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STEM-CELL BIOLOGY FOR TOOTH AND PERIODONTAL REGENERATION (M BARTOLD, SECTION EDITOR)

Stem Cells, Scaffolds and Gene Therapy for Periodontal Engineering

Miguel Padial-Molina · Hector F. Rios

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Abstract The periodontium is continually exposed to a variety of challenges. Over the past decade, preclinical and clinical research has highlighted the complex modulatory role of the periodontal ligament (PDL) as a mediator of tissue repair and homeostasis. Experiments carried out in both human and animal models have highlighted the importance of the PDL as a protective structure. The unique properties of PDL cells endow this tissue with functional attributes that are not replicated by other biological systems. Furthermore, distinct PDL matricellular properties favor a synchronized molecular response to environmental challenges that supports normal dental and alveolar adaptation. Today, the mechanism by which periodontal integrity is restored and maintained is the focus of novel and innovative research. The ability to decipher the molecular mechanisms that support periodontal homeostasis, together with emerging science in biomaterials and stem cell biology, represents a unique opportunity to enhance the predictability of current regenerative surgical approaches and to develop novel treatment strategies for periodontal tissue engineering. This review will focus on the most recent available information concerning cells, gene delivery, and new scaffold technologies that are relevant in periodontal regeneration.

Keywords Periodontal ligament · Periodontal regeneration · Periodontal engineering · Tissue engineering · Stem cells

M. Padial-Molina · H. F. Rios (✉)
Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan, 1011 North University Ave, Office 3349, Ann Arbor, MI 48109-1078, USA
e-mail: hrios@umich.edu

M. Padial-Molina
Research Group CTS#583, University of Granada – Junta de Andalucia, Granada, Spain

Introduction

The tooth-supporting apparatus is composed of the alveolar bone, the periodontal ligament (PDL), the cementum, and the gingiva. The periodontium is a highly specialized, adaptive, and dynamic tissue able to sustain different microbiological, inflammatory, and mechanical challenges through a number of complex molecular events [1]. Alterations of this equilibrium in the form of different periodontal diseases affect a significant percentage of the adult population [2, 3].

Periodontal regeneration has long been the ultimate goal in periodontal therapy [4]. However, treating and re-establishing the diseased periodontium's original structure, properties, and function constitute a significant challenge [5]. Different approaches have been proposed, but the amount of regenerated tissue is oftentimes limited and difficult to predict. By definition, periodontal regeneration implies the regeneration of the cementum, periodontal ligament, alveolar bone in a specific temporal sequence and spatial distribution is based on a number of essential factors (Fig. 1) [6–9].

Although the exact cellular and molecular events are still not clear, we know that specific cells must first migrate to the healing area and proliferate to provide the machinery needed for the new tissue to grow and differentiate. This process is mediated and coordinated by soluble factors, many cell types, extracellular matrix (ECM), and matricellular proteins. Ideally, scaffolds will provide a three-dimensional template structure to support and facilitate these processes. Angiogenic signals and new vascular networks provide the nutritional base for tissue growth and homeostasis, after which normal mechanical stimuli will increase to promote an organized ECM synthesis as well as cementum and bone formation and maturation. Once those structures are established, PDL fibers become organized and connect the tooth to the alveolar bone. Finally, because of the microbial load at the periodontal

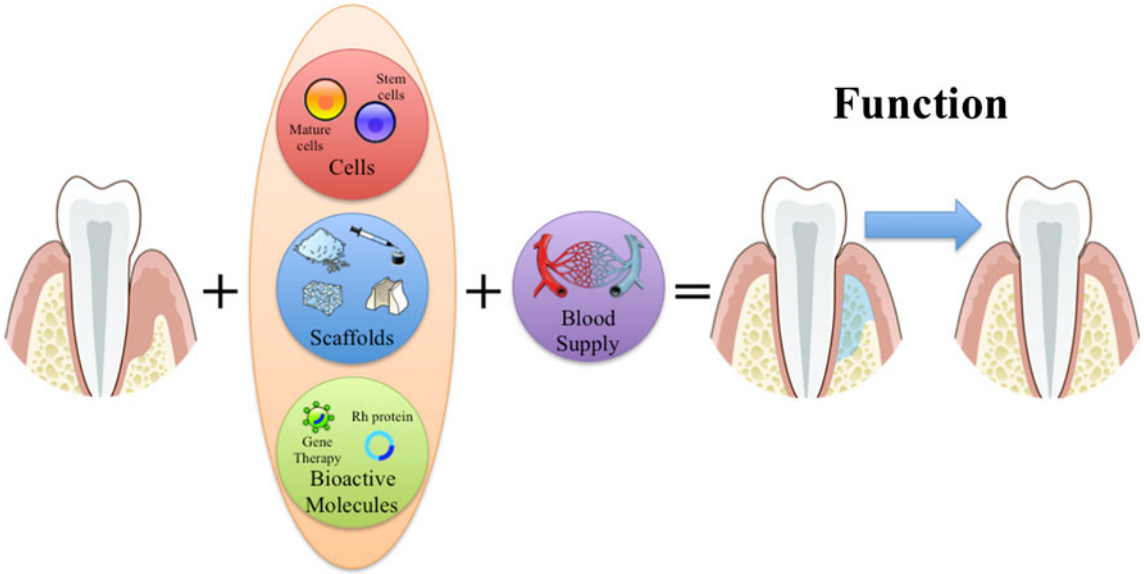

Fig. 1 Schematic illustration of principles and requirements for periodontal engineering

area, strategies to control infection and host response are required to optimize periodontal regeneration [8–10].

This paper focuses on key concepts and the most recently published information in periodontal engineering (2012–2013) concerning early-stage cell and gene delivery by new scaffold technologies. The discussion focuses primarily on three important aspects: 1) cell-based therapy, 2) scaffold fabrication technologies, and 3) gene therapy in periodontal engineering.

Cells for Periodontal Regeneration

Generally, cell therapy involves the transfer of new cells into a tissue to treat diseases or disorders. In regenerative medicine, cells are used to improve the regeneration process by promoting new tissue growth and differentiation [11]. They can be delivered as either promoters of multiple cell types needed in the regeneration process or as carriers to deliver different types of molecules, either directly or by gene therapy [12]. In the context of periodontal regeneration, cells seeded into periodontal defects should be easy to harvest, non-immunogenic, and highly proliferative, and should have the ability to differentiate into various types of cells comprising periodontal tissue [13].

Different types of cells – either mature or stem cells – from both extraoral and intraoral origins have been proposed for periodontal regeneration (Table 1, adapted from [9]). During the last year, important advances have occurred in terms of new strategies to obtain these cells as well as a description of some special properties of particular interest in periodontal regeneration. This includes not only ease of access to the cells, but also initial in vivo applications that demonstrate very promising results. In that context, it is very interesting that the gingiva contains mesenchymal stem cells [GMSC) [14–19]. These cells are both mesoderm- and neural crest-derived, the latter showing an increased capacity to differentiate to neural cells and chondrocytes, as well as to modulate immune cells, when compared to the other [20]. This is of special relevance since neural crest cells, as a transient, migratory, multipotent cell population, participate in the embryonic development of most dental tissues, including the gingiva, dental follicle, periodontal ligament, and alveolar bone, which are, in fact, sources of adult stem cells as well [21]. Mesenchymal stem cells have been also generated from human foreskin iPSC (induced pluripotent stem cells). These cells exhibit the capacity to regenerate periodontal tissues in a

Table 1 Types and uses of cells in periodontal regeneration (9)

Cell type	Origin	Defect type
Bone marrow stromal cells	Autograft	Class III defects
		Periodontal fenestration
		Osteotomy
Adipose stromal cells	Autograft	Periodontal palatal defects
Periodontal ligament cells	Autograft	Class II defects
		Periodontal fenestration
	Allograft/Xenograft	Periodontal fenestration
Periodontal ligament stem cells	Allograft	Ectopic
		Periodontal fenestration
	Autograft	Periodontal defects
Cementoblasts	Allograft	Ectopic
		Periodontal fenestration
Dental follicle cells	Allograft	Ectopic
		Periodontal fenestration

rat periodontal defect model [22••]. Other studies have confirmed the possibility of obtaining mesenchymal stem cells from alveolar bone marrow by slow minimally-irrigated osteotomy [23] or Bichat's fat pad [24].

More and more reports are showing the utility of periodontal ligament stem cells (PDL-SC) for periodontal regeneration [25, 26]. These cells can be effectively isolated from the PDL and are capable of forming cementum and periodontal ligament tissues upon in vivo transplantation, as described almost a decade ago with human cells transplanted into a mouse model [27]. Such potential has been confirmed recently in a dog model, which could be used as another periodontal regeneration large-animal model [28].

The regenerative potential of PDL-SC has classically been assessed in acute rat fenestration models, where transplanted cells are carried by a collagen sponge [29]. Other approaches include chronic class II/III defects (created sometime prior to or concurrently with the regenerative procedure). These studies are usually performed in dogs. Within the last year, a report has confirmed no superiority in class II periodontal regeneration when comparing autogenous cortical bone (ACB), ACB+PRP (platelet rich plasma), or a combination of PRP+MSCs [30]. However, the lack of a MSCs+ACB+PRP group in this particular study skews the comparison. In other cases, the addition of PDL cells makes the regeneration process more effective, especially in combination with collagen sponges and collagen membranes in class III defects [31].

More recently, efforts in periodontal regeneration have begun to utilize cell sheet engineering. The principle of cell sheet engineering is based on the capacity of cells to attach and proliferate on a temperature-responsive polymer surface (usually poly(N-isopropylacrylamide) or PIPAAm). When the cell sheet has grown, cells with extracellular matrix proteins spontaneously detach from the culture dishes by lowering the temperature [32]. This approach is even being used in a clinical study with autologous PDL cells (http://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000005979&language=E). New developments include the possibility of configuring multilayered cell sheets that could carry not only different layers of cells but also abundant extracellular matrix, which would eliminate the adverse effect of cell carriers. The approach was recently tested in a rodent animal model and showed a clear superiority over monolayer cell design [33••].

Mesenchymal stem cells derived from the PDL have been studied more extensively, although it is important to note that some reports are promoting the use of other intraoral sources such as the gingiva. While the potential to differentiate into multiple cell lineages and generate new bone ectopically is more effectively achieved using PDLSCs, some authors highlight drawbacks such as limited cell availability and reduced clinical applicability (sacrifice of a tooth to obtain stem/progenitor cells from their dental pulp or periodontal ligament) [34, 35]. Gingival-derived stem cells have been effectively applied in a recent miniature pig periodontal regeneration study [35]. The combination of autologous gingival-derived stem cells loaded in a scaffold showed better regeneration outcomes (higher changes in clinical attachment level, probing depth, gingival recession, histological attachment level, and lower junctional epithelium length and connective tissue adhesion) compared with unloaded scaffolds and negative controls. Interestingly, this study initially induced periodontal defects over a period of 4 weeks, which indicates some advantage over an acute intrasurgically created defect.

Delivery of Cells for Periodontal Engineering

There are various cell delivery methods in periodontal engineering applications (Table 2, adapted from [36]). In general, scaffolds are used in tissue regeneration to provide and maintain the necessary space for the cells to grow and to physically support the regeneration process. They should 1) provide a three-dimensional architecture that supports a desired volume, shape, and mechanical strength; 2) have a high porosity and surface-to-volume ratio, with a well-interconnected open-pore structure to promote high seeding density and to embrace bioactive molecules; 3) be biocompatible; and 4) degrade at a controlled rate and pattern that allows sufficient support until tissue defects are fully regrown [37].

As mentioned above, scaffolds classically consist of random collagen sponges. However, extensive studies in scaffold engineering have been conducted in recent years, and scaffolds can be combined with cell- or gene-based approaches to serve as supportive carriers conducting a sustained release of bioactive factors, thereby inducing stimuli for tissue formation [9]. Bioactive molecules such as growth factors may also be encapsulated into nanoparticles and microparticles to aid in their sustained release from scaffolds. Other approaches include mimicking stem cell niches to regulate daughter cell proliferation, differentiation, and dispersion into surrounding tissue or attracting useful cells to a desired anatomic site [9, 38]. The feasibility to establish a three-dimensional polarity in scaffolding design constitutes an important advance in creating biomimetic scaffold surfaces that can be applied in gene

Table 2 Types and uses of biomaterial in periodontal engineering (36)

Biomaterial-free	Biomaterial-assisted	Biomaterial-based
Cell suspension	Natural origin	Injectable (soft scaffolds)
Cell sheet engineering	Synthetic polymers	Solid biomaterials
Cell pellets or microtissues	Ceramics	Microencapsulation
		Growth factor
		ECM-composed

and cell therapy strategies [39]. Several other scaffold fabrication technologies have been used recently, including conventional prefabricated scaffolds such as particulate, solid form, and injectable scaffolds. Regardless of scaffold form, their purpose is the same, which is to influence the environment where they are implanted to promote a better outcome [40, 41].

Conventional scaffolds are usually prefabricated from both natural and synthetic polymeric materials. Naturally derived scaffolds include autografts, allografts, and xenografts. Ceramics are another form of naturally derived scaffolds, most commonly used in bone regeneration and implant therapy [42]. Alloplasts and other polymers are synthetically engineered materials consisting of bioactive molecules that serve a purpose similar to that of natural scaffolds.

Natural Polymers

Most biomaterials of natural origin in use today are based on cross-linking or self-assembly properties, and they have an innate ability to interact with and mediate degradation by cells [9]. They form hydrophilic polymers with over 90 % of water. Materials in this category include collagen, chitosan, dextran, alginate, *Aloe vera*, and fibrin.

Some interesting studies in periodontal engineering have been published within the last year where these materials have been used. A novel porcine acellular dermal matrix maintaining a 3D collagen framework has been tested both in vitro and in vivo. Together with hydroxyapatite, the construct has shown appropriate biodegradation patterns and favorable tissue compatibility [43]. Similarly, another new 3D scaffold made of collagen hydrogel, cross-linked to the ascorbate-copper ion system and injected into a collagen sponge, has demonstrated good biocompatibility and biodegradability 2 weeks after implantation in class II furcation defects (of 5 mm depth and 3 mm width) created in beagle dogs. Reconstruction of alveolar bone, cementum, and periodontal ligament was frequently observed as well just 4 weeks after surgery [44].

Platelet-rich fibrin's ability to promote cell proliferation, migration, and wound-healing is well-known and has been recently confirmed in periodontal tissues [45, 46]. PRF is a biodegradable scaffold (replaced with dense collagen even after 2 weeks) [45] that stimulates the synthesis of many growth factors, including vascular endothelial growth factor (VEGF), thrombospondin 1 (TSP-1), connective tissue growth factor (CTGF), hepatocyte growth factor (HGF), and pro-collagen type I [46], but decreases alkaline phosphatase (ALP) activity and expression of bone sialoprotein (BSP) and osteocalcin (OCN) while upregulating collagen I (Col-I) and cementum-derived protein 23 (CP-23) [47]. In vivo, in combination with PDLSC sheet fragments, it has been shown to promote more effective periodontal healing, characterized by regeneration of PDL-like tissues and reduction of ankylosis and inflammation [47].

Finally, another interesting natural polymer that is being studied is based on the *Aloe vera* extract known as acemannan [48]. This polysaccharide successfully stimulated both soft- and hard-tissue healing in class II furcation defects in mongrel dogs. However, little is known about this polymer, and further studies are needed.

Ceramics

The most used and studied ceramic material in the dental field is hydroxyapatite, which has been demonstrated to restore periodontal defects and to carry and deliver growth factors such as BMPs and fibroblast growth factor-2 (FGF2) [49]. Because increase of this material is inhibited by treatment with SB203580, a p38 inhibitor, and phosphorylation of p38, its ability to stimulate BMP-2 expression is dependent on the p38 MAP kinase pathway [50]. In the form of nanocrystals, it has some advantages over other materials. Compared to enamel matrix derivative (EMD), for example, nano-hydroxyapatite stimulates PDL cell proliferation and adhesion more efficiently, although cell migration is higher when using soluble EMD. These effects are mediated by alpha5beta1-mediated adhesion and focal adhesion kinase (FAK) activation [51]. Some studies have failed to prove an in vivo utility in periodontal regeneration when used alone [52]. Fortunately, nano-hydroxyapatite is a versatile material that can be combined with many polymers, either natural or synthetic, including chitosan [53], collagen [54], and gelatin [55], to improve the final results.

Another synthetic inorganic calcium phosphate–based material used as a delivery system is β-tricalcium phosphate. This material is primarily used in osseous defects around teeth or dental implants. Other composites in this group have begun to be used in periodontal engineering and are confirming its applicability as a stimulator of PDL cell proliferation and differentiation [56, 57]. Their value for periodontal regeneration remains to be explored [9].

In this same category, bio-glasses or silicate-based crystals have similar properties and have been widely used in implant surfaces as biomimetic agents [42]. In periodontal regeneration, they may induce cementoblast proliferation [58] and osteogenic/cementogenic differentiation of PDL cells [59], and they are non-toxic, even in the form of nanoparticles [60]. They have been also used in combination with chitosan to create resorbable membranes that directly induce bone regeneration [61].

Synthetic Polymers

Synthetic polymers have been studied extensively in periodontal engineering [62]. They provide greater freedom for property modification, control of macrostructure, and

degradation time, which can help to control the release mechanism and exposure duration of bioactive molecules [63, 64]. Such control provides the ability to maintain the therapeutic level of encoded proteins and to limit unwanted immune response and potential side effects [65].

The main representative agent in this category is the group of poly(lactic-co-glycolic acid)-based biomaterials. When configured as a microsphere, they have excellent properties for encapsulation of genes, antibiotics, and growth factors, as an occlusive membrane for guided tissue regeneration, and for cementum and complex tooth structure engineering. In addition to these promising applications, the use of nano-sized particles has attracted considerable attention [9]. Nano-sized particle-based scaffolds can provide a suitable environment for targeted cells and tissues in controlling the dynamic release of entrapped biologics. Periodontal therapy based on these systems, however, is only in the very early stages.

Custom-Made and Functionalized Scaffolds

Various novel delivery scaffolding systems are being extensively studied and are demonstrating superior capabilities to meet the challenges of current regeneration therapy. There are several techniques and technologies that have been developed and applied to fabrication of scaffold matrices. Only through further research and development in this area, along with cell-based and gene therapy, can tissue engineering continue to advance.

This concept relies upon computer-based scaffold design and fabrication [66]. This type of technology, image-based design, has been used in recent years to define virtual three-dimensional models of anatomic geometry of the defect and to create a template for a scaffold on a global anatomic level [67•]. Furthermore, the scaffold can be designed to mimic the heterogeneous structures to be regenerated: variations in porous microstructures and scaffold surface topography, which will influence the modulus of elasticity, permeability, and cell orientation [39]. This hybrid scaffold concept has proven effective at establishing an adequate periodontal tissue interface that integrates the newly formed cementum, bone, and properly oriented PDL fibers in others studies as well [68].

One of the most important features of the periodontium is PDL fiber orientation from bone to cementum. A tissue-engineering scaffold resembling the structure of the natural extracellular matrix by mimicking nanofibrous features can often facilitate tissue regeneration in bone, cartilage, enamel, dentin, and periodontium [69]. There are methods to create single- and multiple-channeled nanofibrous poly(L-lactic acid) scaffolds [70]. According to the authors of this study, the overall shape, the number and spatial arrangement of channels, the channel wall matrix architecture, the porosity and mechanical properties of the scaffolds are all tunable. The porous channel wall facilitates protein adsorption.

Delivery of Molecules in Periodontal Engineering

The delivery of different molecules, mainly growth factors [71], was the first approach in using a biological agent modifier for periodontal regeneration purposes. Because periodontal regeneration requires these molecules, it is very important to identify them to benefit periodontal engineering. Some are successfully being used in clinical studies [72]. They can be delivered either by recombinant products or gene therapy [64, 73]. Furthermore, gene therapy can be performed by directly delivering the infecting agent or by an indirect or cell-based delivery approach [9]. Table 3 shows various viral and non-viral gene therapy vectors used in tissue engineering (adapted from [9]).

Gene therapy can be defined as the treatment of disease or disorder by transferring genetic materials to introduce, suppress, or manipulate specific genes that direct an individual's own cells to produce a therapeutic agent [9]. Gene therapy has several advantages over traditional treatments, the first of which is greater sustainability than that of a single protein or compound application. Whereas the half-lives of pharmaceutical compounds or recombinant protein usually range from several hours to several days, viral vector genes can be expressed in vivo from weeks to years. Secondly, gene delivery reduces technical challenges associated with ex vivo protein expression and purification such as palmitoylation and glycosylation. Third, transient and controlled delivery of genetic sequences encoding a combinatorial group of regenerative factors could mimic the natural biologic healing response. And, fourth, coupled with tissue-engineering strategies, gene delivery in a spatially controlled and bioavailable fashion offers strong potential for three-dimensional tissue regeneration at the tooth-ligament-bone interface [9].

Various in vitro and ex vitro studies have suggested that PDGF (platelet-derived growth factor), FGF (fibroblast growth factor), IGF (insulin growth factor), BMP (bone morphogenic protein), and other growth factors are able to strongly stimulate periodontal regeneration, including both bone and cementum ([71].

Platelet-Derived Growth Factor

Platelet-derived growth factor (PDGF) is a member of a family of multifunctional polypeptide growth factors and is considered a critical switch to initiate tissue repair process. It is both a potent recruiter of and strong mitogenic factor for cells crucial to musculoskeletal tissue repair, including mesenchymal stem cells (MSCs), osteogenic cells, and tenocytes, and it also upregulates angiogenesis [74].

Recombinant human PDGF-BB in a synthetic scaffold matrix (beta-tricalcium phosphate) promotes long-term stable clinical and radiographic improvements for patients with localized periodontal defects [75]. Interestingly, a study of this

Table 3 Viral and non-viral gene therapy vectors used in tissue engineering (9)

Vector	Type	Advantages	Disadvantages
Retrovirus	Viral	Non-immunogenic	Infects only dividing cells
		Constitutive transgene expression	Insertional mutagenesis
Lentivirus	Viral	Infects dividing and non-dividing cells	Insertional mutagenesis
		Infects wide range of cell types	Potential pathogenicity
		Low immune response	Complex large-scale preparation
Adenovirus	Viral	Infects dividing and non-dividing cells	Potential immunogenicity
		Does not integrate into target cell genome	Transient expression
Adeno-associated virus	Viral	Infects dividing and non-dividing cells	Difficult to produce at high titers
		Low immunogenicity Non-pathogenic in human	Small transgenes
Plasmid	Non-viral	Non-immunogenic Non-pathogenic	Low transduction efficiency
DNA polymer complexes	Non-viral	Infects dividing and non-dividing cells Cell-specific targeting	Low transduction efficiency

growth factor showed a particularly greater effect in the lower-dose group (0.3 mg/mL). No special root preparation was made. A recent in vitro study demonstrated a higher rate of periodontal cell adhesion when using EDTA, PDGF, or a combination of both, compared to SRP (scaling and root-planing) or untreated groups. However, there was no significant difference between PDGF and PDGF+ EDTA groups [76]. Other dimers such as PDGF-AB have also been tested, but clinical application has not yet been approved [77].

There have been numerous gene therapy studies for periodontal regeneration using PDGF [78–82]. Most recently, analysis of the release of this growth factor by nano-sized calcium phosphate particles is showing promising results in terms of biocompatibility and efficient transfection into fibroblasts [83].

FGF

While it has not been used thus far in gene therapy, basic fibroblast growth factor, also known as FGF-2, may be of interest in the near future. In combination with beta-TCP, FGF-2 has proven efficacy in a root coverage study in beagle dogs [84] as well as in class III furcation defects [85].

Although FGF-2 induces proliferation of human periodontal ligament cells [86], it also decreases calcium accumulation and ALP activity, suggesting that it may inhibit terminal differentiation [87]. It has been recently shown to reduce the expression of SDF-1alpha (stromal cell-derived factor-1alpha) [88] via the FGFR1 [89]. SDF-1alpha has been reported as playing a crucial role in stem cell homing and recruitment to injured sites.

Bone Morphogenetic Protein

In dentistry, gene therapy with bone morphogenetic proteins (mainly 2 and 7) has been shown in vivo to stimulate not only cementum with Sharpey's fibers insertion, but also statistically significant quantities of alveolar bone [90, 91]. BMP-2 accelerates bone regeneration but is associated with limited cementum and periodontal ligament regeneration, local root resorption, and ankylosis when used as a recombinant protein. This negative effect in periodontal engineering could be overcome by the use of ex vivo BMP-2-engineered autologous MSCs [92]. Similarly, BMP-7 promotes proliferation, differentiation, and mineralized nodule formation, especially in cementoblasts [93] by inducing PCPE1 and BMP1 responsible for processing of type I collagen [94]. It also downregulates BMP-4, although it upregulates DMP-1, probably more through the IGF-II than the IGF-I pathway [93].

Other Transcription Factors and Regulators

In addition to growth factors, other critical transcription factors and regulators of osteogenesis that may be of great interest in periodontal tissue engineering and alveolar bone augmentation include RUNX2, osterix (Osx), and LIM domain mineralization protein (LMP) [9].

LMPs show potential in the modulation of periodontal progenitor cells (LMP1) [95]. Additionally, adenovirus delivery of LMP3 in hPDL cells can induce osteogenesis in vitro by significantly upregulating ALP (alkaline phosphatase), BSP (bone sialoprotein), and BMP2. Furthermore, when co-delivered with AdBMP7 in vivo, LMPS induce higher new bone formation [96].

Other mediators are being explored as well in the periodontal engineering arena. Specifically, our group is exploring the potential of a matricellular molecule named periostin in periodontal regeneration. Periostin, which is highly specific for the [97], is essential for PDL integrity and function during occlusal loading [12]. Furthermore, periostin is reduced by the proinflammatory cytokine TNF-alpha and the virulence factor *P. gingivalis* LPS in vitro, suggesting a potential role in the pathogenesis of periodontal disease [98]. Not surprisingly, in an animal model, higher inflammatory infiltrate and higher alveolar bone was correlated with lower levels of periostin in the PDL [99]. In this context, we investigated whether recombinant addition of periostin was able to restore the proliferation, migration capacities, and activation of survival signaling pathways of hPDL after being challenged by TNF-alpha and *P. gingivalis* LPS. In this study, periostin significantly increased cell proliferation (by twofold), migration (especially at earlier time points and low dose), and activation of survival signaling pathway PI3K/AKT/mTOR (higher phosphorylation of AKT and PS6) under both non-challenged and challenged conditions [100].

Future Directions in Periodontal Engineering

As discussed in this review, many different approaches and biologic agents are being studied in the area of periodontal engineering. The major challenge today remains the timely organization of the sequence of events that must happen with the healing area in order to promote the cells that are needed at each precise moment and without compromising normal cell function. New materials and signaling molecules – whether delivered by gene therapy or not – are therefore of great interest. Additional evidence and practice standardization are still needed in order to satisfy the regulatory requirements to apply these technologies in the clinical scenario. It is necessary to understand differences in the regenerative process between chronic periodontal pathology and other defects such as implant sites, extraction sockets, etc. The application of periodontal engineering, therefore, requires a thorough understanding of the homeostasis and pathogenesis of these defects.

Conclusions

Periodontal regeneration based on tissue engineering approaches has a solid background for clinical application in human periodontal defects. The cell-based, scaffold, and gene therapy methods interface and complement each other. However, some of these therapies are still at the preclinical level. In the near future, the success of periodontal regeneration will undoubtedly be conditioned on the ability to correctly diagnose clinical situations where these techniques can be predictably applied.

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Compliance with Ethics Guidelines

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- •• Of major importance

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