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The recent advances in scaffolds for integrated periodontal regeneration

Hyun Nyun Woo [1], Young Joon Cho [1], Solaiman Tarafder, Chang H. Lee [*]

Center for Dental and Craniofacial Research, Columbia University Medical Center, 630 W. 168 St., VC12-212, New York, NY, 10032, USA

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ABSTRACT

The periodontium is an integrated, functional unit of multiple tissues surrounding and supporting the tooth, including but not limited to cementum (CM), periodontal ligament (PDL) and alveolar bone (AB). Periodontal tissues can be destructed by chronic periodontal disease, which can lead to tooth loss. In support of the treatment for periodontally diseased tooth, various biomaterials have been applied starting as a contact inhibition membrane in the guided tissue regeneration (GTR) that is the current gold standard in dental clinic. Recently, various biomaterials have been prepared in a form of tissue engineering scaffold to facilitate the regeneration of damaged periodontal tissues. From a physical substrate to support healing of a single type of periodontal tissue to multi-phase/bioactive scaffold system to guide an integrated regeneration of periodontium, technologies for scaffold fabrication have emerged in last years. This review covers the recent advancements in development of scaffolds designed for periodontal tissue regeneration and their efficacy tested *in vitro* and *in vivo*. Pros and Cons of different biomaterials and design parameters implemented for periodontal tissue regeneration are also discussed, including future perspectives.

1. Introduction

The periodontium is an integrated, functional unit of multiple tissues surrounding and supporting the tooth, including but not limited to cementum (CM), periodontal ligament (PDL) and alveolar bone (AB). Periodontal tissues can be destructed by chronic periodontal disease, which can lead to tooth loss [1–3]. In support of the treatment for periodontally diseased tooth, various biomaterials have been applied starting as a contact inhibition membrane in the guided tissue regeneration (GTR) that is the current gold standard in dental clinic. Recently, various biomaterials have been prepared in a form of tissue engineering scaffold to facilitate the regeneration of damaged periodontal tissues [4–7]. From a physical substrate to support healing of a single type of periodontal tissue to multi-phase/bioactive scaffold system to guide an integrated regeneration of periodontium, technologies for scaffold fabrication have emerged in last years. This review covers the recent advancements in development of scaffolds designed for periodontal tissue regeneration and their efficacy tested *in vitro* and *in vivo*. Pros and Cons of different biomaterials and design parameters implemented for periodontal tissue regeneration are also discussed, including future perspectives.

2. Purpose of use of biomaterial scaffolds for periodontal regeneration

2.1. Background of periodontal regeneration therapy

The ultimate purpose of periodontal treatment is to regenerate periodontal tissues in harmony, whereby CM, PDL, and AB are formed simultaneously in their right positions where PDL fibers are oriented longitudinally between CM and AB (Fig. 1A). Periodontitis is initiated by bacterial infection and involved with increased infiltration by neutrophil and macrophages, activation of osteoclasts vis RANKL signaling, followed by bone resorption (Fig. 1B) [8]. Dental plaque and calculus often advance vulnerability to progressive bacterial infection and periodontitis by opening gap to periodontium (Fig. 1B) [9]. The current clinical practices include a non-surgical, conservative treatment removing the causes of periodontitis (e.g. dental plaque and calculus) and a resective surgery performed to reduce periodontal pocket depth (Fig. 1C). However, the form of healing by these techniques is frequently the attachment of long junctional epithelium to root surfaces [10]. Long junctional epithelium is attached to root surfaces by hemidesmosomes of which ability to protect periodontium is inferior to that of connective

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* Corresponding author. Regenerative Engineering Laboratory, Columbia University Medical Center, 630 W, 168 St. VC12-230, New York, NY, 10032, USA.
E-mail address: chl2109@cumc.columbia.edu (C.H. Lee).
[1] Equal contribution.

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tissue (CT) fibers embedded into the CM. Therefore, periodontitis is likely to recur if patients neglect plaque control or if the host immune response is reduced. In order to overcome this problem, the need for regenerative periodontal therapy has emerged. In the past, bone graft procedures were often performed as regenerative therapy, but bone graft alone does not prevent the downgrowth of long junctional epithelium [11]. Therefore, GTR (Fig. 1D), which applied the principle of contact inhibition to regenerate periodontal tissue, has been developed and used over the last 30 years (Fig. 2).

The concept of GTR was started by Karring and Nyman in the 1980s in search of possible regenerative elements in periodontium. Karring et al. reported that root resorption and ankylosis may be occurred by osteoblasts when periodontitis-affected root was inserted into AB in their dog experiment [12]. In the same year, Nyman et al. reported that root resorption occurred when periodontitis-affected root was inserted into gingival connective tissue in their dog and monkey experiment [13]. In 1982, Nyman et al. proposed that PDL cells have a potential for regeneration in monkey experiment [14]. It was also reported that GTR using Millipore filters to treat a periodontitis-affected tooth achieved a new attachment by periodontal ligament without the formation of long junctional epithelium or ankylosis in clinical trials [15]. In spite of successful GTR procedure, there have been occasional reports that root resorption and ankylosis can still happen [16,17]. Moreover, when the regeneration ability of PDL and CM significantly reduced as chronic periodontitis persists over an extended period of time, it is often difficult to orchestrate harmonious regeneration of multiple types of periodontal tissues. Moreover, persistent periodontitis can significantly reduce regeneration of PDL and CM and thus regeneration of the multiple periodontal tissues may be uncertain [18].

As a new solution to overcome such obstacles, tissue engineering approaches have recently been investigated for periodontal regeneration [19]. Various biomaterial scaffolds delivered with cells and/or bioactive can be applied along with GTR (Fig. 1D). Recently, more advanced scaffold systems have been developed to guide integrated regeneration of periodontium (Fig. 1E) [20–22]. These scaffolds are designed to deliver bioactive cues for periodontium regeneration and to undergo degradation to be replaced by new tissues (Fig. 1F).

2.2. Scaffolds to support healing of periodontal tissues

In tissue engineering, scaffolds mainly serve as a substrate for cell attachment, tissue ingrowth, as well as an initial structural support [24]. In GTR, non-degradable or degradable membranes serve a contact inhibition of epithelium growth that in turn allow a relatively slow (4–6 weeks) healing of periodontal connective tissue and PDL [25]. However, a prolonged period of periodontitis may deteriorate the outcome of GTR by deteriorating healing capacity of PDL cells, hindering host immune response or severely denaturalizing CM [18].

Scaffolds used for the regeneration of periodontal tissues can provide a contact guidance that enables timely migration of cells into periodontal defects, followed by promoted regeneration [26]. To further facilitate cell migration and tissue ingrowth, various bioactive cues including growth factors (GFs) and cytokines have also been delivered with the scaffolds [27–29]. To our best knowledge, however, there has been no experimental data strongly suggesting scaffold-mediated periodontal healing as fast as GTR. Evidence is still premature to suggest that scaffold-mediated periodontal healing is comparable to that of GTR.

Most previous studies with biodegradable scaffolds have focused on the guided regeneration of CM and PDL [4,7,30,31]. Cho et al. reported that CM-like tissue structure was formed on the surface of human dentin

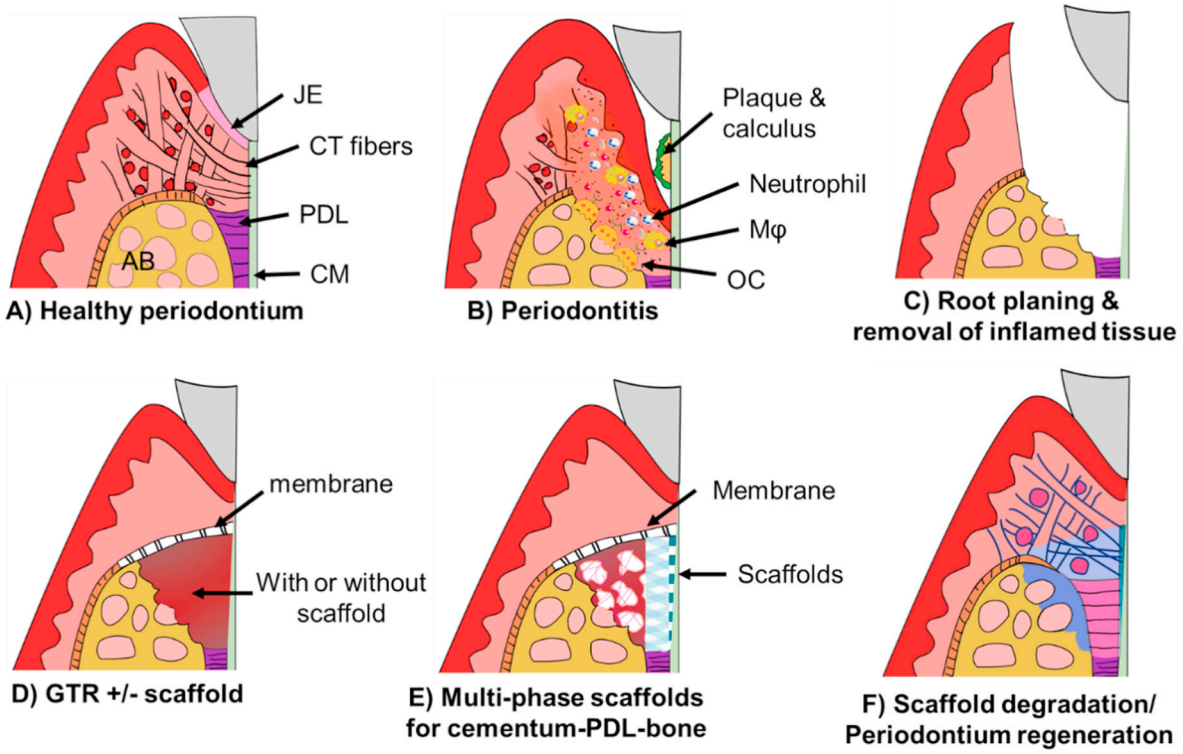

Fig. 1. Illustration of healthy periodontium, periodontitis, and scaffold-based regenerative approaches. A) Healthy periodontium is consisted of junctional epithelium (JE) (0.71–1.35 mm), connective tissue (CT) fibers (1.06–1.08 mm) [23], cementum (CM), periodontal ligament (PDL) and alveolar bone (AB). B) Periodontitis results in bone resorption by activated osteoclasts (OC), formation dental plaque & calculus, and epithelial down growth, as associated with inflammatory responses with increased number of neutrophil and macrophages (Mϕ). C) Surgical procedure involves root planing to remove calculus, necrotic CM and inflammatory granulation tissue. D) As a default treatment option, GTR with contact inhibition membrane is frequently performed that can be combined with filling the periodontal tissue gap with various scaffolds. E) Multi-phase scaffolds with delivery of bioactive cues can be implanted to induce integrative regeneration of multiple periodontal tissues. F) The implanted scaffolds are expected to undergo degradation as new tissues are forming.

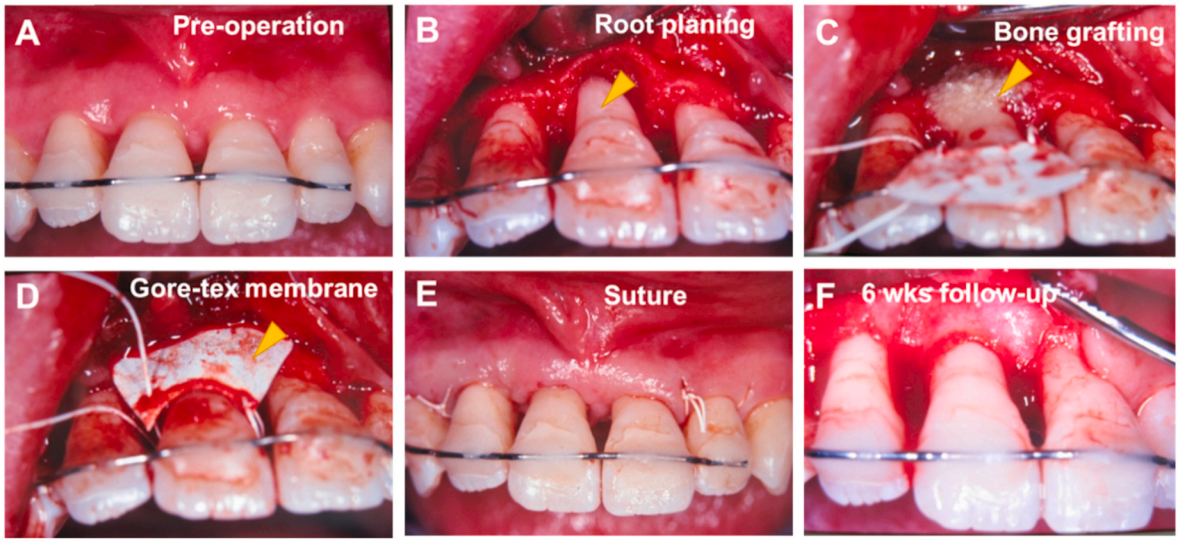

Fig. 2. Clinical case of periodontal regeneration using GTR with supporting bone graft and Gore-tex membrane (Picture credit to Dr. Young Joon Cho's clinic).

when incubated with human PDL stem/progenitor cells (PDLSCs) seeded in 3D-printed poly(ε-caprolactone) PCL scaffolds spatially delivered with connective tissue growth factor (CTGF), bone morphogenic protein 2 and 7 (BMP-2 and BMP-7) [31]. Chen et al. fabricated an electrospun multiphasic scaffold which consisted of PCL, collagen type I (COL-I), and rhCEMP1/ACP that promoted formation of CM-like structure when implanted in rat calvaria with PDLSCs for 8 weeks [32]. Park et al. performed a subcutaneous implantation of micro/macro-porous biphasic calcium (MBCP) blocks seeded with BMP-2 pre-treated PDLSCs into immunocompromised mice [7]. After 4 weeks, BMP-2 pre-treatment group showed formation of mineralized tissue integrated with fibrous tissues [7].

Several previous works also implemented tri-phasic scaffolds to guide an integrated regeneration of CM, PDL and AB [33]. In 2014, Lee et al. reported the reconstruction of periodontium complex using a 3D printed tri-phasic scaffold. They spatiotemporally delivered amelogenin, CTGF and BMP-2 for regeneration of CM, PDL and AB, respectively, by single type of multipotent dental stem/progenitor cells [4]. When implanted in dorsum of immunodeficient mice for 6 weeks, the tri-phasic scaffolds with spatiotemporal delivery of three different bioactive cues and dental stem/progenitor cells successfully promoted integrated formation of periodontium-like multi-tissue construct [4]. Park et al. fabricated tri-phase, 3D-printed scaffolds consisted of regionally different micro-architectures that showed potential in promoting integrated healing of multi-tissue periodontium by PDLSCs [34].

As above-described, various scaffold systems have shown great potential for integrated periodontal regeneration. The recent technical development in micro-precise regional control in design of scaffolds has made important milestone toward integrated regeneration of multi-tissue periodontium. The existing approaches to regenerate integrated periodontium *via* various scaffolds and delivery system are discussed more in-depth below.

2.3. Anti-microbial effect of scaffolds for periodontal regeneration

The major etiology of periodontitis is bacteria (e.g. *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola*), and host immune responses to them manifest the disease [35]. Although the current periodontal treatment involves a removal of dental plaque and calculus, the sources and niche of bacteria causing pathological symptoms, the recurrence rate of periodontitis after the initial periodontal treatment is highly associated with maintenance to prevent secondary infection [36]. In the case of GTR, for example, the maintenance to prevent the deposition of dental plaque around surgical site after surgery is critical for successful surgical outcome over 4–6 week of post-treatment. Since GTR membranes not only inhibits epithelial and CT ingrowth but also blocks blood supply, the recession of the gingival soft tissue happens frequently that in turn leads to exposure of the membrane to the oral cavity, increasing the risk of re-infection. Such membrane exposure occurs more frequently with non-degradable membranes such as e-polytetrafluoroethylene (e-PTFE) [37]. Without a doubt, such bacterial re-infection inevitably likely disrupts the clinical outcome of periodontal tissue healing, as reported in a number of previous studies. Schallhorn et al. reported that delayed and adverse healing patterns, potentially associated with bacterial re-infection, were 8% and 3%, respectively, over 100 GTR treatment at 100 sites [38]. Sanctis et al. reported that bacterial colonization on e-PTFE membranes reduced the regeneration of periodontal tissue by 50% [39]. Unfortunately, biodegradable membranes adopted to overcome the shortcomings of non-degradable membranes are not free from the risk of re-infection. Once exposed to oral cavity, degradable membranes are vulnerable to bacterial re-infection what can cause a structural rupture in a short time [40].

Together, current periodontal treatments hold a certain level of risk for bacterial re-infection. Anti-microbial function, therefore, has been incorporated in various scaffolds designed to support periodontal regeneration as well as to minimize the potential re-infection after the initial periodontal treatment as summarized in Table 1. As a simple and straightforward approach, material components with innate anti-microbial effect have been added to scaffold materials. An example of such component is chitosan, a natural sea shell-derived polymer with antibacterial, antifungal, bio-adhesive and hemostatic effects [41–43]. Several previous studies showed the promising effects of chitosan in bacteria causing periodontitis. For example, Arancibia et al. reported that chitosan particles with a concentration of 5 mg/mL inhibited periodontal pathogens such as *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans* [44]. Similarly, Lee et al. reported that chitosan membrane with grafted epigallocatechin-3-gallate and lovastatin showed a bactericidal effect on periodontopathic bacteria such as *Aggregatibacter actinomycetemcomitans, Prevotella nigrescens, and Porphyromonas gingivalis* [45].

Chitosan also showed its anti-microbial effects when added in scaffolds. Zhou et al. demonstrated that the scaffold composed of fish collagen/bioactive glass/chitosan composite nanofibers had antibacterial effects on *Streptococcus mutans,* leading to promoted regeneration of furcation defects in a dog model [46]. Silver and magnesium (Mg) are another example of anti-microbial components. Abdelaziz et al. reported

Table 1
Summary of scaffolds for periodontal regeneration incorporated with anti-microbial component.

Antimicrobial component	Scaffold construct	Bioactive cue	Research design	Experimental duration	Outcomes	Ref
Chitosan	Chitosan (CS)/poly(vinyl alcohol) (PVA)/ hydroxyapatite (HA) electro-spun composite nanofibrous mats	Piroxicam (PX)	Initial material characteristics, release profile, and *in vitro* cytocompatibility	72 h	Sustained release of PX; appropriate mechanical behavior and minimum cytotoxicity; no direct observation for anti-microbial effect.	[53]
	Chitosan membrane with grafted epigallocatechin-3-gallate (EGCG) and lovastatin	Lovastatin	Initial material characteristics, antimicrobial activity test, and *In vivo* periodontal healing evaluation	21 days *in vitro*; 8 weeks *in vivo*	Lovastatin sustained release promoted osteogenesis; EGCG14-CS exhibited the promising bactericidal activity; improved periodontal healing in dog model.	[45]
	Chitosan/alginate/PLGA hybrid scaffolds	IGF-1, BMP-6	Initial material characteristics, release profile, gene expression, and mineralization assay	30 days	Alginate/PLGA released IGF-1 & BMP-6; hybrid scaffold activated proliferation and osteoblastic differentiation of cementoblasts; no direct observation for antimicrobial effect.	[54]
	Fish collagen/bioactive glass/ chitosan composite nanofibers	N/A	Initial material characteristics, antimicrobial activity assay, and *in vivo* periodontal healing evaluation	60 days	The composite nanofibers had antibacterial effect on *S. mutans*; improved periodontal healing in dog model	[46]
	Chitosan/β-glycerol phosphate (β-GP) hydrogel	TGF-β1, PDGF-BB, IGF-1	Initial material characteristics, release profile, and *in vitro* vitality assessment	2 weeks	Constantly released TGF-β1, PDGF-BB, IGF-1; no direct observation for antimicrobial effect.	[42]
	Injectable chitosan/ β-glycerophosphate hydrogels	BMP-7, ornidazole (ORN)	Initial material characteristics, release profile, antimicrobial assay, and *in vivo* periodontal healing evaluation	21 days *in vitro*; 8 weeks *in vivo*	Constantly released BMP-7 and ORN; the hydrogels loaded with chitosan and ORN showed clearly antimicrobial effect against *P. gingivalis*; improved periodontal regeneration in dog model	[55]
	Mesoporous HA/chitosan scaffolds	rhAmelogenin	Initial material characteristics, release profile, antimicrobial assay, and *in vivo* periodontal healing evaluation	7 days *in vivo*; 8 weeks *in vivo*	HA/chitosan scaffold showed antibacterial activity against *F. nucleatum* and *P. gingivalis*; enhanced formation of CM-like tissue in mouse model	[56]
	Sandwich-like chitosan/ polycaprolactone/gelatin scaffolds	N/A	Initial material characteristics, and *in vivo* subcutaneous implant to evaluate barrier effect	3 months *in vitro*, 4 weeks *in vivo*	Favorable stability and degradation rate; no direct observation for antimicrobial effect; cell occlusive effect in rat model	[57]
Tetracycline	PLGA/gum tragacanth nanofibers	N/A	Initial material characteristics, release profile, and antimicrobial properties	75 days	Nanofibers had a smooth and bead-less structure; tetracycline constantly released for 75 days after burst release during the first 2 h; Bacterial inhibition against G(+)	[51]
Metronidazole	Infection-responsive electrospun nanofiber mat	N/A	Initial material characteristics, release profile, *in vitro* cytocompatibility, and antimicrobial activity	72 h	Good cytocompatibility; the nanofiber mat released metronidazole and showed antibacterial effect.	[49]
	Dual drug loaded coaxial electrospun PLGA/PVP fiber	Naringin	Initial material characteristics, release profile, *in vitro* cytocompatibility, and antimicrobial activity	21 days	Fabricated fiber had adequate properties; metronidazole and naringin loaded fiber inhibited anaerobic bacteria.	[52]
Silver nanoparticles	Electrospinning nanofibrous with HA & silver nanoparticles	N/A	Initial material characteristics, release profile, and antimicrobial activity	32 days	Improved bone regeneration activity; silver nanoparticles enhanced antibacterial effect.	[47]
Doxycycline & simvastatin	Core-Shell poly-(D,L-Lactide-*co*-Glycolide)-chitosan Nanospheres	IL-1β, MMP-8, VEGF	Initial material characteristics, release profile, antibacterial examination, gene expression analysis, and *in vivo* periodontal healing evaluation	28 days	Scaffold constantly released simvastatin and doxycycline and significantly inhibited *P. gingivalis* and *S. sanguinis*; down-regulated IL-1β & MMP-8, up-regulated VEGF, and decreased bone loss in rat model.	[50]
nMgO	Biodegradable multifunctional nanofibrous membrane	N/A	Initial material characteristics, antibacterial effects, osteogenesis evaluation, and *in vivo* periodontal healing evaluation	14 days *in vitro*; 6 weeks *in vivo*	nMgO incorporated membranes enhanced osteogenic property & the antibacterial effect against *E. coli* and *S. aureus*; enhanced periodontal regeneration in rat model.	[48]
Mg doped HA nanoparticles	3D nano bilayered spatially and functionally graded scaffold	Bromelain	Initial material characteristics, release profile, antibacterial effects, and *in vivo* periodontal healing evaluation	12 days	Increased mechanochemical properties; improved antibacterial potential & sustained release; enhanced periodontal regeneration in rat model	[58]

that electrospun nanofibers containing silver nanoparticles had antibacterial effects over 32 days [47]. Liu et al. reported that magnesium oxide (MgO)-conjugated nanofibrous membrane showed antibacterial activities to *E. coli and S. aureus* in a dose-dependent manner and showed promising *in vivo* efficacy in periodontal regeneration in rats [48].

Besides such material components, various anti-microbial medicines have been applied to scaffolds that include tetracycline, doxycycline and metronidazole. Shi et al. reported that metronidazole conjugated

electrospun nanofibers enhanced antibacterial capacity [49]. Chang et al. reported that core-shell poly-(D,L-Lactide-*co*-Glycolide) (PLGA)--chitosan nanospheres encapsulated with doxycycline inhibits *P. gingivalis and S. sanguinis* [50]. Ranjbar-Mohammadi et al. fabricated PLGA/gum tragacanth nanofiber scaffolds and delivered tetracycline through co-axial electrospinning [51]. They demonstrated a sustained release of tetracycline targeting for a long-term anti-bacterial effects in periodontal treatment [51]. Similarly, coaxial electrospun PLGA/PVP fibers were fabricated loaded with metronidazole and naringin and showed potential to inhibit anaerobic bacteria [52].

Despite the promising progress, the aforementioned approaches to incorporate anti-microbial material component or drug compounds have been limited to *in vitro* experiments or *in vivo* periodontal healing mode without clinically relevant re-infection condition.

2.4. Anti-inflammatory effects of scaffolds for periodontal regeneration

Periodontitis is a multifactorial inflammatory disease, characterized by progressive destruction of periodontium [2]. Even though the major etiologic factor of periodontitis seems to be bacterial, the excessive host immune response and/or inadequate resolution of inflammation are the main contributing factors to the pathogenesis of periodontitis [3]. Accordingly, there have been attempts to suppress inflammation using pharmacological agents, such as non-steroidal anti-inflammatory drugs (NSAIDs). However, they are used only as auxiliary means because the effects of intervention using only proinflammatory pathways and/or signaling without removing causative factors are limited [3,59].

Clinically, periodontal treatment starts with removing the causative factors (e.g. dental plaque and calculus) as well as inflammatory granulated tissue. Since biomaterials, either as a scaffold or a contact inhibiting membrane, are applied after a thorough removal of irritants and inflammatory granulation tissues, modulation of inflammation after periodontal treatment may not be a critical issue to be concerned. Nonetheless, inflammation caused by potential foreign body reaction may need an attention with application of biomaterial-based scaffold or membrane. As most wound healing process involve inflammatory stage [60], the treated cited is subjected to mild inflammatory conditions. However, persistent inflammation possibly caused by improper post-operative maintenance and poor systemic conditions of patients may lead to damage of periodontal tissues [61,62]. Accordingly, several biomaterials have been designed to equip with anti-inflammatory function to support periodontal regeneration as summarized in Table 2.

Previous studies reported chitosan-based scaffolds with sustained release of meloxicam (NSAID) [63] or aspirin to mitigate post-treatment inflammation [64]. In another study, PCL scaffolds were delivered with ibuprofen that showed an anti-inflammatory effect [65]. A 3D PCL scaffold combined with tannic acid, an anti-oxidant and has anti-inflammatory cue, suppressed inflammation induced by LPS [66]. Most of the previous biomaterials with anti-inflammatory component were designed for a function of GTR membrane rather than incorporated into architecture of the scaffold used in periodontal regeneration.

3. Scaffold biomaterials for periodontal regeneration

3.1. Natural materials

Natural biomaterials generally have excellent cell affinity and biocompatibility. They are less toxic and rarely cause inflammatory responses or immune reactions. Therefore, natural biomaterials have been widely used as scaffolds for the regeneration of periodontal tissues [69–71]. Collagen and chitosan are two most commonly investigated natural biomaterials for regeneration of periodontal tissues. For

Table 2
Summary of scaffolds for periodontal regeneration incorporated with anti-inflammatory component.

Type of scaffold	Anti-inflammatory component	Research design	Experimental duration	Induction of inflammation	Outcomes	Ref
CS/PVA/HA electrospun fibers and films	Meloxicam (NSAIDs)	*In vitro* material characterization; cytocompatibility test (VERO cell culture)	72 h	N/A	Meloxicam, a selective COX-2 inhibitor, showed a sustained drug release over extended periods of time from CS/HA/PVA composite fibrous membranes and films; no direct observation in ant-inflammatory effect	[63]
Electrospun polycaprolactone (PCL) scaffold	Ibuprofen (IBU)	*In vitro* material characterization; cell vitality & wound closure assay, release profile, and *in vivo* periodontal regeneration evaluation	22 days	LPS	The anti-inflammatory effects of IBU on gingival cells were actively intensified; IBU-PCL scaffold significantly enhanced the clinical attachment and reduced bone destruction in mouse model.	[65]
3D BMP-2-Delivering Tannylated polycaprolactone (PCL) Scaffold	Tannic acid (TA)	*In vitro* material characterization; release profile; antioxidant assay; ROS measurement; anti-inflammatory effect test; ALPase activity assay	28 days	LPS	The BMP-2/TA/PCL scaffold significantly inhibited the mRNA levels of MMP-3, COX-2, IL-6, and TNF-α in LPS; increased osteogenic effect	[66]
Polycaprolactone - (Polyvinyl Alcohol/ Collagen) Hybrid Nanofiber	Ibuprofen (IBU)	*In vitro* material characterization; release profile	N/A	N/A	Both PCL and PVA/COL loaded membranes consistently released IBU; no direct observation in ant-inflammatory effect	[67]
Collagen membrane	Progranulin (PGRN)	*In vitro* coimmunoprecipitation assay; *in vivo* periodontal regeneration evaluation by microCT analysis, histomorphometric analysis & immunohistochemical staining	6 weeks	TNF-α	Collagen membrane containing PGRN had the effects of anti-inflammation, osteoclastogenic inhibition, and osteogenic promotion; PGRN enhanced periodontal regeneration in rat model.	[68]
Chitosan (CS)/b-sodium glycerophosphate/ gelatin hydrogels	Aspirin/ erythropoietin (EPO)	*In vitro* material characterization; release profile; toxicity assay & degradation evaluation; *in vivo* evaluation of anti-inflammatory effect & periodontal regeneration	2 weeks	Ligature wire (& LPS) with methods in Bhattarai 2016	No toxicity; hydrogel scaffold constantly released aspirin & EPO; CS/b-sodium glycerophosphate/ gelatin hydrogel aborted the inflammation and accomplished AB regeneration in rat model.	[64]

example, a 3D collagen scaffold with aligned pores in size of 50–100 μm showed a promising effect on induction of dynamic and rapid migration of PDL [69]. Collagen hydrogel absorbed with BMP-2 produced a significant amount of new PDL and improved periodontal attachment without ankyloses in a dog study [70]. Similarly, collagen sheet with fibroblast growth factor-2 (FGF-2) inhibited epithelial downgrowth into periodontal defect and enhanced periodontal regeneration in a rat model [71].

Chitosan has also been widely used for periodontal regeneration because of its good biocompatibility, biodegradability, non-immunogenicity, and anti-microbial properties against bacteria or fungi [55]. Given its disadvantages as a sole scaffold material such as low solubility and aggregation of particles, chitosan has been frequently mixed into another type of scaffold material to provide anti-microbial function [72]. Chitosan nanoparticles combined with PLA nanofibers showed improved mechanical properties and hydrophilicity as compared to PLA alone [73]. Chitosan, when combined with hydroxyapatite (HA) and amelogenin scaffolds, exhibited enhanced antibacterial activity *in vitro* as well as *in vivo* [56].

3.2. Bioceramics

Bioceramics based materials such as hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and bioactive glass (BG) have been widely used to support healing of AB in periodontium. Bioceramic scaffolds typically provide high mechanical stability and biodegrability suitable for periodontal regeneration [74–76]. The key advantages of bioceramic based scaffolds over other natural and synthetic materials are their outstanding osteoconductive and osteoinductive properties [77]. Moreover, bioceramics can be delivered into periodontal defect in various forms such as granule, paste, and injectable format [78,79]. On the other hand, the slow degradation rate of ceramics can be disadvantageous for periodontal regeneration as remaining ceramic particles can result in mechanical irritation or inflammation [80].

Biodegradable bioceramics such as hydroxyapatite and β-TCP have been widely used as a scaffold material for bone tissue regeneration [75]. A 6-month follow-up clinical trial showed that β-TCP containing BMP-2 and PDGF applied with collagen membrane had high clinical attachment level and radiographic bone gain [27]. Another clinical trial that used β-TCP with 0.1%–0.4% FGF-2 as a scaffold implanted in vertical bony defects of patients [81] demonstrated that FGF-2 combined β-TCP resulted in improved clinical attachment as compared to control β-TCP alone [81]. Scaffolds composed of nano β-TCP combined with FGF-2 also showed enhanced cell infiltration and periodontal hard tissue regeneration in rat and dog model as compared to collagen scaffold [82]. HA combined with vascular endothelial growth factor (VEGF) showed osteoconductive properties with PDLSCs and release of VEGF *in vitro* [83]. Calcium phosphate cement (CPC) when conjugated with metformin, an antidiabetic drug, showed promising function in osteogenic differentiation of PDLSCs [84].

Bioactive glass, another well-known bioceramics with excellent osteogenic property, also widely used for periodontal tissue regeneration. Bioactive glass can be combined with both soft and hard scaffold materials, and its degradation rate is adjustable [85]. A clinical study using bioactive glass putty applied to 15 patients with grade II furcation defects showed promising improvement in vertical probing depth reduction at 9 months in comparison with platelet rich fibrin (PRF) treatment [86].

Outstanding limitations of bioceramic based materials include the mechanical properties; they are often too brittle to form reliable 3D structure with desired shape and dimension. Recent technological advancement enabled a 3D printing of bioceramics into patient-specific anatomic shape and dimension of scaffolds [87,88]. However, high temperature/pressure-based fabrication process often shrink the 3D structure and the low elasticity and extreme brittleness of most bioceramics remain as limitations to be used as scaffold for periodontal tissue regeneration at the sites that require high mechanical stability for longer duration [87,88].

3.3. Synthetic polymers

Synthetic polymers have been predominantly used as materials for the second-generation degradable membrane to replace the non-resorbable membrane-PTFE. Such polymers have also been applied for scaffold materials. Polyester-based polymers such as polylactic acid (PLA), polyglycolic acid (PGA), polylactic-*co*-glycolic acid (PLGA) and polycaprolactone (PCL) have been frequently utilized for periodontal scaffold materials [26]. Polyester's degradation byproduct may be toxic but it has been considered safe given the insignificant amount of residual particles that are released at a very slow rate [89]. Synthetic polymers have a number of unique advantages including highly adjustable physio-chemical properties, controllable biodegradation rate, and simple and straightforward fabrication process allowing mass production [90].

Various polymeric scaffolds have been investigated for periodontal regeneration. Electrospun PLGA/PCL composite scaffolds with FGF-2 and bone marrow mesenchymal stem cells (MSCs) resulted in improved periodontal tissue healing by 6 weeks *in vivo* in a rat model [91]. PLGA scaffold conjugated with plasmid DNA encoding FGF-2 enhanced the proliferation of human PDL cells and formation of PDL-like tissue without root resorption after one month in a dog model [92]. Peng et al. An investigation of 3D printed PCL/PLGA composite scaffolds for their potential application with human PDL cells showed significantly increased adhesion, proliferation, and osteogenic capacity of human PDL cells *in vitro* [93]. A multi-phase composite scaffolds consisted of micro-patterned PCL/PLGA for PDL and amorphous PCL for bone with PDGF-BB and BMP-7 for spatial delivery from the scaffold showed enhanced regeneration of bone-periodontal ligament interface when implanted in a rat fenestration defect model [20].

3.4. Hydrogel

Hydrogel is a network of crosslinked macromolecular polymers with absorption characteristics and hydrophilic properties [94,95]. Various types of biomaterials can be formed as hydrogel [96]. The advantages of a hydrogel form include high-water content, biocompatibility and the flexibility in structural design and formation. Various types of hydrogels have been applied for periodontal tissue regeneration. When collagen membrane with biphasic calcium phosphate (BCP) was compared with hydroxypropylmethyl cellulose (HPMC) hydrogel membrane with BCP HPMC with BCP showed superior outcome including inhibition of soft tissue invasion into periodontal defect as well as significant bone regeneration after 12 weeks in a canine model [97]. Choi et al. demonstrated that proanthocyanidins (PAC)-treated collagen gel showed higher surface roughness and enhanced attachment of PDL cells [98].

Collagen hydrogel scaffold loaded with FGF-2 showed CM-like tissue and PDL-like Sharpey's fibers formation without ankyloses and root resorption when treated a class II furcation defects in a canine model [99]. HydroMatrix, an injectable peptide nanofiber hydrogel, also showed enhanced adherence, migration and proliferation of PDLSCs *in vitro* [76]. Polyethylene glycol (PEG) hydrogel mixed with calcium phosphate (CaPs) and recombinant human cementum protein 1 (rhCEMP1) also improved periodontal regeneration in a rat model [32]. In another study, PEG was co-polymerized with PLGA to form thermosensitive PLGA-PEG-PLGA hydrogel [6]. This hydrogel, when delivered with PDGF-BB, showed the potential to guide periodontal regeneration by promoting angiogenesis, osteogenic differentiation of PDLSCs and bone healing in a rat model [6]. Other types of hydrogel, such as transglutaminase crosslinked gelatins (TG-gels) [21] and self-assembling peptides (P11-4) [100] have also been showed promising outcome in cell migration, adhesion, and metabolic activity when

tested with PDL cells.

The preferred choice of hydrogel as a carrier of growth factors, cytokines and/or cells is most likely due to the simple preparation of growth factor-loaded or cell-encapsulated hydrogel. However, it should be noted that the release of growth factors loaded in hydrogels can be varied depending on the chemistry, degree of crosslinking, and degradation kinetics of the hydrogel(s) as well as interactions between hydrogel and drug or GF molecules. It is worthwhile noting that release rate can vary significantly between *in vitro* and *in vivo*. Thus, it is important to consider targeted controlled release pattern of bioactive cues delivered through hydrogel-based scaffolds [101]. A primary limitation of hydrogel in tissue engineering application is the weak mechanical stability. To circumvent the barrier to some extent, several studies applied chemical modifications or created hybrid scaffolds with structural polymers [102].

3.5. 3D-printed scaffolds

As an emerging technology, 3D printing allows us to better control macro- and micro-structure of tissue engineering scaffolds [103]. Periodontium is a complex structure consisted of multiple tissue types as soft and hard tissues are integrated. To recapitulate such multi-phase tissue compositions, 3D printing technique has recently been adopted to fabricate scaffolds with regionally variant internal microstructures suitable for CM, PDL and/or AB [4,34]. In addition, different growth factors can also be combined with 3D printed scaffolds to help the regeneration of each tissue in periodontium. Beside the internal microstructure, 3D printing with layer-by-layer deposition enables to create custom-designed scaffold in a specific shape and dimension fitting to the anatomic shape of each periodontal defect [4,5,29,31,34,93,104]. Despite a relatively small number of publications to date, application of 3D printing for periodontal regeneration appears to be growing.

4. Approaches for integrated periodontal regeneration

Recent focus of periodontal regeneration has been mainly on three tissues including PDL, AB, and CM and how effectively and simultaneously regenerate the periodontal defects independent from PDL cells using tissue engineering and scaffold technologies [105]. This integrated periodontal regeneration has been possible based on the four principles: scaffolds, blood supply, cells, and signaling molecules along with periodontal therapy that includes removal of biofilm and reduction of inflammation [106]. With the advancement of scaffold fabrication, cell seeding, and delivery strategies for signaling molecules, various approaches are considered to achieve integrated periodontal regeneration [107]. In order to evaluate advancement of scaffolds used in periodontal regeneration, studies that are published only from 2015 to 2020 were searched and selected fort this review. In this article, the efficacy and effectiveness of the multi layered/phasic scaffolds, cell seeded scaffolds, deliveries of bioactive cues, and gene therapy have been reviewed.

4.1. Multi-phasic scaffolds

Multi-phasic scaffolds can be categorized into two groups: bi- and tri-phases (Fig. 3). Each layer/phase is designed to guide a specific target tissue regeneration. In the integrated periodontium regeneration, of which the target tissues are PDL, AB, and CM, bi- or tri-phasic scaffolds are suitable for the purpose of true periodontium regeneration [108]. Bi-phasic scaffolds have two different phases that can simultaneously target two different tissues: PDL-AB, AB-CM, or PDL-CM. Whereas tri-phasic scaffolds have three phases that simultaneously target three different tissues: PDL-AM-CM.

Compared to traditional GTR materials or single phasic scaffolds, there are advantages of applying multi phasic scaffolds to periodontal tissue regeneration. Spatial and temporal releasing of the multiple signaling molecules that are precisely engineered to the target tissue regeneration can allow direct and prolonged initiation of the regeneration pathway [109]. Periodontal tissue regeneration requires three dimensional spaces for new CM, PDL, AB. Multi-phasic scaffolds can provide the spatial niches where new cells and tissues can effectively harbor and communicate between the cells [108]. Moreover, timely releasing of signaling molecules from the multi-phasic scaffolds could offer more controlled approach to guide regeneration each component of the periodontium [110].

Bi-layered/bi-phasic scaffolds are effective in the integrated periodontal tissue regeneration by inducing formation of CM, PDL, and AB. Huang et al. demonstrated periodontium regeneration including 60.13 ± 22.72 μm of new CM, PDL fibers embedded into the CM, and AB formation by utilizing a biphasic cryogel loaded with enamel matrix derivatives (EMD) for CM and PDL regeneration, and BMP-2 for bone regeneration [111]. Another study from Shi et al. showed that a biphasic calcium phosphate composed of 40% HA and 60% β-TCP seeded with osteogenically induced PDLSC can effectively regenerate periodontal defects [112]. Pilipchuk et al. applied different micro-patterns on surface of PCL film to guide bone and PDL formation and reported that 30 μm groove depth significantly enhanced collagen reorientation suggesting its potential application for PDL [113]. The same group applied the micropatterned scaffolds as combined with delivery of growth factor genes to promote regeneration of bone and PDL-like structure [20].

Tri-phasic scaffolds are a complete set of the integrated periodontium to promote regeneration of CM, PDL, and AB. Chen et al. utilized a multiphasic scaffold consist of rhCEMP1/ACP/PCL/COL and showed that CEMP1 plays an important role for regeneration of CM like tissues in the integrated periodontium regeneration [32]. Sowmya et al.

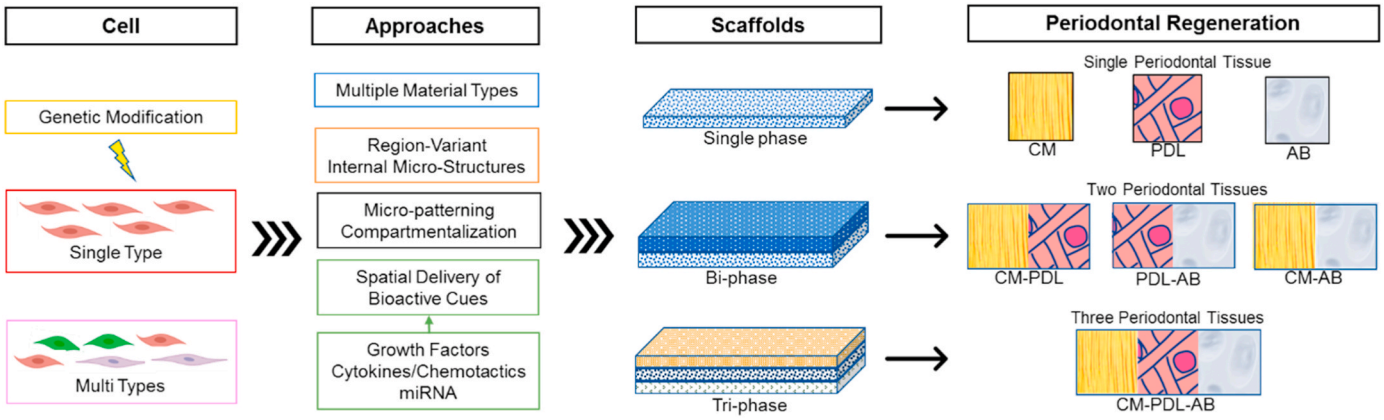

Fig. 3. Approaches for scaffold-based periodontal tissue regeneration in recent published studies from 2015 to 2020 (CM: cementum, PDL: periodontal ligament, AB: alveolar bone).

suggested that tri-layered nanocomposite hydrogel scaffolds that are made of chitin–PLGA/nBGC/CEMP1, chitin–PLGA/FGF2, and chitin–PLGA/nBGC/PRP layers designated for CM, PDL, and AB can effectively perform the integrated periodontal tissue regeneration [114]. Instead of a layered structure, a few previous works applied scaffold compartmentalization to guide formation of CM, PDL and AB using computer-designed structures with different patterns (33).

4.2. Scaffolds seeded with multiple types of cells

Single or multiple cells seeded directly into the multi-phasic scaffold is a viable option to increase success of integrated periodontal regeneration. Cells that are most widely applied in integrated periodontal tissue regeneration are periodontal ligament stem cells (PDLSC) and bone marrow mesenchymal stem cells (BMMSC) as they are directly involved with target tissues [115]. Recent studies showed adipose-derived mesenchymal stem cells (AMSC) and gingival mesenchymal stem cells (GMSCs) also have the potential for periodontal tissue regeneration [24,116]. Since periodontium or periodontal tissue regeneration depends on four key factors (i) cells, (ii) scaffold, (iii) blood supply, and (iv) signaling molecules, advancement of tissue engineering strategies is an important step forward to the successful periodontal tissue regeneration.

Cell loaded scaffolds could have advantages in periodontal tissue regeneration over only scaffolds that relies on natural cells in the periodontium. Zhao et al. showed *in vitro* that when hPDLSCs were seeded into calcium phosphate cement (CPC) scaffold, osteogenic regeneration can be expected [117]. Liu et al. demonstrated the integrated periodontal tissue regeneration that include CM, PDL, and AB by using BMSCs seeded collagen-hydroxyapatite (CH)-based scaffold [118]. Shi et al. showed that human PDLSC seeded biphasic calcium phosphate scaffolds can regenerate the periodontal defects with a new CM, PDL, and AB [112]. Sari et al. showed high level of cell adhesion and cell viability of rat ADMSC with bovine teeth scaffolds for osteogenic bone formation [24]. Diniz et al. suggested that human GMSCs and bone hBMMSCs can be seeded into silver lactate (SL)-containing RGD-coupled alginate hydrogel scaffold to induce osteogenic formation leading to regeneration of AB defects [116]. Pan et al. showed that PLGA-PEG-PLGA hydrogel can be optimal for delivering human PDLSCs that upregulates PDGF-BB [6].

4.3. Delivery systems for bioactive cues

One of the four key factors of the integrated periodontal tissue regeneration is signaling molecules. Bioactive cues such as growth factors can be locally delivered for the regeneration of target tissue [106]. Host immune responses and inflammatory regulations are integral part of homeostasis and regenerative therapy of periodontium and involve various kinds of bioactive cues. Therefore, delivering bioactive cues *via* scaffolds can create a niche for stem cells to be effectively differentiated and increase chances of producing desired periodontal tissue regeneration [109].

Notable advancement of the integrated periodontal tissue regeneration has been demonstrated by recent studies with the discovery of new bioactive cues and better understanding of the mechanism of function of new and existing ones as well. Most widely studied bioactive cues for the periodontal tissue regeneration include amelogenin, BMP-2, and PDGF-BB but also BMP-6, BMP-7, FGF-2, TGF-β1, and IGF-1 [42,54,119–121]. The role of microRNA has also been investigated for its potential use for periodontal tissue regeneration [122]. Delivery of these molecules has also advanced and diversified as the bioactive cues become critical in the periodontal regeneration. Efficacy and effectiveness of various scaffolds with different bioactive cues needs careful examination and evaluation.

Cochrane et al. demonstrated periodontal regeneration by using β-TCP scaffolds as a delivery vehicle for rhFGF-2 in 88 human subjects. Radiographic effectiveness of β-TCP with rhFGF-2 for periodontal regeneration was confirmed in 6 months [81]. Ammar et al. showed that chitosan derived thermo sensitive hydrogel scaffold can be used as a delivery scaffold system for freeze dried platelet concentration from which sustained release of PDGF-BB, IGF-β1, and TGF-β1 were noticed. The gel viscosity reached its highest at 37.5 °C and thus potential use in periodontal tissue regeneration in clinical setting was implied [42]. Duruel et al. demonstrated that chitosan/alginate/PLGA hybrid scaffold can be used as a delivery system and could provide sustained release of IGF-β1 and BMP-6. The study tested efficacy on cementoblast for osteogenic and CM regeneration [54]. Ding et al. studied a scaffold system for *in situ* periodontal tissue regeneration where they demonstrated fast FGF-2 release from the PLGA shell and slow BMP-2 release from the PLLA core [120]. Liu et al. demonstrated periodontal regeneration through Treg pathway using miR-10a and IL-2/TGF-β *via* a scaffold composed of polylactic acid-*co*-glycolic acid microspheres (PLGA MS) for miR-10a/hyperbranched polymer (HP) and mesoporous silica nanoparticles (MSN) for IL-2/TGF-β integrated into PLLA nanofibrous spongy microspheres (NF-SMS) as an injectable carrier [122].

4.4. Gene therapy

Genes of the interests, viral or non-viral vector system, can be transduced or transfected into the target cells such as PDL, osteoblast, fibroblast cells. Plasmids are non-viral vector and considered less invasive since they do not incorporate into cell chromosomes, however effectiveness may be unfavorable [106]. There are two main strategies in gene therapy of the integrated periodontal tissue regeneration: *in vivo* and *ex vivo* delivery. Although *in vivo* delivery is one-step protocol, its therapeutic efficiency is low and possible host immune response is high. On the other hand, *ex vivo* is two-step protocol where cells of interest are biopsied and modified *in vitro* setting, validity of the transduced cells and effectiveness can be high but more costly and labor intensive [123]. Although gene therapy in periodontal tissue regeneration is at the beginning stage and requires more evidence for clinical trials, therapeutic effectiveness of gene therapy in periodontal tissue regeneration can extend possible treatment options and expand chances of integrated functional periodontal tissue regeneration.

Zhang et al. showed efficacy of *in vivo* delivery of mesoporous silk scaffolds loaded with adenovirus for PDFG-B and BMP-7 into periodontal defects of beagle dogs. The result in 8 weeks showed higher degree of regeneration of CM, AB, and PDL than PDGF-B or BMP-7 alone but mostly it was seen horizontal with little vertical regeneration [124]. Jiang et al. demonstrated PDL regeneration *in vivo* from PLGA scaffolds loaded with plasmid FGF-2 for specifically PDL regeneration in the replanted teeth of beagle dogs in 4 weeks [125]. Xie et al. demonstrated that plasmid BMP-2 *via* electronspun PLGA and core PEI scaffold can induce hPDLSC to produce prolonged BMP-2 expression [126]. Pilipchuk et al. showed PDL and bone regeneration *via in vivo* gene delivery of adenovirus transduced BMP-7 and PDGF-BB with micropatterned dual PLGA/PCL scaffold system in 6 weeks of rat models, although no CM regeneration was observed [20].

5. Application targets of scaffolds

Periodontal health is outcome of constant homeostasis between microbiome and host immune response, and the periodontal disease arises from dysbiosis and become manifested in progressive AB loss along with the frequent inflammation [127,128]. More than 40% of population in the U.S. may suffer from periodontitis, and a prevalence of about 796 million cases of periodontal disease in the world has been reported [129,130]. Untreated periodontal disease may lead to persistent gum bleeding and inflammation, chronic or acute periodontal abscess, progressed AB loss, increased tooth mobility, and ultimately tooth loss, which can be detrimental to overall oral and dental health and significantly affect the quality of life [131,132]. Although dental implants have been a promising solution for any tooth replacement,

maintaining natural dentition should be prioritized and thus regeneration of periodontal tissue loss should be promoted when intervention is appropriate [133].

PDL cells are the origin cells of traditional guided tissue regeneration (GTR) technique. From the healthy intact surfaces of the roots, PDL cells slowly migrate over the decontaminated roots where GTR is performed. The cells will become differentiated into cementoblasts, fibroblasts, and osteoblasts [12,13]. However, the conventional GTR is technique sensitive and predictability of successful GTR is largely dependent on bone defect types as PDL cells are the only capable of regenerative initiation [134].

Simplified surgical techniques including minimally invasive surgical techniques have been proposed to overcome the difficulty of periodontal regeneration with a perspective of wound stability and blood supply along with applying biological cues in the recent periodontal regeneration [135–137]. Therefore, multiple application targets incorporating multi strategies of tissue engineering, biological cues, and gene therapies can be a promising solution for clinicians to deliver the precise and reliable periodontal regeneration method and ultimately prevent tooth loss due to periodontal diseases [106].

In this review, total 203 studies were selected out of 873 studies. Searches were from Pubmed, Google Scholar, Web of Science, and EMBASE from 2015 to 2020. As summarized in Fig. 4A, Total 47 studies were identified for multi target application of periodontal regeneration. It was found that 17 studies focus on PDL-AB, one study on PDL-CM, one study for CM-AB, and 28 studies on PDL-CM-AB. The result indicates that limited studies were found for CM-AB or CM-PDL studies, but most application targets have been moved toward to PDL-AB and PDL-CM-AB, implying that efforts and trends are shifting for complete periodontal regeneration as periodontal regeneration therapy begins to incorporate multiple strategies and methods.

6. Animal models in periodontal regeneration

Periodontal disease is unique in which not all teeth or dental implants undergo the same progression of the disease in one's mouth, meaning that the disease is rather tooth and site specific than patient specific unlike other systemic diseases [1]. This complexity of the disease arises from biological consequences between homeostasis and dysbiosis in respect to host immune response and microbiome, and other environmental, behavioral, genetic, and epigenetic risk factors may contribute to this process [127]. Due to its multifaceted nature, establishing a reliable and clinically relevant pre-clinical animal model is critical for development of regenerative strategies for periodontal tissues [138–141].

Kantarci et al. reviewed advantages and disadvantages of animal models for periodontal and peri-implant disease studies [141]. In general, smaller animals have advantages such as low cost, tendency to have higher inflammatory response, and accessibility to transgenic manipulation. Disadvantages include dissimilarities of dentition and dental/oral anatomy compared to human. Popular small animals are rats, mice and rabbits. On the other hand, lager animals have advantages include similarities of dentition and dental/oral anatomy to those of human. Disadvantages include high cost, necessity for special facility, susceptibility to other diseases and infections, resistance and predisposition to the periodontal and peri-implant disease, and more restrictions and regulation for ethical reasons compared to smaller animals [141].

Non-human primates have been the first choice for periodontal regeneration studies. Caton et al. suggested non-human primate models as a periodontal regeneration study model due to their anatomical and physiological similarities to human. For histologic studies, non-human primates, especially *Rhesus* monkeys, are in advantageous to demonstrate quantifiable analysis of CM, PDL, and AB regeneration [139]. However, they are prone to other infectious diseases, less control over post-operative trauma, and high cost of maintenance [138]. Amir et al. demonstrated potential use of PDL cell sheets and RGD chitosan scaffold for periodontal regeneration including PDL, AB, and CM in *M. nemestrina* monkey model [142].

Dogs, mostly Beagles, have been largely used as a periodontal pathogenesis and regeneration model [9,143,144]. Beagle dogs have anerobic microbiome similar to human and thus prone to periodontitis [145,146]. Wikesjo et al. demonstrated that dogs can be used as a surgical periodontal disease model [140]. Various biomaterials including BMPs, EMD, and graft materials have been studied in dog models [147–150]. However, Giannobile et al. demonstrated that dogs have a faster bone remodeling rate compared to non-human primates, and thus this could be a limitation of dog models in periodontal regenerative studies [151].

Miniature pig is a good alternative animal model to dogs due to the similarities of its physiology and periodontium structure to human [152]. Its bone remodeling and composition are the most similar to human compared to all the other existing animal models [153]. Miniature pig models have been utilized for applications and studies including EMD, platelet concentration, growth factors such as rhBMP-7, implant related guided bone regeneration using alloplasts, autogenous grafts, and replantation [154–158]. Miniature pigs are considered relatively more expensive than small animals and more affordable than large animals, but there are still less studies available [146]. Fawzy El-Sayed et al. demonstrated periodontal regeneration of PDL, CM, and AB with gingival margin derived stem cell (G-MSC) in conjunction with IL-1ra loaded hydrogel synthetic extracellular matrix in miniature pig model [159].

Rabbit has been widely used in periodontal regeneration and peri-implant studies. However, its bone composition and remodeling rate is somewhat different to human and most studies were done in long bone which shares less common to AB and periodontal regeneration and thus more suitable for bone healing study model [153,160]. Also, Oortgiesen et al. demonstrated that rabbit is less suitable for regeneration of PDL due to its rapid occlusal eruption [161].

Rat model was proposed as a suitable periodontal surgical model for periodontal regeneration studies using fenestration defects of their molars [162,163]. However, due to continuous eruption and hard tissue

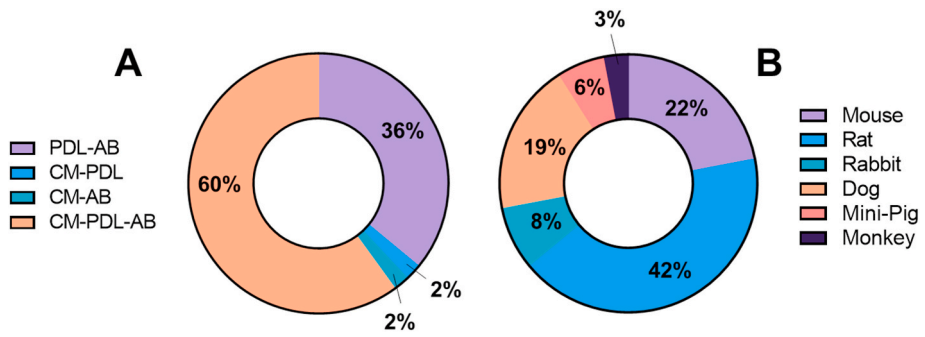

Fig. 4. Distribution of application targets (**A**) and animal models (**B**) in recent periodontal regeneration studies from 2015 to 2020.

formation at the root surface in rats predispose results of actual periodontal regeneration [160]. Mice, another rodent model, is a popular animal model in periodontal regeneration studies. They are cost-effective, the full genome sequence is available, and thus it is easier to manipulate genetic encodings for specific target genes [141]. Nevertheless, similar to rat model, occlusal wearing and eruption, and hard tissue formation at the root surface may affect results of periodontal regeneration studies and therefore not a suitable periodontal regeneration research model [160] Moreover, small size of the anatomy makes surgical intervention difficult [146]. Key advantages and disadvantages of each animal model are summarized in Table 3.

Of the search from 2015 to 2020, the majority of studies were based on small animal models including rat, mice, dog, rabbit, and miniature pig in descending order (Fig. 4B). Rodent was used in 64% of the searched studies, and most studies were dog or rodent models. The proportion of animal model used in periodontal regeneration studies in use of scaffolds shows that although rodents may not be most suitable for periodontal regeneration studies, they are still considered most popular study model. Only small number of studies had large animals such as monkey even though this animal model is considered the most suitable study model for periodontal regeneration. Dog model has been still widely used, and miniature pig model becomes an emerging animal model for periodontal regeneration research.

Table 3
Comparison of animal study models in periodontal regeneration.

Animal Model	Structural similarity to human	Cost-Effectiveness	Suitability for periodontal regeneration	Remarks
Non-human primate	High	Low	High	Most similar dental structure and bone remodeling rate to human and thus most suitable model for periodontal regenerative studies; Highest level of cost and care for the animals; Ethical issues
Miniature Pig	High	Medium	High	Similar physiology and periodontium structure, similar bone composition and remodeling rate to human; Relatively high cost
Dog	Medium	Medium	Medium	Similar anerobic microbial composition to human; Faster bone remodeling rate than non-human primates; Ethical issues
Rabbit	Low	Medium	Low	Relatively low cost; Dissimilar bone composition and remodeling rate to human, Less suitable for PDL regeneration due to rapid teeth eruption
Rat and Mice	Medium	High	Low	Lowest level of cost and care; Accessible genetic manipulation; Similar periodontium structure to human; Less suitable for periodontal regeneration due to constant occlusal wear and hard tissue apposition to root surface.

7. Peri-implant regeneration

Peri-implant disease has gained attention due to increased prevalence of peri-implantitis in the recent years [164,165]. Total 10–40% of implants are at risk of peri-implantitis, which involves progressive bone loss around the implants and ultimately leading to implant loss [166,167]. Risk factors of peri-implantitis including smoking habits, diabetes and existing other systemic conditions, lack of prophylactic dental care, and a history of periodontitis may increase risk of peri-implantitis [168]. Current treatment of peri-implantitis is based on guided bone regeneration (GBR) technique because dental implants lack PDL and CM but only AB tissue is osseointegrated into the dental implant surfaces [169] Success in non-surgical or surgical aspects of peri-implantitis treatments depends on detoxification and decontamination of the contaminated rough implant surfaces, and this has been the most challenging part of the treatment, and thus re-osseointegration through GBR technique has been still unpredictable and questionable [170–173].

Dental implants are vulnerable to bacterial penetration through the gingival adhesion in peri-implant tissue and to attachment of bacterial endotoxins onto the rough implant surfaces when implant surfaces become contaminated [164,174,175]. This may be due to lacking periodontal fibers directly attached to CM and thus progression of bone loss is more aggressive in peri-implantitis compared to periodontitis [176,177]. Due to this limitation of current dental implants, ideas of peri-implant regeneration including PDL, CM, and AB using scaffold technologies have been explored, but evidence and clinical implementation are limited at the preliminary stage [178,179].

Few previous studies targeting peri-implantitis include Zhu et al. that investigated efficacy of a layered collagen-cell aggregate scaffold technique with periodontal ligament stem cells (PDLSCs) and Jawbone-derived Mesenchymal Stem Cells (JBMSCs) on regeneration of PDL- and CM-like tissues in mice and miniature pig models [178]. However, *de novo* PDL-like tissue was parallel to the titanium surface rather than forming perpendicular attachment, and formation of CM-like structure and bone regeneration appears to warrant further improvement [178]. Similarly, Kammerer et al. used GBR technique for bone regeneration of circumferential defects in peri-implantitis sites created in a miniature pig model [179]. As result, the delivery of PDLSCs mixed with collagen powder and PDGF revealed lower bone regeneration in term of new bone height than collage powder alone on Ca-P coated implants. Also, there was no significant difference in new bone height between control and experimental groups [179]. Despite the suboptimal *in vivo* outcome from PDLSCs and selected biomaterials, the above-mentioned previous works represent significant progress in our efforts to develop a regenerative therapy for peri-implantitis.

8. Clinical application of scaffolds for periodontal tissue regeneration

The ultimate goal of research on all biomaterials is for safe and effective clinical use. Although significant efforts have been made to develop clinically functional scaffold for periodontal regeneration, there are only few reports of application of biomaterials in clinical practice for periodontitis treatment (Table 4).

In 2015, Rasperini et al. investigated the first clinical case in which a 3D printed scaffold was applied to a patient's periodontal defect [29]. A

Table 4
Clinical applications of scaffolds for periodontal tissue regeneration.

Type of scaffold	Bioactive agents	Research design	Follow-up duration	Number of patients	Outcomes	Ref
3D printed PCL scaffold	rhPDGF-BB	Clinical case report, scaffold implantation after scaling and root planning	14 months	1	The implanted 3D scaffold served to fill the human periodontal osseous defect without signs of chronic inflammation or dehiscence. However, the implanted scaffold became exposed at 13 months, followed by a graft exposure 3 mm below the gingival margin. After removal of the exposed part of the graft, the site showed a larger dehiscence and wound failure, necessitating entire scaffold removal.	[29]
3D woven-fabric PLLA scaffold	MSC and PRP	Monocenter clinical trial; Implantation of scaffolds with MSC and PRP after phase I/II therapy	36 months	10	Clinical attachment level, pocket depth, and linear bone growth (LBG) were improved during the entire follow-up period. No clinical safety problems attributable to the investigational MSCs were identified.	[180]
β-TCP	rhFGF-2	Multicenter randomized controlled clinical trial; double-blinded; randomized to 1 of 4 treatment groups—β-TCP alone (control) and 0.1% recombinant human FGF-2 (rh-FGF-2), 0.3% rh-FGF-2, and 0.4% rh-FGF-2 with β-TCP—following scaling and root planing with EDTA	6 months	88	0.3% and 0.4% rh-FGF2/β-TCP groups showed significant improvements and 71% success rate at 6 months. Percentage bone fills of control, 0.1%, 0.3% and 0.4% group were 61%, 63%, 73% and 71%, respectively. No serious adverse events related to the products were reported.	[81]
Zn-substituted monetite-based scaffold	None	Randomized controlled clinical trial (split-mouth, double-blind); test group - open flap debridement (OFD) with Sil-Oss®, and control group - OFD with hydroxyapatite (HA) bone graft.	9 months	30	Zn-substituted monetite-based scaffold group (Sil-Oss®) exhibited a significant bone fill and the percentage of tissue mineralization compared to HA at 3 and 6 months. However, there were no significant differences in clinical attachment level and probing depth at 6 months	[182]
β-TCP, Autologous PDL-derived cell sheets	None	A single-arm and single-institute clinical study; bony defects were filled with β-TCP granules & 3-layered PDL-derived cell sheets following standard flap surgeries	6 months	10	Mean reduction of periodontal probing depth was 3.2 ± 1.9 mm. Mean clinical attachment gain was 2.5 ± 2.6 mm, and average increase of radiographic bone height was 2.3 ± 1.8 mm. Clinical improvements were maintained during a mean follow-up period. No serious adverse events were observed.	[183]
PCL scaffold, human umbilical cord mesenchymal stem cells	None	Randomized control clinical study; A patient of multiple gingival recession (Miller's Class II) was selected	6 months	N/A	Root recession was significantly reduced (over 80% root coverage).	[181]
β-TCP	None	Randomized clinical and biochemical trial; group I: β-calcium triphosphate (β-TCP) with collagen membrane, group II: cultured gingival fibroblasts (GF) on the β-TCP scaffold with collagen membrane.	6 months	20	Group II showed significantly higher reduction in vertical pocket depth (VPD), clinical attachment level (CAL) gain and radiographic bone gain than group I. The concentration of PDGF-BB in group II was significantly higher on 1, 3, 7 days than group I.	[27]
Demineralized porcine bone matrix (DPBM)	Enamel matrix derivatives (EMD)	Randomized clinical trial; group1: DPBM with EMD, group 2: DPBM only	24 months	42	Although both groups showed clinically and radiographically significant improvement, there were no statistically significant differences between 2 groups.	[28]

3D printed PCL scaffold containing rhPDGF-BB was implanted in a patient's one wall bony defect and the outcome was followed up by 14 months. Unfortunately, the implanted scaffold was exposed intraorally at 13 months so the exposed site was covered with amelogenin and cyanoacrylate after the removal of a few scaffold remnants. Finally, they reported a 3 mm attachment gain and 75.9% of the scaffold remained after 14 months with mainly connective tissue healing and minimal evidence of bone repair [29]. Although the clinical outcome needs further improvement, the use of 3D printed scaffold with different spaces for PDL and AB was a meaningful attempt to suggest the direction to go forward for complete periodontal regeneration. The following year, a biodegradable 3D woven fabric composite scaffold containing autologous mesenchymal stem cells with platelet rich plasma was applied for the treatment of infrabony defects in 10 patients. The clinical outcome by 36 months indicated an average 4.7 mm of linear bone growth [180]. More recently, Kadam et al. treated multiple gingival recession using PCL scaffold and human umbilical cord delivered MSCs and reported an 80% root coverage and bone regeneration after 6 months [181].

The aforementioned clinical studies are mostly focused on AB regeneration [27,28,81]. Given the complexity of periodontium as an integrated multi-tissue unit, it is expected to be more challenging for clinical translation of regenerative strategies for periodontium as compared to approaches focused on AB healing. Nonetheless, we envision the significant recent research progress to be translated into clinically available treatment option for patients with periodontitis in near future.

9. Outstanding challenges and future perspectives

As discussed above, our efforts to improve the clinical outcome of periodontitis treatment have made meaningful progress in recent years. The tremendous research accomplishment made in tissue engineering and regenerative medicine for various tissues and organs has allowed the dental research community to adopt many of the potential approaches for development of regenerative strategies for periodontal

regeneration. These ongoing efforts have shown potential in development of clinically applicable strategies to regenerate not only single type of periodontal tissue but also integrated periodontium with multiple soft and hard tissues that is likely critical for successful function restoration of teeth affected by periodontitis.

A notable progress in periodontal regeneration through tissue engineering approaches is the recent advances in design and construction of efficient biomaterial scaffolds. Traditionally, most biomaterials in periodontics have been limited to GTR membrane. Current state-of-art in periodontal scaffolds is beyond the enhanced alternative to the traditional GTR membrane and rather moving forward to guiding formation of multiple tissues in periodontium with the ultimate goal to regenerate functional periodontium after significant tissue damages by periodontitis. Advanced scaffold fabrication technologies such as 3D printing enabled us to apply different micro-structures in different regions of micro-scale scaffolds suitable for guiding formation of each type of tissues. The research progress in controlled delivery system has shown potential to provide a timely and spatially controlled delivery of multiple bioactive cues as incorporated in scaffolds.

Despite the promising improvement, to date, there has been no scaffold system that resulted in successful clinical outcome in periodontal regeneration. One of the outstanding challenges in clinical translation of the scaffolds for periodontal regeneration is the limited understanding of *in vivo* degradation of implanted materials. The abovementioned scaffold biomaterials are mostly biodegradable that means the implanted scaffolds undergo degradation in body as replaced by newly forming tissue. Most of hydrogels degrade relatively fast and synthetic polymers such as PLA, PGA, PLGA and PCL shows slower degradation with some degree of controllability [184–188]. The degradation rate of those biomaterials has been well characterized *in vitro* but such degradation can be quite different *in vivo* due to biochemical environment associated with blood supply, inflammation and metabolism [184–188]. However, no previous studies examined *in vivo* degradation of implanted scaffolds in order to balance the degradation with regeneration. Fortunately, a couple of advanced imaging modalities incorporated with biomaterial scaffolds are being developed that potentially allows non-invasive tracking of *in vivo* degradation of scaffolds [188,189]. Application of such imaging technology will be beneficial to optimize the scaffold composition and structure that lead to *in vivo* degradation balanced with periodontal regeneration.

Our limited understanding of periodontal biology can be another translational hurdle. Many previous scaffolds were incorporated with bioactive cues including growth factors and cytokines. Some of the bioactive cues were to recruit and stimulate host cells (e.g. PDLSCs) into scaffolds and/or to induce their differentiation. Although many studies focused on PDLSCs, known to differentiate into CM as well as PDL, the endogenous PDLSCs likely had long been exposed to severe inflammation caused by periodontitis that may have alternated their regenerative potential. Moreover, the current periodontitis treatment, as a default, involves the removal of affected periodontal tissues including PDL. Thus, it is important to consider what endogenous cell sources need to be targeted to scaffold-guided periodontal regeneration. Recently emerged CRISPR technology may have potential to circumvent this hurdle. CRISPR gene activation (CRSIPRa) can be used to activate specific transcriptional factors which are deliverable *in vivo* through biomaterial scaffolds. Delivery of CRISPRa agents with specifically designed guide RNA have potential to induce endogenous transcriptional networks in adjacent cells *in vivo*, consequently transforming them into PDLSCs [190,191].

In addition, a more precisely controlled delivery may be necessary to better orchestrate the regeneration process for multiple periodontal tissues. It is critical to control the timing of the bioactivities of anti-inflammatory cues as inflammation is a necessary step in the timely controlled tissue healing process. Similarly, growth factors to simulate differentiation and tissue formation needs to be timely controlled. The previous delivery modes used in periodontal scaffolds such as simple adsorption and loaded in polymer are not suitable for precise release control. We may consider the advanced control-delivery systems such as layer-by-layer fabricated nanofilm [192] and core-shell nanoparticles [193] providing sequentially controlled release of multiple factors. A number of emerging hydrogel-based delivery systems also allowed timely and/or stimulation-sensitive release of multiple factors that can be incorporated into various scaffold structures [194].

Periodontium is a complex multi-tissue structure and a need for its regeneration is inevitably linked with severe/chronic inflammation by periodontitis. Despite these challenging features, our continuous attempts have made significant progress in development of bioactive scaffolds with potential to regenerate periodontal tissues. Active interdisciplinary collaborations between biomaterial scientist, biologists, chemical engineers and clinicians will serve as a key catalyst potentially leading us toward functional regeneration of periodontal tissues.

Author contribution

H.N.W. wrote section 4–8, Y.J.C. wrote sections 2–3, S.T. edited entire manuscript in perspective of biomaterials, and C.H.L. was responsible for overall construction of manuscript, write-up for sections 1 and 9, and edit and finalization of manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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