Research and Prospect: 3D Printing of Gradient Porous Bioceramic Scaffolds Combined with Directed Differentiation of Mesenchymal Stem Cells Promotes Vascular Repair of Large Bone Defects

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Abstract. The repair of large-segment bone defects currently poses a major challenge in the field of orthopedics, as traditional repair methods have various limitations. The 3D printing of gradient porous bioceramic scaffolds, combined with the directed differentiation of mesenchymal stem cells (MSCs), provides a new avenue for simultaneously promoting bone regeneration and vascularization. This review summarizes the design principles of gradient porous scaffolds (material selection, pore size gradient, porosity, and connectivity), 3D printing manufacturing techniques such as low-temperature deposition modeling and 3D plotting, and the regulatory mechanisms of scaffold physicochemical properties on the osteogenic/angiogenic differentiation of MSCs. Through a comparative analysis of existing strategies, this paper discusses the current challenges and future development directions. Designing systems capable for the precise and sustained release of pro-angiogenic factor or scaffold with active immunomodulatory functions are able to cell migration and growth, which have benefits for bone repair. The advancement of this field relies on the deep integration of materials science, stem cell biology, and advanced manufacturing technologies.

Keywords: Gradient porous bioceramic scaffolds, directed differentiation of MSCs, bone defect repair.

1. Introduction

Large-segment bone defects are generally defined as the minimum size of a bone defect that cannot heal through the body's own repair mechanisms, and they are primarily caused by high-energy trauma, tumor resection, infection, or congenital deformities. Traditional repair methods typically include autologous and allogeneic bone grafting. Autologous bone grafting has significant disadvantages, such as limited supply, high donor site morbidity, and the trauma of a secondary surgery. Allogeneic bone grafting, on the other hand, faces issues such as immune rejection, the risk of disease transmission, and poor osseointegration. Therefore, there is an urgent need to develop effective alternative therapies.

Bone tissue engineering aims to mimic the natural microenvironment for bone regeneration by combining biomaterial scaffolds, cells, and bioactive factors. 3D-printed gradient porous bioceramic scaffolds have become a strategy for promoting vascularized repair due to their ability to accurately mimic the dense cortical and porous cancellous layers of natural bone, combined with the self-renewal, osteogenic, and paracrine pro-angiogenic capabilities of mesenchymal stem cells. This review aims to systematically summarize the design principles, biological mechanisms, prospects, and challenges of this technological approach based on existing academic research, so as to provide a reference for future studies.

2. Design and Manufacturing of Graded Porous Scaffolds

2.1. Scaffold Materials

The advantages of bio ceramics lie in their biocompatibility, osteoconductivity/ Oste inductivity, and similarity to the inorganic components of natural bone.

2.1.1. Bioactive calcium phosphate-based ceramics

Calcium phosphate materials are the main inorganic components in human bone tissue and possess good biocompatibility. Among them, the most widely used are hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) and beta-tricalcium phosphate (β-TCP, β-$Ca_3(PO_4)_2$). HA bioceramics exhibit excellent biocompatibility but have deficiencies in biodegradability and mechanical properties; β-TCP exhibits excellent biodegradability but has lower bioactivity. By combining HA and β-TCP to prepare biphasic calcium phosphate ceramics, the unique advantages of both materials can be exhibited to a certain extent [1].

2.1.2. Calcium silicate-based ceramics

The advantage of calcium silicate materials is their ability to release bioactive silicon ions. Research has shown that silicon ions can significantly promote the osteogenic differentiation of MSCs through the bone morphogenetic protein-2 (BMP-2) pathway and the AMP-activated protein kinase (AMPK) pathway, and promote angiogenesis by upregulating the expression of the hypoxia-inducible factor-1α (HIF-1α) signaling pathway through the inhibition of prolyl hydroxylase 2 [2]. However, pure calcium silicate degrades too quickly and has low mechanical strength, so it is often combined with CaP or polymers to balance its properties [3].

2.1.3. Composite materials

Single ceramics have limitations, so they are composited with natural or synthetic polymers to form collagen-ceramic composites, improving cell adhesion, proliferation, and differentiation, and enhancing the scaffold's mechanical properties and bioactivity.

2.2. Graded Structure Parameter Design

Natural bone tissue has a complex, hierarchical porous structure, including Haversian and Volkmann's canals in cortical bone, and the trabecular network in cancellous bone. This porous structure not only provides mechanical support but also offers channels for blood vessel growth and nutrient exchange. Porous scaffolds can promote cell migration, proliferation, and the exchange of nutrients and metabolic waste, thereby accelerating the process of angiogenesis and bone tissue regeneration. Higher porosity and larger pore sizes promote angiogenesis and bone tissue ingrowth, while lower porosity and smaller pore sizes are beneficial for protein adsorption and cell adhesion. The pores also significantly impact the mechanical properties of the scaffold's internal structure. Therefore, only by balancing the contradictory effects of different parameters and optimizing the scaffold's design structure can an ideal environment for bone tissue regeneration be provided. Taking the effect of the porosity of porous tantalum scaffolds on cell proliferation as an example (Table 1), the 400-600 μm group of scaffolds showed higher cell proliferation ability than the other groups [4].

Table 1. The relative growth rate of each group (RGR)

	Time Limit	Group			
		100-200μm	200-400μm	400-600μm	600-800μm
RGR(%)	Day 3	98.2	81.7	84.5	94.5
	Day 5	137.4	157.3	175.6	179.1
	Day 7	138.5	153.9	179.3	167.9

Different gradient porous scaffold models were designed, as shown in Fig. 1: The Un group had a uniform pore size distribution (350 μm). The pore size distribution of the GI group gradually

increased from 200 μm at the center to 500 μm at the periphery. The pore size distribution of the GII group gradually decreased from 500 μm at the center to 200 μm at the periphery [5].

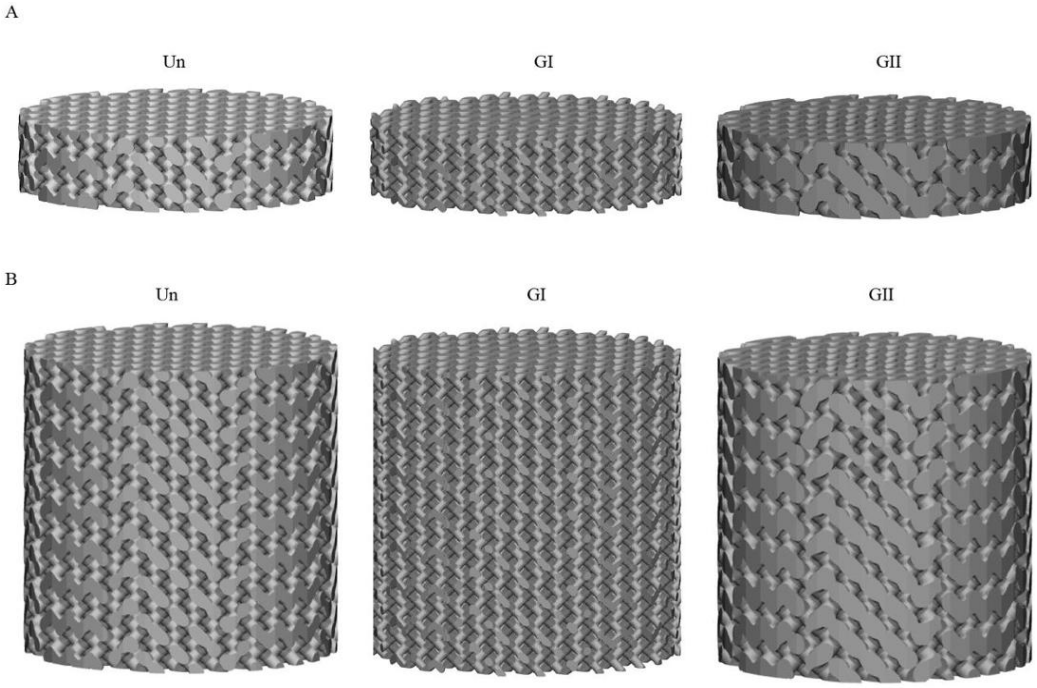

Figure 1. CAD models of porous scaffolds with a homogeneous structure and different gradient structures (A is disc-shaped, B is cylindrical)

2.3. 3D Printing Manufacturing Technology

The 3D printing technologies currently used to fabricate bioceramic scaffolds primarily include Digital Light Processing (DLP), Selective Laser Sintering (SLS), and extrusion-based printing. These technologies enable precise control over the internal structure and external morphology of the scaffolds, allowing for personalized customization. A manufacturing example diagram for repairing personalized bone defects is shown in Figure 2.

Rotational printing technology, combined with the sponge replication method, can be used to fabricate composite structure scaffolds with high-fidelity human bone features. This approach allows for the tailoring of complex geometric shapes based on data obtained from the defect site, enabling the efficient production of high-fidelity bone-mimicking scaffolds. As shown in Fig. 1, the copper-doped diopside/biphasic calcium phosphate composite scaffold fabricated by this method simultaneously mimics the three-dimensional porous network of cancellous bone and the Haversian system in compact bone. Its axial compressive strength reaches up to 149.68 MPa, which is comparable to that of natural human bone [6].

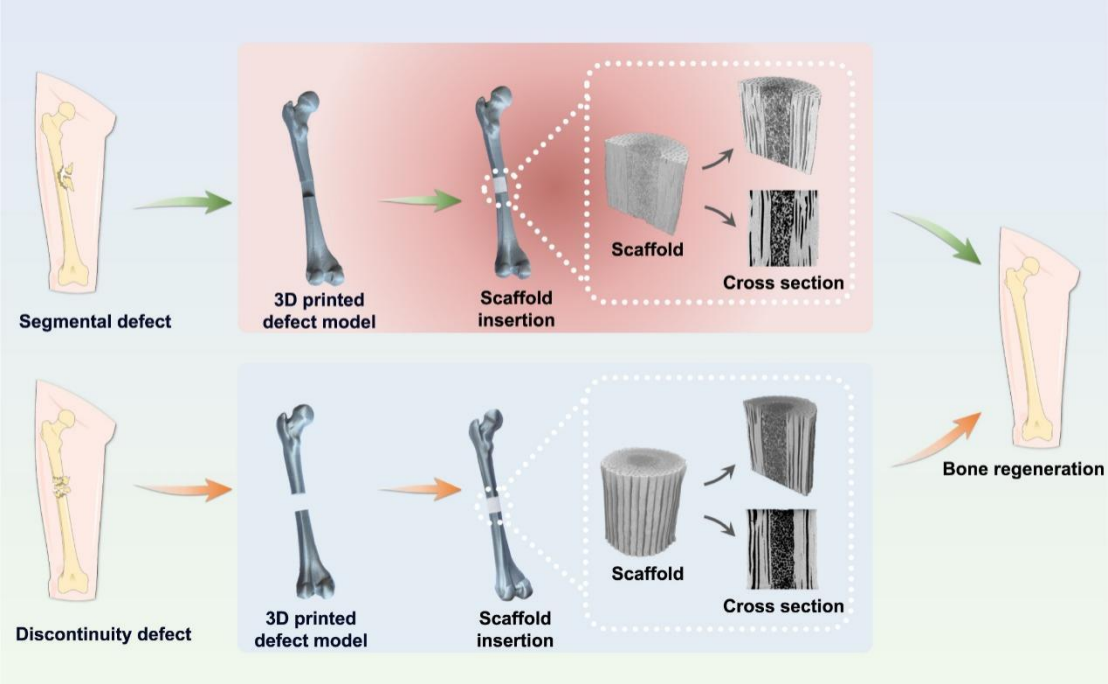

Figure 2. Schematic diagram of the manufacturing strategy for repairing personalized bone defects. The upper panel shows the case of a segmental defect, while the lower panel shows the case of a discontinuity defect.

3. Regulation of Directed Differentiation of Mesenchymal Stem Cells on Gradient Scaffolds

3.1. Mesenchymal Stem Cell-Loaded Scaffolds

After the gradient scaffold is printed, mesenchymal stem cells (MSCs) in the growth phase are densely seeded onto the scaffold surface and co-cultured with a complete medium under suitable conditions. Scanning electron microscopy is used to observe whether the cells are growing well on the scaffold. Further tests confirming that cell viability significantly increases with culture time indicate that the scaffold possesses excellent biocompatibility, successfully achieving stem cell loading and survival [7].

3.2. Molecular Mechanisms of Scaffold Physicochemical Properties-Induced Differentiation

Gradient scaffolds not only provide physical support but also actively regulate the fate of MSCs through various signaling pathways via their physicochemical properties

3.2.1. Chemical signals

Calcium ions released from the degradation of CaP-based scaffolds can activate calcium-sensing receptors on the surface of MSCs, triggering downstream signals, promoting the expression of osteogenic genes, and the formation of mineralized nodules [8]. Silicon ions released from CaSi-based scaffolds are potent osteogenic and pro-angiogenic stimulating factors. They activate the Wnt/β-catenin signaling pathway, upregulating key osteogenic transcription factors; stabilize hypoxia-inducible factor-1α (HIF-1α), inducing high expression of its downstream target genes such as vascular endothelial growth factor (VEGF), thereby promoting endothelial cell migration, proliferation, and vessel formation [9]. Phosphate ions are involved in energy metabolism and the phosphorylation of osteogenesis-related proteins. Ions such as strontium, magnesium, and zinc also have specific functions in promoting osseointegration or providing antibacterial properties.

3.2.2. Physical signals

Different pore sizes affect differentiation by regulating cell morphology. Small pores restrict cell spreading, often inducing a spindle-like shape and activating the RhoA/ROCK pathway to promote osteogenesis; large pores allow for sufficient spreading, which is more conducive to pro-angiogenic phenotypes. The effective stiffness of the scaffold can influence the differentiation direction of MSCs. An environment with stiffness close to that of natural bone tissue is generally more favorable for osteogenic differentiation. Gradient scaffolds can achieve stiffness gradients in different regions through structural design [10].

3.3. Factor-Binding Related Effects

3.3.1. Biofactor delivery

Loading different growth factors into different regions of the scaffold or using carriers with different release kinetics to achieve sequential controlled release: Pro-angiogenic factors are loaded into the large-pored outer layer of the scaffold or in fast-release carriers to promote early vascular invasion and neovascularization. Osteogenic induction factors are loaded into the small-pored inner layer of the scaffold or encapsulated in sustained-release microspheres to provide continuous osteogenic stimulation, promoting bone matrix deposition and mineralization. Developing carrier systems that can simultaneously load and control the release of multiple factors to achieve multifunctional composite delivery.

3.3.2. Genetically modified MSCs

Enhancing specific functions of MSCs through genetic engineering. For example, MSCs overexpressing RUNX2 and BMP-2 can enhance their in vivo and in vitro osteogenic capabilities, while MSCs overexpressing VEGF and stable HIF-1α can continuously secrete pro-angiogenic factors in the hypoxic core of the defect, improving local blood supply [1, 2].

4. Challenges and Future Perspectives

Developing standardized and highly reproducible bioinks and printing processes to reduce batch-to-batch variations, especially for systems containing nanomaterials and bioactive factors. Improving the precision of design and printing control for complex gradients. Establishing a quality evaluation standard system for gradient porous active scaffolds. Conducting in-depth research on the long-term biocompatibility, in vivo distribution, and potential systemic effects of novel biomaterials and their degradation products. Developing more advanced non-invasive or minimally invasive monitoring technologies for long-term dynamic assessment of cell activity within the scaffold, tissue regeneration progress, and material degradation [11].

Designing smarter responsive delivery systems for the precise and sustained release of pro-angiogenic factor combinations at the defect site. Designing scaffold materials with active immunomodulatory functions through scaffold-immune cell interactions, to guide macrophages and other cells to polarize towards a pro-regenerative/pro-angiogenic phenotype.

Utilizing smart materials to enable implanted scaffolds to undergo pre-programmed morphological or functional changes in response to specific in vivo stimuli, better adapting to complex defects and minimally invasive surgical needs. Applying machine learning algorithms to analyze clinical and experimental data to optimize patient-specific scaffold designs and predict repair outcomes [12].

5. Conclusion

3D printing technology for gradient porous bioceramic scaffolds, combined with the directed differentiation of mesenchymal stem cells (MSCs), offers a solution to the challenge of vascularized repair for large-segment bone defects. This review deeply elucidates the pivotal role of gradient design in coordinating osteogenesis and vascularization, and reveals the molecular mechanisms

through which the physicochemical properties of scaffolds regulate MSC fate. Calcium phosphate-based and calcium silicate-based bio ceramics, along with their composites, are at the forefront of current research. Advanced 3D printing technologies have laid the foundation for the precise fabrication of complex gradient structures.

However, the widespread clinical application of this technology still faces significant challenges, including manufacturing standardization, long-term biosafety assessment, precise personalized matching for complex defects, and the rigorous clinical translation pathways for cell-laden products. Future breakthroughs will rely on the deep integration and convergence of multiple disciplines, including materials science, stem cell biology, biomanufacturing, and artificial intelligence. The development of smart-responsive scaffolds, AI-driven personalized design, and multifunctional integrated scaffolds represents highly promising directions for future development. Through continuous innovation in basic research and efficient collaboration in clinical translation, the technology of gradient scaffolds combined with stem cells holds the promise of ultimately becoming an effective clinical strategy for repairing large-segment bone defects, thereby significantly improving patients' quality of life.

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