



Recent advances in periodontal regeneration: A biomaterial perspective

Yongxi Liang^a, Xianghong Luan^b, Xiaohua Liu^{a,*}

^a Department of Biomedical Sciences, Texas A&M University College of Dentistry, Dallas, TX, 75246, USA

^b Department of Periodontics, Texas A&M University College of Dentistry, Dallas, TX, 75246, USA



ABSTRACT

Periodontal disease (PD) is one of the most common inflammatory oral diseases, affecting approximately 47% of adults aged 30 years or older in the United States. If not treated properly, PD leads to degradation of periodontal tissues, causing tooth movement, and eventually tooth loss.

Conventional clinical therapy for PD aims at eliminating infectious sources, and reducing inflammation to arrest disease progression, which cannot achieve the regeneration of lost periodontal tissues. Over the past two decades, various regenerative periodontal therapies, such as guided tissue regeneration (GTR), enamel matrix derivative, bone grafts, growth factor delivery, and the combination of cells and growth factors with matrix-based scaffolds have been developed to target the restoration of lost tooth-supporting tissues, including periodontal ligament, alveolar bone, and cementum. This review discusses recent progresses of periodontal regeneration using tissue-engineering and regenerative medicine approaches. Specifically, we focus on the advances of biomaterials and controlled drug delivery for periodontal regeneration in recent years. Special attention is given to the development of advanced bio-inspired scaffolding biomaterials and temporospatial control of multi-drug delivery for the regeneration of cementum-periodontal ligament-alveolar bone complex. Challenges and future perspectives are presented to provide inspiration for the design and development of innovative biomaterials and delivery system for new regenerative periodontal therapy.

1. Introduction

Periodontium are tooth-supporting tissues that are composed of gingiva, cementum, periodontal ligament (PDL) and alveolar bone. Periodontitis is an inflammatory disease that leads to degradation of periodontal tissues, causing tooth movement and eventually tooth loss [1]. Currently, clinical treatments for periodontitis focus on plaque removal and local inflammation control, such as scaling and root planing and surgical treatments [2–5]. Those therapies attempt to minimize symptoms and prevent disease progression, but cannot restore the attachment of periodontal tissues to teeth and the original periodontal tissues. Therefore, the functions of teeth and dentition remain impaired after the treatments. Some regenerative approaches, such as guided tissue regeneration (GTR) and bone grafts, were developed to achieve periodontal tissue formation. However, clinical outcomes of those approaches are variable and unpredictable [6–9]. Therefore, it is imperative to develop alternative regenerative strategies to restore the structures and functions of periodontal tissues for periodontitis patients.

During tooth development, niche-resident dental follicle cells differentiate into cementoblasts, fibroblasts, and osteoblasts that form cementum, PDL, and alveolar bone, respectively [10]. The niche (microenvironment) that induces the formation of tooth supporting tissues, however, is not retained after tooth development, leading to the

difficulty of restoring damaged/lost periodontium after maturity. As a bioengineering approach, tissue engineering is capable of recapitulating the microenvironment in certain aspects and regenerating functional tissues [11]. When tissue-engineering strategy is adopted for periodontal regeneration, it is pivotal to consider two important elements: scaffold (biomaterials and scaffolding design) and controlled drug delivery (bioactive molecules and methods for controlled delivery) (Fig. 1). Several review articles presented excellent summaries of periodontal regeneration a decade ago [8,12–16]. However, their work mainly focused on stem cells, biological evaluation, and the characteristics of biomaterials or bioactive molecules, but did not pay particular attention to scaffolding design and drug delivery. More importantly, periodontal regeneration is a rapidly expanding research field. A number of new biomaterials, approaches, and technologies have been developed for regenerative periodontal treatment over the past ten years. Therefore, there is a need to summarize recent progresses on periodontal regeneration from a biomaterial perspective.

In this paper, we first overview periodontal tissue regeneration strategies, including GTR and tissue engineering. Next, we discuss the biomaterials and drug delivery systems that have been developed for periodontal regeneration. We highlight the development of advanced bio-inspired scaffolding biomaterials and temporospatial control of multi-drug delivery. Furthermore, we summarize the applications of the biomaterials and drug delivery systems for the regeneration of

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* Corresponding author. Department of Biomedical Sciences Texas A&M University College of Dentistry, 3302 Gaston Ave, Dallas, TX, 75246, USA.

E-mail address: xliu1@tamu.edu (X. Liu).

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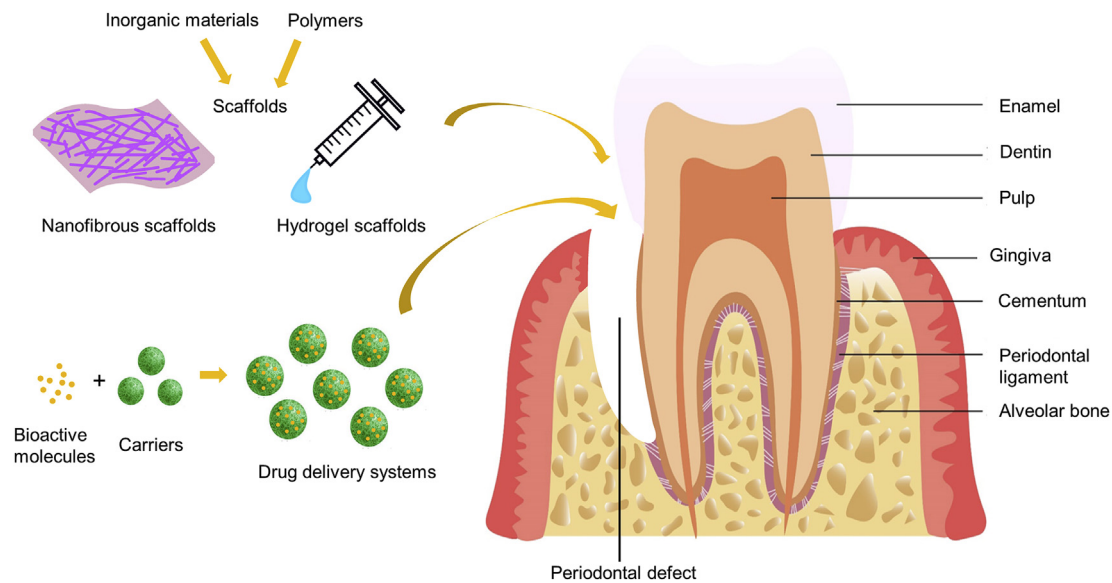


Fig. 1. Schematic illustration of the anatomy of periodontal tissues, periodontal defect, scaffolds of tissue engineering approach and drug delivery system.

periodontal tissues that include PDL, cementum, and alveolar bone. Special attention is given to the regeneration of PDL-cementum-alveolar bone complex. Finally, our perspectives on the use of bio-inspired materials and drug delivery systems to reconstruct the hierarchical and functional periodontium are provided as signposts for the future advancement of this field.

2. Biomaterials and scaffolding design for periodontal regeneration

2.1. Strategies for periodontal regeneration

There are two strategies for periodontal regeneration: GTR and tissue engineering approaches. GTR has been widely used for periodontium regeneration in clinic for decades. It is a regenerative surgical technique that involves the procedure of raising mucogingival flap around affected teeth, scaling and planing root surfaces and placing barrier membranes temporarily under gingiva [17]. The biological basis of the GTR technique is to block the apical growth of epithelium to the space over the denuded root surface by using barrier membrane, therefore facilitating PDL cells and osteoblast to form PDL tissues and alveolar bone [18]. Numerous clinical research have confirmed the benefits of GTR treatments, including greater clinical attainment level (CAL) gain, probing pocket depth (PPD) reduction and bone regeneration compared to open flap debridement (OFD) treatment [7,19–21].

Although GTR has positive treatment outcomes, there are limitations of GTR treatment for periodontal regeneration. First, the regenerative benefits of the GTR treatment vary from case to case [22]. Many factors, such as diabetes, smoking, dental plaque control, tooth anatomy and morphology, affect the results of the GTR treatment [6,7]. Therefore, the outcomes of GTR treatment in clinical practice may not be as successful as those in clinical trials. A quantitative analysis showed that GTR was a predictable method for narrow intrabony defects and class II mandibular furcation defects [6]. The outcomes of GTR treatment for other types of periodontal defects, however, are limited and unpredictable [6]. There are two types of barrier membranes for GTR: non-absorbable and absorbable membranes. For non-absorbable membranes, a second surgery is necessary to remove it from the defect area, which increases the risk of infection as well as surgical burden [22].

Tissue engineering strategy uses stem/progenitor cells, scaffolds and bioactive molecules to build biomimetic systems to induce new tissue

formation. Depending on whether biomaterials are used, tissue engineering strategy for periodontal regeneration can be categorized into scaffold-free and scaffold-based approaches. For the scaffold-free approach, cells or cell aggregates are transplanted to a defect area without a cell carrier. Several types of cells, including bone marrow derived mesenchymal stem cells (BMSCs) [23–25], adipose-derived stem cells (ADSCs) [26], periodontal ligament stem cells (PDLSCs) [27,28], and dental pulp stem cells (DPSCs), have been tested for the potential to form periodontal tissues. Direct cell implantation faces the problem of cell diffusion out of the defect area. Cell sheet technique, which entraps cells in the extracellular matrix (ECM) secreted by the cells themselves, is capable of preventing cell migration. It was reported that cell sheet therapy induced more bone formation than cell suspension in swine periodontal defects [29].

Cell sheet technology, however, is capable of regenerating only a layer of tissue with a simple structure. Given the complicated architecture of periodontium which includes two hard tissues (alveolar bone and cementum) and a soft tissue (PDL), the use of scaffold-based approach is the only choice for the regeneration of PDL-cementum-alveolar bone complex. Multiphasic scaffolds with distinctive characteristic in each layer are required to imitate the periodontal structures. Specifically, the architecture, chemical composition, cellular/biochemical composition in each layer need to be tailored to achieve periodontal complex regeneration [30–32]. Details of biomaterials and scaffolding design are discussed in the following sections.

2.2. GTR biomaterials

GTR barrier membranes prevent the ingrowth of epithelial cells and provide space to regenerate PDL and alveolar bone. The barrier membranes should have basic properties, including biocompatibility, cell-occlusiveness, tissue integration, space maintenance, and clinical manageability [33–35]. GTR barrier membranes are classified into non-absorbable and absorbable membranes. Generally, non-absorbable membranes have superior space maintenance compared to absorbable membranes. Gore-tex® is the first GTR barrier membrane that is made of polytetrafluoroethylene (PTFE) with high mechanical properties [17]. The titanium-reinforced PTFE membrane further increased the compressive strength, resulting in better outcomes compared to the PTFE membrane [36]. The ultrathin (0.01 mm) titanium-reinforced PTFE membrane occupied minimal space, therefore, provided more room for new tissue formation [37]. The smoother surface of the

titanium-reinforced PTFE also lowered immunological reaction in vivo [37]. However, a second surgery is required for the removal of non-absorbable barrier membranes, which increases the risk of infection, delays wound healing, and impairs the regenerative outcomes.

Absorbable membranes are gradually degraded in vivo and avoid the drawback of secondary surgery after implantation. Both natural and synthetic biomaterials are tested as absorbable GTR membranes. Generally, natural biomaterials have excellent biocompatibility with cellular binding sites, but have low mechanical strength [38]. On the other side, synthetic biomaterials have tunable degradation rates and mechanical properties, but lack biological recognition (cellular binding motif). The degradation property of membranes affects the ability of space maintenance and new tissue formation. As a rule, the degradation rate should be moderate: fast degradation leads to premature mechanical loss while slow degradation prevents new tissue ingrowth. Generally, compared to non-absorbable membranes, absorbable membranes have a limitation of low mechanical strength.

Recent development of GTR barrier membranes focus on the optimization of mechanical and degradation properties and the incorporation of new functions into GTR membranes. For example, GTR membranes were prepared from composites that combined the advantages of different biomaterials [38]. The combination of natural and synthetic polymers integrated the bioactive recognition of natural materials and improved mechanical properties of synthetic materials [38–40]. GTR membranes were also used as carriers for drug delivery to enhance tissue regeneration [41,42]. Anti-bacteria drugs were loaded into GTR membranes to inhibit local infection and inflammation, therefore, facilitating PDL tissue formation [35]. Multi-layered GTR membranes with different functions in each layer were also developed to enhance periodontal tissue regeneration [40,43,44].

While many GTR membranes have been developed for periodontal regeneration in recent years, most of them are in laboratory stages. To evaluate the biosafety and effectiveness of those GTR membranes, more in vivo studies and clinical trials are required. As discussed above, the outcomes of GTR treatment are unpredictable in several types of periodontal defects. In other words, GTR membranes with improved properties is insufficient to acquire successful treatment outcomes. The combination of GTR membranes with other approaches, such as bone grafts, may provide better regenerative results.

2.3. Tissue engineering biomaterials

Table 1 lists the biomaterials that are used recently for periodontal tissue regeneration. Briefly, polymeric materials that have relative low mechanical strengths (e.g. collagen, gelatin, and chitosan) are the candidate materials for PDL regeneration, while inorganic materials with relative high mechanical strength, such as hydroxyapatite (HA), tricalcium phosphate (TCP), biphasic calcium phosphate (BCP), and bioactive glass (BG) are used for cementum and alveolar bone regeneration. To regenerate cementum-PDL-alveolar bone complex, composite biomaterials that contain polymers and inorganic components have to be used (Table 1).

In conclusion, most materials used in periodontal regeneration are traditionally used in other regenerative research, such as inorganic materials (HA, tricalcium phosphate, BG ect) and polymers (gelatin, collagen, PCL, PLGA ect). Those traditional biomaterials are modified or combined together into composite materials to make suitable microenvironment and scaffold systems to induce periodontal regeneration in previous research. However, even though these biomaterials can resemble the compositions in certain aspects, they cannot mimic the fine structures of the natural periodontal tissues. Therefore, novel and bio-inspired materials that are designed to closely mimic the architecture of periodontal tissues at micro and nanoscale levels are prerequisite to achieve functional periodontal tissue regeneration.

2.4. Scaffolding design for periodontal regeneration

Several aspects need to be considered when designing a scaffold for periodontal regeneration. These are scaffold compositions, structure, architecture, and ease of use (injectability). Generally, scaffolding materials should mimic the compositions of the ECM of periodontal tissues, therefore, recapitulating the ECM microenvironment of periodontium. As periodontium is composed of cementum, PDL, alveolar bone and gingiva, scaffolding design for each of the components is different. For example, alveolar bone is a hard tissue, and the scaffold for alveolar bone regeneration should induce mineralized tissue formation; while PDL is a fibrous tissue, and the scaffold for PDL regeneration should facilitate soft tissue formation and prevent mineralization. Based on that, inorganic biomaterials, such as hydroxyapatite and calcium phosphate, are the scaffolding components to enhance biomineralization, while polymeric biomaterials are widely used for PDL regeneration [88,90,91].

A scaffold needs to provide structural guidance for the formation of periodontal tissues with proper structures. For example, the ECM of PDL are composed of nanofibrous network, and the nanofibrous scaffold simulates the architecture of the natural ECM [92–99]. Electrospinning is a simple and effective method to prepare nanofibrous matrix, which presents high surface area and porosity to facilitate cell attachment, migration, and proliferation, so it is used to fabricate PDL scaffolds. Besides, PDL has well-organized collagen fibers that are classified into five groups with each group having various locations and directions. The scaffold for PDL regeneration should provide biophysical guidance to regenerate the well-organized PDL fibers. By restoring the proper structure, the regenerated periodontium can perform the functions of supporting tooth and bearing occlusal force, similar to the natural counterparts.

Given PDL is anchored to root cementum and alveolar bone, the regeneration of PDL alone is not sufficient to perform PDL function. Instead, cementum and alveolar bone regeneration should also be included when designing scaffolds for periodontal regeneration. Therefore, a cementum-PDL-alveolar bone multilayer scaffold is often used for periodontal regeneration.

Besides the compositional, structural and functional requirements for periodontal scaffolding design, the shape of scaffolds is another factor to be considered. Hydrogels can be injected into defect areas and be crosslinked in situ, making it desirable for irregular periodontal defects. The drawback of a hydrogel scaffold is the relatively low mechanical strength. The incorporation of components with high mechanical strength, such as calcium phosphate, improved the mechanical property of the hydrogel [100,101]. However, the injectability of the hydrogel was affected as well. In contrast to hydrogels, pre-formed scaffolds have pre-designated size and morphology prior to the implantation [95,96]. Pre-formed scaffolds are fabricated by different methods, such as lyophilization, direct casting, and 3D printing. Among them, 3D printing can precisely present more biophysical cues inside scaffolds to guide tissue regeneration and is the most promising technique. For example, 3D printing was used to fabricate scaffolds with microgroove and microchannel structures to guide fiber orientation [31,75,89,90]. In addition, a 3D printing scaffold can be customized to match the shape of individual patient using cone beam computer tomography (CBCT) images [102]. While 3D printing method has many advantages, it cannot fabricate scaffolds that mimic the ECM architecture at a nanoscale level. To address this issue, the resolution of 3D printing has to be greatly improved.

3. Controlled delivery systems for periodontal regeneration

3.1. Drugs and growth factors used for periodontal regeneration

A general tissue engineering approach involves in the combination of scaffold, cells and bioactive molecules (drugs and growth factors) to

Table 1
Summary of biomaterials used for periodontal tissue regeneration.

Biomaterials	Target tissue	Characteristics	References
Inorganic biomaterials			
Hydroxyapatite (HA)	Alveolar bone; cementum	Similar chemical composition and structure to the inorganic phase of bone Osteoconductive Direct bonding effect to natural bone Slow degradation	[45–48]
Tricalcium phosphate (TCP)	Alveolar bone; cementum	Similar chemical composition to the inorganic phase of bone Bioabsorbable Osteoconductive TCP α and TCP β	[49–53]
Biphasic calcium phosphate (BCP)	Alveolar bone	Mixture of HA and TCP in various ratios to adjust degradation rate and biological activity Similar chemical composition and structure to the inorganic phase of bone	[54–56]
Bioactive glass (BG)	Alveolar bone; cementum	Compositions of bioactive glasses vary Ions dissolved from BG promote angiogenesis, osteogenesis and antibacterial activity Degradation rate vary over a wide rage	[57–63]
Natural polymers			
Collagen	PDL	Most abundant protein in the ECM of alveolar bone, PDL and cementum Biocompatible Low mechanical strength Safety problems: pathogen transmission, immune reaction	[64–66]
Gelatin	PDL; alveolar bone; cementum	Hydrolysis product of collagen No pathogen transmission and immune reaction Easily modified for chemical and light crosslinking	[67–70]
Chitosan	Alveolar bone; PDL; cementum	Derived from chitin Biocompatible Antibacterial property	[71–74]
Synthetic polymers			
Poly (lactic-co-glycolicacid) (PLGA)	Alveolar bone; PDL	Biocompatible Tunable degradation rate No cell recognition motif	[75–78]
Polycaprolactone (PCL)	Alveolar bone; PDL	Biocompatible Slow degradation rate No cell recognition motif	[79–82]
Composite biomaterials			
PLGA + CaP	Alveolar bone	Fabricated into two layers (smooth outer layer and rough microporous inner layer) Designed to support GTR membrane and promote alveolar bone regeneration in dogs	[83]
Collagen + HA	Alveolar bone	Fabricated by freeze-drying of both collagen and HA or precipitating HA to collagen BMSCs seeded into the scaffold to promote alveolar bone formation in a dog' periodontal defect	[84]
Chitosan + β -TCP	Alveolar bone	Fabricated by freeze-frying HPDLC seeded into the scaffold to recruit host cells and promote osteoblast differentiation	[85]
PLGA + Magnesium	Alveolar bone	Mg in the PLGA increased mechanical strength of composite materials, buffered the acidic by-product of PLGA degradation, and enhanced osteogenic capacity and bone formation in vivo	[86]
Gelatin methacrylate + HA	Alveolar bone	Methacrylate was introduced for photo-crosslinkable The composite induced hPDLSCs to differentiate into osteoblast and promoted new bone formation in nude mice	[67]
Gelatin + β -TCP	Alveolar bone	Gelatin and β -TCP were mixed in homogenizer and freeze dried New bone tissue and some fibers parallel to bone surfaces were formed in a dog periodontal defect	[87]
PCL + β -TCP + CaP coating	PDL; Alveolar bone	PCL electrospun scaffold was fabricated as the PDL layer. A thin layer of CaP was coated on the surface of PCL- β -TCP scaffold to improve the osteogenic capacity CaP coating induced more bone formation	[88]
PGA	PDL; Alveolar bone	Microchannels in the PDL layer were designed to guide fibers formation	[89]
PCL		Porous structure was fabricated to allow cell proliferation No organized fiber insertion in PDL and bone interface	
PCL + HA	Alveolar bone; PDL; cementum	Three layers of scaffold design was used to mimic the architecture of periodontium No organized fiber insertion in PDL and bone interface	[90]
Chitin + PLGA + BCG	Alveolar bone; cementum; PDL	PLGA was added to increase degradation time and improve mechanical stability. BCG enhanced osteogenic capacity in bone and cementum layers.	[91]

induce tissue regeneration. Among them, bioactive molecules control diseased conditions, stimulate innate regenerative capacity, and provide signals for tissue formation. A number of drugs and growth factors have been tested for periodontal regeneration, and their results are summarized in Table 2.

3.2. Controlled delivery systems for periodontal regeneration

Most growth factors have short half-lives and narrow therapeutic windows. Therefore, controlling the release patterns of drugs and growth factors from carriers to achieve stable long-term effects and avoid side effects is highly desirable. Particularly, the spatial and temporal controlled delivery of drugs or growth factors is essential for periodontal regeneration. Based on the number of drugs (or growth

factors) to be delivered, the release systems can be divided into monodrug and multidrug delivery systems.

In a monodrug delivery system, a drug or growth factor is applied to fulfill particular functions. For example, stromal cell-derived factor 1 (SDF-1) was used to recruit host mesenchymal stem cells and hematopoietic stem cells to wound areas, and those cells promoted vasculature and new bone formation in rat periodontal defects [127,128]. Bone morphogenetic protein 2 (BMP-2) was used to enhance bone regeneration and accelerate mandibular bone defect repair [141]. The bioactive agents can be incorporated into biomaterials via non-covalent methods, such as physical entrapment, surface adsorption, and ionic complexation [142]. However, these methods cannot control burst release, which is undesirable for drug delivery [142]. In addition, the bioactivity of bioactive agents often reduces after the fabrication

Table 2
Summary of drugs and growth factors used for periodontal regeneration.

Bioactive Molecules	Characteristics	Functions	Applications in periodontal regeneration	References
Drugs Statins: Simvastatin (SMV); Atorvastatin (ATV)	Inhibitors of 3-hydroxy-2-methyl-glutaryl coenzyme A (HMG-CoA) reductase, commonly used for arteriosclerosis and hyperlipidemia	Inhibiting osteoclasts activity Increase BMP-2 level Inhibiting HMG-CoA level	Gels loaded with SMV induced significantly PD reduction, CAL gain and more bone formation ATV exerted better regenerative results than SMV	[103–108]
Metformin	Anti-hyperglycemic biguanide to treat type II diabetes;	Promote osteogenic differentiation and bone formation via LKB1/AMPK pathway	Greater PD reduction and CAL gain when applied to periodontitis patients	[109–111]
Growth factors Platelet derived growth factor (PDGF)	Four isomeric composed of dimer of A, B and C chain: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC Two receptors: α and β receptors 22 subfamily proteins Activate tyrosine kinase activity	Chemotaxis Enhance cell proliferation and differentiation Enhance Angiogenesis Promote wound healing Enhance cell mitogen Enhance angiogenesis Promote cell differentiation	Promoted periodontal bone regeneration in various clinical trials, with the best concentration of 0.3 mg/ml	[112–116]
Fibroblasts growth factors (FGF)		Recruit stem/progenitor cells to defect areas to promote tissue regeneration Promote collagen formation	Significantly promoted bone regeneration in periodontal defects in patients.	[117–122]
Stromal-cell-derived factor-1 (SDF-1)	Also named C-X-C motif ligand 12 (CXCL12) Receptors: C-X-C motif receptor 4 (CXCR4)	Recruit stem/progenitor cells to defect areas to promote tissue regeneration	Promote bone and fibrous tissues regeneration	[123–128]
Bone morphogenic proteins (BMP) family	Belongs to TGF- β super family Contains more than 20 proteins Proteins are homodimeric or heterodimeric to each other BMP-2 and BMP-7 are the most widely used	Stimulate bone and cartilage formation Recruit bone progenitor cells Promote osteoblasts and the other cells differentiation Wound healing Angiogenesis	BMP-2: Clinical trials confirm its bone regenerative effects, but may induce root resorption and ankylosis BMP-6: Induce bone, PDL and cementum regeneration in periodontal defects of rats and dogs BMP-7: pleiotropic functions for osteogenic and cementogenesis in Class III furcation defects in dogs	[112,129–140]

processes. Some natural biomaterials, such as heparin and heparan sulfate glycosaminoglycans in ECM have binding domains that exert strong interaction with bioactive molecules. The binding of these glycosaminoglycans to bioactive molecules protects the bioactive molecules (e.g. growth factors) from denaturation and proteolytic degradation, and subsequently prolongs the sustained release. Based on this principle, we recently developed a hierarchical nanosphere-encapsulated microspheres system for bone tissue regeneration [143]. In this system, BMP-2 bound with heparin and was encapsulated into heparin-conjugated gelatin nanospheres, which were further immobilized in nanofibrous microspheres (Fig. 2). This system allows the integration of nanofibrous architecture with controlled growth factor delivery into one injectable microsphere. BMP-2 possesses binding domains with heparin, and the binding of BMP-2 to heparin stabilizes the BMP-2 and controls its sustained release. In addition, the heparin-binding BMP-2 was encapsulated in gelatin nanospheres and entrapped by the microsphere nanofibers, which mimicked the architecture of collagen fibers and had many unique properties including superior surface area, high porosity, low density, and controllable degradation rate. The ECM-mimicking nanofibrous architecture enhanced bone marrow-derived mesenchymal stem cell adhesion, proliferation, differentiation, and new tissue formation [144–147]. The in vivo study showed that the BMP-2-loaded hierarchical nanofibrous microsphere was an excellent carrier and significantly enhanced bone regeneration [143]. The combination of controlled growth factor delivery with an injectable biomimetic scaffold provides new insights into the design of cell-instructive scaffolds.

Periodontal regeneration is a complicated process that involves multiple aspects including the control of infection and inflammation, recruitment of stem/progenitor cells, promotion of cell proliferation and differentiation, as well as new tissue formation. Besides, periodontium is composed of distinct layers of tissues and different biological cues should be provided to guide PDL, cementum, and alveolar bone formation. Therefore, the delivery of multiple bioactive reagents is necessary for periodontal tissue regeneration. For temporal control of bioactive reagents release in periodontal regeneration, it is essential to understand normal healing process. In terms of periodontitis, the first step is to control local infection and inflammation, and create a healthy and stable environment for new tissue formation. Therefore, antibacterial drugs should be released first to control local bacterium infection in a periodontal release system [148]. Next, drugs/growth factors that regulate local inflammation level, promote cell proliferation and differentiation are applied in sequence [148].

There are several methods to achieve temporal control of multidrug releases: direct presentation, multiphase loading and particulate-based delivery [149]. Direct presentation incorporates two or more bioactive agents into carriers via physical entrapment or absorbance. While this strategy is simple, it has little control of sequential releases of the bioactive agents. Multiphase loading adds each bioactive reagent in different phase of biomaterials to form a multiphased drug delivery carrier. For example, lovastatin was loaded into PLGA microsphere, which was encapsulated in tetracycline-loaded chitosan (Fig. 3A). The core-shell structured microspheres first released tetracycline from the shell to control local infection, followed by the release of lovastatin from the PLGA core to reduce inflammation and oxidation and promote new bone formation (Fig. 3B) [150]. In another multiphase delivery system, four drugs were loaded separately in different layers of a surface-eroding polymeric system composed of cellulose acetate phthalate and Pluronic F-127 (Fig. 3C). The drugs were entrapped from the surface to the deep layers, and blank layers were used to control the lag time between the releases [148]. When the polymer was eroded from the surface to the deep layers, drugs were released in order (Fig. 3D). Particulate-based delivery incorporates bioactive reagents in different particulates, which are further incorporated into a scaffold. In a study, IGF-1 was loaded in alginate microparticles and BMP-6 was loaded in PLGA microparticles, respectively, which were further incorporated

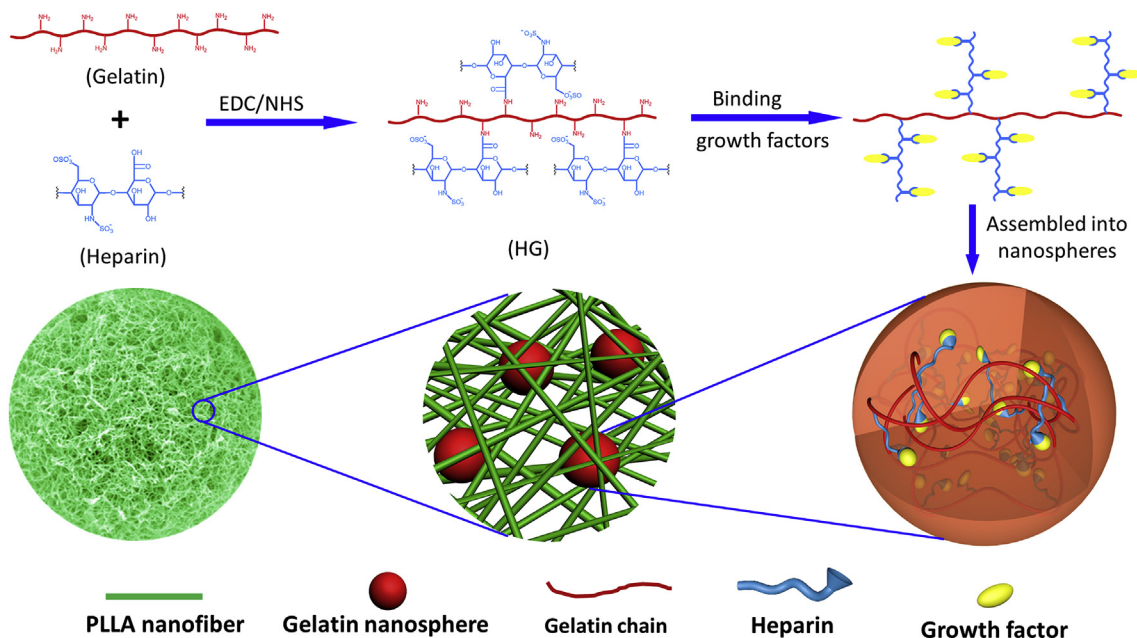


Fig. 2. Schematic illustration of the design of hierarchical injectable nanofibrous microspheres for bone regeneration. BMP-2 is encapsulated into heparin-conjugated gelatin nanospheres, which are further immobilized in nanofibrous PLLA microspheres. Adapted with permission from Ref. [143].

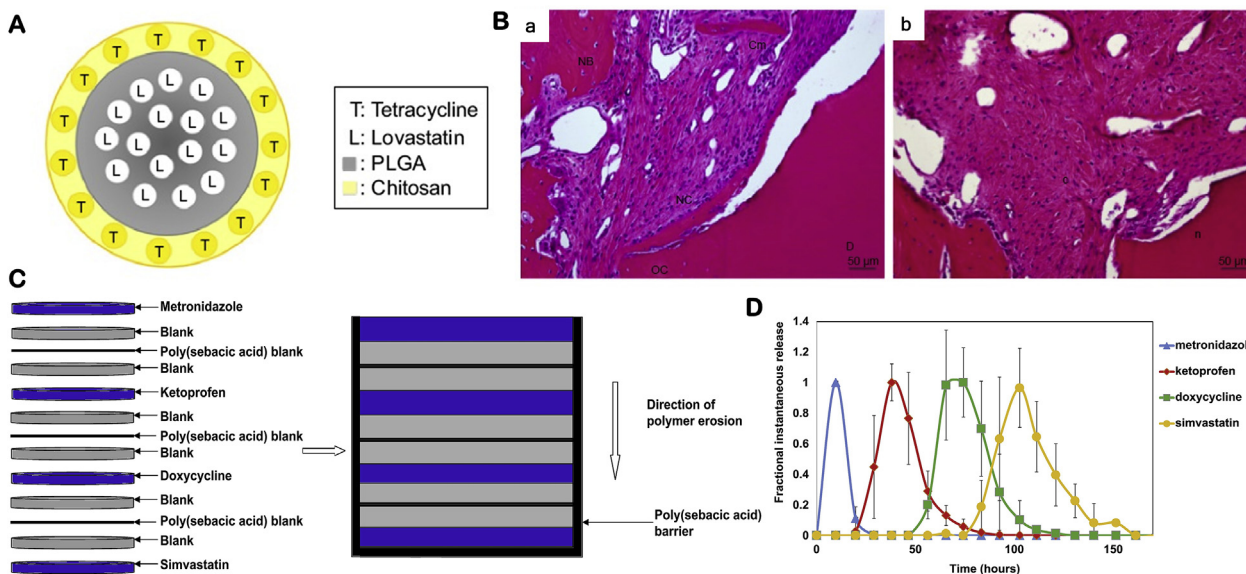


Fig. 3. Development of multi-drugs delivery systems. (A) A PLGA-lovastatin-chitosan-tetracycline release system, in which tetracycline was loaded in chitosan and lovastatin was loaded in PLGA microparticles. (B) The sequential release of tetracycline and lovastatin effectively controlled local infection and promote new bone and cementum regeneration. Adapted with permission from Ref. [150]. (C) The design of four drugs delivery system using a layer-by-layer technique. (D) The release profiles confirmed the sequential releases of the drugs from the system in vitro. Adapted with permission from Ref. [148].

into a chitosan scaffold [151]. The fast release of IGF-1 from the alginate microparticles induced osteoblast and cementoblast proliferation and differentiation, while the slow release of BMP-6 from the PLGA microparticles induced periodontal tissue regeneration. Spatial control of drug releases is achieved via the addition of different growth factors in different part of the scaffold. For example, cementum protein 1, FGF-2 and platelet-rich plasma were added to cementum, PDL and bone layer of the chitosan-based scaffold, respectively [91].

4. Applications of biomaterial and controlled delivery systems for periodontal regeneration

4.1. PDL regeneration

PDL is a fibrous tissue between alveolar bone and cementum. A number of PDL scaffolds were fabricated into nanofibrous fibrous matrices to mimic the architecture of the ECM of PDL. The nanofibrous scaffolds provided ECM-like environment to facilitate fibroblastic differentiation and PDL markers expression [152,153]. The structure of PDL is characterized by the directions of the PDL primary fibers. Thus, imitation of fiber alignment is critical for PDL regeneration. Several methods were developed to induce PDL fiber alignment, such as

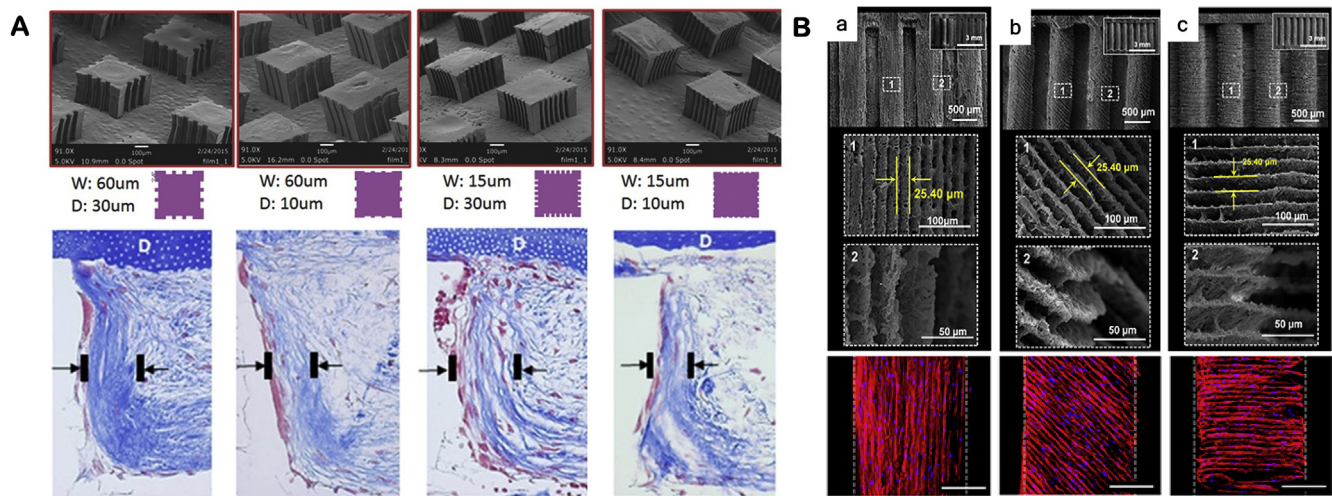


Fig. 4. Development of micropatterned scaffolds to guide collagen fiber orientation of PDL. (A) The 3D printed micropatterned matrix with various geometry (width, W and depth, D of the grooves) controlled the orientation of collagen deposited on the matrix. (B) The 3D printing technology was used to create angulated microgroove patterns that were used control cell orientations such as parallel (0°), oblique (45°) and perpendicular (90°) angulations. Adapted with permission from Refs. [31,79].

electrospinning and channel containing scaffold. Electrospinning is a simple method to fabricate oriented matrices, which showed the aligned fibers guided cell elongation and collagen fibers direction on the surface of the matrices [30]. The electrospinning method, however, only controls cell orientation on the surfaces of the matrix. Channel-containing scaffold is an alternative design to guide PDL cells and fiber directions in three-dimension [89]. In another study, 3D printing micro-channel fiber-guiding scaffolds were developed to induce the partial alignment of the fibers [75]. However, *in vivo* results showed that the fibers were deposited not inside the channels, but parallel to the dentin surface [89]. By introducing microfabrication techniques into scaffolding fabrication, micropatterned scaffolds with various micro-patterns and sizes (width and depth) were fabricated and tested for aligned tissue formation (Fig. 4A) [79].

Given the directions of PDL fibers vary in different groups, a freeze-drying scaffold was prepared to mimic fibers directions of apical, horizontal and oblique groups [32]. However, no *in vitro* or *in vivo* data showed the effectiveness of the scaffold. Similarly, one study used 3D printing process to produce microgroove structure, which controlled cell orientations in parallel (0°), oblique (45°) or perpendicular (90°) angulations (Fig. 4B) [31]. Similarly, no *in vivo* results confirmed the guiding function of the 3D printing scaffold.

Besides the reconstruction of PDL structure, functional regeneration of PDL is another issue to be considered. In the body, PDL is continuously subjected to mechanical stress caused by occlusal force. An *in vitro* study showed that PDL cells sensitively responded to mechanical stress for proliferation and differentiation, and increased the expressions of tendon-related markers such as periostin and tenascin [154]. After the PDL cells were treated with mechanical stress on aligned fibrous scaffolds and transplanted into rat premaxillary periodontal defect, histological results showed more aligned PDL-like fibrous tissue formation, indicating mechanical stimulus influenced fiber regeneration [154]. This *ex vivo* mechanical stress model, however, cannot mimic the complicated mechanical stress *in vivo*. A better mechanical stress model that more closely emulates occlusal force *in vivo* will assist the regeneration of functional PDL.

4.2. Alveolar bone and cementum regeneration

Since alveolar trabecular bone and cementum are both mineralized tissues and have similar compositions, they are discussed together in this section. To induce hard tissue formation, inorganic materials, like

calcium phosphate, are included in the scaffold for alveolar bone or cementum regeneration [46,155]. To overcome the limitations of fabrication difficulty and brittleness, natural or synthetic polymers are often incorporated in the scaffold [90,91].

BMP family proteins and amelogenin are widely used to induce alveolar bone regeneration [129,133,156,157]. While these proteins were also tested for cementum regeneration, other bioactive molecules that specifically induce cementum formation were examined. For example, cementum protein 1 (CEMP1) is a specific cementum marker that plays important roles in inducing cementum repair and formation. CEMP1 facilitates PDL cells attachment, proliferation and cementoblast differentiation rather than osteoblast differentiation [158]. In addition, CEMP1 can promote hydroxyapatite crystal and mineralized tissue formation [159,160], making it a potential candidate to induce cementum regeneration. When CEMP1 was loaded into chitin-PLGA/nBCG scaffold for cementum regeneration, a thin layer of mineralized tissue on the surface of root dentin was observed [91]. E-aminocaproic acid (ACA) is another molecule to induce cementum repair. ACA is a proteinase inhibitor that can slow down the fibrinolysis procedure during periodontitis and reduce cementoblast apoptosis. A chitosan particles carrier system loaded with ACA in fibrin matrix promoted cementum regeneration in a dog periodontal defect [161].

Cementum is a thin layer of mineralized tissue with a thickness of a few hundred micrometers. Therefore, scaffolds for bone regeneration are not suitable for cementum regeneration; instead, cell sheet can better fit the requirements. Various cells, such as PDL cells, BMSC and alveolar periosteal cells, were fabricated into cell sheets for cementum regeneration. Results showed that the cell sheets formed a thin layer mineralized cementum-like tissue on the surface of roots with the expression of the CEMP1 marker [162–165]. Because of the regenerative capacity, cell sheet was combined with PDL and bone scaffolds to regenerate cementum-PDL-bone complex [166,167].

Recently, a new immunomodulatory strategy was developed for alveolar bone regeneration [168]. This strategy targeted on macrophages that play a central role in inflammation activation and resolution. Macrophages can mainly be divided into two phenotypes: classically activated pro-inflammatory M1 macrophages and alternatively activated pro-healing M2 macrophages. Macrophages are capable of dynamically switching from one phenotype to the other depending on the surrounding microenvironment. For example, interleukin 4 (IL-4) is an effective cytokine to change the pro-inflammatory M1 into an anti-inflammatory M2 phenotype. Based on these biological findings, a

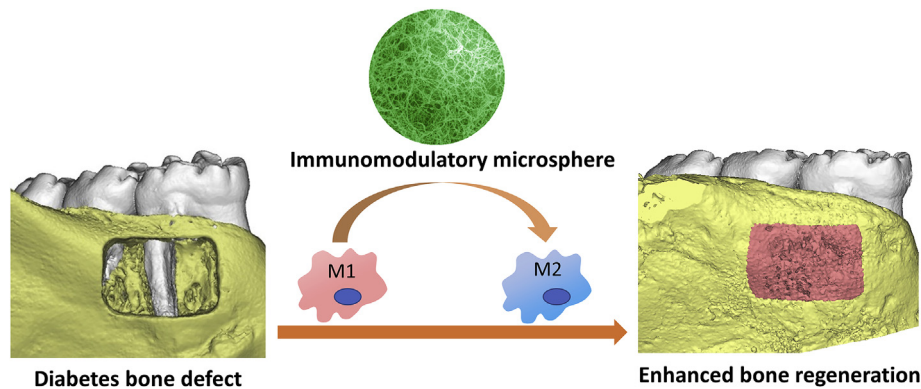


Fig. 5. An immunomodulatory approach to enhance alveolar bone healing and regeneration. The nanofibrous microsphere mimic the architecture of bone ECM and switched the transition of macrophages from M1 to M2 phenotypes; therefore, enhanced alveolar bone healing. Adapted with permission from Ref. [168].

unique bio-inspired injectable microsphere as an osteoimmunomodulatory scaffolding biomaterial was developed [168]. The microsphere was self-assembled with heparin-modified gelatin nanofibers that mimic the architecture of natural bone ECMs and provided an osteoconductive microenvironment for bone cells. Interleukin 4 (IL4), which has binding domains with heparin, was incorporated into the nanofibrous heparin-modified gelatin microsphere. Binding IL4 to heparin protected the IL4 from denaturation and degradation, and controlled its sustained release. The *in vivo* study showed the osteoimmunomodulatory microspheres switched the pro-inflammatory M1 macrophage into a pro-healing M2 phenotype, efficiently resolved the inflammation, and subsequently enhanced osteoblastic differentiation and new bone regeneration (Fig. 5). The development of immunomodulatory biomaterials, therefore, is a promising approach for bone healing.

It is worth mentioning that the regeneration of periodontal horizontal bone loss has been neglected for a long time. According to a report in 2010, the prevalence of horizontal bone loss is much higher than vertical bone loss in population [169]. However, the majority of alveolar bone regeneration research has been focusing on vertical bone loss regeneration. Guided bone regeneration (GBR) or GTR combined with bone graft materials were examined for horizontal bone augmentation [169–171]. However, as mentioned in previous sections, the outcomes were unpredictable. Currently, there are few studies on horizontal bone regeneration using tissue engineering strategy. One work reported the use of calcium-alginate scaffold for the repair of dog supraalveolar bone defect [172]. The result showed that the buccal-lingual bone thickness remained thinner than normal, and the shape and structure of regenerated bone were not restored as normal alveolar crest [172]. Consequently, tooth function and gingiva appearance were not restored. To date, it is still a major challenge to achieve horizontal bone loss regeneration, which is a direction for future periodontal research.

4.3. Alveolar bone-PDL-cementum complex regeneration

After decades of research on periodontal regeneration, it has been well accepted that alveolar bone, PDL and cementum are a structural and functional entity and the restoration of only one or two portions of the periodontium cannot restore normal periodontal functions [173]. For instance, a biphasic scaffold with only bone and PDL compartments could not form cementum on the root surface of a periodontal defect [75,152]. Without cementum, the connection between PDL and tooth was loose and the periodontium could not play the supporting role. The addition of a thin layer of cell sheet to the biphasic scaffold induced cementum-like tissue formation [88,166]. Since each layer of the periodontium has distinctive characteristics, the scaffold for each part should be designed accordingly to achieve a multi-tissue regeneration.

A chitin-PLGA-bioglass composite scaffold was fabricated to regenerate alveolar bone, PDL and cementum [91]. The incorporation of

bioactive glass was to enhance mineralized tissue formation on the cementum and bone portion. Bioactive molecules were encapsulated in different layers of the scaffold, separately, to induce bone (platelet-rich plasma derived growth factors), PDL (fibroblast growth factor 2) and cementum (cementum protein 1) regeneration. Another example is the use of cell sheet as the cementum layer combining with bone and PDL compartments (Fig. 6). The multilayer scaffold was held together with a dentinal slice and subcutaneously transplanted *in vivo*. PDL-like and cementum-like tissues were observed after 8 weeks [166]. The bone portion of the scaffold was further coated with calcium phosphate to increase osteoconductivity. While cell sheets could be generated from different cells sources, bone marrow mesenchymal stem cell sheet and PDL cell sheet had better results compared to gingival cells in promoting bone, PDL and cementum regeneration [167].

Although the above scaffolds regenerated periodontal tissues with similar structures to natural periodontal tissues, they failed to regenerate Sharpey's fibers, a pivotal structure of periodontium. Sharpey's fibers are the end part of the PDL collagen fiber bundles embedded in cementum and bone [10]. Sharpey's fibers connect cementum and alveolar bone to PDL, therefore, are essential for the integrity and function of periodontal tissues. Without the formation of Sharpey's fibers, there is no functional connection between cementum/alveolar bone and PDL, and the regenerated periodontium complex has no function. To rebuild the interface between PDL and bone, a study used 3D printing to fabricate PDL and bone scaffolds that provided channels to allow regenerated tissues to penetrate each other [89]. However, this scaffold only guided PDL fiber aligned in the channels of PDL portion, but no Sharpey's fibers inserted into the bone portion. The failure is likely because the large diameter of the guiding structure (0.8 mm in diameter) was ineffective to guide the direction of Sharpey's fibers (several microns in diameter). Multi-layers cell sheets were used and the close connection between the PDL and cementum enabled the fibers from each layer to mingle together to form Sharpey's fiber-like structure [163]. However, the inserted fibers were randomly distributed. To date, there are no feasible and effective ways to regenerate Sharpey's fibers of periodontium complex.

Periodontal mineralization homeostasis is a key factor for the long-term stability of newly formed periodontium complex. Ideally, the regenerated alveolar bone and cementum tissues should be mineralized and PDL be non-mineralized for a long term. However, the regenerated PDL tissue can be influenced by the surrounding hard tissues, leading to gradually mineralization, becoming thinner, and finally disappeared to cause tooth ankyloses. Therefore, maintenance of periodontal mineralization homeostasis is critical for the long-term stability of regenerated tissues. One possible solution is the introduction of moderate mechanical stimulation to the regenerative periodontium, which was believed to prevent dentoalveolar ankylosis and PDL mineralization [174], even though the mechanism is unclear yet. Another way is the

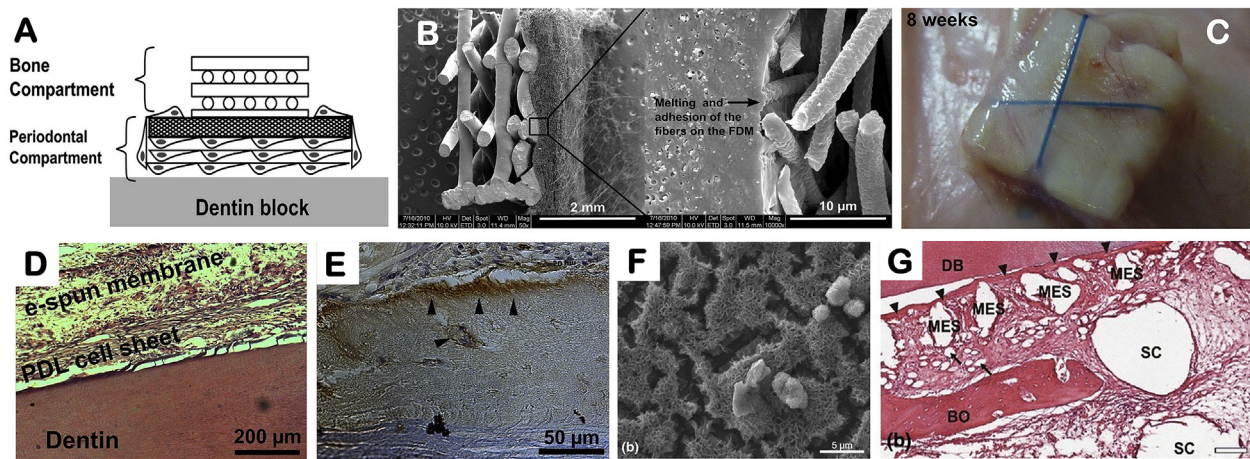


Fig. 6. Combination of cell sheet technology with bone and PDL scaffolds to regenerate cementum-PDL-alveolar bone. (A) Fabrication scheme showing the combination of PDL and bone compartments with a dentin slice. The bone and PDL layers were fabricated using fused deposition and electrospinning, respectively. (B) SEM image of the biphasic scaffold showing the fusion of the electrospun fibers onto the fused deposition component. (C) Subcutaneously transplanted the construct to induce periodontal regeneration in vivo. (D) PDL-like and (E) cementum-like tissues formation after transplantation for eight weeks. (F) CaP was coated on the PCL fused deposition compartment. (G) The CaP-coated scaffold enhanced bone formation. Adapted with permission from Refs. [88,166].

controlled delivery of mineralization inhibitors in the PDL portion and mineralization inducers in the alveolar bone and cementum portions of the periodontal scaffold. However, the key mineralization regulators in periodontium warrant to be clarified to fulfill this strategy. In addition, controlled delivery of the regulators for a long period of time is another challenge. Overall, the periodontal mineralization homeostasis is of great significance for long-term stability of regenerated tissues, but how to regulate and achieve the expected results need further exploration.

5. Conclusion and future perspective

Biomaterials used in periodontal regeneration include inorganic materials, polymeric materials and composites. While inorganic biomaterials are components used for bone and cementum regeneration due to their similar compositional and mechanical property, polymeric biomaterials are used for PDL regeneration. The combination of inorganic and polymeric materials is used fabricate biomimetic scaffolds for bone and cementum regeneration. To provide an ECM-mimicking microenvironment, biomimetic nanofibrous and multilayer scaffolds have been developed for periodontal tissue regeneration in recent years. A few studies attempted to regenerate periodontal tissues with the proper structures, such as the oriented PDL fibers, but achieved limited success. Some of the main challenges for regenerating functional periodontal tissues are listed below:

From material perspective, most of the biomaterials used for periodontal regeneration are traditional biomaterials, such as HA, β -TCP, PLLA, PCL, and PLGA. Even though these biomaterials can resemble the compositions in certain aspects, they cannot mimic the fine structures of the natural periodontal tissues, such as the different groups of PDL fibers, cellular and acellular cementum structure. One specific challenge is the regeneration of Sharpey's fibers between cementum-PDL-alveolar bone. Currently, none of the scaffolding systems regenerated functional Sharpey's fibers. Without these fibers, the connections between cementum-PDL-alveolar bone is unstable and cannot support teeth or bear occlusal force. Therefore, novel and bio-inspired materials that are designed to closely mimic the architecture of periodontal tissues at micro and nanoscale levels are prerequisite to achieve functional periodontal tissue regeneration.

To regenerate the hierarchical architecture of periodontal tissues, spatially and temporally controlled delivery of biophysical and biochemical cues is indispensable. For temporal regulation, the time point and sequence for cell migration, proliferation, differentiation and tissue

formation need to follow the natural process. As for spatial regulation, inducing the distinguished alveolar bone, PDL, and cementum formation at the same time requires a well-designed regeneration system. While there have been a variety of multidrug delivery systems developed, none of them can achieve precise control to guide periodontal tissue regeneration. There are a number of barriers that hinder optimized tissue regeneration. One essential issue is the lack of complete understanding of basic biology of periodontal tissue repair and healing. Factors that determine periodontal tissue formation have not been clarified, therefore, the selected drugs/growth factors cannot achieve the expected regenerative effects. Another knowledge gap is the proper concentrations of bioactive molecules, because overuse or insufficient drugs/growth factor compromises the outcomes. Therefore, an in-depth understanding of the basic biology is indispensable to provide more detailed information to guide the fabrication of biomimetic materials.

It was reported that mechanical cues plays a role in restoration of functional periodontal tissues [154]. However, the mechanical cues were rarely included in periodontal tissue regeneration. Therefore, integration of mechanical cues to biomaterial design and evaluation of the regenerative effect will be inspiring for future studies.

Other challenges for periodontal regeneration include the restoration of horizontal alveolar bone loss and long-term stability of regenerated periodontal tissues. Addressing these problems will tremendously advance the progresses of periodontal tissue regeneration.

Despite all these challenges, periodontal tissue regeneration is an exciting and rapidly growing field. The advances of this field provide the promising potential to improve the health of dental patients in the near future.

CRediT authorship contribution statement

Yongxi Liang: Writing - original draft. **Xianghong Luan:** Conceptualization, Funding acquisition. **Xiaohua Liu:** Conceptualization, Writing - review & editing, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare no conflict of interests.

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