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Cell- and Gene- Based Therapeutics for Periodontal Regeneration

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ABSTRACT

Periodontitis is a disease of the periodontium, characterized by loss of connective tissue attachment and supporting the alveolar bone. Therefore, to regenerate these lost tissues of the periodontium researchers have included a variety of surgical procedures including grafting materials growth factors and the use of barrier membranes, ultimately resulting into regeneration that is biologically possible but clinically unpredictable. Recently a newer approach of delivering DNA plasmids as therapeutic agents is gaining special attention and is called gene delivery method. Gene therapy being considered a novel approach have a potential to channel their signals in a very systematic and controlled manner thereby providing encoded proteins at all stages of tissue regeneration. The aim of this review was to enlighten a view on the application involving gene delivery and tissue engineering in periodontal regeneration.

Keywords: Gene, growth factors, periodontal regeneration, stem cells, tissue engineering, vectors

INTRODUCTION

Functional regeneration of periodontal apparatus is one of the most important aims of research in current periodontal regenerative therapy. The successful periodontal regeneration is a highly challenging tasks since careful histological assessment has revealed that allogenic bone grafts and even fresh autologous bone grafts, generally become encased in a dense fibrous connective tissue in periodontal defects.[1] Thus, a current challenge faced by clinicians is the complete, reliable and reproducible regeneration of the periodontal tissues, paying way to novel treatments that utilize a cell and gene-based approaches. This current review enlightens the clinicians with the application of cell and gene-based therapeutics in periodontal tissue regeneration.

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PERIODONTAL TISSUE ENGINEERING

The goal of the tissue engineering is to regenerate the functional tissue through a series of key events that occur during periodontal tissue formation and growth, by means of delivering signaling molecules, cells, and scaffold/matrix to periodontal defects.[2-4]

Signaling molecules therapeutics (protein-based therapeutics)

With the introduction of a new era of tissue engineering whereby biological mediators such as partially purified protein mixture from developing teeth (such as Emdogain®) and growth factors from recombinant technology can be used to accelerate to periodontal regeneration. Apart from these biological mediators, platelet-rich plasma, which represents an autologous growth factor cocktail can be harvested from patients

with minimal invasiveness and can be used for regenerative therapy.[4] However, the major drawbacks of this protein-based approach is the short half-life and also the instability of these proteins resulting in multiple delivery dosage and also delivery of milligram quantities of factor in routine clinical practices, thus leading to risk of unwanted side effects.[5] Limitations such as, limited control overdose administration, loss of bioactivity, nontargeted delivery and/or lack of availability has led to newer safe, and effective modes of periodontal regeneration through cell-based approach.

Cell-based therapeutics

As stated first by Rudolph Virchow in 1855, "All cells come from other cells." Cell-based therapy involves the direct delivery of cells at the diseased site for tissue regeneration.[6] This approach is beneficial in periodontal tissue engineering because of following possibilities (i) direct participation of the transplanted cells in the regenerative process, (ii) genetic modification with therapeutic genes, and (iii) ability to get differentiated into a variety of periodontal tissue prototypes (bone, cementum, and periodontal ligament [PDL]).[6] Autologous cells appear to be a most appropriate source of cells for tissue engineering due to their high cellular activity. Whereas allogenic and xenogenic cells being heterogenous are potent agents for immunogenic reactions requiring immunosuppressive therapy when they are utilized for tissue engineering.[1,2,4]

Although six types of stem cells have been isolated in humans, they have been categorized mainly into embryonic stem cells (ES cells) and adult stem cells.[7]

Embryonic stem cells

Embryonic stem cells have an advantage of having greater differentiation potential when compared to multipotent or unipotent cells [Figure 1].[8] They exhibit stage-specific embryonic antigens 3 and 4, proteins TRA-1-60 and TRA-1-81, alkaline phosphatase (ALP), and also maintain their chromosomal length through the high expression of telomerase.[9]

Adult stem cells

Tissues such as skin, hematopoietic system, bone, and liver have the capacity to repair and renew indicating presence of stem or progenitor cells[10] [Figure 1]. Adult stem cells appear more mature with a finite lifespan and only multipotent differentiation capacity when compared to ES cells.[11] Hematopoietic stem cells were the first adult stem cells that were isolated from bone marrow.[12] Apart from hematopoietic adult nonhematopoietic stem cells also resides in the bone marrow microenvironment.[13] Which are known as bone marrow stromal stem cells or mesenchymal stem cells (MSCs) and characteristically express: CD44,

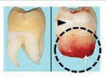
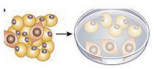
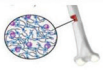
Stem cells	Sources	Regenerative potential
Embryonic stem cell	 The inner cell mass of blastocysts	Capable of differentiating into cells of all three germ layers of the adult body
Adult stem Cell	Dental Origin	
	 Dental pulp tissue of extracted third-molar teeth (DPSCs)	Odontoblast-like cells and forming dentin/pulp-like complex
	 Exfoliated deciduous tooth (SHED)	Bone and dentin formation
	 Periodontal ligaments	Clonogenic clusters resembling fibroblasts, cementoblast, osteoblast
	 Dental follicle around 3rd molar	Generate periodontal ligament- like tissue Cementum
	 Apical papilla of tooth	Capable of differentiating into adipocytes and odontoblasts/osteoblasts
	 Jaw bone MSCs	Clonogenic and have potent osteogenic potential in vitro and in vivo
	Nondental origin	
	 Adipose tissue	New periodontal ligament-like and alveolar bone-like structures
	 Appendicular bone marrow MSCs	Ability to form bone and cartilage in vivo cementum, periodontal ligament and alveolar bone

Figure 1: Stem cell sources and their regenerative potential

CD105 (SH2; endoglin), CD106 vascular cell adhesion molecule-1, CD166, CD29, CD73 (SH3 and SH4), STRO-1, CD90 (Thy-1), CD117, and Sca-1.[14,15] Bone marrow is considered to be the main source of MSCs. Other than the bone marrow, MSCs can also be located in tissues such as adipose tissue, umbilical cord blood, chorionic villi of the placenta, amniotic fluid, peripheral blood, fetal liver, lung; and even in exfoliated deciduous teeth, dental pulp, and PDL.[16]

Induced pluripotent stem cells

In recent years, bone marrow, dental, and gingival epithelium-derived MSC have evolved as a promising new therapeutic approach for the regeneration of lost periodontal tissues.[17] Major factors inhibiting their use in routine clinical treatment are their limited accessibility and complex invasive methods to procure

it. The possible reprogramming of adult somatic cells into a pluripotent stem cell state, through the addition of certain transcription factors, represents an appealing alternative to obtain from large source of adult-derived stem cells for use in regenerative medicine.[17] These cells have opened up the possibility of "fixing" a particular genotype (either normal or diseased) in pluripotent stem cells and thus attempting at developing robust *in vitro* disease models. The advantage of induced pluripotent stem cells (iPSC) over conventional MSC is that they can be obtained from any tissue type in the body, and also exhibit an unlimited growth capacity that can serve as an inexhaustible source of stem cells.[17]

Various animal studies have shown potential of different oral tissues to generate iPSC, including stem cells from dental pulp, exfoliated human deciduous teeth, apical papilla, oral mucosa, third molar mesenchymal stromal cells, PDL fibroblasts, and gingival fibroblasts. Most of these *in vitro* and *in vivo* studies were conducted using mice iPSC. However, recently, a study has shown successful induction of integration-free human urine iPSCs into the intact epithelial sheet, which differentiated into ameloblasts in a tooth-like structure thus indicating a possible role of human iPSCs in tooth regeneration.[18]

Gene delivery-based therapeutics

Gene delivery-based therapeutics is based on transferring of genetic materials to alter specific genes in individual's cells to produce a therapeutic effect.[19] To overcome the problems associated with protein and cell-based approach, gene therapy has been developed which modifies function of cells to provide long-term exposure of multiple growth factors and thereby maintains constant protein levels at the site of the defect.[3-5]

VECTORS FOR GENE DELIVERY

Gene delivery vectors are divided into two groups: Viral and nonviral vectors.[20]

Viral vectors

- Adenoviral vectors (adeno-associated virus [AAV]): These vectors possess a characteristic feature of (a) infecting a variety of cell types (b) being purified at high titers and (c) results in a high level of gene expression.[21] Some of the disadvantages of adenovirus include the inability to express the gene for a long-term and also results in inducing host immunogenic response. To overcome the problems involving immunogenicity, the newer generation that is the second generation of adeno vectors have been introduced[22]
- Retroviral and lentiviral vectors: These vectors have the advantage over adenoviral vectors due to their low-grade immunogenicity and also for expressing genes for a long-term. They have a good capability of delivering genes to stem cells and also cytotoxic genes to cancer cells[23]
- AAVs: AAV known for their superior safety feature due to their nonimmunogenic nature and are thought to be useful in periodontal regeneration.[24] They carry a unique character of expressing and delivering gene throughout life in both dividing and nondividing cells.

Nonviral vectors

Nonviral vectors consist of naked DNA alone or in conjunction with a carrier. They are superior when compared to viral vectors due to their nonimmunogenicity, low toxicity, and less likely of being introduced into the host cell genome.[25] These vectors also have low gene transfer efficiency and in some cases *in vivo* instability and transient gene expression.[25,26]

GENE DELIVERY APPROACHES

Genetic alteration of somatic cells could be achieved by manipulating the cells residing naturally within the individual's body that is, *in vivo* approach or it could be by manipulating the cells obtained from individual's body and subsequently returning it to the host that is, *ex vivo* approach.

In vivo approaches

In *In vivo* approach, DNA plasmids are directly injected into desired sites. These injected gene constructs are taken by the host cells and then start to produce the encoded protein for therapeutic purpose.[5]

Ex vivo approaches

In *ex vivo* approach, a specific population of cells (example PDL cells) is obtained from tissues, followed by transferring of genes to these cells under *in vitro* conditions. This gene coded cells are subsequently transferred back into periodontal defects. Thus, resulting into the selective genetic manipulation of the desired cell type and the regeneration of respective lost tissues.

TARGET GENES FOR PERIODONTAL TISSUE ENGINEERING

Transforming growth factor-beta family members and bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) and transforming growth factor-betas (TGF-βs) belong to the same peptide superfamily, they are pleiotropic factors, which play an important role in the control of somatic tissue development and renewal.[27] TGF-1 stimulates apposition of bone matrix and also bone cell replication and increases proliferation in cementoblasts.[28] BMP-3 and BMP-7 (i.e., osteogenic protein-1) play an important role in cementogenesis and the assembly of PDL,[29] whereas

BMP-2 and BMP-7 stimulate human PDL fibroblasts to differentiate into osteoblastic phenotypes by increasing the production of ALP, resulting in an accelerated bone regeneration.

Platelet derived growth factors

Platelet-derived growth factor (PDGF) is an active growth factor, which is a product of two distinct genes PDGF-A and PDGF-B. PDGF stimulates DNA synthesis and cell replication in osteoblasts,[30] as well as increases bone collagen synthesis and the rate of bone matrix apposition.[31]

Insulin-like growth factor-1

These biologic mediators having single-chain peptides are of two types insulin-like growth factor-1 (IGF-1) and IGF-2 and are primarily involved in periodontal wound healing and regeneration by affecting the PDL cell turnover.[32] IGF-1 stimulates human PDL fibroblast proliferation in a dose- and time-dependent manner[33] and along with PDGF-BB and TGF-β induce periodontal soft and hard tissues regeneration.[34]

Sonic hedgehog

The sonic hedgehog gene also encodes a regulator protein of embryonic osteogenesis and the repair of bone fractures, which may also have significant effects on periodontal bone regeneration and is found during embryogenesis.[4]

Wingless

These are a family of 19 secreted glycoproteins that play a role in embryonic development and postdevelopmental physiology by regulation of cell proliferation, differentiation, and apoptosis. The role of wingless (WNTs) in homeostasis and regeneration of periodontal tissues remains largely unknown. Since the utilization of WNTs is still in its infancy, further studies are required to prove its role in periodontal regeneration.[19]

GENE DELIVERY SYSTEMS/DEVICES

A controlled gene delivery systems, which act as localized depot of genes, provide an extended sustained release of genes, delivering controlled maintenance of the therapeutic level of encoded proteins. They also limit the DNA degradation in the nuclease rich extra-cellular environment *in vivo*. A wide range of synthetic polymers, natural origin polymers, composites, and inorganic materials may be used as platforms for the delivery of genes naked plasmid DNA or a viral particle.[35]

Microparticulate systems

The function of particulate systems for gene delivery was promptly considered as a mode to protect DNA during tissue regeneration and to provide adequate control over release rate. After releasing, DNA can transfect the cells at the delivery site. Hence, much attention has been focused on such as Food and Drug Administration approved poly (lactic-co-glycolic acid) (PLGA), for the encapsulation of genes.[36] Other materials used to encapsulate DNA are natural chitosan[37] and gelatin.[38]

Polymeric hydrogel systems

Polymeric hydrogel systems are formed by cross-linking of a natural or synthetic polymer. Commonly used hydrogels are poly (ethylene glycol), polylactic acid (PLA), polyglycolic acid, PLGA, and copolymers poly (epsilon-caprolactone)-poly (ethylene oxide)-poly (epsilon-caprolactone).[39]

Implantable scaffolds

These delivery systems are based on GTR technology, cell scaffolding, and drug delivery of growth factors and/or genes. Currently used scaffolds include (i) porous collagen/chitosan scaffold, (ii) gelatin sponge system, (iii) biodegradable PLA barrier device (Atrisorb), (iv) space-providing macroporous expanded polytetrafluoroethylene device, and (v) hydroxyapatite-based biomimetic matrix.[39]

Gene delivery for host modulation of periodontal disease

Major cause of periodontal destruction in periodontal disease is the host response against periodontal pathogenic bacteria. New approaches have been developed to target host-derived inflammatory mediators such as MMPs, cathepsins, and other osteoclast-derived factors leading to bone resorption.[40] Gene therapy has the potential for long-term maintenance of therapeutic proteins. Gene therapy using AAV to deliver the tumor necrosis factor receptor-immunoglobulin Fc (TNFR: Fc) fusion gene to experimental *Porphyromonas gingivalis* – lipopolysaccharide-mediated bone loss resulted in sustained therapeutic levels of serum TNFR protein for ≥3 months, and inhibition of *P. gingivalis* – lipopolysaccharide-mediated bone loss.[41] Patil *et al*.[42] showed that increased expression of tristetraprolin, (a key cytokine-regulating RNA-binding protein) by an adenoviral vector significantly reduces the levels of interleukin-6, TNF-α, and prostaglandin E2 *in vitro* which in turn may reduce inflammation-induced bone loss in an experimental periodontitis model. Recently, an *in vivo* study demonstrated that mitogen-activated protein kinase phosphatase-1 has potential to prevent alveolar bone loss.[19] These findings suggest the possible role of gene-based approach in the modulating host response to periodontal pathogens.

CONCLUSIONS

Genetically modified cell therapy is considered to be the recent advances in periodontal regeneration but with its

limitations it has rarely found its way into clinical practice. Interdisciplinary approaches are needed to develop effective strategies to move from repair to regeneration involving clinicians as well as cell biologists and material scientists. Thus, cell and gene-based approaches may complement each other in the predictable regeneration of the lost periodontal apparatus by overcoming the limitations of current treatment approaches.

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Conflicts of interest
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