

Research on 3D Printed Scaffolds Loaded With Extracellular Vesicles to Regulate the Microenvironment of Periodontitis Microbiota and Promote Tissue Regeneration

Xun Ma

School of Stomatology and Optometry, Hubei University of Science and Technology, Xianning 437100, China

ABSTRACT

In recent years, extracellular vesicles have been widely recognized as important mediators of communication between cells, as they contain bioactive molecules and have the potential to regulate immunity and promote regeneration. 3D printed scaffolds can simulate extracellular matrix structures to provide directional spatial guidance for regenerating microenvironments. The purpose of this study is to develop a three-dimensional printed scaffold system with extracellular vesicles and evaluate its intervention in the microenvironment of periodontitis microbiota and promotion of periodontal tissue regeneration; Simultaneously improve the extraction and characterization methods of extracellular vesicles, and screen suitable biomaterials and their 3D printing processes to obtain composite scaffolds with appropriate porosity, mechanical strength, and release characteristics. Evaluate the inhibitory effect of the scaffolds on periodontal pathogens and their ability to regulate bacterial homeostasis at the in vitro level, and observe whether they affect macrophage polarization and the expression of inflammatory factors; Then, by establishing an animal periodontitis model, the repairing effect of the stent on the alveolar bone defect area and the fusion status of the newly formed tissue were observed. The results showed that the composite scaffold can significantly inhibit the number of pathogenic bacteria, rebuild microbial diversity, alleviate inflammatory reactions, and significantly promote the formation of alveolar bone and periodontal ligament like tissues. In summary, this article provides a new approach for the comprehensive treatment of periodontitis: microbial microenvironment regulation combined with tissue engineering technology.

KEYWORDS

3D printing bracket; Extracellular vesicles; Periodontitis; Microbial microenvironment; Tissue regeneration

1. INTRODUCTION

In recent years, microbiome studies have found that the occurrence and development of periodontitis are closely related to the disorder of the entire microbial community composition. Simply sterilizing without considering the reconstruction of microbial diversity is difficult to achieve lasting therapeutic effects. Therefore, from the perspective of microbial microenvironment, reshaping a healthy microbial ecosystem has become a new goal in the treatment of periodontitis. Meanwhile, extracellular vesicles are naturally formed membrane coated particles in the nanoscale range, which play a crucial role in loading bioactive substances such as proteins, fatty acids, DNA, and RNA during intercellular interactions [1]. EVs derived from MSCs or immune regulatory cells have anti-inflammatory, angiogenic, and tissue differentiation promoting effects. The application of EV in periodontal tissue engineering can coordinate the control of inflammatory response and repair process. 3D printing technology endows the design of tissue engineering scaffolds with great flexibility.

Porous scaffolds with different porosity, pore size, and mechanical strength can be obtained by adjusting printing parameters and material formulations, providing suitable space for cell migration, nutrient exchange, and new tissue growth; In addition, 3D printed scaffolds can also serve as slow-release carriers for extracellular vesicles, avoiding their rapid degradation and prolonging their local residence time [2]. Therefore, encapsulating extracellular vesicles into 3D printed scaffolds to form a composite system that integrates microbial intervention, immune intervention, and tissue induction has important research significance and potential clinical translational application prospects for solving the current problems in periodontitis treatment. The purpose of this study is to prepare a composite 3D printed scaffold loaded with extracellular vesicles and evaluate its impact on the microenvironment of periodontal disease in implants and its ability to regenerate periodontal tissue *in vivo*; Elucidate the mechanism by which this composite scaffold material improves microbiota anti-inflammatory bone regeneration, and provide a basis for targeted therapy of periodontitis in clinical practice [3].

2. KEY ISSUES OF IMBALANCED MICROENVIRONMENT OF PERIODONTITIS MICROBIOTA AND TISSUE REGENERATION DISORDERS

Periodontitis is not caused by a single pathogen, but by an imbalance in the overall bacterial population structure. In a healthy periodontium, the oral microbiota is mainly composed of symbiotic bacteria such as streptococcus and actinomycetes, forming a stable and resistant ecological environment in the mouth; Once the oral hygiene condition is poor, the body's immune system is weakened, or the environment changes, the proportion of symbiotic bacteria decreases [4]. G-A anaerobic bacteria overgrow and become highly invasive pathogenic biofilms, causing local dysbiosis. At the same time, they also produce various toxic factors (mainly LPS) to activate the host immune response, leading to the secretion of large amounts of inflammatory mediators such as TNF - α , IL-1 β , and IL-6, attracting PMNs and monocytes. Release MMP and ROS, promote collagen degradation and bone resorption. In the positive feedback of dysbiosis and inflammatory response, the local periodontal microenvironment gradually deteriorates. The function of osteoblasts is reduced while osteoclasts are activated, decoupling bone formation from bone resorption. The proliferation and osteogenic differentiation ability of periodontal ligament SCs and alveolar bone derived MSCs are weakened, and the regenerative potential is hindered. Secondly, in the inflammatory microenvironment, vascular leakage syndrome leads to poor local blood flow, exacerbates nutrient and oxygen supply deficiencies, and greatly weakens tissue self-healing ability. Therefore, simple bactericidal and bacteriostatic treatment or only administering exogenous regenerative factors cannot achieve satisfactory results. Not only do we need to consider how to restore the normal microbiota, but we also need to consider how to inhibit inflammatory reactions while activating endogenous repair processes [5].

3. CONSTRUCTION AND CHARACTERIZATION OF EXTRACELLULAR VESICLES LOADED ON 3D PRINTED SCAFFOLDS

3.1. Extraction and Biological Function Analysis of Extracellular Vesicles

This experiment selected bone marrow mesenchymal stem cells as extracellular vesicle derived cells, which have good immune regulation and osteogenic differentiation abilities. When the cell growth reaches about 80%, replace the serum containing exosomes with serum without exosomes and culture for 48 hours. Collect the supernatant and use differential centrifugation combined with ultracentrifugation to isolate extracellular vesicles. Firstly, remove cell debris at 300g for 10 minutes, dead cells at 2000g for 20 minutes, large vesicles at 10000g for 30 minutes, and finally obtain a

precipitate at 100000g after ultracentrifugation for 70 minutes. The precipitate is resuspended in phosphate buffer and purified by ultracentrifugation; Using a nanoparticle tracking analyzer to detect particle size and concentration; Observe its morphological characteristics under transmission electron microscopy; Western blot was used to detect the expression of CD9, CD63, and TSG101. After identification, it was found that the extracted extracellular vesicles had a typical cup-shaped or spherical shape, with a maximum diameter of about 120nm, which is within the normal range of extracellular vesicle sizes; All marker proteins showed positive expression; In terms of biological activity, the effect of this type of extracellular vesicle on macrophage polarization under LPS stimulation was studied in vitro, and it was confirmed that extracellular vesicles can significantly reduce the expression of pro-inflammatory macrophage markers iNOS and CD86, and upregulate the expression of anti-inflammatory markers Arg1 and CD163. In addition, extracellular vesicles can also promote ALP activity and mineralization nodule formation in BMSCs, indicating osteogenic induction [6].

3.2. Material Selection and Structural Design of 3D Printing Bracket

The selection of scaffold materials is mainly considered from the aspects of biocompatibility, printability, mechanical properties, and degradation rate. This study used gelatin methacryloyl/polycaprolactone composite materials. Among them, gelatin methacryloyl can provide more adhesion sites for cell growth and control the degree of cross-linking; However, polycaprolactone can provide sufficient mechanical strength and has a long degradation time. Mix them in different mass ratios and select a formula that achieves both printing accuracy and appropriate mechanical strength simultaneously [7]. Using extrusion 3D printing technology to prepare a bracket with a printing nozzle size of 200 μ m and a layer height of 150 μ m. The printing rate is adjusted in conjunction with extrusion pressure to ensure continuous and uniform lines. The design of the bracket adopts a mesh structure with 0°/90° stacking and crossing, and the theoretical pore size is about 300-400 μ m. This design ensures that cells migrate into the scaffold and has a sufficiently large specific surface area to support extracellular vesicles. Using scanning electron microscopy to analyze the surface morphology and inner wall structure of the stent, and proving that the pores have good penetration performance; The mechanical compression test shows that its elastic modulus is within the level range of cancellous bone, which can provide corresponding mechanical support without causing stress shielding phenomenon. The swelling experiment proves that the scaffold has a certain degree of water absorption performance, and this degree is suitable for later extracellular vesicle loading and release processes.

3.3. Preparation Process and Release Characteristics of Extracellular Vesicles Loaded on Scaffolds

In order to ensure the uniform load and slow release of extracellular vesicles on the scaffold, this paper adopts the method of physical adsorption combined with hydrogel embedding: the extracted extracellular vesicles are suspended in phosphate buffer solution, and dropped onto the surface of the three-dimensional printed scaffold, and placed at 4 °C for 12h to allow the extracellular vesicles to be adsorbed on the fiber surface of the scaffold; Then a cross-linking operation of low concentration gelatin methacryloyl hydrogel was performed on the scaffold. Some extracellular vesicles were embedded in the water gel network [8]. This dual loading can generate different types of release kinetics: extracellular vesicles adsorbed on the surface are rapidly released to achieve early anti-inflammatory and antibacterial effects; The extracellular vesicles encapsulated by hydrogel are slowly released to provide long-term signal stimulation to promote tissue repair. By fluorescence labeling the extracellular vesicles and tracking their distribution on the scaffold, it was confirmed that the vesicles were mainly distributed along the scaffold fibers and formed uniform fluorescence signals in the hydrogel area. The release kinetics experiment showed that in the phosphate buffer solution system, about 30% of extracellular vesicles were released in the first 24 hours, and then entered the

sustained release platform phase. After 14 days, about 70% were released cumulatively. The released extracellular vesicles still had good morphological integrity and biological activity, and could be internalized and exerted by target cells; The difference in porosity and mechanical properties of the scaffold before and after loading is not significant, indicating that the loading process will not damage the main structure of the scaffold.

4. MECHANISM OF REGULATING THE MICROENVIRONMENT OF PERIODONTITIS MICROBIOTA

4.1. Inhibition of Pathogenic Bacterial Growth

In order to evaluate the effect of composite scaffolds on periodontal pathogens, we selected *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Actinobacterium parahaemolyticus* as representative periodontal pathogens. The scaffold materials were co cultured with them, and their growth status was observed by measuring the optical density of the culture medium and plate colony count. The results showed that a simple 3D printed scaffold did not have a significant antibacterial effect, while a composite scaffold loaded with extracellular vesicles could effectively slow down the growth rate of various strains and reduce their final bacterial count. Among them, the impact on *Porphyromonas gingivalis* was the most significant, with a decrease of nearly 2 orders of magnitude in CFU within 24 hours [9]. Further use SEM to observe the adhesion of bacteria on the scaffold and the formation of biofilms. The control group had a high bacterial density and a large number of bacteria secreted extracellular matrix to form a dense biofilm; The bacterial density in the composite scaffold group was low, and no complete biofilm was formed, with only a few scattered bacteria. At the same time, the total amount of biofilm was quantitatively detected using crystal violet staining, and it was found that the amount of biofilm formed in the composite scaffold group was about 60% lower than that in the control group. These results suggest that extracellular vesicles may function in various ways, such as interfering with bacterial adhesion, disrupting formed biofilms, or directly inhibiting bacterial metabolism.

4.2. Regulatory Effects on Microbial Diversity and Homeostasis

A single strain experiment cannot represent the microbial environment in the oral cavity. This article adopts the method of co culturing multiple strains in vitro. Different types of bacteria were mixed and cultured in a certain proportion, including *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Actinobacillus actinomycetes*, and *Streptococcus pyogenes*. The 16S rRNA gene sequencing method was used to detect the changes in the microbial community before and after co culturing with the composite scaffold. The results showed that with the passage of time, the trend of dominant bacteria changing from pathogenic bacteria to *Streptococcus pyogenes* reversed in the control group, and the diversity index of the microbial community decreased; When a composite scaffold is present, the percentage of pathogenic bacteria decreases, the proportion of *Streptococcus* increases, and the diversity of the microbiota increases. It is speculated that this may be due to the inhibition of microbial growth, rather than directly affecting bacteria, but indirectly affecting their ecological niche. EVs or their released substances may interfere with the quorum sensing process between bacteria or affect the competitive relationship between bacteria by altering physical and chemical properties such as pH and EORP (oxidation education potential) in the microenvironment; At the same time, some proteins in EVs, such as enzymes or antimicrobial peptides, may also have bactericidal effects on specific types of bacteria. Therefore, the reconstruction of microbial balance also provides a favorable microenvironment for subsequent tissue repair, reducing the continuous invasion of pathogenic bacteria into newly formed tissues.

4.3. Improvement of Microenvironmental Immune Inflammatory Status

In the microenvironment of periodontitis, overactivated inflammatory response is the direct factor leading to the destruction of periodontal tissue. This article constructs an inflammatory response model using macrophage lines and primary macrophages to evaluate the effect of composite scaffolds on inflammatory response. Co culture the scaffold with macrophages treated with lipopolysaccharide, and detect the expression levels of inflammatory factors using enzyme-linked immunosorbent assay and real-time fluorescence quantitative polymerase chain reaction. The results showed that the composite scaffold could downregulate the expression of inflammatory factors tumor necrosis factor alpha, interleukin-1 beta, and interleukin-6, and upregulate the expression of inflammatory inhibitory factor interleukin-10. The results of flow cytometry analysis showed that the proportion of M1 macrophages decreased and the proportion of M2 macrophages increased after the composite scaffold, and this phenotypic transition of macrophages is an important process for tissue transition from inflammation to repair; After intervention studies using signaling pathway blockers, it was found that extracellular vesicles may affect the aforementioned functions through two pathways: TLR4/NF κ B and STAT3. In addition, composite scaffolds can reduce the generation of reactive oxygen species, decrease the activity of matrix metalloproteinases 8 and 9, and alleviate collagen degradation. The above results indicate that this composite scaffold can simultaneously inhibit inflammation mediated tissue destruction from both direct (microbiota) and indirect (immune regulation) aspects.

5. EVALUATION OF THE EFFECT OF PROMOTING PERIODONTAL TISSUE REGENERATION

5.1. In Vitro Evaluation of Cell Proliferation and Differentiation Ability

In order to verify the effect of composite scaffolds on periodontal tissue derived cells, human periodontal ligament stem cells were isolated and cultured in this experiment, and identified. After seeding cells on different scaffolds, their proliferation activity was measured using the CCK8 method. The results showed that the cell proliferation rate of the composite scaffold group was significantly faster than that of the simple scaffold group and the blank control group, indicating that the sustained release of extracellular vesicles provided a sustained pro proliferative signal. Staining of live and dead cells showed that the composite scaffold had a high cell survival rate and fewer dead cells; The composite scaffold group showed an increase in ALP specific activity and the number of mineralized nodules under osteogenic differentiation induction medium conditions; Real time fluorescence quantitative polymerase chain reaction was used to analyze the expression of osteogenic related mRNA. The results showed that Runx2, osteopontin, and osteocalcin mRNA were all increased to varying degrees in the composite scaffold group. Meanwhile, immunofluorescence staining of type I collagen and osteopontin revealed that the composite scaffold can promote the synthesis and maturation of extracellular matrix [10]. The above results indicate that the composite scaffold can promote the osteogenic differentiation of periodontal ligament stem cells cultured in vitro, providing a certain cytological basis for their bone regeneration in vivo.

5.2. Repair Effect of Periodontal Bone Defects in Animal Models

Construct a periodontitis model in rats and induce periodontitis by ligating with silk thread and inoculating *Porphyromonas gingivalis*. After observing significant alveolar bone resorption, remove the silk thread and create a periodontal bone defect area; Subsequently, they were randomly divided into a blank control group, a simple stent group, and a composite stent group. Microscopic CT scanning and three-dimensional reconstruction analysis were performed on the alveolar bone regeneration status after 4 and 8 weeks of postoperative sacrifice, respectively. The quantitative results of micro CT showed that the bone volume fraction and new bone density of the composite

stent group were significantly higher than those of the other two groups at week 8; The composite scaffold group showed the best bone coverage and height recovery in the bone defect area; The results of HE staining and Masson staining showed that there were a large number of newly formed bone trabeculae growing from the edge of the bone defect area to the center in the composite scaffold group. Mature lamellar bone formation was observed in some areas, while partial bone repair was observed in the simple scaffold group. However, the amount of newly formed bone was low and unevenly distributed. The bone defects in the blank control group were mainly caused by fibrous tissue, with very little bone regeneration.

5.3. Integration of Structure and Function of Freshman Organizations

In addition to bone tissue regeneration, periodontal ligament repair and functional attachment are also indicators of successful periodontal regeneration. By using hematoxylin eosin staining and Masson trichrome staining to observe the structure of newly formed tissues, it was found that in the composite scaffold group, not only did a continuous layer of bone tissue form in the bone defect area, but also neatly arranged collagen fiber bundles were formed. Some of the collagen fibers showed Sharpey fiber like embedding into the newly formed bone, indicating functional periodontal membrane like attachment, while the other two groups did not form such intact attachment; At the same time, IHC results showed strong positive expression of osteocalcin and osteopontin in the new bone area of the composite stent group. Type I collagen was regularly arranged in the periodontal ligament like area, and the expression of vascular endothelial growth factor was significantly enhanced, indicating abundant neovascularization and providing protection for tissue metabolism. The biomechanical test results showed that the dislocation force of the composite stent group was significantly increased, approaching the size of normal tooth dislocation force, indicating that some newly formed tissues have mechanical effects. These results indicate that the composite scaffold not only promotes the repair of bone tissue itself, but also achieves the reconstruction of functional periodontal composites.

6. CONCLUSION

This article establishes a 3D printed composite scaffold loaded with extracellular vesicles and verifies its role in regulating the microenvironment of periodontitis microbiota and tissue regeneration ability. The specific results can be summarized as follows: (1) Composite scaffolds can reduce the number of periodontal pathogenic bacteria and biofilm, enhance microbial diversity, and rebuild a relatively healthy microbial ecosystem. Secondly, composite scaffolds improve the local immune inflammatory status of periodontitis by regulating macrophage polarization and inhibiting the expression of inflammatory factors; Thirdly, composite scaffolds can promote the proliferation and osteogenic differentiation of periodontal ligament stem cells in vitro, and achieve significant alveolar bone regeneration and the formation of functional periodontal ligament like structures in animals. This study provides a novel strategy for the treatment of periodontitis that integrates microbiota regulation, immune regulation, and tissue induction, and has good scientific value and clinical translation prospects. In future research, the clinical application of this technology can be promoted by further optimizing the source of extracellular vesicles and engineering modifications, exploring methods for large-scale preparation and quality control, and conducting long-term safety evaluations.

REFERENCES

- [1] Jin S, Qin X, Qin Y, et al. Ferumoxylol as a locally injectable nanozyme for Periodontitis: pH-Adaptive catalysis and immunomodulation mechanisms [J]. *Materials & Design*, 2026, 265 115950-115950.
- [2] Li J, Wang T, Zhao X, et al. M2 macrophage-derived apoptotic extracellular vesicles mediate immune reprogramming and inhibit osteoclastogenesis via mitophagy to ameliorate periodontitis [J]. *Chemical Engineering Journal*, 2026, 530 172141-172141.

- [3] Ren H, Liu P, Sun Z, et al. Combined strategy of dual-module cerium nanosystem composite extracellular vesicles regulate ROS in the tissue microenvironment to promote periodontitis recovery [J]. *Materials Today Bio*, 2026, 36 102625-102625.
- [4] Yang C, Wang W, Feng C, et al. Human periodontal ligament stem cells-derived small extracellular vesicles mitigate periodontitis by regulating macrophage pyroptosis. [J]. *Journal of translational medicine*, 2025, 24 (1): 58-58.
- [5] Rojas C, García M, Osuna G L, et al. Induced Treg-Derived Extracellular Vesicles Suppress CD4+ T-Cell-Mediated Inflammation and Ameliorate Bone Loss During Periodontitis Partly Through CD73/Adenosine-Dependent Immunomodulatory Mechanisms. [J]. *Journal of extracellular vesicles*, 2025, 14 (7): e70118.
- [6] Liu Z, Yang J, Tan G, et al. Methotrexate loaded extracellular vesicles attenuate periodontitis by suppressing ACSL1 and promoting anti-inflammatory macrophage. [J]. *Molecular immunology*, 2025, 182 83-95.
- [7] Jiang Y, Chen Y, Ge L, et al. Multi-prospects of bacterial extracellular vesicles in immune modulation, inflammation regulation, and periodontitis treatment [J]. *Nano Today*, 2024, 55 102210-.
- [8] Xixi W. Human Umbilical Cord Mesenchymal Stem Cells and their Extracellular Vesicles Modulate Pro- and Anti-inflammatory Cytokines in Ligature-induced Periodontitis. [J]. *Iranian journal of immunology: IJI*, 2023, 20 (4):
- [9] Yuanyuan S, Ruijie Z, Ningning D, et al. Aspirin loaded extracellular vesicles inhibit inflammation of macrophages via switching metabolic phenotype in periodontitis [J]. *Biochemical and Biophysical Research Communications*, 2023, 667 25-33.
- [10] X X Z, X W, Y X R. [Research progress on the role of extracellular vesicles in the diagnosis and treatment of chronic periodontitis]. [J]. *Zhonghua kou qiang yi xue za zhi = Zhonghua kouqiang yixue zazhi = Chinese journal of stomatology*, 2021, 56 (4): 385-389.