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Concise Review: Periodontal Tissue Regeneration Using Stem Cells: Strategies and Translational Considerations

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ABSTRACT

Periodontitis is a widespread disease characterized by inflammation-induced progressive damage to the tooth-supporting structures until tooth loss occurs. The regeneration of lost/damaged support tissue in the periodontium, including the alveolar bone, periodontal ligament, and cementum, is an ambitious purpose of periodontal regenerative therapy and might effectively reduce periodontitis-caused tooth loss. The use of stem cells for periodontal regeneration is a hot field in translational research and an emerging potential treatment for periodontitis. This concise review summarizes the regenerative approaches using either culture-expanded or host-mobilized stem cells that are currently being investigated in the laboratory and with preclinical models for periodontal tissue regeneration and highlights the most recent evidence supporting their translational potential toward a widespread use in the clinic for combating highly prevalent periodontal disease. We conclude that in addition to in vitro cell-biomaterial design and transplantation, the engineering of biomaterial devices to encourage the innate regenerative capabilities of the periodontium warrants further investigation. In comparison to cell-based therapies, the use of biomaterials is comparatively simple and sufficiently reliable to support high levels of endogenous tissue regeneration. Thus, endogenous regenerative technology is a more economical and effective as well as safer method for the treatment of clinical patients. STEM CELLS TRANSLATIONAL MEDICINE *2019;8:392–403*

SIGNIFICANCE STATEMENT

Stem cells play a crucial role in the regeneration of the tooth/supporting tissue that is lost or damaged because of the periodontal diseases. The review summarizes the translational potential of the developed regenerative approaches using either culture-expanded or host-mobilized stem cells for periodontal regeneration. To facilitate the translation of ex vivo manipulated stem cells into clinical use, concerted research endeavors should be placed on making cell therapy and tissue engineering more practical and economical as well as safer. To enable the use of endogenous cells for therapeutics, the current and future designs should focus on directing more cells to the site of injury and making the target site more suitable for cell differentiation and new tissue growth.

INTRODUCTION

Periodontitis, an oral disease with a high prevalence worldwide, affects the function of teeth and constitutes one of the main oral health burdens [1]. An epidemiological survey has suggested that more than half of all adults are affected by periodontal disease to varying degrees [2–4], and a remarkable surge (25.4% increase) in the prevalence rates of periodontal disease was observed from 2005 to 2015 [5, 6]. Periodontitis can consistently disrupt tooth-investing tissues and lead to tooth loss if left untreated [5, 7]. Periodontitis is also closely associated with the occurrence and prognosis of various systemic diseases, including cardiovascular diseases, cancer, obesity, diabetes, and chronic nephritis [8–11]. Therefore, the exploration of effective and safe periodontal therapies that can be translated into the clinic is an urgent health need worldwide.

The ambitious purpose of periodontal therapy is to regenerate multiple periodontal tissues, including the alveolar bone, cementum, and periodontal ligament (PDL) in the damaged periodontium [12]. Although nonsurgical periodontal therapies (e.g., scaling and root planning) can prevent disease progression by

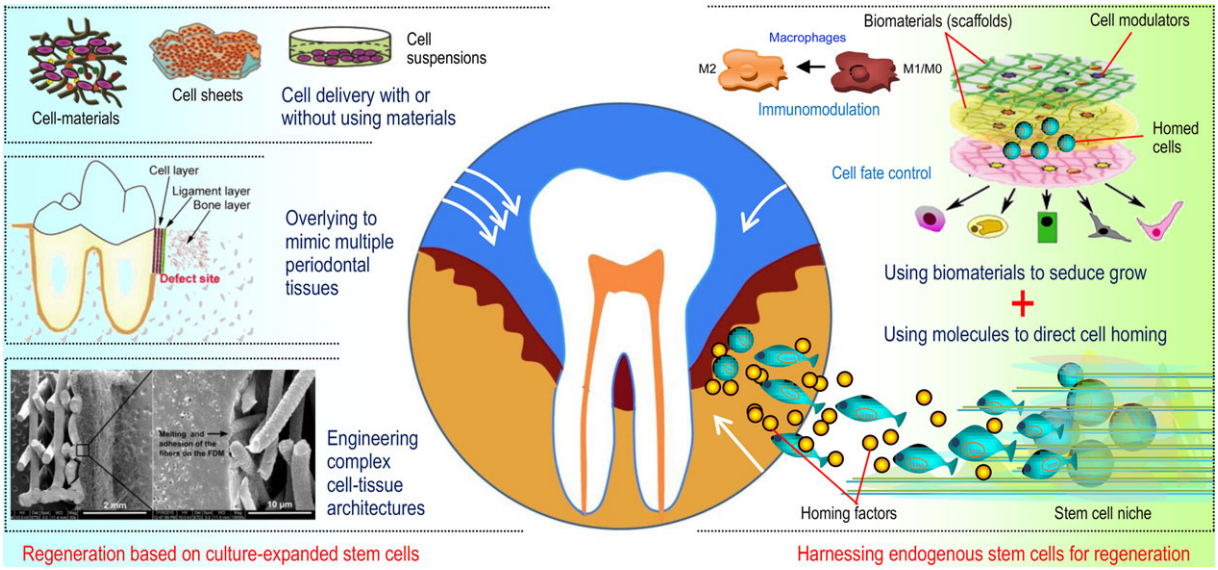

Figure 1. Periodontal regeneration can potentially be achieved via either in vitro designed cell-material constructs for transplantation to the area of damage, where the transplants undergo remodeling and revascularization to integrate with the host tissue, or in vivo manipulation of the cell-material interplay at the target site, where biomaterials and molecules coax the recruitment of endogenous stem cells to regrow new tissues.

physically removing the pathogens and necrotic tissues, only a small amount of periodontal tissue can be regenerated at the treated sites [7]. The application of technologies such as guided tissue regeneration (GTR) for periodontal surgery can erratically restore the alveolar bone and soft tissues, but the overall outcomes are not necessarily satisfactory and show a lack of clinical predictability [13]. Although new biomaterials and growth factors have enriched the methods for managing periodontal defects, clinical trials have revealed that their efficacy is still controversial, and the structural and functional regeneration of lost periodontal structures remains challenging [12].

Stem cells can self-renew and differentiate into multiple cell types and thus have tremendous therapeutic potential. The identification of stem cells from human PDL tissues, termed PDL stem cells (PDLSCs), in 2004, led to a new era of research on periodontal regeneration [14]. Since then, other stem cells have been found to possess the ability to form multiple periodontal tissues under appropriate induction conditions [15]. In addition to their regenerative potential, the ability of stem cells to undergo immunomodulation plays an equally important role in achieving a successful outcome (reviewed in [16]). Today, the use of stem cells is considered as a mainstream strategy for periodontal treatment, particularly for complete regeneration of the periodontal complex, which implies not only the reconstruction of appropriate alveolar bone but also the induction of cementogenesis along the root surfaces with the oriented insertion of newly formed PDL tissue [13, 17, 18].

Based on therapeutics using ex vivo-expanded stem cells, the regeneration of the periodontal complex has been demonstrated to be feasible in a variety of models tested (reviewed in [17, 18]). However, in vitro cell culture places a heavy financial burden on patients and is associated with multiple other difficulties, including an insufficient stem cell source that is available for use, time-consuming culture procedures, and safety issues [19, 20]. To accelerate the clinical use of stem cell technology, the mobilization/homing of resident stem cells for regeneration based on endogenous healing mechanisms has become a new concept in regenerative medicine, which we herein definitively term endogenous regeneration medicine (ERM) [21–24]. ERM is particularly promising in periodontal research because of the high incidence rate of periodontitis, and mounting evidence indicates that endogenous stem cells can be directed to the periodontium to exert regenerative and immunomodulating functions; this strategy is similar to or more effective than the use of transplanted foreign stem cells (e.g., see [25, 26]). In the future, ERM could offer a safer as well as more effective and economical method for periodontal regeneration than current cell-based therapies. In this concise review, we summarize the current periodontal regenerative approaches based on either in vitro cell-material design (cell delivery and transplantation) or in vivo cell-material interactions (cell recruitment and homing; Fig. 1) and highlight the most recent evidence supporting their translational potential toward widespread use in the clinic for combating highly prevalent periodontal diseases.

Stem Cell Delivery Shows Promise for Periodontal Healing

Any cell type with an enormous proliferative capacity and a multipotent nature, particularly stem cells, can be used to replenish destroyed cells under certain conditions [27, 28]. The discovery and therapeutic application of stem cells have offered a new concept for periodontal regeneration. The current stem cell-based therapies in periodontics rely mainly on the delivery of culture-expanded cells to the periodontal defect to enhance wound healing, and many elegant studies have documented positive outcomes using either intraoral or extraoral stem cells (reviewed in [29, 30]).

Single-cell suspensions prepared in vitro or cells suspended in medium can be directly injected into the site of injury easily,

and this method is simple and minimally invasive [31]. Bone marrow-derived mesenchymal stem cells (BMMSCs) locally injected into mouse periodontal defects exert anti-inflammatory and immunomodulatory effects at the target site and contribute to the regeneration of new tissue [32]. In addition, an early-phase study also showed that in the clinic, the transplantation of expanded autologous fibroblasts might be efficacious for curing papillary insufficiency following a papilla priming procedure [33]. However, there are drawbacks associated with the injection of cell suspensions, such as an insufficient cell supply following an injection, poor engraftment, spread of injected cells to surrounding healthy tissue, and loss of cell fate control (reviewed in [31]).

The therapeutic outcomes of delivered cells at the transplanted sites could potentially be improved with the aid of cell sheet/pellet technology, a scaffold-free approach for cell transplantation (reviewed in [20, 34]). In this context, confluent cells that are cultured/expanded are harvested as intact cell sheets, and monolayer or stacked cell sheets are notably easier to deliver and transplant than fluid cells and result in minimal cell loss/damage (reviewed in [35]). Because stem cells are delivered together with their extracellular matrix (ECM) produced during ex vivo culture, cells with cell-cell and cell-matrix contacts are expected to remain engrafted for a long time after their introduction into the body without changes in their viability and function, which would lead to enhanced tissue regeneration compared with that observed with the injection of cells alone. In fact, the transplantation of cell sheets instead of fluid cells has revealed improvements in animal periodontitis with bone defects (e.g., see [36, 37]).

In many cases, stem cell sheets are transplanted into the damaged periodontium with bone substitutes, such as hydroxyapatite/tricalcium phosphate (TCP; e.g., see [38, 39]), bovine bone (e.g., see [40]), synthetic hydrogels (e.g., see [41]), and their composites (e.g., see [42]), to increase the stability of the cells within the defect (reviewed in [35]).

Irrespective of the animal model used and cell/defect type, substantial evidence from preclinical animal studies indicates that the local delivery of foreign cellular materials can benefit the outcome of periodontal disease management (reviewed in [43]). Although human case studies (e.g., see [44]) and randomized clinical trials (e.g., see [40]) have demonstrated the feasibility and safety of stem cells, more well-designed human studies should be performed to examine the beneficial effects of stem cells on periodontal regeneration. In addition, future translational research is still needed to identify cell populations with highly regenerative potential and to optimize the delivery methods. In addition, the therapeutic procedure should be sufficiently practical for use in a clinical setting.

Periodontal Tissue Engineering Via in vitro Cell-Material Design

Given that the periodontium represents a unique complex architecture surrounding and supporting the tooth, the complete regeneration of hybrid periodontal tissues in humans remains limited and unpredictable, at least in current clinical practice, which shows that this achievement is a hard-fought goal even with the aid of stem cell transplantation. To achieve complex periodontal regeneration, several advanced engineering approaches based on in vitro cell-material design have paved the way for preclinical animal studies and offer state-of-the-art therapeutic avenues for functional periodontal regeneration (reviewed in [18]). Without a doubt, the combination of stem cells with well-organized biomaterials that present and release robust signaling molecules might support a higher level of tissue regrowth than the use of cells alone.

Inspiration From the Bone-PDL-Cementum Apparatus

Insight into the physiogenesis and anatomical structure of the periodontium provides a solid basis for understanding the process of tissue destruction as well as for advancing the design of more effective cell-based therapeutic strategies [45]. The periodontium, which supports and anchors the teeth in their alveolar sockets, has a well-arranged structure and can be divided into four main vital components, that is, gingiva, root cementum, PDL, and alveolar bone [45, 46] (Fig. 2). As the main part of this apparatus, the periodontal complex (i.e., bone-PDL-cementum) is hierarchically organized into the final three components affiliated with periodontal tissues [18] and characterized by periodontal fibers that are inserted into the root cementum and adjoining alveolar bone. Because of the interfacial interconnection of this multitissue complex, the periodontium can support and stabilize the tooth by distributing and absorbing forces as well as serve as a barrier to defend against dissimilar invading pathogens [45, 47–49]. Consequently, the periodontal complex is considered the cornerstone of the periodontal structure and plays pivotal roles in restoring and maintaining the function of periodontal tissues. When the integrity of the periodontal complex is compromised by periodontitis, complete regeneration of the complex is very difficult to achieve through the local administration of stem cells alone because of the multiple tissue types and complex structures involved [50, 51]. Taking inspiration from the anatomical structure of the periodontium, researchers hypothesize that the regeneration of the periodontal complex can benefit from specific cell-material designs and have used the concept of tissue engineering to recreate the native bone-PDL-cementum apparatus.

Overlaying to Mimic Multiple Periodontal Tissues

Given that the veritable regeneration of periodontal tissue involves reestablishment of the bone-PDL-cementum apparatus, the simple combination of in vitro-cultured stem cells and biomaterials cannot realistically be used to achieve this goal [13, 52]. However, it is possible to regain the periodontal hybrid tissues by layering materials and cells to mimic the different tissue layers involved in the periodontium [18]. The vertical stacking of three-layered PDLSC sheets, woven poly(glycolic acid), and porous β-TCP in an orderly manner based on this concept and their placement into the three-wall periodontal defects of a canine resulted in newly formed bone and cementum interspersed with the aligned collagen fibers (Fig. 3A) [53]. Similarly, cell sheets comprising PDLSCs and/or jaw BMMSCs have been multilayered to regenerate a complex periodontium-like architecture [54]. Recently, a "sandwich" tissue engineering complex was constructed by adding a layer of mineralized membrane on each side of the collagen membrane. After seeding with gingival fibroblasts, this complex was implanted into periodontal defect areas in

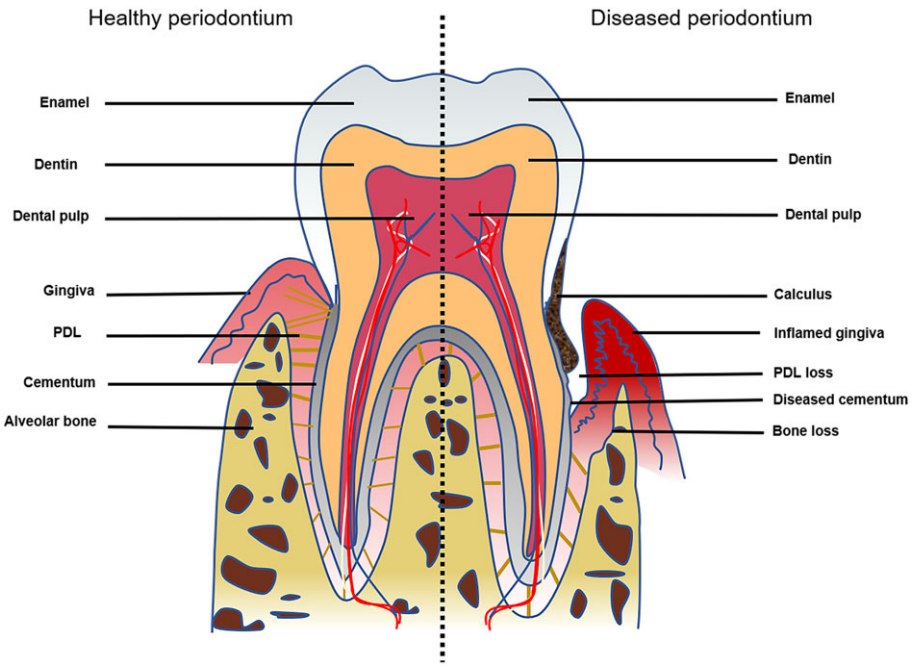

Figure 2. Schematic representation of the periodontium containing the intact periodontal complex (i.e., bone-PDL-cementum apparatus). As a result of disease (e.g., periodontitis), damage to the periodontium leads to the loss of multiple periodontal tissues surrounding and supporting the tooth. Abbreviation: PDL, periodontal ligament.

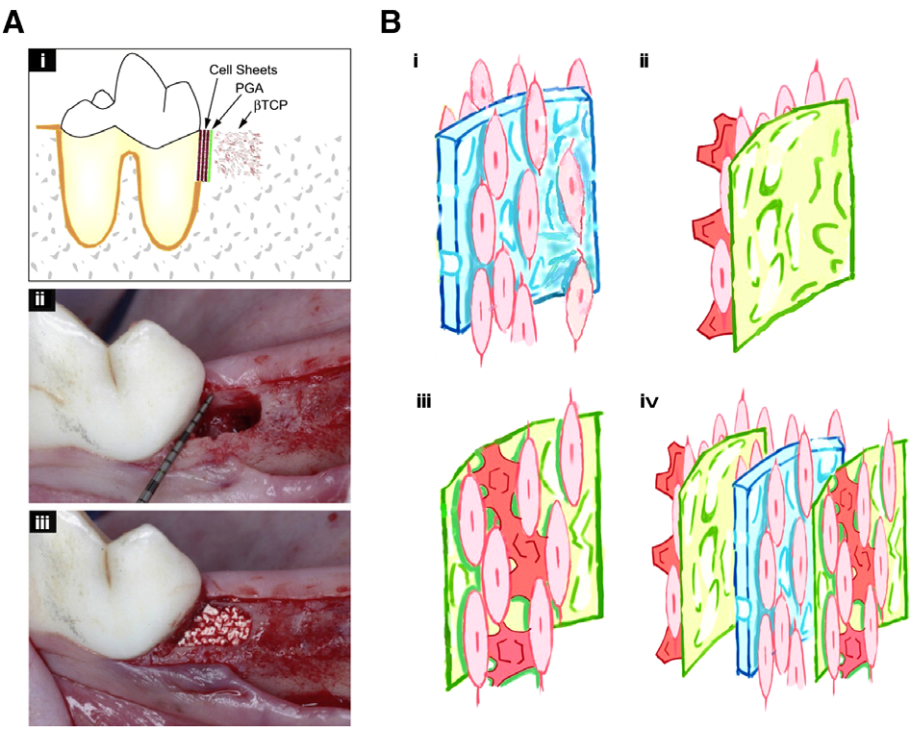

Figure 3. Overlaying cell sheets and biomaterials to mimic multiple periodontal tissues. **(A):** Three-layered cell sheets together with woven PGA and porous β-TCP were used to repair three-wall infrabony defects in dogs [53] (please refer to the original source for more information). **(B):** Schematic representation of a generated sandwich complex including (i) an engineered membrane (Bio-Gide collagen membrane seeded with cells on both sides and cultured without the addition of mineralization-induction medium) and (ii) two mineralized membranes (a cellular small intestinal submucosa in which cells are seeded on one side and cultured in mineralization-induction medium for 8 days); (iii and iv) a sandwich structure was obtained by placing a cell-seeded periodontal membrane between the two cell-seeded/mineralized membranes [54] (please refer to the original source for more details). Abbreviations: PGA, polyglycolic acid; β-TCP, β-tricalcium phosphate.

dogs, and simultaneous neogenesis of ligamentous and osseous structures was achieved (Fig. 3B) [55]. Although the development of bilayered cell constructs serve as a promising strategy to simultaneously regenerate multiple periodontal tissues, the orchestrated use of multiple regenerated tissues to reconstruct the periodontal complex with micron-scaled tissue compartmentalization as well as osseous and ligamentous interfacial structures with systematic tooth-supporting functions remains a major clinical challenge.

Engineering Approaches to Reconstruct Periodontal Complex Interfaces and Architectures

Recent advances in biomaterials technology have enabled the engineering of periodontal scaffolds with triphasic tissue interfaces and structures (e.g., see [56]). In particular, computer-aided design and three-dimensional (3D) printing now allow the generation of compartmentalized hybrid scaffolds with biomimetic interfaces in 3D space (e.g., see [57]). These scaffolds with spatial constructs can specifically regulate cell behaviors and influence the orientations of multiple tissues and thus provide an essential foundation for the formation of oriented ligamentous tissues and their successful incorporation into the new bone and cementum (reviewed in [17]). In this context, biomimetic hybrid scaffolds containing bone-specific and PDL-specific polymer compartments are used to engineer human tooth-ligament interfaces [56], and the engineering of bone-ligament complexes has successfully been achieved with fiber-guiding scaffolds (Fig. 4A) [56]. Biphasic scaffolds that mimic the bone compartment by using a fused deposition modeling scaffold and mimic the PDL compartment by using an electrospun membrane have been reported, and the combination of these scaffolds and multiple PDLSC sheets allows the in vivo regeneration of complex periodontal structures (Fig. 4B) [58]. Furthermore, the coating of the bone compartment with a calcium phosphate layer increased the osteoconductivity of seeded osteoblasts [59].

Using 3D printing and directional freeze-casting techniques, a fiber-guiding hybrid scaffold has been designed to spatiotemporally control the morphogenesis, integration, and functionalization of various tissues [60]. Animal experiments have shown that this customized fiber-guiding scaffold can accurately adapt to defect sites and thus successfully guide cell/tissue directionality during regeneration and facilitate the formation of a more stable ligament-ligand complex with a rapidly maturing matrix [61]. More recently, a similar "3D-patterned, periodontal mimic multiphasic architecture" approach was reported, taking his technology one step closer to clinical translation [62]. The aforementioned fiber-guiding scaffold has been approved by the U.S. Food and Drug Administration for clinical use [60]. Collectively, 3D-patterned multiphasic complexes have enabled the reconstruction of periodontal complex architectures for periodontal tissue engineering strategies, but when translated to clinical use, the complexity of tissue-engineered constructs must be kept to a minimum to ensure cost-effectiveness and ease of production.

Crucial Barriers to Progress

In vitro stem cell-material designs can mimic the anatomy of the periodontium, and different biomaterials, such as the bone graft materials and cell sheets, particularly barrier membranes, could be applied for this strategy. The goal of periodontal tissue engineering is to reinstate the normal function of the diseased periodontium to support the teeth, whereas the triad would require stem cells, biomaterials, and infection control. The available data demonstrate the capacity of stem cells to regenerate lost periodontal tissues in animal models (Table 1); however, the efficacy of cell-based interventions in humans remains to be confirmed [43]. Although experimental data have allowed the initiation of clinical trials in periodontal cell therapy, proper consideration of the cell source, material type, and regulatory concerns is crucial to facilitate clinical translation [63]. For periodontal tissue engineering, regeneration in response to overengineered constructs should be investigated in larger animals, including rodents, because the anatomy of the dentoalveolar architecture in larger animals more closely resembles that of humans [64]. In addition, the experimentally generated defects need to accurately mimic the pathophysiology of periodontitis in humans. In many cases, the periodontal defects generated in the currently used animal studies do not sufficiently reflect the inflammatory status of human periodontitis.

Harnessing Endogenous Stem Cells Via in vivo Cell-Material Interaction

Based on the body's self-regeneration potential, a new avenue for enhancing periodontal regeneration is to use endogenous stem cells. This concept makes periodontal regenerative therapy safer, simpler and easier to accept [21], reduces the high cost of cell culture, storage, and transplantation, and provides a bright future for the clinical application of new regenerative approaches. However, under most circumstances, the regeneration ability of diseased tissue is quite weak, and the lost/damaged periodontium might not be able to repair itself [65]. The overscale defect and impaired regenerative capacity caused by aging and other systemic diseases also add to the local inflammatory microenvironment. Harnessing endogenous stem cells to defect sites via in vivo cell-material interactions is the first goal of ERM. Based on this concept, multiple scaffolds combined with growth factors, antibodies, and drugs have been designed and applied to enhance cell homing and tissue regeneration [24, 66].

Self-Healing Potential of Periodontal Tissues

The periodontium possesses a staggering self-healing potential with the help of proper treatment and guidance. For example, at the early stage of periodontitis, removing the dental plaque can cure the infected/damaged periodontal tissues [8]. Various degrees of periodontal repair/regeneration have been observed after GTR treatment, but the outcome is unpredictable [13]. Thus, endogenous regeneration of the periodontium is feasible under certain circumstances. The factors affecting periodontium regeneration are quite complex, and the correct type and a sufficient number of cells, a beneficial microenvironment with biological signals and suitable scaffolding matrices, are all critical for regeneration. Therefore, the current strategies for enhancing self-healing focus on directing resident cells for target trafficking and on coaxing these cells to grow new tissues [24].

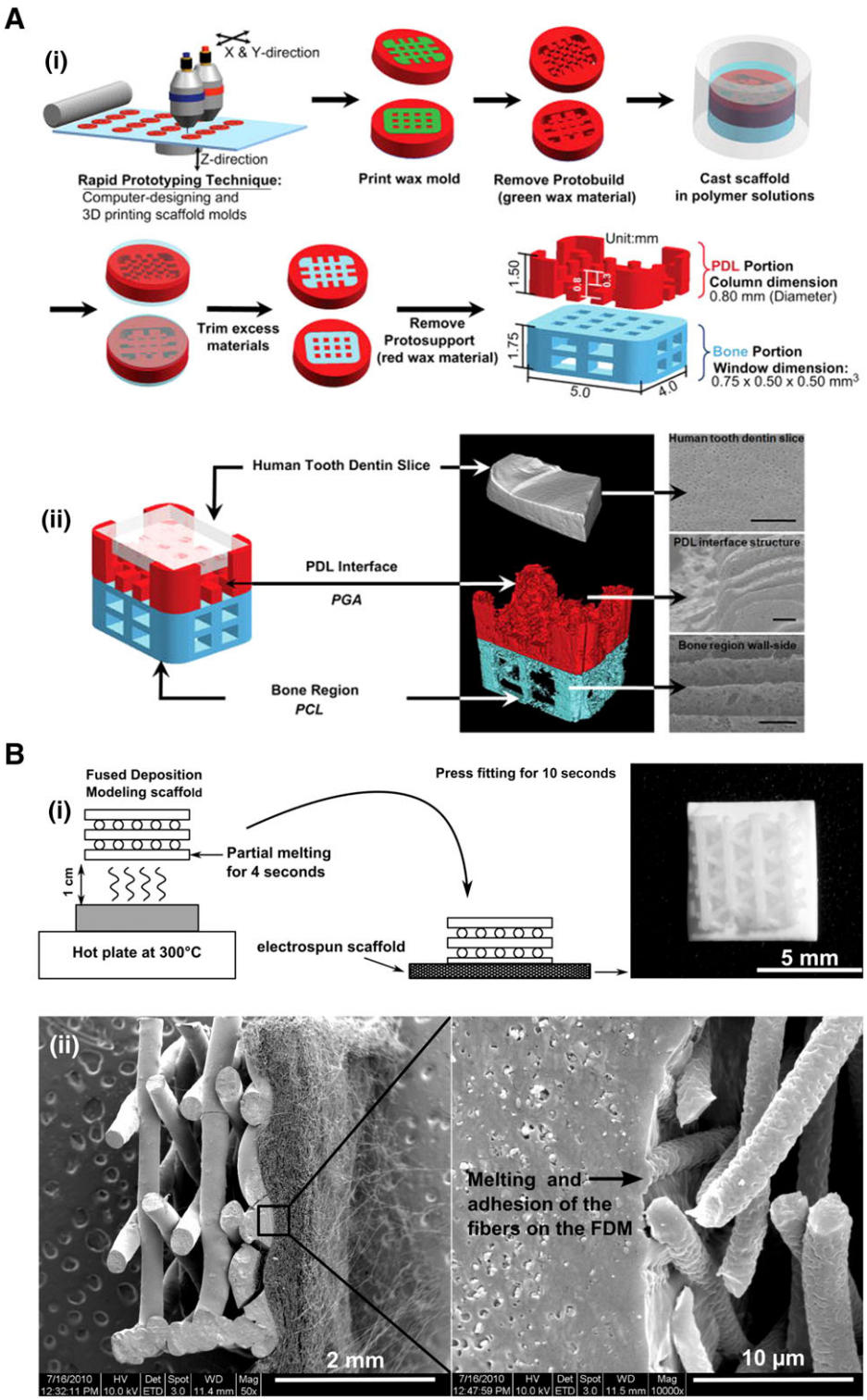

Figure 4. Engineering of biomimetic materials and architectures to reconstruct the periodontal complex. **(A):** Graphical illustration of the fabrication of a biphasic scaffold mimicking the tooth-ligament-bone complex using multiscale computational design and polymeric architecture manufacturing [56] (please refer to the original source for fabrication details). **(B):** Schematic representation of the fabrication (i) of a biphasic scaffold (ii) composed of the bone compartment (left side, a FDM scaffold) and periodontal compartment (right side, a membrane with electrospun fibers); this scaffold can be applied for the simultaneous regeneration of the bone-ligament complex when combined with stem cell sheets [58] (please refer to the original source for more information). Abbreviations: 3D, three-dimensional; FDM, fused deposition modeling; PCL, polycaprolactone; PGA, polyglycolic acid; PDL, periodontal ligament.

Table 1. Selected examples based on culture-expanded cells and/or biomaterial design toward in vivo periodontal regeneration

Concept	Cells	Materials	Animal models	Cell-material construct and transplantation	Outcomes	References
Cell transplantation without exogenous biomaterials	Allogeneic BMMSCs	No materials	Experimental periodontitis in rats	Injection of cell solution without additional materials	Cell injection significantly enhanced periodontal tissue regeneration.	[32]
	Allogeneic DPSCs	No materials	Experimental periodontitis in miniature pigs	Transplantation of cell sheets without additional materials	Cell sheet treatment led to more bone regeneration compared with that obtained with the injection of cells alone	[36]
Overlaying to mimic multiple periodontal tissues	Autologous PDLSCs	HA/TCP	Experimental periodontitis in miniature pigs	Transplantation of a combination of cells and materials	Materials containing PDLSCs significantly improved periodontal tissue regeneration, as determined through clinical and radiological evaluations	[37]
	Autologous GMSCs	HA-sECM	Experimental periodontal defects in miniature pigs	Transplantation of a combination of GMSCs and IL-1ra-releasing HA-sECM	GMSCs in conjunction with HA-sECM (either with or without IL-1ra) showed significant potential for periodontal regeneration.	[41]
	Autologous PDL-derived cells	Woven PGA (cell sheet support) and β-TCP (defect fill)	Three-wall periodontal defects in canines	Multilayered cell sheets supported with woven PGA were applied to the dental root surfaces, and the remaining defect area was filled with porous β-TCP	The transplantation of three-layered cell sheets resulted in the regeneration of both new bone and cementum connecting with well-oriented collagen fibers	[53]
	Allogeneic PDLSCs and jaw BMMSCs	TDM/CBB	Immunodeficient mice	Ectopic transplantation of TDM/CBB material-coated composite cell sheets	The combination of PDLSC and JBMMSC sheets facilitated the regeneration of complex periodontium-like structures.	[54]
	Autologous gingival fibroblasts	Bio-Gide collagen membrane and SIS segments	Experimental periodontal defects in beagles	Transplantation of a sandwich tissue-engineered complex (Fig. 2B)	Periodontal defects were completely repaired by the sandwich tissue-engineered complex 10 days after the operation	[55]
Engineering to reconstruct periodontal complex interfaces and architectures	Heterogenic gingival fibroblasts	Composite hybrid PCL/PGA scaffolds (Fig. 3A)	Immunodeficient mice	Ectopic transplantation of biomimetic hybrid scaffolds (with or without ad-BMP7-modified cells)	In vivo regeneration of tooth dentin-ligament-bone complexes	[56]
	Heterogenic DPSCs	Multiphase scaffolds (PCL/HA)	Immunodeficient mice	Ectopic transplantation of DPSC-seeded multiphase region-specific microscaffold with spatiotemporal delivery of BMP2, CTGF, and amelogenin	In vivo regeneration of multiphase periodontal tissues	[57]

(Continues)

Table 1 (*Continued*)

Concept	Cells	Materials	Animal models	Cell-material construct and transplantation	Outcomes	References
	Heterogenic osteoblasts and PDLSCs	A biphasic scaffold composed of an FDM scaffold (PCL/β-TCP) and an electrospun membrane (PCL) (Fig. 3B)	Immunodeficient rats	Ectopic transplantation of a biphasic scaffold composed of a bone compartment (seed with osteoblasts) and a periodontal compartment (combined by PDLSC sheets)	In vivo simultaneous regeneration of the alveolar bone/PDL complex	[58]
	Heterogenic osteoblasts and PDL cells	Second-generation biphasic scaffold incorporating an osteoconductive bone compartment through a CaP coating and replacing the periodontal compartment with a thin melt electrospun scaffold with larger pores	Immunodeficient rats	Ectopic transplantation of a modified biphasic scaffold (optimized from Fig. 3B) with osteoblasts and PDL cell sheets	In vivo regeneration of the complex hierarchical periodontal structure	[59]

Abbreviations: β-TCP, β-tricalcium phosphate; ABSCs, alveolar bone stem/progenitor cells; ad-BMP7, adenovirus-encoding murine bone morphogenetic protein-7; BMMSCs, bone marrow mesenchymal stem cells; CaP, calcium phosphate; CBB, ceramic bovine bone; CEMP-1, cementum protein 1; CTGF, connective tissue growth factor; DPSCs, dental pulp stem cells; FDM, fused deposition modeling; GMSCs, gingival mesenchymal stem cells; HA, hydroxyapatite; JBMMSC, jaw bone marrow mesenchymal stem cell; PCL, polycaprolactone; PDL, periodontal ligament; PDLSCs, periodontal ligament stem cells; PGA, polyglycolic acid; sECM, synthetic extracellular matrix; SIS, small intestinal submucosa; TCP, tricalcium phosphate; TDM, treated dentin matrix.

Directing Endogenous Stem Cells to the Periodontium

The directing of endogenous cells to an injury site in response to biological signals (including but not limited to chemokines, growth factors, cytokines, and cell-adhesive molecules) is a process known as directed stem cell homing [67]. Clearly, the recruitment of sufficient cell populations to a diseased site is the first step to achieve successful regeneration. Many chemoattractants and growth factors, such as stromal cell-derived factor-1α (SDF-1α) and stem cell factor, can be used to attract stem cells and recruit the patient's own stem cells for self-repair (reviewed in [22]). However, directly injected factors have a very short half-life and high expenditure, and using carriers or scaffolds for growth factor delivery is a practical strategy [68]. In vitro results have shown that SDF-1α-loaded gelatin sponges could release their cargo for up to 35 days and enhance bone/PDL regeneration [69]. Because the therapeutic activity of SDF-1α is negatively affected by dipeptidyl peptidase-IV (DPP-IV), the DPP-IV inhibitor parathyroid hormone (PTH) has been used for SDF-1α protection, and PTH/SDF-1α cotherapy has been found to increase the migration of reparative cells to rat periodontal defects [70]. In addition to the growth factor-loaded construct, platelet-rich plasma (PRP) and platelet-rich fibrin are warehouses of various native growth factors that can also be used for facilitated stem cell homing and periodontal regeneration [71]. The combination of platelet-rich preparation and native matrix has been used for tooth root regeneration, and the results showed that the cementum-PDL complex can be regenerated on the construct placed in a tooth socket by homing stem cells [72]. Moreover, decellularized ECM, which retains the structural components of native tissue and contains an abundant variety of signals and growth factors, can also direct endogenous cell homing and manipulate stem cell differentiation (reviewed in [40, 73]). Compared with artificial materials, native matrices induce less inflammatory responses and make therapies safer and more effective (reviewed by [73, 74]). In fact, many biomaterials or their modified forms have already paved the way for clinical periodontal regeneration. The creation of more specific designs or the incorporation of selected homing factors would largely enhance their capacity to recruit host cells and thus further induce endogenous tissue regeneration.

Inducing Growth of the Periodontium

After the stem cells reach their target site, the promotion of their differentiation into the periodontium through growth factor delivery and biomaterial design is another basic step required for periodontal regeneration (reviewed in [24]). In a local microenvironment targeted for regeneration, macrophages (Mϕs) are one of the predominant immunological regulators [75]. Mϕs have two different phenotypes, M1 and M2: during the endogenous regenerative process, M1 plays an elimination role in the initial inflammatory stage, whereas M2 plays an inflammation regulatory role in the later stage. Hence, immunomodulatory cytokines, such as interleukin-4 (IL-4), have been used to promote M1-to-M2 transformation [76]. For example, the systemic injection of IL-4 has been found to regulate the polarization of Mφs during the orthodontic process and hence attenuate tooth root resorption [77]. To

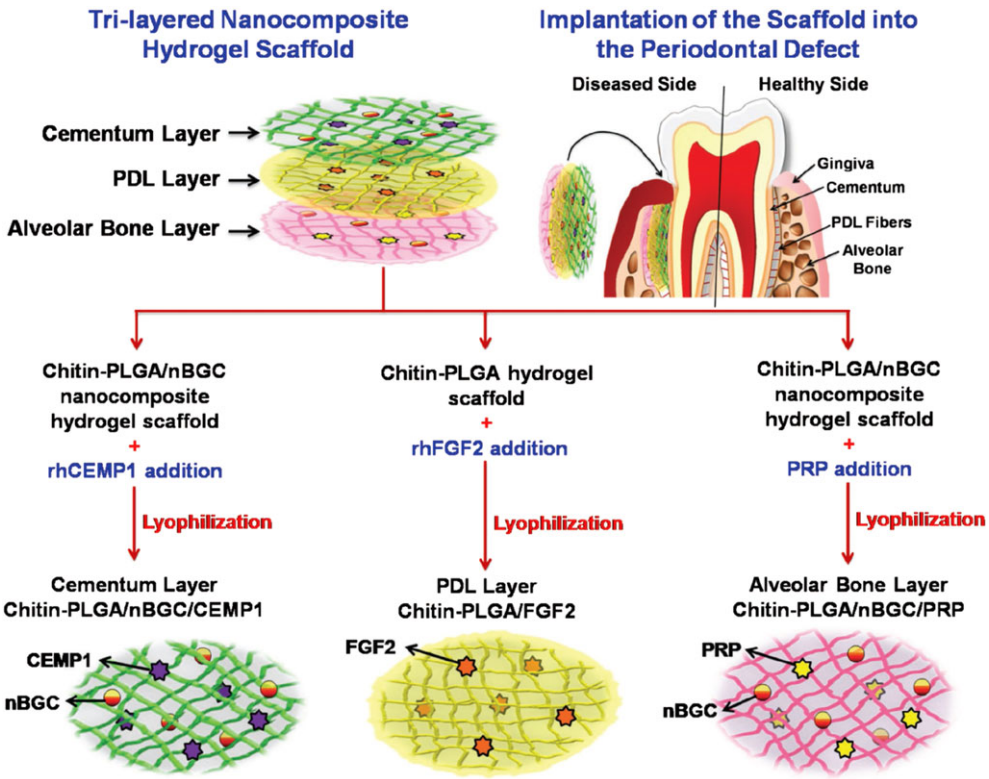

Figure 5. Schematic representation of the formation of a trilayered nanocomposite hydrogel scaffold (each layer incorporates different growth factors or preparation-containing growth factors) for the simultaneous regeneration of multiple periodontal tissues [87] (please refer to the original source for more information). Abbreviations: CEMP1, cementum protein-1; FGF-2, fibroblast growth factor-2; PDL, periodontal ligament; PLGA, poly(lactic-*co*-glycolic acid); PRP, platelet-rich plasma; nBGC, nanobioactive glass ceramic; rhCEMP1, recombinant human cementum protein-1; rhFGF, recombinant human fibroblast growth factor.

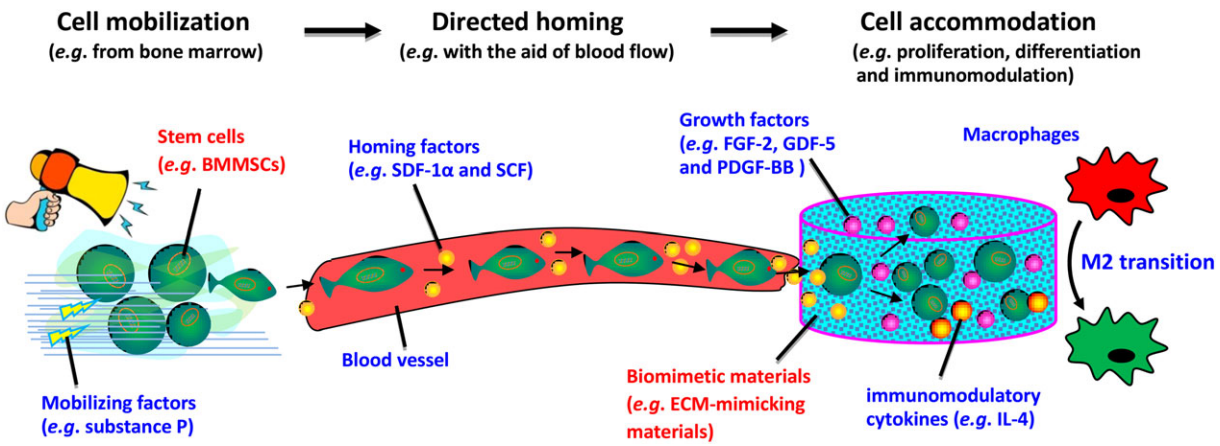

Figure 6. Schematic representation of the mobilization of stem cells from their niche (e.g., bone marrow) using cell mobilizing factors such as substance P, directed cell movement with the aid of blood flow, and cell homing factors such as SDF-1α and SCF and the regulation of stem cell fate (e.g., cell proliferation and differentiation) once they reach the site of injury, normally via the design of materials such as the generation of ECM-mimicking biomaterials and the presentation of a wide variety of growth factors, such as FGF-2, GDF-5, and PDGF-BB and immunomodulatory cytokines, such as IL-4. Abbreviations: BMMSCs, mesenchymal stem cells derived from bone marrow; ECM, extracellular matrix; FGF-2, fibroblast growth factor-2; GDF-5, growth/differentiation factor-5; IL-4, interleukin-4; PDGF-BB, platelet-derived growth factor-BB; SCF, stem cell factor; SDF-1α, stromal-derived factor-1α.

achieve a better effect, a specifically devised carrier has been used to stabilize this cytokine and protect it from denaturation and degradation, leading to a prolonged modulation of macrophage polarization. This strategy has resulted in an enhanced repair of the rat mandibular periodontal fenestration defect [78].

In fact, the polymorphism of immune-regulating cytokines (IL-4 and IL-13) has been demonstrated to be associated with the susceptibility of chronic periodontitis [79, 80].

As mentioned above, biomaterials mimicking the properties of the host tissue to avoid immune detection could

benefit periodontal regeneration. For example, ECM-mimicking devices, such as an electrospun nanofibrous scaffold [81] or a nanocomposite hydrogel [82], could benefit bone regeneration. In prospect, these ECM-mimicking biomaterials not only provide a scaffold for cell homing but also create a friendly immune environment for correct periodontal regeneration.

The presentation and release of signaling molecules (such as growth factors) are also important for facilitating stem cell homing, proliferation, and differentiation. In this regard, growth/differentiation factor-5 (e.g., see [83]), fibroblast growth factor-2 (FGF-2; e.g., see [84]), platelet-derived growth factor-BB (e.g., see [55]), and many other factors have already been used alone or in combination with various biomaterials for local cell manipulation and periodontal regeneration. Throughout the native regenerative response, different growth factors play multiple roles in specific release sequences and dosages; thus, the sequential release of multiple growth factors to mimic the normal biology of periodontal regeneration can lead to an enhanced outcome (e.g., see [85, 86]). The use of growth factors to modulate the local regenerative microenvironment and enhance the endogenous healing process of the periodontium has been reviewed elsewhere [22, 24]; please refer to those articles for more information.

Along with the required growth factors, the implantation of tissue-/structure-mimicking biomaterials would also benefit the recreation of a suitable local matrix microenvironment. An interesting example that supports the proof-of-concept that anatomically shaped that tooth scaffolds can be used for periodontal regeneration by cell homing [25] is the trilayered scaffold reported by Sowmya et al.[87] for concurrent regeneration of the three types of periodontal tissues; each layer was specifically designed to contain chitin/poly(lactic-*co*-glycolic acid) (chitin-PLGA) and/or nanobioactive glass ceramic (nBGC) components. A layer composed of chitin-PLGA/nBGC loaded with recombinant human cementum protein-1 was applied to generate cementum, and similar components combined with PRP were included to regenerate the bone. For PDL regeneration, a chitin-PLGA hydrogel was loaded with recombinant human FGF-2. The assessment of this scaffold using a rabbit periodontal defect model showed that the cell-free construct induced complete regeneration of the hybrid tissues in the periodontium (Fig. 5) [87]. Based on available data, chemoattractive constructs have been proven effective in the treatment of periodontal defects, and further research should consider the translation of these proof-of-concept studies to clinical use.

Collectively, the data show that periodontal regeneration via in vivo cell-material interactions motivates the self-healing potential of the host. The biomaterials themselves and the incorporated signaling molecules that are used to recreate a proper in vivo milieu are all key to initiating the process of self-regeneration (reviewed in [24, 66]). The cascade of the mobilization of stem cells from their niches, directed cell homing, and the propagation and differentiation of the recruited cells once they reach the site of injury can potentially result in the functional regeneration of multiple periodontal tissues and their unique architectures (Fig. 6; e.g., see [78, 88]). Substantial work using developmental biology insights is still needed to direct the design of next-generation biomaterials that actively work together with nature's own regeneration and healing mechanisms based on biological disciplines.

Conclusion

Based on the principles of tissue engineering, the use of cells, biomaterials, and growth factors for periodontal regeneration has been proposed as a new concept for the treatment of periodontal disease. Although bioengineered cellular materials represent the current state-of-the-art treatment and have resulted in curative successes in preclinical scenarios, the effective regeneration of human periodontal tissue lost/damaged because of periodontitis needs continuous improvements. Endeavors aiming to bridge the gap between the promising results obtained with animal models and the reality of clinical treatments indicate that clinical success can be facilitated by avoiding the use of external stem cells. Therefore, studies have established endogenous regenerative techniques involving material design and/or cytokine presentation that permit the patient's own cells to be harnessed for therapeutics [89]. An evolving body of work in this topic corroborates that the recreation of a suitable microenvironment at the injured site can instruct the homing of resident stem cells and can induce the periodontium itself to regenerate. In the future, better control of the local microenvironment for stem cell homing and accommodation will most likely render high levels of periodontal tissue regeneration a clinical reality, even for mature periodontal teeth.

Periodontal tissue regeneration is a complex healing cascade that arises from a coordinated interplay among stem cells, biomaterials, and the host immune system [90]. In this concise review, we illustrate the possible approaches based on the therapeutic potential of stem cells that might enhance periodontal wound healing and regeneration and highlight the advantages and disadvantages of culture-expanded and host-mobilized cell populations in clinical translation. However, stem cell therapies and tissue engineering in the arena of periodontics remain in their infancy, irrespective of the cell source or route from which the cells are obtained. Although a number of clinical observations have delivered promising outcomes, there is still no perfect cell regenerative paradigm ready for translation to the clinic. The translation of a laboratory-based periodontal regenerative approach to the clinic requires a thorough understanding of the mechanism underlying the cell-based regeneration progress, a targeted cell-material design for a given task, and a potential dichotomy between the need for a biomimetic design and cost-effective production, because sophisticated constructs are normally required for producing complex tissue.

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Author Contributions

X.-Y.X., X.L., and F.-M.C.: conception/design, collection and/or assembly of data, structure and figure design, data analysis

and interpretation, manuscript writing and revision, final approval of manuscript; J.W., X.-T.H., and H.-H.S.: data collection, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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