	• Autogenous bone graft
	  • Material to be grafted can be obtained from the same individual
	  • Graft harvested from both the intra-oral (osseous coagulum, bone blend, cancellous bone marrow transplants, and bone swaging) and extra-oral sites (iliac cancellous marrow)
	• Allograft
	  • Material to be grafted is obtained from different individuals of the same species
	  • Antigenic properties of the graft suppressed by chemicals, radiation, and freezing
	  • Used both undecalcified freeze–dried bone allograft and decalcified freeze–dried bone allograft
	• Xenografts
	  • Material to be grafted obtained from different species
	  • Currently anorganic bovine-derived bone is used, which is an osteoconductive porous bone mineral matrix from bovine cancellous or cortical bone

Non-graft materials	• Used historically but not in current trend. • Includes sclera, cartilage, plaster of Paris, plastic materials, calcium phosphate biomaterials, and coral-derived materials.
Collagen carriers	• Collagen is the main structural protein for tissue support. It also plays an essential role in wound healing by providing a biologic scaffold for cellular activities such as cell attachment, migration, and proliferation. • Produced to create an atelocollagen scaffold where both constructs were effective in supporting recombinant human bone morphogenetic protein-induced bone formation.

Table 3: Characteristics of stem cells in periodontal tissue engineering

Stem Cell	Characteristics
Mesenchymal stem cells	• Mesenchymal stem cells (MSCs) are self-renewable and can differentiate into all cell lineages that form mesenchymal and connective tissues. • First, stem cell populations that generate native craniofacial structures, such as the mandibular joint, are heterogeneous and probably include both mesenchymal and hematopoietic stem cells.
Dental pulp stem cells	• Dental pulp contains proliferating cells that are analogous to bone cells because they express osteogenic markers and respond to many growth factors for osteo/odontogenic differentiation. Dental pulp cells are capable of forming mineral deposits with distinctive dentin-like crystalline structures. • Dental pulp stem cells (DPSCs) have been isolated from extracted human third molars and DPSC-derived cells developed long cytoplasmic processes, a departure from their usual bipolar fibroblastic appearance.
Human exfoliated deciduous teeth	• The exfoliated deciduous tooth houses living pulp remnants consisting of connective tissue, blood vessels, and odontoblasts. • These stem cells express early cell-surface markers for bone marrow-derived mesenchymal stem cells and a variety of osteoblast/odontoblastic markers, including Runx2, alkaline phosphatase (ALP), matrix extracellular phosphoglycoprotein (MEPE), and bone sialoprotein (BSP).
Periodontal ligament stem cells	• Stem cells in human PDL (PDLSCs) and PDLSCs implanted into nude mice generated cementum/PDL-like structures that resemble the native PDL as a thin layer of cementum that interfaced with dense collagen fibers, similar to Sharpey's fibers. • PDLSCs have the potential of forming periodontal structures, including cementum and PDL.

stem cells that have unique capabilities to self-renew, grow indefinitely, and differentiate or develop into multiple types of cells and tissues. Coating the surface of every cell in the body are specialized proteins, called receptors, which have the capability of selectively binding or adhering to other "signaling" molecules. There are many different types of receptors that differ in their structure and affinity for the signaling molecules. Normally, cells use these receptors and the molecules that bind to them as a way of communicating with other cells and to carry out their proper functions in the body. These same cell surface receptors are the stem cell markers. The importance of this new technique is that it allows the tracking of stem cells as they differentiate or become specialized. Scientists have inserted into a stem cell a "reporter gene" called green fluorescent protein or GFP. These discovery tools are commonly used in research laboratories and clinics today, and will probably play important roles in advancing stem cell research. There are, however, limitations to this research. One of them is that a single marker identifying pluripotent stem cells, those stem cells that can make any other cell, has yet to be found. As new types of stem cells are identified and their research applications become increasingly complex, more sophisticated tools will be developed to meet investigators' needs. For the foreseeable future, markers will continue to play a major role in the rapidly evolving world of stem cell biology.

DISCUSSION AND CONCLUSION

Regeneration of a functional and living tooth is one of the most promising therapeutic strategies for the replacement of a diseased or damaged tooth.[8-10] Recent advances in dental stem cell biotechnology and cell-mediated murine tooth regeneration have encouraged researchers to explore the potential for regenerating living teeth with appropriate functional properties.[11-13] Murine teeth can be regenerated using many different stem cells to collaboratively form dental structures *in vivo*.[11,12] In addition, dentin/pulp tissue and cementum/periodontal complex have been regenerated by human dental pulp stem cells and periodontal ligament stem cells, respectively. However, owing to the complexity of human tooth growth and development, the regeneration of a whole tooth structure, including enamel, dentin/pulp complex, and periodontal tissues, as a functional entity in humans is not possible given the available regenerative biotechnologies. The end goal of tissue engineering is to develop products capable of healing diseased or lost tissues and organs; thus, representing a departure from conventional biomedical research, whose primary focus is an understanding of mechanisms. This does not imply that the understanding of mechanisms is unimportant in tissue engineering. Instead, an understanding of the mechanisms of interactions among cells, growth factors, and biomaterials undoubtedly will advance the end goal of developing cell-based therapies and off-the-shelf tissue-engineering products;[7] conversely, craniofacial tissue engineering could not have advanced to the current stage without the incorporation of interdisciplinary skill sets of stem cell biology, bioengineering, polymer chemistry, mechanical engineering, robotics, etc. Thus, craniofacial tissue engineering and regenerative dental medicine are integral components of regenerative medicine.[7] Despite the accumulation of molecular information and our understanding of the regulation of tooth development, it is not clear how teeth could be grown in practice. Perhaps, one day, we will be able to isolate cells that have the capacity to form teeth and then tooth development could be initiated *in vitro*. Such multipotential stem cells could be obtained by some of the methods described above. After initiation, the tooth germ could either be transplanted into the mouth

or it could be cultured *in vitro*. This approach would be the most difficult as it would require a thorough knowledge of all processes that govern the formation of the proper three-dimensional structure of the tooth. Alternatively, it is possible that tooth development could be initiated *in vivo* by applying specific growth and differentiation factors.[14] In summary, these are still early days of periodontal tissue regeneration and more recent developments in basic science indicate that these approaches are unquestionably practical and, given their promise, worth exploring.

REFERENCES

1. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med 2002;13:474-84.
2. Saini R, Marawar PP, Shete S, Saini S. Periodontitis a true infection. J Global Infect Dis 2009;1:149-51.
3. Kao RT, Murakami S, Beirne OR. The use of biologic mediators and tissue engineering in dentistry. Periodontol 2000 2009;50:127-53.
4. Bartold PM, Xiao Y, Lyngstaadas SP, Paine ML, Snead ML. Principles and applications of cell delivery systems for periodontal regeneration. Periodontol 2000 2006;41:123-35.
5. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, *et al*. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1061-2.
6. Saini R, Saini S, Sharma S. Stem cell therapy: The eventual future. Int J Trichol 2009;1:145-6.
7. Mao JJ, Giannobile WV, Helms JA, Hollister SJ, Krebsbach PH, Longaker MT, *et al*. Craniofacial tissue engineering by stem cells. J Dent Res 2006;85:966-79.
8. Chai Y, Slavkin HC. Prospects for tooth regeneration in the 21st century: A perspective. Microsc Res Tech 2003;60:469-79.
9. Thesleff I. Developmental biology and building a tooth. Quintessence Int 2003;34:613-20.
10. Yen AH, Sharpe PT. Regeneration of teeth using stem cell-based tissue engineering. Exp Opin Biol Ther 2006;6:9-16.
11. Duailibi MT, Duailibi SE, Young CS, Bartlett JD, Vacanti JP, Yelick PC. Bioengineered teeth from cultured rat tooth bud cells. J Dent Res 2004;83:523-8.
12. Ohazama A, Modino SA, Miletich I, Sharpe PT. Stem-cell-based tissue engineering of murine teeth. J Dent Res 2004;83:518-22.
13. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 2005;8:191-9.
14. Thesleff I, Tummers M. Stem cells and tissue engineering: Prospects for regenerating tissues in dental practice. Med Princ Pract 2003;12:43-50.

How to cite this article: Saini R, Saini S, Sharma S. Therapeutics of stem cells in periodontal regeneration. J Nat Sc Biol Med 2011;2:38-42.

Source of Support: Nil. **Conflict of Interest:** None declared.