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GROWTH FACTOR LOADED BIOMATERIALS FOR PERIODONTAL TISSUE REGENERATION: A SYSTEMATIC REVIEW OF PRECLINICAL STUDIES ON ANIMAL MODELS

Z RASTNIMI FAKTORJI OBOGATENI BIOMATERIALI ZA REGENERACIJO OBZOBNIH TKIV: SISTEMATSKI PREGLED PREDKLINIČNIH RAZISKAV NA ŽIVALSKIH MODELIH

Urška Romih[1,2*], Rok Schara[1,2]

[1]University medical center Ljubljana, Department of Oral Medicine and Periodontology, Hrvatski trg 6, 1000 Ljubljana, Slovenia
[2]Unversity of Ljubljana, Faculty of Medicine, Department of Oral Medicine and Periodontology, Vrazov trg 2, 1000 Ljubljana, Slovenia

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Over the past decade, numerous biomaterials have been developed for periodontal tissue regeneration. The aim of this systematic review was to evaluate the application of growth factor–loaded biomaterials for the regeneration of the periodontal complex in preclinical animal studies. The review was conducted in accordance with PRISMA guidelines. A computerized search of SCOPUS, PubMed, and Web of Science was performed, including English-language articles published between 2015 and 2025. From an initial yield of 173 articles, 12 studies met the inclusion criteria after screening titles, abstracts, and full texts. All included studies demonstrated successful regeneration of the three key periodontal tissues – alveolar bone, cementum, and periodontal ligament – in animal models. With one exception, the studies were short-term, with histological evaluations performed up to 12 weeks. The biomaterials used ranged from relatively simple systems to complex multiphasic scaffolds. Six studies employed biomaterials loaded with a single growth factor, while the remaining six used combinations of two or more growth factors. Fibroblast growth factor (FGF) was the most frequently used growth factor, followed by bone morphogenetic proteins (BMPs). The findings indicate that scaffolds are the most commonly used biomaterial platform, and that FGF- and BMP-based systems are predominant. Notably, simpler biomaterial and growth factor combinations often achieve regenerative outcomes comparable to more complex scaffold designs but remain robust, especially when the goal is functional regeneration.

Keywords: biomaterials, tissue regeneration, growth factor, scaffold

V zadnjem desetletju je bilo razvitih veliko novih biomaterialov za regeneracijo obzobnih tkiv. Avtorja v tem članku zato predstavljata sistematični pregled in analizo uporabe biomaterialov obogatenih z rastnimi faktorji, za regeneracijo obzobnih tkiv, v predkliničnih študijah na živalskih modelih. Pregled sta avtorja izvedla v skladu s smernicami PRISMA. Računalniško iskanje literature sta opravila v bazah SCOPUS, PubMed in Web of Science, ki so vključevale članke v angleškem jeziku, objavljene med letoma 2015 in 2025. Od začetnih 173 najdenih člankov, sta po pregledu naslovov, povzetkov in celotnih besedil je merila za vključitev izpolnjevalo 12 raziskav. Vse vključene raziskave so pri živalskih modelih pokazale uspešno regeneracijo alveolarne kosti, cementa in parodontalnega ligamenta. Z izjemo ene so bile študije kratkoročne, s histološko analizo po 12 tednih. Uporabljeni so bili tako enostavni biomateriali kot kompleksna, večfazna ogrodja. Šest študij je uporabilo biomateriale z enim rastnim faktorjem, preostalih šest pa kombinacijo dveh ali več rastnih faktorjev. Najpogosteje uporabljen biomaterial je bilo ogrodje, najpogosteje uporabljen rastni faktor pa fibroblastni rastni faktor (FGF), sledili so kostni morfogenetski proteini (BMP). Enostavnejše kombinacije biomaterialov in rastnih faktorjev so pogosto dosegle primerljive regenerativne učinke kot kompleksnejša ogrodja, vendar manj idealne, ko je bil cilj funkcionalna regeneracija.

Ključne beside: biomateriali, tkivna regeneracija, rastni faktor, ogrodje

1 INTRODUCTION

Periodontium is an integrated and functional unit of alveolar bone (AB), periodontal ligament (PDL) and cementum (CM). Various biomaterials and methods have been used to support the treatment of periodontal defects.

Most commonly used tissue regeneration procedure in clinical practice is guided tissue regeneration (GTR), with the use of barrier membranes. These membranes prevent epithelial tissue ingrowth and provide space for the tissue to regenerate. We can use them together with scaffolds. Scaffolds are used in tissue engineering primarily as substrates for cell attachment, tissue ingrowth and initial structural support. Various scaffolds can also exhibit membrane like properties. Scaffolds can be made from natural materials (i.e., collagen, chitosan, silk), bioceramics and synthetic polymers (i.e., PLLA, PLGA). The use of bioactive ceramics (bioceramics), such as hydroxyapatite (HA), bioactive glass ceramic (BGC) and β tricalcium phosphate (βTCP), in polymer membranes has a positive impact on mineralization and cellular activity. In addition, bioceramics can improve the mechanical properties and neutralize the acidic derivates produced by the degradation of polymers.[1]

*Corresponding author's e-mail:
urska.skof.02@gmail.com (Urška Romih)

For integrated periodontal regeneration, bi- or triphasic scaffolds are a suitable solution because they provide spatial niches, in which new cells can harbour and communicate as regeneration requires three-dimensional spaces for CM, PDL and AB. Different techniques are available to fabricate such products. Among them are the established cryogel technique for macroporous scaffolds and electrospinning method for the fabrication of microporous structures. To enhance tissue regeneration, cells or bioactive molecules such as antibiotics or growth factors can be seeded on these engineered constructs.[2] To overcome problems with irregular periodontal defects, new scaffold biomaterials are being fabricated using different methods, with hydrogels being a popular option. Hydrogels are cross-linked polymers that can be injected into defect areas.[3] They are easily prepared as carriers of bioactive molecules and/or cells.[2] Recent studies showed that induced pluripotent stem cells (iPSCs) derived mesenchymal stem cells may facilitate the repair of periodontal defects by increasing regeneration and the production of newly formed mineralized tissues.[4] Furthermore, an effective alternative for delivery of growth factor proteins is gene therapy. In this field, experiments utilize viral or non-viral vector systems that can be used to transduce or transfect genes of interest into target cells.[2] Adenovirus vectors (Ad) may circumvent many of the limitations of protein delivery by exhibiting high in vivo transduction efficiency with a relatively short expression period[5] making them well suitable for regeneration without eliciting long-term health concerns.[6]

Growth factors (GFs) play an essential role in tissue formation. They modulate cellular activity as they can provide cells with a stimulus for proliferation, migration and differentiation. Periodontal tissue regeneration can be enhanced by exposing the treated area to GFs, such as platelet-derived GF (PDGF), fibroblast GF-2 (FGF-2), transforming GF1 (TGF1), and bone morphogenetic proteins (BMPs).[7] Concentrated growth factor (CGF) belongs to a novel generation of platelet concentrate products. Made from platelet-rich plasma (PRP), it is rich in various endogenous growth factors and can potentially participate in the regeneration of soft tissues. Most GFs have short half-lives and narrow therapeutic windows. Natural biomaterials such as heparin and heparan sulfate glycosaminoglycans in the ECM have binding domains that exert a strong interaction with bioactive molecules. This binding protects bioactive molecules from denaturation and proteolytic degradation, consequently prolonging sustained release.[3]

To our knowledge, this is the first comprehensive review of preclinical studies on animal models to synthesize current research on growth factor-loaded biomaterials for periodontal tissue regeneration. It offers researchers foundation for preclinical optimization and facilitates the translation of these models into human clinical trials.

Our questions are: Which are the latest growth factor-loaded biomaterials that have successfully regenerated all three tissues of the periodontal complex (alveolar bone, cementum and periodontal ligament) in animal models with experimental periodontal defects? Which primary materials and which growth factors were used?

The aim of this systematic review is to guide researchers in potential new preclinical studies and in the evaluation of biomaterials in human clinical trials. This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[8]

2 EXPERIMENTAL PART

2.1 Inclusion and exclusion criteria

Inclusion criteria: articles written in English, publication date between 2015–2025, animal models with experimental periodontal defects, control group, application of biomaterials with growth factors, simultaneous regeneration of alveolar bone, cementum and periodontal ligament

Exclusion criteria: review articles, book chapters, studies without a control group, the three periodontal tissues (periodontal ligament, alveolar bone, cementum) not being regenerated, in vitro studies, pilot studies, animal studies without experimental periodontal defects, human trials, nonrelevant studies

2.2 Information sources

In order to identify the most up-to-date research regarding the growth factor-loaded biomaterials for periodontal tissue regeneration in animal models, we used selected databases: PubMed, Web of Science and Scopus. The following search algorithms were used: for Scopus TITLE-ABS-KEY ((alveolar_bone) AND (periodontal_ligament) AND (cementum) AND (regeneration) AND (growth_factor OR growth_factors OR FGF OR BMP OR PDGF)) AND PUBYEAR > 2014 AND PUBYEAR < 2026 AND PUBYEAR > 2014 AND PUBYEAR < 2026 AND (LIMIT-TO (LANGUAGE, "English")); for PubMed: ("periodontal ligament"[MeSH Terms] OR "periodontal ligament" [Title/Abstract]) AND ("alveolar bone" [MeSH Terms] OR "alveolar bone"[Title/Abstract]) AND ("cementum" [MeSH Terms] OR cementum [Title/Abstract]) AND ("regeneration" [MeSH Terms] OR regeneration [Title/Abstract]) AND ("growth factor*" [MeSH Terms] OR "growth factor*" [Title/Abstract] OR "BMP" [MeSH Terms] OR "BMP" [Title/Abstract] OR "FGF" [MeSH Terms] OR "FGF" [Title/Abstract] OR "PDGF" [MeSH Terms] OR "PDGF" [Title/Abstract]); and for Web of Science: TS=("alveolar bone") AND TS=("periodontal ligament") AND TS=(cementum) AND TS=(regenerat*) AND TS=(growth factor* OR BMP OR FGF OR PDGF).

2.3 Research selection and recording methods

The study selection was assessed by two reviewers (U.Š., R.S.) separately. Studies were screened by title, abstract and ultimately by full text reading. Qualified articles were chosen on the basis of inclusion and exclusion criteria and imported in the software Rayyan®. Both reviewers (U.Š., R.S.) contributed in the data processing stage. The following information was retrieved from each study: animal species, animal sex, number of animals, number of groups, number of periodontal defects in each animal, types of periodontal defects, types of biomaterials used, types of control groups, duration from application to histologic evaluation, methods for evidence control, results.

2.4 Risk of bias assessment

The methodological quality and risk of bias of the included studies were independently evaluated by two reviewers, who were blinded to the authors' names and institutional affiliations. Any disagreements were resolved through discussion and consensus. The risk of bias was assessed using the SYRCLE risk of bias tool, specifically designed for animal studies.

3 RESULTS

3.1 Study selection

The computerized search strategy yielded 173 citations, 38 of which were articles from PubMed, 70 from Scopus, and 65 from Web of Science. No additional studies were retrieved through manual searches of the reference lists or internet searches in Google Scholar. 173 records were screened by title and abstract. Furthermore, full text was screened in ordered to check the inclusion/exclusion criteria. 26 articles remained, of which 14 records were duplicates. 12 articles met the full reading criteria.

The flowchart used for the selection of the studies is shown in **Figure 1**.

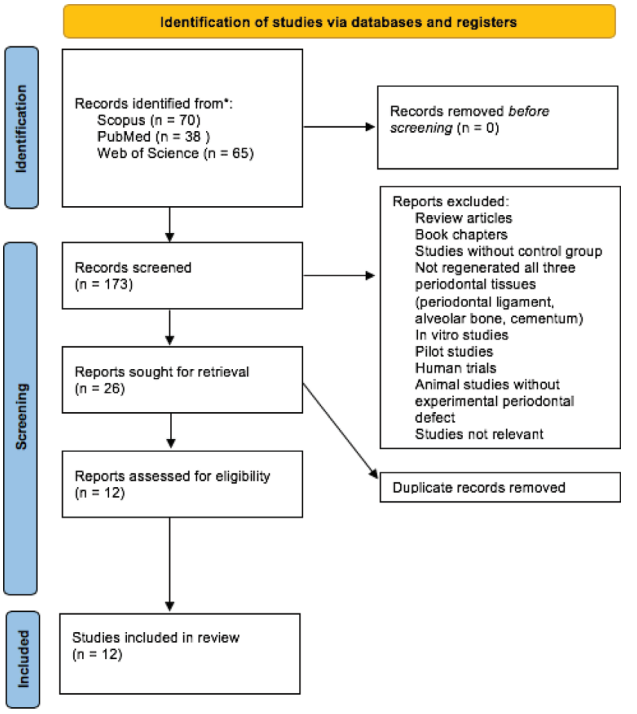

Figure 1: Simplified search strategy

3.2 Quality assessment

Based on the SYRCLE risk-of-bias assessment, the reporting quality of randomization, blinding, and housing remains limited in many preclinical animal studies, consistent with trends in the field. Most studies avoided attrition and selective reporting biases, strengthening the confidence in their reported outcomes. Only a few studies (e.g., Zhang et al., 2015; Matsuse et al., 2017; Momose et al., 2016; Ding et al., 2020) consistently scored low risk across most domains, making them relatively more robust methodologically. Risk of bias is presented in **Table 1**.

3.3 Study characteristics

All twelve studies successfully demonstrated the regeneration of all three tissues of the periodontal complex

STUDY (AUTHOR, YEAR)	1. Random Sequence Generation	2. Baseline Characteristics	3. Allocation Concealment	4. Random Housing	5. Caregiver Blinding	6. Random Outcome Assessment	7. Outcome Assessor Blinding	8. Incomplete Data	9. Selective Reporting	10. Other Bias
Zhang et al., 2015	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Matsuse et al., 2017	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Momose et al., 2016	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Sowmya et al., 2017	Low	Low	Unclear	High	Unclear	Unclear	Unclear	Low	Low	Low
Chien et al., 2018	High	Low	High	Unclear	Unclear	Unclear	Low	Low	Low	Low
Hua et al., 2023	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Low
Yu et al., 2022	Unclear	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Anzai et al., 2016	Unclear	Low	Unclear	Unclear	High	Unclear	High	Low	Low	High
Nagayasu-Tanaka et al., 2015	Unclear	Unclear	Unclear	Unclear	High	Unclear	Low	Low	Low	High
Huang et al., 2020	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Kiyota et al., 2024	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Ding et al., 2020	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Low

Table 1: Risk of bias

Table 2: Study characteristicsBiomaterials tested

STUDY	SPECIES		N AN.	N GR.	N DEF/ AN.	DEFECT TYPE	BIOMATERIAL		CONTROL GR.	TIME to histo.	EVIDENCE	RESULTS
	Animal	Sex				Primary	Growth factor					
Anzai et al. 2016	Beagle	F	24	3	2	2-wall intrab. (surgically created model)	3% hydroxyl propyl solution (HPC)	FGF-2	1: negative control – only 3% HPC 2: blank group: animals without defect	13mo	histology (Azan staining), micro–CT	Height of the newly formed bone, length of the newly formed PDL, length of the newly formed cementum, and distance to the junctional epiyhelium of the FGF-2 group were significantly higher than those of the control group (p = 0.003, p = 0.001, p = 0.004, and p = 0.010).
Yu et al. 2022	Sprague-Dawley rats	M	40	4	1	fenestration defect	intrafibrillarly mineralized collagen (IMC) scaffold + Bio-Gide	CGF (concentrated growth factor)	1: CGF + Bio-Gide 2: CGF-DBBM + Bio-Gide (deproteinized bovine bone minerals) 3: blank gr.: Bio-Gide – self healing	8w	histology (HE and Masson's trichrome staining), micro–CT	The amount of new alveolar bone in the distal root defect region of the first molar in the CGF/IMC group (61.49% ± 7.39%) was significantly greater than those in the CGF–DBBM (32.37% ± 5.14%), CGF (35.41% ± 8.75%), and blank (1.84% ± 3.19%) groups. The trend for newly formed cementum was consistent with that for alveolar bone. More new PDL (71.29% ± 19.12%) was observed in the CGF/IMC group than in the CGF-DBBM (55.72% ± 5.23%), CGF (47.77% ± 3.89%), and blank (6.34% ± 10.99%) groups. The proportion of OCN, VEGFR1, TGFb1 and Smad3 was much higher in the CGF/IMC group than in controls.
Matsuse et al. 2017	Beagle	U	6	2	3	2-wall intrab.	porous á-TCP + heparin	bFGF	unmodified porous á-TCP	2w, 4w, 8w	histology (HE, Azan staining), micro–CT	The ratios of new bone area/total area (BA/TA), new cementum (NC)/total height (TH) (%), and new PDL (NP)/ total height (TA) (%) of each group were measured at 4 weeks, and they were significantly higher in the bFGF group compared to controls. (p < 0.05).
Nagayasu-Tanaka et al. 2015	Beagle	F	4	2	2	3-wall intrab.	3% HPC	FGF-2	3% HPC	3d, 7d, 14d, 28d	histology (HE, Azan staining), immunohistochemistry	Promotion of normal tissue formation induced by FGF-2 at only 7 days leads to enhanced new AB, C, and PDL. Height of the new bone in the FGF-2 group was after 7 days significantly larger and the amount of new cementum and PDL in the FGF-2 group was also higher compared to control group. BMP-2, osterix, ALP and OC expression were increased significantly in the FGF-2 group compared with that in the control group at 7 and 14 days.
Zhang et al. 2015	Beagle	M	5	5	4	buccal dehiscence	mesoporous bioactive glass (MBG)/silk scaffold	AdPDGFB or AdBMP7 or AdPDGFB + AdBMP7	1: control non-filled defects, 2: scaffold alone	8w	histology (HE, Azan staining), micro–CT for bone quality	Scaff. + adPDGF-B demonstrated a significant new formation of the PDL. Scaff + adBMP7 demonstrated a significantly greater new bone formation. Scaff.+ adPDGF-B + adBMP7 demonstrated qualitative features similar to those of native periodontal structures.
Kiyota et al. 2024	Beagle	F	15	3	3	3-wall intrab.	atelocollagen sponge	BDNF 25 ìg/mL, BDNF 50 ìg/mL	1: atelocolag. sponge 2: control non-filled defects	1w, 2w, 4w	histology (Azan staining), immunohisto, real time PCR	4 weeks after application, in the BDNF group, a significant amount of new bone and an adequate width of PDL were observed. In control group, PDL tissue regeneration was insufficient, and cementum regeneration was limited. In the BDNG group, the denuded root surface was almost completely covered with new cementum. OPN expression was much higher in the defect area and along the root surface in the BDNF group compared to control group. Higher concentration (50 µg/mL) is considered to be effective for periodontal tissue regeneration.
Momose et al. 2016	Beagle	F	6	3	6	buccal Class II furcation defects	collagen hydrogel	FGF-2	1: collagen hydrogel 2: control non-implantation	10d, 4w	histology (HE and Masson's trichrome staining)	The mean value of the new bone area of the FGF-2 receiving group was 2 times greater compared with the control group. Reforming well-developed PDL-like tissue and an acellular cementum-like structure with inserted Sharpey's fibers was significantly stimulated by FGF application. Implantation of the FGF-2 loaded scaffold resulted in 70% regeneration of periodontal tissue. In addition, the FGF-2 loaded scaffold consistently suppressed aberrant healing (epithelial down-growth, ankylosis, root resorption).
Sowmya et al. 2017	White New Zealand rabbit	U	12	4	1	unspecified defect	tri-layered nanocomposite hydrogel scaffold: 2 layers of chitin-PLGA/nBGC (for AB and C) and 1 layer of chitin-PLGA for PDL	CEMP1, FGF-2, PRP	1: negative control 2: GTR membrane 3: scaffold without GF	3m	histology, immunohistochemistry, micro–CT	In vivo, complete defect closure and healing with the formation of new cementum, fibrous PDL, and alveolar bone with well-defined trabeculae were more pronounced in defects treated with the tri-layered nanocomposite hydrogel scaffold with growth factors in comparison to the other three groups.
Chien et al. 2018	Sprague-Dawley rats	M	16	4	1	unspecified defect	thermosensitive CHC hydrogel (chytosan/gelatin/glycerol phosphate) + iPSC	BMP6	1: hydrogel + BMP6 2: BMP6 only 3: negative control	42d	histology (HE staining), micro–CT	BMP6 only: new AB; BMP6 + hydrogel: new AB + C; BMP6 + hydrogel + iPSCs: new AB + C + PDL. Only the iPSCs-BMP-6-hydrogel showed significantly greater bone volume refraction relative to the other groups. This group provided significantly higher trabecular numbers, and similar results were observed for the trabecular thickness. Markers of osteoblasts, osteoclasts and PDL formation were all significantly upregulated in the iPSCs-BMP-6-hydrogel group compared to the other groups. IL-8, TNF-á and IL-1 b were down regulated in the iPSCs-BMP-6-hydrogel group compared to the hydrogel-only and BMP-6-hydrogel groups.
Huang et al. 2020	Beagle	M	6	6	6	2-wall intrab.	biphasic cryogel scaffold (BCS): gelatin + gelatin/BTCP/HA (BH), functionally graded membrane (FGM): PLLA on conventional membrane (CM)	EMD-loaded scaffold, BMP2, PDGF-loaded membrane	1: BH-CM 2: BCS-CM 3: BBC-CM 4: BH-FGM 5: BCS-FGM 6: BBC-FGM	12w	histology (staining Alizarin Red and Stevenel's Blue), micro–CT	The biphasic scaffold with GFs (BBS) showed significantly greater osteogenesis ($P < .01$) and early defect fill ($P < .05$) relative to the mono-phasic scaffold of bone phase (BH). FGM significantly promoted osteogenesis ($P < .05$) in BH-treated sites but showed limited benefit in BBS-treated sites. On denuded roots, cementum deposition was evident in BBS-treated sites. Neither ankylosis nor root resorption was noted in any specimen. The ligament-like fibers were obliquely inserted to the root surface in the BBS-CM and BBS-FGM groups but were parallel to the root surface in other groups.
Ding et al. 2020	Wistar rats	U	36	3	1	buccal cortical defect	electrospun PLLA/PLGA framework	bFGF + BMP2	1: PLLA/PLGA 2: neg. control	4w, 8w	histology (HE staining), immunohistochemistry, micro–CT	Bone volume/total volume (BV/TV) of the PLLA/PLGA framework with the GF group was much higher than that of the other two groups at 1 week ($P < 0.05$), 2 weeks ($P < 0.05$), 4 weeks ($P < 0.01$) and 8 weeks. The bone surface/total volume (BS/TV) value showed a similar tendency at 1, 2, 4, and 8 weeks postoperatively ($P < 0.05$). Compared with the angulation of the native mature ligament (47.59 ± 2.99°), all groups demonstrated less angulation at week 4 (P < 0.001), while at week 8, the angulations in the native ligament tissue and PLLA/PLGA with the GF group were very similar (49.87 ± 5.69° vs. 47.59 ± 2.99°) and there was no significant difference between the two groups (P > 0.05). The newly formed cementum was observed in PLLA/PLGA with the GF group at 8 weeks postoperatively; however, no formation of the cementum was observed in the other groups at any time. PLLA/PLGA with the GF group could attract more host-derived MSCs to defects in the early stage of wound healing. Furthermore, this group showed more ALP expression after 1 and 2 weeks and more OCN expression after 4 and 8 weeks of bone repair compared with the other two groups (P < 0.05). The expression level of Col I exhibited similar trends with the OCN expression.
Hua et al. 2023	Sprague-Dawley rats	F	25	4	4	rectangular perio. defect	electrospun PLA–PCL nanofibrous mats + chitosan hzdrogel + nanoparticles	CHI–NPs–CTGF, PLA–PCL NPs–BMP2, PLA–PCL NPs–rhCEMP1	1: PLA–PCL without GF 2: porous CHI control 3: blank control	12w	histology (HE, Mason staining), micro–CT	The bone volume/tissue volume (BV/TV) value in the triphasic scaffold group with GFs was higher than those of the other groups ($P < 0.05$). The deposition of newly formed hyperchromatic cementum-like tissue around the root surface was also recognized. The average angle between PDL-like tissue and root surface in the triphasic scaffold with GFs was nearly perpendicular, closely resembling that of the physiological PDL in the normal control, which was much larger than those of the triphasic scaffold without GFs and monophasic CHI scaffold (P < 0.05). BMP2 staining was significantly positive in both triphasic scaffold groups.

– alveolar bone, cementum, and periodontal ligament in animal models.[9–20] Seven studies[9–11,17–20] were conducted on Beagle dogs, four studies[12,14–16] were conducted on Sprague Dawley or Wistar rats, and one study[13] was conducted on white New Zealand rabbits. The minimum number of Beagle dogs involved in a study was 4,[18] and the maximum number was 24.[17] The minimum number of rats involved in a study was 16,[14] and the maximum was 40.[16] In the study on New Zealand rabbits, 12 animals were included.[13]

The created periodontal defects varied between studies. The most common defects were two-wall periodontal defect[10,17,19] and fenestration defect.[9,12,16] Two studies demonstrated three-wall periodontal defect,[18,20] two studies demonstrated non-specified periodontal defect,[13,14] one study covered rectangular defect,[15] and one covered Class II furcation defect.[11]

The shortest period between the material application and the last histologic evaluation was 28 days[18] and the longest period was 13 months.[17] However, apart from the mentioned study, no other study performed the last histologic check-up after 12 weeks. In ten studies, histologic evidence was evaluated using HE staining. Some studies additionally evaluated histologic evidence with other types of staining (Azan, Massons, Alizarin Red and Stevenel's Blue). Furthermore, most studies evaluated the bone quality with micro-CT.[9,10,12–17,19] Immunohistochemistry was performed in four studies.[12,13,18,20]

Study characteristics are summarized in **Table 2**.

3.4 Primary materials

Two studies used 3% HPC.[17,18] Matsuse et al. used porous α-TCP + heparin.[10] Collagen was used as a primary material in three studies.[11,16,20] Yu et al. used an intrafibrillary mineralized collagen (IMC) scaffold together with Bio-Gide. A periodontium-like biphasic scaffold was fabricated in two steps: biomimetic self-assembly of collagen fibrils and nanohydroxyapatites were used to form a porous IMC scaffold, followed by microstamping of CGF (concentrated growth factor) arrays on its surface.[16] Kiyota et al. used atelocollagen sponge,[20] while Momose et al. used collagen hydrogel.[11]

Most studies used multiphasic scaffolds made from a combination of natural and synthetic polymers and/or bioactive glass ceramics. Hua et al. used a triphasic scaffold made from electrospun PLA-PCL nanofibrous mats and chitosan hydrogel with an orderly aligned microporous channel structure fabricated via directional freeze-drying technology. Chitosan-stabilized bovine serum albumin (BSA) nanoparticles were used to load three different GFs in the target layer.[15] Chien et al. used thermosensitive CHC (chitosan/gelatin/glycerol phosphate) hydrogel + iPSC.[14] Sowmya et al. developed a tri-layered nanocomposite hydrogel scaffold by assembling chitin and PLGA for each layer, with an addition of nBGC for mineralized layers (alveolar bone and cementum).[13] In the study by Huang et al., a biphasic cryogel scaffold (BCS) was made of gelatin for the ligament phase and gelatin with beta-tricalcium phosphate/hydroxyapatite (BH) for the bone phase. Growth factors were applied to formulate a biomolecule-aided BCS (BBS). A functionally graded membrane (FGM) was designed as a tested barrier membrane by adhering PDGF-encapsulated poly(L-lactide-co-D/L-lactide) nanofibers to the conventional membrane (CM).[19] Zhang et al. used mesoporous bioactive glass (MBG) on a silk scaffold with a well-ordered nanochannel structure.[9] One study used a scaffold made solely from synthetic materials. Ding et al. used an electrospinning super-assembly technique to develop a fibrous framework with a poly(L-lactic acid)/poly(lactide-co-glycolide) (PLLA/PLGA) core/shell structure that could sequentially release two different GFs (PLLA/PLGA electrospun framework).[12]

3.5 Growth factors

The most commonly used growth factor in the studies was FGF,[10–13, 17,18] followed by BMP.[9,14,15,19] Six studies used biomaterials with a single GF and six studies used biomaterials with a combination of two or more GFs, as described in next section. In two studies, FGF was used in combination with other factors, such as BMP[12] or CEMP and PRP.[13] Furthermore, BMP was used in combination with PDGF[9,19] or with a combination of CTGF and CEMP.[15] BDNF was used in only one study.[20] Yu et al. used CGF.[16]

3.6 Outcomes of individual studies

Biomaterials with a single growth factor

In the studies conducted by Anzai and Yaganasu Tanaka, the use of 3 % HPC combined with FGF resulted in a greater height of the newly formed bone, cementum, and periodontal ligament compared to the control groups. Anzai et al. demonstrated that the newly formed bone exhibited a denser structure with richer trabeculae than the intact bone, regardless of the FGF-2 treatment. FGF-2 enhanced periodontal tissue regeneration achieved similar quality to that seen with natural physiological healing in terms of bone density, extracellular matrices, and connective tissue attachment.[17] Nagayasu Tanaka additionally performed an immunohistochemical analysis, revealing that the peak number of OCN-positive cells (OCN – osteocalcin, a key marker of osteoblast activity) appeared earlier and was higher in the bone defect area and on the root surface of the FGF-treated group. Expression levels of BMP-2, osterix (a transcription factor involved in preosteoblast maturation), ALP (alkaline phosphatase, produced by osteoblasts), and OCN were all significantly higher in the FGF-2 group compared to the control group at both day 7 and day 14.[18]

Matsuse et al. and Momose et al. also used FGF. In the study by Matsuse et al., bFGF was immobilized on heparin-modified a-TCP particles, which featured a con-

tinuous porous structure. Momose et al. used FGF-2 embedded in a collagen hydrogel. Both studies reported significantly increased formations of new bone, cementum-like tissue, and periodontal tissues in comparison to control groups.[10] In the study by Momose, downgrowth of epithelial tissue was observed in all control groups but was rarely present in the FGF-2 and hydrogel-treated groups. The collagen hydrogel scaffold effectively prevented epithelial invasion and was completely degraded in all groups by week 4.[11]

Chien et al. developed a thermosensitive chitosan/gelatin/glycerol phosphate (CHC) hydrogel loaded with iPSCs and BMP-6. Six weeks after transplantation, qualitative assessments showed that only the iPSCs-BMP-6 hydrogel group demonstrated significantly greater bone volume regeneration compared to the other groups. This group also exhibited significantly higher trabecular numbers and similar improvements in the trabecular thickness. New bone and cementum were observed in both the BMP-6-only and BMP-6-hydrogel groups; however, only the iPSCs-BMP-6 hydrogel group also promoted new periodontal ligament (PDL) formation. Markers of osteoblasts (ALP), osteoclasts (TRAP), and PDL formation (Masson's trichrome staining) were all significantly upregulated in the iPSCs-BMP-6 hydrogel group compared to the other groups. Furthermore, inflammatory cytokines (IL-8, TNF-á, and IL-1b) were significantly downregulated in this group compared to the hydrogel-only and BMP-6-hydrogel groups.[14]

Kiyota et al. investigated the application of BDNF on an atelocollagen sponge and observed a significant amount of new bone formation, adequate width of periodontal ligament, and nearly complete coverage of the denuded root surface with new cementum. In the control group, periodontal tissue regeneration was insufficient. Additionally, the OPN expression in the defect area and around the root surface was markedly higher than in the control group.[20]

Biomaterials with a combination of growth factors

The use of the IMC/CGF scaffold by Yu et al. demonstrated significant gains in new alveolar bone, cementum, and periodontal ligament compared to the control groups. After 8 weeks, small amounts of incompletely degraded scaffold were still present; however, the tissue structure closely resembled that of natural periodontal tissue. Two mesenchymal stem cell (MSC) recruitment markers were highly expressed in the periodontal defects of the CGF/IMC group at 4 weeks. At 8 weeks, markers such as BMP2-positive cells, OCN-positive cells, VEGFR1 (a marker of angiogenesis), TGFβ1 (a marker for cell proliferation, differentiation, and ECM synthesis), and Smad3 (an intracellular signalling molecule activated by TGFβ1) were all significantly higher in the CGF/IMC group compared to the control group.[16]

Sowmya et al. utilized CEMP1 for the cementum layer, FGF2 for the PDL layer, and PRP for the alveolar bone layer. They demonstrated that a tri-layered scaffold, with each layer containing a specific GF that defined the anatomical tissue of the periodontal complex, was effective in complete periodontal regeneration, wound healing, and defect closure with the formation of cancellous tissue. Significant increases in the new tissue formation were observed compared to the other groups.[13]

In the study by Ding et al., a core/shell structure allowed for the initial release of FGF, followed by the release of BMP2. At 8 weeks, significantly more bone was formed compared to the other groups, and cementum tissue was observed, which was not seen in the other groups. Furthermore, the angulations in the native ligament tissue and the PLLA/PLGA with GFs were very similar. This group also showed higher ALP expression after 1 and 2 weeks, and higher OCN expression after 4 and 8 weeks of bone repair. The expression of collagen type I (Col I – the most common bone matrix collagen that plays a vital role in bone formation and structure) followed trends similar to the OCN expression.[12]

Hua et al. used a triphasic PLA/PCL/chitosan scaffold with GFs, resulting in a higher mineralized bone volume and collagen density than in the control group. The deposition of newly formed hyperchromatic cementum-like tissue around the root surface was also observed. BMP2 staining in the triphasic scaffold with GFs indicated that this group had the best osteoinductive capacity and periodontal repair efficacy. BMP2 staining was significantly positive in both triphasic scaffold groups.[15]

Zhang et al. used a bioactive glass scaffold loaded with adenoviruses for BMP and PDGF, which resulted in periodontal tissue formation that was significantly greater than in the controls. Scaffolds containing adPDGF-B demonstrated significant new formation of the periodontal ligament (PDL), achieving 70 % of the original height, with no extra alveolar bone gain. Scaffolds containing adBMP7 resulted in greater new bone formation but with little vertical height increase. Scaffolds containing both adPDGF-B and adBMP7 led to PDL regeneration that approached 90 % of its original height, along with controlled regeneration of both alveolar bone and cementum (9).

Huang et al. similarly showed that a biphasic scaffold with GFs (BBS with EMD-BMP-2) had potential for reconstructing the alveolar ridge, periodontal ligament, and cementum. BBS demonstrated significantly greater osteogenesis ($P < 0.01$) and earlier defect fill ($P < 0.05$) compared to the monophasic bone scaffold (BH). While cementum thickness was not significantly different between BH- and BBS-treated sites, the coverage of a functionally graded membrane (FGM) consisting of PDGF/PLLA nanofibers on a conventional membrane resulted in significantly thicker newly formed cementum in the BBS-FGM group compared to the BCS-FGM group (BBS scaffold without GFs). Ligament-like fibers were obliquely inserted into the root surface in the BBS-CM and BBS-FGM groups, while they were parallel to the

root surface in the other groups. The combination of FGM and BBS provided limited additional benefits.[19]

Complications

No studies reported complications related to material application, such as ankylosis, or root resorption. Epithelial downgrowth was rarely seen in the study by Momose et al. in the hydrogel scaffold with FGF-2 treatment,[11] and was not reported in other studies.

4 DISCUSSION

5.1 Primary biomaterials

Using monophasic primary biomaterials for periodontal tissue engineering is simple and cost-effective, especially when composed of a single component. Atelocollagen sponge, made from type I collagen, is biocompatible and widely used in clinical practice for wound healing. Momose et al. combined collagen sponge with collagen hydrogel to retain more material in periodontal defects.[11] Hydroxypropyl cellulose (HPC), a cellulose derivate used by Anzai et al. and Nagayasu Tanaka et al., is biocompatible, water-soluble, adhesive, and highly viscous, making it practical for medical use. Matsuse et al. utilized α-TCP, a thermodynamically stable and degradable material with potential as a space-making scaffold and drug delivery system due to its porous structure that promotes blood vessel formation and progenitor cell harboring.[10] Zhang et al. developed a monophasic mesoporous scaffold from bioactive glass and silk fibrin, effective for delivering adenovirus-loaded growth factors. Our study demonstrates that hydrogels are among the most commonly used materials. Hydrogels, which are cross-linked polymer carriers, are easy to prepare and handle since they are injectable, and suit irregular periodontal defects.[2,3] Chien et al. created a thermosensitive chitosan/gelatin/glycerol hydrogel that solidifies at body temperature, enabling 3D cell and BMP7 immobilization and sustained release.[14]

Our study also confirms that multiphasic scaffolds loaded with GFs can regenerate periodontal tissues effectively and precisely, although their design is more complex and costly. Common scaffold fabrication methods include electrospinning for producing nanoscale structures, which allows good fiber alignment but limits vascular and cell ingrowth,[21] and cryogel techniques that create macroporous, flexible structures, but with lower surface area for adhesion and poorer control of fiber alignement.[22] Multiphasic scaffolds that combine nano- and macroporous features can overcome these limitations.

Huang et al. developed a bone-phase scaffold using b-TCP/hydroxyapatite and dispersing it in gelatin with cross-linkers at subzero temperatures. The gelatin meshwork redistributed bone phase to achieve favorable interparticle distance that was beneficial for osteogenesis and improved cell affinity, thereby promoting osteogenic potential. The scaffold promoted osteogenesis and sustained molecule release via gelatin degradation.[19]

Yu et al. highlighted the importance of precise micro/nano-architecture for spatial regulation, migration, and orientation of cells, leading to the formation of distinct tissue phases. Their CGF/IMC biphasic scaffold closely resembled natural periodontal tissues. The CGF layer featured a periodontal ligament-like parallel structure that promoted endogenous stem cell migration and alignment of new collagen fibers. The mineralized IMC layer had a bone-like microstructure with uniform, interconnected pores that supported stem cell migration, vascular ingrowth, and new bone formation.[16]

Furthermore, it is important that scaffolds exhibit sufficient mechanical strength that promotes selective cell repopulation, enables space/wound stability and prevents undesirable tissue invasion. For example, PLA/PCL nanofibers paired with chitosan hydrogel balance biocompatibility, exploit the mechanical strength of synthetic biomaterial, and harness the antibacterial activity of chitosan. Moreover, densely stacked electrospun nanofibers on both sides of the triphasic scaffold simulate the lamellar structure of cementum and alveolar bone while the chitosan hydrogel in the middle guides PDL regeneration through a directional microporous structure.[15]

Sowmya et al. created a three-layer porous scaffold using chitin-PLGA blends and nBGC to target tissue-specific regeneration by combining the advantages of chitin, which provides ECM similarity, with those of PLGA, which offers mechanical stability and slower degradation. nBGC was added to the cementum and alveolar bone layers where mineralization was desired.[13]

Moreover, temporal release of multiple bioactive agents is crucial for periodontal healing. Ding et al. fabricated PLLA/PLGA core-shell fibers delivering GFs sequentially. PLGA was selected as the shell material because of its adjustable and faster degradation rate, while PLLA was selected as the core material because of its long degradation time. The fibrous framework furthermore mimicked the native ECM and provided a suitable microenvironment for cell proliferation and differentiation.[12]

5.2 Application of growth factors

FGF2 is a heparin-binding growth factor with strong angiogenic and mitogenic effects on mesenchymal cells, playing a key role in bone regeneration by stimulating the proliferation and differentiation of undifferentiated bone marrow cells.[11] It also promotes the proliferation of multipotent cells in the periodontal ligament, fibroblasts, and vascular endothelial cells, and induces VEGF secretion from PDL cells. These actions support new bone formation, angiogenesis, and healing. Additionally, FGF2 contributes to the regeneration of cementum and the periodontal ligament.[10]

Concentrated growth factor (CGF) and platelet-rich plasma (PRP) are autologous platelet concentrates, with CGF containing higher levels of growth factors.[23] Rich in TGF-b1, VEGF, PDGF-BB, IGF-1, and bFGF, CGF plays a key role in tissue regeneration. PDGF-B promotes cell proliferation (particularly in fibroblasts and smooth muscle cells), angiogenesis and acts as chemoattractant. TGF-b1 supports the ligament-fibroblastic differentiation of PDLSCs. Furthermore, TGF-b1 enhances osteogenesis and angiogenesis through Smad3 signaling, and upregulates downstream genes involved in osteogenesis and angiogenesis (BMP2, OCN, VEGFR-1). This contributes to new bone and blood vessel formation during periodontal tissue repair.[16]

Bone morphogenetic protein-2 (BMP-2) is a potent promoter of bone formation. It supports Sharpey's fiber development and drives stem cells like MSCs, PDLSCs, and DFSCs toward osteoblast and cementoblast differentiation, aiding osteogenesis, cementogenesis, and PDL realignment.[11,14] BMP-6 also enhanced periodontal regeneration in rat models, and in vitro, it was shown to induce even stronger osteogenic differentiation than BMP-2.[14]

Brain-derived neurotrophic factor (BDNF), vital for neuron function, is also found in non-neural tissues, including osteoblasts and immune cells. In periodontal tissue, it promotes cementogenesis, increases osteopontin (OPN) expression, stimulates gingival epithelial and PDL cell growth, enhances fibroblast activity and has anti-inflammatory effects. BDNF has a short half-life and is easily degraded, which makes its delivery challenging. A collagen sponge has been identified as a suitable carrier to stabilize and deliver it effectively.[20]

In this paragraph, the combinations of growth factors used in the mentioned studies are examined. Zhang et al. explored the combination of a potent chemotactic growth factor, PDGF-B, recruiting mesenchymal stem cells (MSCs), with an osteoinductive growth factor, BMP7, for their differentiation into osteoblasts. This combination demonstrated a synergistic effect in enhancing the repair of periodontal defects.[9] Sowmya et al. utilized three different bioactive substances, each incorporated into a distinct layer of a triphasic scaffold. CEMP1 was used to promote cementogenesis, FGF2 facilitated periodontium regeneration, and PRP supported alveolar bone regeneration.[13] In a study by Ding et al., the initial release of FGF provided a sufficient number of recruited cells and an angiogenic environment. The subsequent release of BMP2 activated osteogenic and cementogenic differentiation and enhanced the formation of Sharpey's fibers.[12] Chien et al. and Huang et al. used BMP in combination with enamel matrix derivatives (EMD). In their study, EMD supported cementogenesis and ligament fiber formation, while BMP facilitated osteogenesis.[13,18] Additionally, Huang et al. incorporated induced pluripotent stem cells (iPSCs) into an injectable hydrogel. BMP served as an effective vehicle for cell delivery, addressing the issue of limited cell retention and survival. iPSCs, known for their potential to generate patient-specific, multi-lineage functional cells and tissues without immune rejection, further enhanced the regenerative effect. The combination of iPSCs with EMD showed better outcomes compared to EMD alone, suggesting that iPSCs and their microenvironment may play a key role in periodontal tissue differentiation.[19] Hua et al. employed chitosan-stabilized bovine serum albumin (BSA) nanoparticles to stabilize and deliver growth factors in a controlled and sustained manner. BMP2 was used for bone development, CEMP for cementum formation, and CTGF for the periodontal ligament (PDL) component.[15]

6 CONCLUSION

In the last decade, various GF-loaded biomaterials have been developed for periodontal tissue regeneration, successfully demonstrating simultaneous regeneration of periodontal ligament, alveolar bone and cementum on animal models. The major challenge with most developed biomaterials is the expensive and time-consuming fabrication process. Among new biomaterials, scaffolds have been the most common choice as the primary biomaterial, and the most frequently used growth factors have been FGF and BMP. Scaffold-based, multi-GF complex systems are desirable because the periodontium is a highly complex and hierarchical structure. These advanced materials enable spatial and temporal regulation of tissue regeneration, which may lead to improved tissue organization and functional integration by better mimicking the natural healing environment. Nevertheless, well-chosen single growth factor approaches delivered via appropriate biomaterial platforms can also result in robust periodontal regeneration and therefore remain effective and clinically relevant. Based on the available evidence, the superiority of complex systems cannot be confirmed, as the included studies exhibited substantial heterogeneity in terms of animal models, defect configurations, evaluation time points and tissue analysis methods. Only one study was a long-term trial that evaluated tissue regeneration after 13 months, whereas all the other studies were short-term. Thus, more long-term preclinical trials are desirable. Some biomaterials that have been tested on rats have yet to be evaluated on non-rodent species animals. Other biomaterials are currently progressing toward clinical trial testing. Additionally, only a small number of studies included immunohistochemical analysis, thus limiting the possibility to elucidate the underlying biological mechanisms and to precisely evaluate tissue maturation, cellular differentiation pathways, angiogenesis, and functional integration of regenerated tissues. Further studies incorporating such methods are necessary to enhance the biological interpretation and translational relevance of preclinical findings. A major knowledge gap remains in the complete understanding of basic biology of periodontal tissue repair and healing.

Periodontal regeneration is a rapidly evolving, dynamic field, with an enormous potential for research.

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