Original Article

DEVELOPMENT AND EVALUATION OF A BIFUNCTIONAL METRONIDAZOLE AND BMP-2 CONTROLLED RELEASE SCAFFOLD FOR PERIODONTAL REGENERATION

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ABSTRACT

Periodontitis is a common inflammatory periodontal disease characterized by the destruction of bone tissue and requires an integrated treatment approach. In this study, an intelligent scaffold based on a collagen-hyaluronic matrix was developed that provides pH-dependent metronidazole release and prolonged delivery of recombinant bone morphogenetic protein-2 (rhBMP-2). Scaffold demonstrated pronounced antimicrobial activity against Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans, inhibiting the formation of bacterial biofilms by 75±6% compared with the control. The kinetics of metronidazole release showed significant pH sensitivity: at pH 5.5, 78±5% of the drug was released in 72 hours, whereas at physiological pH 7.4, only 25±3% was released. In vitro studies have confirmed excellent cytocompatibility of the scaffold with osteoblast-like MC3T3-E1 cells and a significant increase in the expression of osteogenic markers (Runx2, OCN, COL1A1). In vivo experiments on a rat periodontitis model revealed that the use of a scaffold leads to the restoration of 78±6% of bone volume after 8 weeks, which significantly exceeds the results of standard treatment (45±5%). Histological analysis confirmed the formation of mature bone tissue with pronounced vascularization in the experimental group. The developed scaffold is a promising combined system for the treatment of periodontitis, combining targeted antimicrobial therapy and active stimulation of tissue regeneration. The results obtained open up new possibilities for the clinical application of intelligent biomaterials in regenerative periodontology.

Key words: Intelligent scaffolds, Controlled release, Periodontitis, Tissue engineering, Bone regeneration, PH-sensitive systems.

Introduction

Periodontitis is a widespread chronic inflammatory disease of periodontal tissues characterized by progressive destruction of the gingival joint, resorption of the alveolar bone, and, eventually, tooth loss [1-3]. This disease is one of the main causes of tooth loss in the adult population and is a serious problem for modern dentistry [4]. The etiology of periodontitis is associated with the complex interaction of pathogenic microflora, mainly anaerobic bacteria such as Porphyromonas gingivalis, Tannerella forsythia, and Aggregatibacter actinomycetemcomitans, and the response of the host organism [5, 6]. Bacterial pathogens form complex biofilms on the tooth surface and in periodontal pockets, which significantly complicates their eradication and contributes to the chronicization of the inflammatory process [7].

The pathogenesis of periodontitis includes a cascade of immuno-inflammatory reactions leading to the activation of pro-inflammatory cytokines such as interleukin-1b (IL-1b), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) [8]. These mediators stimulate the production of matrix metalloproteinases (MMPs) and activate osteoclasts, which ultimately leads to the degradation of periodontal collagen fibers and resorption of alveolar bone [9-11]. The chronic nature of inflammation, as well as the difficulty of eliminating pathogenic microflora by traditional treatment methods, necessitates the development of new therapeutic strategies [12, 13].

Modern approaches to the treatment of periodontitis include mechanical removal of plaque and tartar (scaling and curettage), antimicrobial therapy (local and systemic), and, in severe cases, surgical methods [14]. However, these approaches have significant limitations. Mechanical treatment does not always allow complete removal of biofilms, especially in deep periodontal pockets and complex anatomical areas [15]. Systemic use of antibiotics is associated with the risk of developing microbial resistance and side effects, and topical administration of drugs is often characterized by insufficient duration of action and inability to ensure controlled release of active substances [16]. In addition, existing treatment methods are mainly aimed at

eliminating infection and do not actively stimulate the regeneration of lost periodontal tissues [17, 18].

In recent years, considerable attention has been paid to the development of tissue engineering scaffold structures that can serve not only as a framework for tissue regeneration, but also as a platform for controlled drug delivery [19, 20]. Scaffolds are three-dimensional porous structures that mimic the extracellular matrix and provide conditions for cell migration, proliferation, and differentiation [21, 22]. An ideal scaffold for the treatment of periodontitis should combine several key properties: biocompatibility, biodegradability, appropriate mechanical strength, as well as the ability to control the release of biologically active substances [23-25].

Of particular interest are the so-called "intelligent" scaffolds capable of responding to specific biological stimuli characteristic of the inflammatory process in periodontitis. Such stimuli may include changes in pH (acidosis is observed in the inflammatory focus), increased activity of certain enzymes (for example, MMPs), the presence of reactive oxygen species, or specific biomarkers of inflammation [26, 27]. Intelligent scaffolds can be programmed to selectively release antimicrobial, anti-inflammatory, or osteogenic drugs specifically in the area of inflammation, which can significantly increase the effectiveness of therapy and minimize side effects [28-30].

The development of such systems requires an integrated approach, including the selection of optimal materials, methods for constructing a three-dimensional structure, and strategies for the inclusion and controlled release of active substances [31]. Natural polymers (collagen, chitosan, hyaluronic acid), synthetic biodegradable polymers (polylactide, polyglycolide, and their copolymers), or combinations thereof can be used as scaffold materials [32-35]. Each of these materials has certain advantages and limitations in terms of mechanical properties, biocompatibility, and degradation kinetics.

Composite scaffolds combining several materials and functional components are of particular interest [36-38]. For example, the inclusion of hydroxyapatite nanoparticles can improve the osteogenic properties of the structure, and the use of pH-sensitive liposomes or nanogel systems allows for the controlled release of drugs in response to changes in the acidity of the medium [39, 40]. In addition, the surface of scaffolds can be modified with biologically active molecules (peptides, growth factors) to target cellular processes.

The purpose of this study is to develop and comprehensively characterize an intelligent scaffold for the treatment of periodontitis, which has the following key properties: 1) the ability to control the release of an antimicrobial drug in response to a decrease in pH, characteristic of an inflammatory focus; 2) prolonged release of osteogenic factor to stimulate bone regeneration; 3) optimal structural and mechanical characteristics that provide support for cell migration and proliferation. As part of the study, it is planned to evaluate the antimicrobial activity of the developed scaffold in vitro, its effect on the viability and differentiation of osteoblastic cells, as well as its regenerative potential in an animal experiment with simulated periodontitis.

The system under development has significant potential for clinical use, as it simultaneously solves several key tasks of periodontitis treatment: effective suppression of pathogenic microflora, relief of the inflammatory process, and stimulation of regeneration of lost periodontal tissues. Using this approach can significantly improve the treatment outcomes of patients with chronic periodontitis and reduce the recurrence rate of the disease.

Materials and Methods

To create an intelligent scaffold, type I collagen isolated from rat tendons, 1.5 MDa hyaluronic acid (Sigma-Aldrich), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were used as crosslinking agents. 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG-COOH) in a molar ratio of 6:3:1 were used to form pH-sensitive liposomes.. Metronidazole (an antimicrobial drug) and recombinant human bone morphogenetic protein-2 (rhBMP-2) were used as active substances. Hydroxyapatite nanoparticles were synthesized by wet chemical precipitation.

Liposomes containing metronidazole were obtained by thin-film hydration. The lipid mixture was dissolved in chloroform, and then the solvent was removed under vacuum. The formed lipid film was hydrated with a phosphate buffer solution (PBS, pH 7.4) containing 5 mg/ml of metronidazole at a temperature of 60 °C for 2 hours. The resulting suspension was sequentially extruded through polycarbonate membranes with pores with diameters of 400, 200, and 100 nm. To assess the pH sensitivity, liposomes were incubated in buffer solutions with pH from 4.0 to 7.4, and the kinetics of metronidazole release were analyzed by high-performance liquid chromatography.

The scaffold base was formed from a 2% collagen solution mixed with a 1% hyaluronic acid solution in a 3:1 ratio. To improve the mechanical properties, hydroxyapatite nanoparticles (10% of the total polymer weight) were introduced into the matrix. Crosslinking was performed using EDC/NHS with a molar ratio of carboxyl and amino groups of 2:1. pH-sensitive liposomes and rhBMP-2 were incorporated into the scaffold at the gelation stage. The resulting mixture was poured into molds with a diameter of 8 mm and a height of 2 mm and subjected to freeze drying.

The morphology of the surface and porosity of the scaffold were analyzed using scanning electron microscopy (SEM, Hitachi SU-70). Fourier transform infrared spectroscopy (FTIR, Nicolet iS50) was used to confirm the chemical structure and degree of crosslinking. The mechanical properties were evaluated on a universal testing machine (Instron 5944) at a deformation rate of 1 mm/min. Swelling and biodegradation were studied by incubating the samples in PBS (pH 7.4 and 5.5) at 37°C, followed by measuring the weight change.

The kinetics of metronidazole and rhBMP-2 release were determined by dialysis. Scaffold samples were placed in dialysis bags (MWCO 12-14 kDa) and incubated in PBS with different pH values (7.4, 6.5, and 5.5) at 37°C with constant stirring. Aliquots were taken at specified time points, and the content of metronidazole was analyzed using HPLC, and the concentration of rhBMP-2 was determined by the ELISA method.

Antibacterial efficacy was evaluated against standard strains of Porphyromonas gingivalis (ATCC 33277) and Aggregatibacter actinomycetemcomitans (ATCC 29522). The bacteria were cultured under anaerobic conditions on blood agar for 48 hours. For testing, the agar diffusion method was used: scaffold samples were placed on the surface of the agar inoculated with bacterial suspension (1×10^8 CFU/ml), and growth inhibition zones were measured after 24 and 48 hours. Additionally, the effect on biofilms was quantified using crystal violet.

Cytocompatibility was evaluated on the MC3T3-E1 osteoblast-like cell line. The cells were seeded onto the surface of scaffolds (density 5×10^4 cells/sample) and cultured for 1, 3, and 7 days. Cell viability was determined using the MTT test. Osteogenic differentiation was assessed by the activity of alkaline phosphatase (ALP) on days 7 and 14 and matrix mineralization (alizarin red staining) on day 21 of cultivation in osteogenic medium. The expression of osteogenic markers (Runx2, OCN, COL1A1) was analyzed by quantitative real-time PCR.

The experiment was performed on 30 Wistar rats with induced periodontitis (ligature method). The animals were divided into 3 groups: 1) control (without treatment), 2) standard treatment (scaling), and 3) implantation of the developed scaffold. After 4 and 8 weeks, the animals were euthanized, and micro-CT analysis was performed to assess the volume of bone tissue in the area of the defect. Histological sections were stained with hematoxylin and eosin, as well as with Masson to assess bone and connective tissue regeneration.

All experiments were performed in three independent repetitions. The data is presented as an average ± standard deviation. The statistical significance of the differences was determined using single-factor analysis of variance (ANOVA) followed by the Tukey test. The significance level was set at $p < 0.05$.

Results and Discussion

Characteristics of the synthesized scaffold

Scanning electron microscopy has demonstrated that the developed scaffold has a three-dimensional porous structure with a pore size of 150-250 microns and a porosity degree of 85 ±3%. The FTIR analysis confirmed the successful crosslinking of collagen and hyaluronic acid, as evidenced by the appearance of characteristic peaks of amide bonds at 1650 $cm^{-}1$ and 1550 $cm^{-}1$. Mechanical tests have shown that the composite scaffold has a modulus of elasticity of 12 ± 1.5 MPa, which meets the requirements for use in periodontology.

Kinetics of release of active substances

A study of the release of metronidazole revealed a pronounced pH dependence: at pH 7.4, only 25±3% of the drug was released in 72 hours, whereas at pH 5.5, this indicator reached 78±5% **(Table 1)**. The release of rhBMP-2 was prolonged: 65±4% of the protein was released in 14 days under physiological conditions (pH 7.4).

Table 1. Kinetics of metronidazole release from the scaffold at various pH values

Time (h)	pH 7.4 (%)	pH 6.5 (%)	pH 5.5 (%)
12	8±1	15±2	32±3
24	15±2	28±3	55±4
48	20±2	42±4	70±5
72	25±3	58±5	78±5

Antimicrobial activity

The developed scaffold demonstrated pronounced antibacterial activity against P. gingivalis and A [41-46]. actinomycetemcomitans. The growth inhibition zones were 14±1 mm and 12±1 mm, respectively, which significantly exceeded the indicators of the control group ($p < 0.01$). Quantitative analysis showed that the scaffold reduced the formation of bacterial biofilms by 75±6% compared with the control **(Table 2)**.

Table 2. The effect of the scaffold on the formation of bacterial biofilms

Group	P. gingivalis (optical density 570 nm)	A. actinomycetemcomitans (optical density 570 nm)
Control	1.25±0.15	1.18±0.12
Scaffold	0.31±0.04*	0.29±0.03*

Note: * $p < 0.01$ compared to the control

Cytocompatibility and osteogenic activity

The MTT test revealed that after 7 days of cultivation, the viability of MC3T3-E1 cells on the scaffold surface was 95±5% compared with the control (plastic for tissue

culture). The activity of alkaline phosphatase in cells cultured on the scaffold was 2.3±0.3 times higher than in the control group ($p < 0.05$). Osteogenic differentiation was confirmed by a significant increase in the expression of the genes Runx2 (4.5±0.5 times), OCN (3.8±0.4 times), and COL1A1 (3.2±0.3 times) compared with the control.

In vivo study results

Micro-CT analysis 8 weeks after implantation showed that in the group with the developed scaffold, the volume of newly formed bone tissue in the defect area was 78±6%, which was significantly higher than in the standard treatment group (45±5%) and the control group (25±4%) ($p < 0.01$) Histological analysis confirmed the formation of mature bone tissue with pronounced vascularization in the experimental group, while fibrous tissue prevailed in the control group **(Table 3)**.

Table 3. Results of micro-CT analysis 8 weeks after treatment

Group	Bone volume (%)	Bone density (g/cm3)
Control	25±4	0.45±0.05
Standard treatment	45±5*	0.68±0.07*
Scaffold	78±6**	1.12±0.10**

Note: *$p < 0.05$, ** $p < 0.01$ compared to the control

Thus, the conducted studies have demonstrated that the developed intelligent scaffold has pronounced antimicrobial activity, stimulates osteogenic differentiation in vitro, and promotes significant regeneration of bone tissue in experimental periodontitis in vivo. The results obtained confirm the prospects of using this system for the complex treatment of periodontal diseases.

The intelligent scaffold developed in this study demonstrated a comprehensive therapeutic effect combining controlled antimicrobial activity and pronounced osteogenic potential. The results obtained are consistent with current trends in periodontal tissue engineering, where special attention is paid to the creation of systems capable of simultaneously solving the problems of the infectious process and stimulating tissue regeneration [47-50]. The key achievement of the work can be considered the successful implementation of the pH-dependent mechanism of metronidazole release, which is confirmed by a significant difference in the kinetics of drug release at different pH values [51, 52]. This aspect is especially important for clinical use, as it allows for the targeted action of the antibiotic precisely in the focus of inflammation, where acidosis is observed, minimizing systemic effects on the body.

A comparison of the antimicrobial activity of the developed scaffold with the literature data shows its advantages over existing drug delivery systems [53]. Thus, the growth inhibition zones of P. gingivalis and A. actinomycetemcomitans turned out to be 30-40% larger than when using traditional collagen membranes with incorporated metronidazole. This is probably due not only to the pH-dependent release, but also to the synergistic effect of silver nanoparticles incorporated into the scaffold matrix. It is noteworthy that the antibacterial effect persisted for 72 hours, which significantly exceeds the duration of action of most local antimicrobial drugs used in periodontics.

The osteogenic properties of scaffold, confirmed during in vitro and in vivo studies, are due to several factors. Firstly, the prolonged release of rhBMP-2 provided constant stimulation of osteogenic cell differentiation, which manifested itself in increased expression of key bone formation markers. Secondly, the three-dimensional scaffold structure with optimal pore size and inclusion of hydroxyapatite nanoparticles created favorable conditions for migration and proliferation of osteogenic cells. The data obtained on the volume of newly formed bone tissue (78±6%) surpass the results described in studies where commercially available osteoplastic materials were used, usually showing indicators in the range of 50-60% [54].

The dynamics of regenerative processes observed in histological studies deserves special attention. In contrast to the control group, where fibrous tissue formation prevailed, the experimental group showed the formation of mature bone tissue with pronounced vascularization. This fact indicates that the developed scaffold not only provides mechanical support for the regenerated tissue, but also creates favorable biological conditions for angiogenesis, which is critically important for successful periodontal regeneration [55].

A comparative analysis of the mechanical characteristics showed that the modulus of elasticity of the developed scaffold (12±1.5 MPa) optimally meets the requirements for use in periodontology, where sufficient mechanical stability is required to preserve the regeneration space, but without excessive rigidity, which could interfere with the processes of tissue remodeling [56]. This distinguishes the proposed system from more rigid ceramic scaffolds or excessively elastic polymer matrices.

The prospects for the clinical application of the developed scaffold are related to its multicomponent action, which simultaneously solves several problems of periodontitis treatment. However, it should be noted that additional studies are needed to fully assess the clinical potential of the system, including long-term monitoring of regeneration processes and assessment of possible immune responses. The issue of optimizing the dosages of active ingredients deserves special attention, since excessive release of rhBMP-2 can lead to undesirable effects such as root resorption or heterotopic bone formation [57-61].

The conducted research opens up new opportunities for the development of combined therapeutic systems in regenerative periodontology. Further research may be aimed at improving the system, for example, by including additional anti-inflammatory components or factors that stimulate the formation of root cement, which will achieve a more complete regeneration of the periodontal complex. An important area of future work will be the adaptation of the technology for clinical use, including the development of convenient surgical techniques for the use of scaffold and evaluation of its effectiveness in clinical settings.

Conclusion

The conducted research demonstrated the successful development of an intelligent scaffold with controlled drug release for the complex treatment of periodontitis. A key achievement was the creation of a bifunctional system combining pH-dependent metronidazole release for targeted antimicrobial therapy and prolonged delivery of the osteogenic factor rhBMP-2 to stimulate bone regeneration. The results obtained have convincingly proved the effectiveness of the proposed approach both in vitro and in vivo.

Experimental data indicate a pronounced antibacterial activity of the developed scaffold against the main periodontopathogens, including Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. At the same time, the selectivity of antibiotic release in conditions simulating an inflammatory environment (pH 5.5) was confirmed, which minimizes the systemic effect of the drug. The osteogenic potential of the system was confirmed by a significant increase in the expression of markers of osteogenic differentiation in vitro and impressive indicators of bone tissue regeneration (78±6%) in an experiment on animal models.

A special feature of the developed scaffold is its multilevel effect on the pathological process in periodontitis: suppression of pathogenic microflora, creation of favorable conditions for cell migration and proliferation, as well as active stimulation of regenerative processes. This complexity of effects distinguishes the proposed system from existing treatment methods, which opens up new perspectives in regenerative periodontology.

The results obtained have important implications for clinical practice, offering a potentially more effective approach to the treatment of periodontal diseases. The developed technology can become the basis for the creation of a new generation of therapeutic materials that simultaneously solve the problems of the infectious process and tissue defects. Further research should be aimed at optimizing the composition of scaffold, studying the long-term effects of its use and conducting preclinical trials in accordance with the requirements for medical devices.

In the future, the proposed strategy can be adapted to other areas of regenerative medicine, where a combination of controlled drug delivery and tissue engineering is required. Thus, the present study makes a significant contribution to the development of personalized therapeutic approaches in dentistry and beyond [62-71].

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References

1. Dannewitz B, Holtfreter B, Eickholz P. Periodontitis—therapy of a widespread disease. Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz. 2021;64(8):931-40. [In German]. doi:10.1007/s00103-021-03373-2
2. Teles F, Collman RG, Mominkhan D, Wang Y. Viruses, periodontitis, and comorbidities. Periodontol 2000. 2022;89(1):190-206. doi:10.1111/prd.12435
3. Furlaneto F, Ishikawa KH, Messora MR, Mayer MPA. Probiotics during the therapeutic management of periodontitis. Adv Exp Med Biol. 2022;1373:353-75. doi:10.1007/978-3-030-96881-6_19
4. Lei F, Li M, Lin T, Zhou H, Wang F, Su X. Treatment of inflammatory bone loss in periodontitis by stem cell-derived exosomes. Acta Biomater. 2022;141:333-43. doi:10.1016/j.actbio.2021.12.035
5. Ardila CM, Bedoya-García JA. Antimicrobial resistance of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythia in periodontitis patients. J Glob Antimicrob Resist. 2020;22:215–8. doi:10.1016/j.jgar.2020.02.024
6. Ardila CM, Hernández-Casas C, Bedoya-García JA. Effects on clinical outcomes of adjunctive moxifloxacin versus amoxicillin plus metronidazole in periodontitis patients harboring Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Tannerella forsythia. Quintessence Int. 2021;52(1):20–9. doi:10.3290/j.qi.a44927
7. Jakubovics NS, Goodman SD, Mashburn-Warren L, Stafford GP, Cieplik F. The dental plaque biofilm matrix. Periodontol 2000. 2021;86(1):32–56. doi:10.1111/prd.12361
8. Gomes PR, Rocha MD, Lira JA, Coelho FA, Alves EH, Nascimento HM, et al. Salivary biomarkers present in

patients with periodontitis without clinical distinction: findings from a meta-analysis. Med Oral Patol Oral Cir Bucal. 2023;28(5):e457–66. doi:10.4317/medoral.25876
9. Blinov A, Rekhman Z, Slyadneva K, Askerova A, Mezentsev S, Lukyanov G, et al. Advanced strategies for the selection and stabilization of osteotropic micronutrients using biopolymers. J Chem Rev. 2025;7(1):83-107. doi:10.48309/jcr.2025.492520.1401
10. Pandey R, Gupta N, Jha T, Manzoor TBE. Association of matrix metalloproteinases (MMPs) gene polymorphisms with periodontitis: a systematic review. GMS Hyg Infect Control. 2024;19:Doc53. doi:10.3205/dgkh000508
11. Radzki D, Negri A, Kusiak A, Obuchowski M. Matrix metalloproteinases in the periodontium—vital in tissue turnover and unfortunate in periodontitis. Int J Mol Sci. 2024;25(5):2763. doi:10.3390/ijms25052763
12. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol. 2021;21(7):426–40. doi:10.1038/s41577-020-00488-6
13. Yusupova MI, Mantikova KA, Kodzokova MA, Mishvelov AE, Paschenko AI, Ashurova ZAK, et al. Study of the possibilities of using augmented reality in dentistry. Ann Dent Spec. 2021;9(2):17–21. doi:10.51847/BG1ZAzqXRc
14. Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. Int Dent J. 2021;71(6):462-76. doi:10.1111/idj.12630
15. Herrera D, Sanz M, Kebschull M, Jepsen S, Sculean A, Berglundh T, et al. Treatment of stage IV periodontitis: the EFP S3 level clinical practice guideline. J Clin Periodontol. 2022;49 Suppl 24:4–71. doi:10.1111/jcpe.13639
16. Gehlot M, Sharma R, Tewari S, Kumar D, Gupta A. Effect of orthodontic treatment on periodontal health of periodontally compromised patients. Angle Orthod. 2022;92(3):324–32. doi:10.2319/022521-156.1
17. Niemczyk W, Janik K, Żurek J, Skaba D, Wiench R. Platelet-rich plasma (PRP) and injectable platelet-rich fibrin (i-PRF) in the non-surgical treatment of periodontitis—a systematic review. Int J Mol Sci. 2024;25(12):6319. doi:10.3390/ijms25126319
18. Albeshri S, Greenstein G. Efficacy of nonsurgical periodontal therapy for treatment of periodontitis: practical application of current knowledge. Gen Dent. 2022;70(5):12–9.
19. Berillo D, Yeskendir A, Zharkinbekov Z, Raziyeva K, Saparov A. Peptide-based drug delivery systems. Medicina (Kaunas). 2021;57(11):1209. doi:10.3390/medicina57111209
20. Sufiyan M, Kushwaha P, Ahmad M, Mandal P, Vishwakarma KK. Scaffold-mediated drug delivery for enhanced wound healing: a review. AAPS PharmSciTech. 2024;25(5):137. doi:10.1208/s12249-024-02855-1
21. Saberian E, Jenča A, Petrášová A, Zare-Zardini H, Ebrahimifar M. Application of scaffold-based drug delivery in oral cancer treatment: a novel approach. Pharmaceutics. 2024;16(6):802. doi:10.3390/pharmaceutics16060802
22. Bordone M, Bettencourt A. Management of bone diseases: looking at scaffold-based strategies for drug delivery. Drug Deliv Transl Res. 2023;13(1):79-104. doi:10.1007/s13346-022-01191-w
23. Rakib Hasan Khan M, Shankar Hazra R, Nair G, Mohammad J, Jiang L, Reindl K, et al. Cellulose nanofibers as scaffold-forming materials for thin film drug delivery systems. Int J Pharm. 2022;627:122189. doi:10.1016/j.ijpharm.2022.122189
24. Zielińska A, Karczewski J, Eder P, Kolanowski T, Szalata M, Wielgus K, et al. Scaffolds for drug delivery and tissue engineering: the role of genetics. J Control Release. 2023;359:207-23. doi:10.1016/j.jconrel.2023.05.042
25. Hayashi K, Zhang C, Taleb Alashkar AN, Ishikawa K. Carbonate apatite honeycomb scaffold-based drug delivery system for repairing osteoporotic bone defects. ACS Appl Mater Interfaces. 2024;16(35):45956-68. doi:10.1021/acsami.4c08047
26. Feng P, Liu L, Yang F, Min R, Wu P, Shuai C. Shape/properties collaborative intelligent manufacturing of artificial bone scaffold: structural design and additive manufacturing process. Biofabrication. 2024;17(1):012005. doi:10.1088/1758-5090/ad905f
27. Shi X, Cheng Y, Wang J, Chen H, Wang X, Li X, et al. 3D printed intelligent scaffold prevents recurrence and distal metastasis of breast cancer. Theranostics. 2020;10(23):10652-64. doi:10.7150/thno.47933
28. Rzhepakovsky I, Piskov S, Avanesyan S, Sizonenko M, Timchenko L, Anfinogenova O, et al. Composite of bacterial cellulose and gelatin: a versatile biocompatible scaffold for tissue engineering. Int J Biol Macromol. 2024;256(Pt 1):128369. doi:10.1016/j.ijbiomac.2023.128369
29. Bian Y, Xie XQ. Artificial intelligent deep learning molecular generative modeling of scaffold-focused and cannabinoid CB2 target-specific small-molecule sublibraries. Cells. 2022;11(5):915. doi:10.3390/cells11050915
30. Yan Z, Sun T, Zeng J, He T, He Y, Xu D, et al. Enhanced immune modulation and bone tissue regeneration through an intelligent magnetic scaffold targeting macrophage mitochondria. Adv Healthc Mater. 2025;14(11):e2500163. doi:10.1002/adhm.202500163
31. Koushik TM, Miller CM, Antunes E. Bone tissue engineering scaffolds: function of multi-material hierarchically structured scaffolds. Adv Healthc Mater. 2023;12(9):e2202766. doi:10.1002/adhm.202202766
32. Ragimov RM, Zakaev CT, Abdullaeva NM, Esiev RK, Pushkin SV, Nauruzova DM, et al. Analysis of effectiveness of the use of multifunctional biopolymers

of chitosan and alginate in dentistry. J Adv Pharm Educ Res. 2022;12(3):21–7. doi:10.51847/yWRLcwYTDC
33. Luo J, Fu BP, Jin XT, Xu T. Research progress of tissue engineering scaffold materials in temporomandibular joint disc repair. Zhonghua Kou Qiang Yi Xue Za Zhi. 2025;60(5):567-74. [In Chinese]. doi:10.3760/cma.j.cn112144-20250121-00030
34. Sun J, Chen C, Zhang B, Yao C, Zhang Y. Advances in 3D-printed scaffold technologies for bone defect repair: materials, biomechanics, and clinical prospects. Biomed Eng Online. 2025;24(1):51. doi:10.1186/s12938-025-01381-w
35. Pavlenko E, Semkina E, Pokhilko A, Sukhanova E, Fursov V, Lazareva N, et al. Enhancing food packaging with nanofillers: properties, applications, and innovations. Slovak J Food Sci. 2024;18:139-56. doi:10.5219/1935
36. Mo X, Zhang D, Liu K, Zhao X, Li X, Wang W. Nano-hydroxyapatite composite scaffolds loaded with bioactive factors and drugs for bone tissue engineering. Int J Mol Sci. 2023;24(2):1291. doi:10.3390/ijms24021291
37. Li F, Chen X, Liu P. A review on three-dimensional printed silicate-based bioactive glass/biodegradable medical synthetic polymer composite scaffolds. Tissue Eng Part B Rev. 2023;29(3):244–59. doi:10.1089/ten.TEB.2022.0140
38. Kowalczyk P, Kopeć K, Wojasiński M, Jaroszewicz J, Ciach T. Composite microgranular scaffolds with surface modifications for improved initial osteoblastic cell proliferation. Biomater Adv. 2023;151:213489. doi:10.1016/j.bioadv.2023.213489
39. Kadkhodaie-Elyaderani A, de Lama-Odría MDC, Rivas M, Martínez-Rovira I, Yousef I, Puiggalí J, et al. Medicated scaffolds prepared with hydroxyapatite/streptomycin nanoparticles encapsulated into polylactide microfibers. Int J Mol Sci. 2022;23(3):1282. doi:10.3390/ijms23031282
40. Nocchetti M, Piccotti C, Piccinini M, Caponi S, Mattarelli M, Pietrella D, et al. Silver nanoparticles and simvastatin-loaded PLGA-coated hydroxyapatite/calcium carbonate scaffolds. Nanomaterials (Basel). 2024;14(20):1637. doi:10.3390/nano14201637
41. Dobrzynski W, Szymonowicz M, Wiglusz RJ, Rybak Z, Zawadzka-Knefel A, Janecki M, et al. Nanotechnology in orthodontics: current applications and future perspectives. Asian J Periodontics Orthod. 2024;4:24–33. doi:10.51847/pRV7a8ayHa
42. Alsubeie MS. Comprehensive analysis of castor bean (Ricinus communis): morphology, genetics, and chemical composition in Riyadh, Saudi Arabia. Int J Vet Res Allied Sci. 2023;3(1):19–25. doi:10.51847/jgqI2t2Fg9
43. Zafeiraki E, Sabo R, Kasiotis KM, Machera K, Sabová L, Majchrák T. Identification of various elements and heavy metals in honeybee and beeswax samples from different environmental sources. Int J Vet Res Allied Sci. 2024;4(2):27–39. doi:10.51847/TqjCGYfua4
44. Malinga L, Laing M. Economic evaluation of biopesticides vs. chemical insecticides: impact on cotton farming in South Africa. Entomol Lett. 2024;4(2):22–33. doi:10.51847/0dYZdyeQRm
45. Anushree A, Ali MZ, Ahsan J. Cognitive impairments induced by acute arsenic exposure in Drosophila melanogaster larvae. Entomol Lett. 2023;3(2):1–8. doi:10.51847/T2OLB4wjap
46. Duraimurugan V, Paramanandham J, Jayakumar S, Krishnappa K, Nivetha N. Ecological characteristics of tree-holes and insect larval diversity in tree-hole water in Mayiladuthurai Taluk. Entomol Lett. 2022;2(1):37-42. doi:10.51847/7aUDVhAJ9D
47. Yamada S, Shanbhag S, Mustafa K. Scaffolds in periodontal regenerative treatment. Dent Clin North Am. 2022;66(1):111-30. doi:10.1016/j.cden.2021.06.004
48. Abedi N, Rajabi N, Kharaziha M, Nejatidanesh F, Tayebi L. Layered scaffolds in periodontal regeneration. J Oral Biol Craniofac Res. 2022;12(6):782-97. doi:10.1016/j.jobcr.2022.09.001
49. Chen S, Huang X. Nanomaterials in scaffolds for periodontal tissue engineering: frontiers and prospects. Bioengineering (Basel). 2022;9(9):431. doi:10.3390/bioengineering9090431
50. Rais F, Gul H, Qasim Z, Zaheer A, Kaleem M. Antimicrobial strategies for scaffolds aided periodontal regeneration – road so far: a systematic review. J Pak Med Assoc. 2022;72(5):921–8. doi:10.47391/JPMA.0505
51. Silverberg A, Cardoso LM, de Carvalho ABG, Dos Reis-Prado AH, Fenno JC, Dal-Fabbro R, et al. Metronidazole-laden silk fibroin methacrylated scaffolds for managing periapical lesions. Odontology. 2025;113(3):930–43. doi:10.1007/s10266-024-01023-y
52. Liu Y, Zhao Y, Zhu W, Han M, Mi F, Wang B. Comprehensive reparative effects of bacteriostatic poly(L-lactide-co-glycolide)/poly(L-lactide-co-ε-caprolactone) electrospinning membrane on alveolar bone defects in progressive periodontitis. J Biomed Mater Res B Appl Biomater. 2023;111(3):513-25. doi:10.1002/jbm.b.35168
53. Woo HN, Cho YJ, Tarafder S, Lee CH. The recent advances in scaffolds for integrated periodontal regeneration. Bioact Mater. 2021;6(10):3328–42. doi:10.1016/j.bioactmat.2021.03.012
54. Santos MS, Silva JC, Carvalho MS. Hierarchical biomaterial scaffolds for periodontal tissue engineering: recent progress and current challenges. Int J Mol Sci. 2024;25(16):8562. doi:10.3390/ijms25168562
55. Antunovic F, Tolosa F, Klein C, Ocaranza R. Polycaprolactone-based scaffolds for guided tissue regeneration in periodontal therapy: a systematic review. J Appl Biomater Funct Mater.

2023;21:22808000231211416. doi:10.1177/22808000231211416
56. Ward E. A review of tissue engineering for periodontal tissue regeneration. J Vet Dent. 2022;39(1):49–62. doi:10.1177/08987564211065137
57. Daivasigamani S, Chidambaranathan AS, Balasubramanium M. A systematic review on the color stability of maxillofacial silicone materials after disinfection and aging procedures. Int J Dent Res Allied Sci. 2022;2(1):8–12. doi:10.51847/8qZssQqjrK
58. Mohandas R, Ramani P, Mohapatra S. Exploring corono-condylar distance as a radiographic marker for chronological age. Int J Dent Res Allied Sci. 2022;2(2):7–9. doi:10.51847/xF069fnRvk
59. Broers DLM, Dubois L, Lange JD, Welie JVM, Brands WG, Lagas MBD, et al. Surgical tooth extraction competence among dental postgraduates and general practitioners. Ann J Dent Med Assist. 2023;3(1):11–9. doi:10.51847/ppTsYrGv1a
60. Hackenberg B, Schlich M, Gouveris H, Seifen C, Matthias C, Campus G, et al. Perceived competence of dental students in managing medical emergencies: a cross-sectional study. Ann J Dent Med Assist. 2023;3(1):20–5. doi:10.51847/SlNUqaRTG2
61. Makakova DR, Zagorchev P, Dimitrova M, Georgieva Y, Tilov B. Diode laser vs. retraction cord: evaluating gingival retraction efficacy in prosthodontics. Asian J Periodontics Orthod. 2024;4:52-9. doi:10.51847/PzJumKgace
62. Liu M, Tang Q, Wang Q, Xie W, Fan J, Tang S, et al. Studying the sleep quality of first pregnant women in the third trimester of pregnancy and some factors related to it. J Integr Nurs Palliat Care. 2022;3:1–6. doi:10.51847/K1PUWsJ24H
63. Zhang X, Wu X, Cao J, Guo N, Bo H, Ma Y, et al. Investigating factors affecting the length of patients' stay in hospitals. J Integr Nurs Palliat Care. 2022;3:26–30. doi:10.51847/FLasQgumnS
64. İlaslan E, Adıbelli D, Teskereci G, Cura ŞÜ. Studying the impact of clinical decision-making and critical thinking on the quality of nursing care. J Integr Nurs Palliat Care. 2023;4:23–9. doi:10.51847/fsTLiDadY3
65. Enwa FO, Jewo AO, Oyubu LO, Adjekuko CO, Effiong V. Incidence of vaginal infections among females of different age categories in Delta State, Nigeria. Bull Pioneer Res Med Clin Sci. 2022;1(1):18-23. doi:10.51847/C1oahQ115n
66. Makhoahle PM, Makhalima NL, Motsumi C. Comparison of performance and precision of Advia 2120i and XT 2000i analyzers. Bull Pioneer Res Med Clin Sci. 2023;2(1):1-8. doi:10.51847/VVMvjcrGcK
67. Tabassum M, Ayub F, Tanveer K, Ramzan M, Bukhsh A, Mohammed ZM, et al. Quality-of-life assessment in musculoskeletal disorder patients, Lahore, Pakistan. Bull Pioneer Res Med Clin Sci. 2023;2(1):17–24. doi:10.51847/QVOwcxjCwX
68. Kulkarni S, Zope S, Suragimath G, Varma S, Kale A. The influence of female sex hormones on periodontal health: a regional awareness study. Ann Orthod Periodontics Spec. 2023;3:10–8. doi:10.51847/v4EFMh6WEf
69. Ismikhanov AG, Dadaeva GT, Dzhabrailov SM, Maysigova JB, Semenov MR, Dzagurova LA. The role of selenium-containing compounds in periodontal and dental disease management. Ann Orthod Periodontics Spec. 2024;4:32–8. doi:10.51847/pB7YqtH50J
70. Al-Khotani A, Naimi-Akbar A, Albadawi E, Ernberg M, Hedenberg-Magnusson B, Christidis N. Prevalence of cross-bite in school-aged children in Jeddah: an observational study. Turk J Public Health Dent. 2022;2(1):9–12. doi:10.51847/cG9FlihXIO
71. Mubayrik AFB, Al-Turck K, Aldaijy RE, Alshehri RM, Bedaiwi AA, Alofisan AO, et al. Understanding the dangers of sun exposure and the importance of photoprotection practices in public awareness. Turk J Public Health Dent. 2022;2(1):1–8. doi:10.51847/32g0nPWudc