3D-Printed Functional Materials for Cell Culture and Tissue Engineering: Fabrication Strategies and Applications

Yikang Zhang *

School of Qingdao University, Qingdao, China

* Corresponding Author Email: zhangyikang007zyk@163.com

Abstract. 3D printing technology offers a precise and highly customizable approach for constructing biomimetic three-dimensional tissue engineering scaffolds, largely overcoming the limitations of traditional manufacturing methods in balancing complex microstructures with individual needs. This article systematically reviews the latest advancements in 3D-printable functional materials for cell culture and tissue engineering, focusing on three levels: fabrication concepts, functionalization strategies, and practical applications. Firstly, it examines the key "inks" for 3D printing, comparing the printing characteristics and biocompatibility of natural and synthetic polymers, reversible-dynamic hydrogels, and various inorganic materials, and discusses how to balance printability with scaffold mechanical strength through blending in different ratios or layered structures. Subsequently, the article summarizes strategies to actively regulate cell adhesion, proliferation, differentiation, and even the immune microenvironment by fine-tuning physical structures (pore size gradient, channel orientation, surface roughness, etc.) and chemical modifications (covalent immobilization of peptides, growth factors, or calcium/phosphorus ions, etc.). Based on this, it further illustrates the core application pathways of this technology in constructing highly biomimetic in vitro models—such as tumor microenvironment-on-a-chip, liver or intestinal organoids—and in accelerating the regeneration and repair of bone defects, skin, muscle, and other soft tissues. Although challenges remain regarding the balance between printing resolution and speed, scaffold functional maturation cycles, large-scale quality control, and clinical translation pathways, deep integration with smart responsive materials and immunomodulatory design concepts holds the potential for 3D printing to push "tailor-made, truly functional" artificial tissues into the clinic, bringing substantial breakthroughs to regenerative medicine.

Keywords: 3D printing, Cell culture, Tissue engineering, porous materials, Bone repair.

1. Introduction

The core goal of tissue engineering is to create truly functional tissue substitutes. The key lies in the three-dimensional scaffold and its ability to support cell adhesion, proliferation, migration, and differentiation. An excellent scaffold requires not only basic biocompatibility and mechanical strength but also the ability to mimic the details of the in vivo microenvironment: the composition, structure, and dynamic signals of the extracellular matrix (ECM). However, traditional methods like electrospinning and phase separation face significant limitations in constructing complex porous architectures and personalized anatomical structures. Moreover, conventional 2D culture dishes cannot replicate the three-dimensional interactions between cells and between cells and the matrix, leading to substantial discrepancies between in vitro research findings and in vivo reality. In recent years, to build authentic and accurate cell culture platforms and scaffolds, researchers have continuously developed new methods for constructing cell culture platforms and scaffolds for tissue cultivation: selective laser sintering, stereolithography, fused filament fabrication, micromachining, phase separation, electrospinning, and the recent surge in 3D printing.[1] Among these technologies, 3D printing—especially bioprinting—has pioneered a new approach. Guided by computer models, it layer-by-layer deposits biomaterials, living cells, and even active molecules with precision, allowing for logical and precise control over both the external shape and internal details (porosity, pore size, interconnectivity) of the scaffold. This not only enables customization of the model shape to match a patient's defect but also allows for the spatial patterning of different cells and signals within the same scaffold, making feasible printing strategies for complex transitional tissues like the osteochondral

interface.[2] This article focuses on 3D-printable functional materials for cell culture and tissue engineering, covering the properties of polymers, hydrogels, and inorganic non-metallic materials, their processing methods, and the required surface functionalization modifications; finally, it examines the performance of these materials and techniques in real-world scenarios such as cell culture and bone repair.

2. Preparation Strategies for 3D-Printable Functional Materials

2.1. Polymeric Materials: Natural/Synthetic

Polymeric materials are the most widely used base materials for bioinks, broadly categorized into natural and synthetic based on origin. The most common approach is compositing to offset material weaknesses. Natural polymers like gelatin, chitosan, collagen, silk fibroin, and hyaluronic acid are highly favored due to their inherent biocompatibility, cell recognition sites, and degradability. Their drawbacks, however, are also evident: generally weak mechanical strength and sometimes uncontrollable degradation rates. Taking methacrylated gelatin (GelMA) as an example, this photocurable gelatin derivative offers excellent cytocompatibility, but its printable concentration window is narrow (7%–13%), and it has poor antibacterial capability. Research attempts to composite it with chitosan not only lowered the printable concentration threshold to 4% but also enhanced the scaffold's antibacterial properties: as chitosan content increased from 0% to 5%, the inhibition zone diameter against E. coli expanded from 0 mm to 17.3 mm, while mechanical properties like compressive modulus also improved.

Currently, antibiotics are still commonly used clinically to control infection, but precise dosage control is often difficult in practice. How to select or design a tissue engineering scaffold that can both provide cell support and possess inherent antibacterial capability has become a recurring focus in the field.[15] Chitosan is a naturally positively charged polysaccharide derived mainly from insect shells or shellfish residues. It not only promotes rapid hemostasis and accelerates wound healing but, more crucially, inhibits a variety of bacteria, giving it broad prospects in chemical and pharmaceutical fields. [12-14] This experiment first methacrylated gelatin, then mixed it with chitosan to form a composite bioink. The 3D-printed scaffold, compared to traditional bulk materials, not only offers more controllable internal channel arrangement but also possesses good antibacterial properties, potentially improving the survival rate of subsequent implants.[3]

Silk fibroin is another star natural material that has gained significant attention in recent years. Extracted from silk, its unique internal β-sheet crystalline structure grants its excellent mechanical performance, with an elastic modulus reaching 1–3 GPa. Utilizing various additive manufacturing techniques such as material extrusion, photopolymerization, and even two-photon polymerization, silk fibroin can be shaped into various forms, from macroscopic porous scaffolds to nanoscale fine structures, showing promising application prospects in bone defect and cartilage injury repair. Synthetic polymers such as poly(ε-caprolactone) (PCL), polylactic acid (PLA), and poly (lactic-co-glycolic acid) (PLGA) possess well-defined and finely tunable molecular structures. Their mechanical strength and degradation profiles can be designed according to needs, making them suitable for fabricating load-bearing bone scaffolds requiring long-term mechanical support. PCL scaffolds printed using fused deposition modeling (FDM) have been repeatedly validated in bone tissue engineering experiments, accumulating extensive research data. To further endow these synthetic materials with bioactivity or even smart responsiveness, researchers have developed various novel composite systems in recent years. For example, blending PCL with thermoplastic polyurethane (TPU) produces filaments with thermally responsive shape memory properties. Scaffolds printed from this material can undergo programmed morphological changes near body temperature, better fitting the "deform-and-conform" requirements of minimally invasive surgery for implants. If further functionalized by introducing a polydopamine (PDA) coating on the surface, the scaffold's hydrophilicity and cytocompatibility—measured by MG-63 cell proliferation rate—can be significantly improved.[4]

2.2. Hydrogels

Hydrogels have extremely high-water content and a soft texture closely resembling the feel of natural ECM, making them ideal carriers for encapsulating living cells. However, current research hotspots have moved beyond traditional "A qualitative test" static crosslinked hydrogels towards "dynamic bioinks." These inks rely on reversible physical interactions or dynamic chemical bonds—such as Schiff base bonds, boronate ester bonds, or host-guest interactions—to build a network structure that can be constantly remodeled, mimicking the "remodel-as-you-go" characteristic of natural ECM.[5] Their advantages primarily include smooth printing, self-healing capability after filament breakage, and the ability to form complex 3D structures in one go. Secondly, they provide ample room for cellular remodeling, allowing cells to gradually migrate and reorganize their surrounding matrix, ultimately forming functional tissue. For instance, a team from the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, developed a "dual-crosslinked" dynamic bioink: a photopolymerizable network locks the macroscopic shape, while dynamic covalent crosslinks allow for continuous microscopic remodeling, enabling cells to move freely within it. Using this ink, vascular endothelial cells and smooth muscle cells could spontaneously arrange into a bi-layer tube structure, eventually capable of contracting like natural blood vessels.[6]

Traditional bioink formulations typically have low cell density, differing significantly from real tissues. Recent advancements focus on developing tissue-specific, high-cell-density bioinks. For example, one study incorporated gelatin microspheres loaded with growth factors into a high-cell-density bioink and printed it into a degradable hydrogel microgel bath. The bath acts as both support and nutrient reservoir, allowing controlled slow release of growth factors. Cells were arranged layer-by-layer according to the preset bone-cartilage interface, resulting in a multiphase tissue strip with high resolution, clear boundaries, and functionality closer to the natural structure.[2]

2.3. Inorganic Non-Metallic Materials

Inorganic materials, particularly bioactive ceramics (like hydroxyapatite, β-tricalcium phosphate) and bioglass, are widely used in bone defect repair due to their compositional similarity to bone mineral and inherent osteoconductive and osteoinductive capabilities. However, their inherent brittleness and processing difficulties often hinder clinical scale-up. 3D printing, especially slurry-based extrusion methods, enables the fabrication of complex shapes from composite inks containing otherwise difficult-to-process inorganic powders. Research commonly mixes them with polymers to create organic-inorganic composite inks: Shanghai University developed a biomimetic bone-matrix bioink by crosslinking self-synthesized hydroxyapatite with gelatin and sodium alginate. This ink was used to directly print scaffolds seeded with bone marrow mesenchymal stem cells, which ultimately grew into vascularized bone-like organoids in rats.[7] On another front, magnesium alloys among degradable metals have recently attracted considerable attention. Their elastic modulus is closer to human bone, avoiding stress shielding effects, and the magnesium ions released during degradation can promote osteogenesis. Peking University used 3D printing to fabricate porous WE43 magnesium alloy scaffolds. Implanted into rabbit femoral defects, these scaffolds exhibited good biocompatibility and osteogenic performance, with a degradation rate matching the pace of new bone ingrowth.[8]

Table 1. Four Material Category comparing

Material Category	Representative Materials	Main Advantages	Main Limitations	Typical Application Fields	Key References
Natural Polymers	Gelatin-Chitosan, Silk Fibroin	Excellent biocompatibility, cell affinity, degradable	Weak mechanical properties, potentially rapid degradation	Brittleness (ceramics), difficult degradation rate control (Mg)	[3]
Synthetic Polymers	PCL,PLA,PCL/TPU shape memory materials	High mechanical strength, controllable degradation, good processability	Lack of bioactivity, hydrophobic	Load-bearing bone defect repair, smart implants	[4]
Dynamic Hydrogels	Dual-crosslinked ECM-mimetic inks, dynamic-bonded hydrogels	Adaptable microenvironment, promote cell self-assembly, good printability	Challenges in long-term stability, typically low mechanical strength	Vascular network construction, soft tissue engineering, organoids	[5],[6]
Inorganic Materials	Hydroxyapatite composites, WE43 Mg alloy	High osteoconductivity/ osteoinductivity, mechanical support, degradable (Mg)	Brittleness (ceramics), difficult degradation rate control (Mg)	Bone defect repair, bone-like organoids	[7],[8]

3. 3D Printing Functionalization and Modification

3.1. Surface Physical Modification

Using 3D printing, people can precisely control the macroscopic parameters of the printed framework's pores (porosity, pore size, interconnectivity) as well as microscopic surface details (e.g., how fibers are arranged, micro-topography). These physical cues can directly guide cell orientation, migration, and differentiation. For instance, fibroblasts can be guided to repair tendons, and scaffolds can be designed to mimic the anisotropic 3D lattice of trabecular bone, allowing new tissue to mechanically simulate the stress pathways of real bone.

3.2. Chemical Modification

Chemical modification is still regarded as the most direct and easily scalable route to impart bioactivity to materials. The specific approach involves modifying the material surface with signaling molecules, common examples include the arginine-glycine-aspartic acid (RGD) peptide, bone morphogenetic protein-2 (BMP-2), vascular endothelial growth factor (VEGF), or anticoagulant heparin. For example: coating the scaffold surface with a layer of polydopamine not only improves the hydrophilicity of PCL surfaces but also allows its active catechol groups to immobilize various biomolecules on the surface, simultaneously boosting cell adhesion and proliferation. Alternatively, signaling ions can be introduced into the material matrix, such as incorporating strontium or magnesium ions into the hydroxyapatite crystal lattice; in vitro data show that the strontium-doped group can elevate the transcription levels of osteogenesis-related genes in adipose-derived stem cells by an order of magnitude, leading to more continuous and denser bone tissue regeneration in animal models.

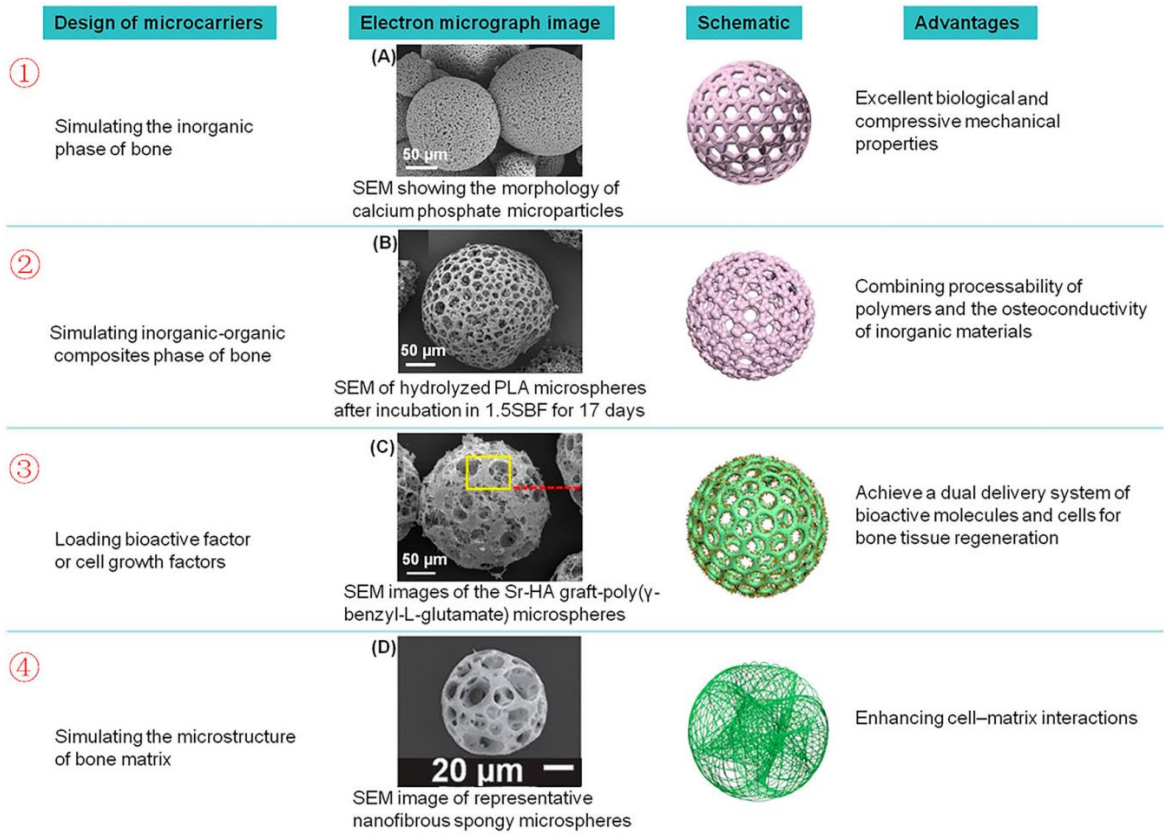

Figure 1. Summarizes the preparation and optimization pathways for porous microcarriers in tissue engineering and regenerative medicine scenarios. The conventional process typically involves first ① simulating the inorganic phase of bone tissue; then ② constructing a composite phase with both inorganic-organic characteristics, the image (B) in parentheses; subsequently ③ loading bioactive factors or cell growth factors, ; and finally ④ replicating the micro-scale hierarchical structure of the bone matrix.

Biofunctional microcarriers are typically obtained by incorporating bioactive factors into natural or synthetic polymers, followed by a step of solidification or crosslinking. Figure 1③ illustrates this optimization process.[22] For instance, to endow microcarriers with 'osteoinductive' properties, a team grafted strontium ions (Sr) directly onto the PBLG backbone, creating a novel nanocomposite Sr-HA-PBLG. They then printed osteoinductive porous microcarriers using Sr-HA-PBLG and co-cultured them with adipose-derived stem cells (ADSCs) for bone regeneration. Compared to the Sr-free control group, ADSCs on the Sr-HA-PBLG microcarriers exhibited higher expression levels of osteogenesis-related genes; implanting the cell-loaded constructs into bone defects for 8 weeks resulted in significantly better defect filling on the Sr-doped side. Doping with bioactive ions like strontium (Sr) and magnesium (Mg) in bone repair materials has been repeatedly verified to significantly improve the material's osteoinductive efficiency and may offer additional benefits such as anti-inflammatory and antibacterial effects. The latest functionalization strategies are shifting focus not only onto parenchymal cells but also towards the immune microenvironment of the implantation site—regulating immune cells through material design. As mentioned earlier, certain hybrid scaffolds can push macrophages towards the M2 phenotype, switching them to a pro-repair, pro-regeneration mode,[2] thereby creating a favorable, mild microenvironment for regeneration at the defect site. This is currently a crucial strategy for the complete repair of large tissue defects.

4. Applications in Advanced Cell Models and Tissue Engineering

3D bioprinting technology overcomes the inherent geometric and mechanical limitations of traditional two-dimensional cell culture, providing an unprecedented revolutionary tool for

constructing highly biomimetic, structurally finely tunable, and patient-specific 3D in vitro models.[16]

4.1. Constructing Physiological/Pathologically Relevant In Vitro Models

3D bioprinting has turned the goal of "high biomimicry" into a reproducible unified framework: simply loading patient-derived cells and custom-formulated bioink into the printhead allows the assembly of personalized tumor micro-models ex vivo. Multi-material printheads switching back and forth can precisely "trace" complex boundaries, such as bone-cartilage interfaces or vascular-nerve bundles, which naturally connect in vivo, layer by layer. 3D-printed hydrogel scaffolds have been widely used to construct models of tumors, liver lobules, and neural tissues. Gou et al. used GelMA to print a liver lobule model, replicating the complete drug metabolism process in a culture dish, opening a new experimental platform for high-throughput drug screening.[10] Printing a patient's own tumor cells with biomimetic bioink not only recapitulates tumor heterogeneity but also, through multi-material printing integration, can incorporate stromal components like vascular endothelial cells and fibroblasts to assemble tumor-like tissues with vasculature. Such 3D models hold broader prospects in drug sensitivity testing compared to traditional 2D cultures or animal models, offering more accurate predictions of chemotherapy or targeted drug efficacy and overcoming the limitations of traditional 2D culture and animal models in predicting human responses.[17] For example, research using 3D printing constructed a prostate cancer organoid model containing specific ECM components. This model successfully simulated the influence of the tumor microenvironment on the therapeutic response to drugs (like EZH2 and DRD2 inhibitors), providing a new platform for precision medicine.

Many critical functions in the human body rely on precise localization and seamless integration at interfaces between different tissues, such as the osteochondral junction, muscle-tendon junction (MTJ), or vascular-nerve bundles; misalignment directly affects movement or metabolism. 3D printing, especially multi-material printing capable of depositing several materials simultaneously, can create gradients or regional patterns of bioink within a single construct, allowing the 'tracing' of these complex interfaces in one print. A team from Zhejiang University proposed an innovative electrochemical molecular lock strategy, linking 3D printing, electrochemical crosslinking, and mechanical training into a pipeline to fabricate a gelatin hydrogel scaffold (ETH scaffold) with continuous stiffness variation from the nano to centimeter scale.[18] This scaffold remarkably mimics the gradient structure of the MTJ: in vitro experiments showed that tendon stem cells and C2C12 myoblasts not only expressed functional proteins normally but also reorganized their own ECM; after implantation in rabbits for 8 weeks, the repaired MTJ almost fully recovered in terms of structure and mechanics, with a tensile modulus reaching 24.89 MPa, far surpassing the control group. In the realm of organoids, 3D bioprinting first locks in the initial spatial arrangement of cells and microenvironmental parameters, significantly reducing batch-to-batch variability, facilitating scale-up, and enabling increasingly complex structures. Technologies like volumetric bioprinting greatly enhance organoid batch consistency, scalability, and structural complexity. Cells also avoid shear forces associated with layer-by-layer printing, opening new avenues for high-throughput fabrication of functionally mature organoids (e.g., liver lobules, kidney organoids) for drug metabolism and toxicity assessment.

4.2. Tissue Regeneration and Repair

In the interdisciplinary frontier of regenerative medicine, functional scaffolds prepared by 3D printing technology have long ceased to be merely laboratory models; they have become key tools for repairing various tissue defects and guiding the body's regenerative processes. The application landscape continues to expand, from early simple bone block fillers, to precise shaping for geometrically complex bone defects, to regenerative support for large-area soft tissue loss, and further upgrading to smart repair systems with immunomodulatory capabilities.

4.2.1 Soft Tissue Regeneration

3D printing technology is also highly anticipated in the regeneration of tissues demanding both mechanical and biological performance, such as skin, cartilage, and blood vessels. For example, a composite scaffold spun from PLGA and gelatin, when applied to a full-thickness skin defect, continuously releases gelatin degradation fragments as it degrades. These fragments recruit fibroblasts to the wound site in batches while stimulating the sprouting of surrounding microvessels, promoting the regrowth of capillary networks in ischemic areas.[11]

An ideal bone repair scaffold should not only possess reliable osteoconductive and osteoinductive functions but also rapidly promote vascular ingrowth post-implantation. The hierarchical fiber arrangement within natural bone provides design inspiration for simultaneously satisfying these three requirements. Researchers from Shanghai University proposed a 3D printing strategy based on this microstructure that requires no additional solvent. They formulated a composite ink from poly(γ-benzyl L-glutamate) (PBLG) and hydroxyapatite nanoparticles (nHA). During extrusion, ethanol vapor induced the formation of a stronger hydrogen bond network between PBLG chains and completed self-assembly, resulting in a biomimetic fibrous framework in one step. The resulting substitute's compressive strength increased to about 36.6 MPa, significantly outperforming traditional methods mechanically. More importantly, this bone-like microstructure significantly accelerated cell migration and upregulated the expression of osteogenesis-related functional proteins in vitro. Rat calvarial defect models also confirmed its promising repair efficiency. Clinically, irregularly shaped bone defects are common. If a scaffold could be compressed for delivery through a minimally invasive channel and then automatically expand to fill the cavity in vivo, it would greatly reduce surgical trauma.[19] A team from the University of New South Wales, Australia, targeting this need, combined the flexibility of PCL with the shape memory properties of TPU to spin a temperature-sensitive composite filament capable of recovering its original shape. They then used FDM to print a monolithic scaffold with interconnected pores. At room temperature, the scaffold can be rolled or compressed into a slender strip for delivery through a small incision. When the temperature rises to near body temperature (~37°C), the cooperative deformation of hard and soft segments within the material causes the entire structure to rapidly rebound, conforming almost seamlessly to the defect contour. To further improve the biointerface, researchers added a polydopamine (PDA) coating to the surface. This modification not only adjusted the water contact angle to a suitable range but also significantly improved the early adhesion rate and subsequent proliferation rate of MG-63 cells, without compromising the scaffold's excellent shape recovery ability (specific structure recovery rate up to 98%), providing strong support for its practical application in bone tissue engineering.[20]

4.2.2 Bone Repair

In soft tissue regeneration scenarios such as blood vessels, skin, and cartilage, 3D printing technology currently focuses on addressing three main issues: first, how to safely encapsulate living cells within the scaffold; second, whether the printed structure can maintain its shape long-term without collapsing in the physiological environment; and third, whether the new tissue can truly integrate with the host and function properly. Hydrogel scaffolds encapsulating living cells often suffer from structural collapse post-implantation due to cell contraction and ongoing compression from surrounding tissues. To mitigate this common challenge, researchers have proposed composite bioink strategies. For example, one study uniformly incorporated highly elastic poly (octamethylene maleate (anhydride) citrate) (POMaC) microparticles into conventional biohydrogels like fibrin and GelMA to formulate a composite bioink with both elasticity and plasticity. The introduction of elastic particles allows the printed construct to more effectively dissipate the mechanical tension generated by cells. In vitro experiments showed that human pluripotent stem cell-derived cardiac tissue patches and vascular structures achieved more complete functional assembly and maintained morphological stability for longer periods. In vivo transplantation results further indicated that constructs containing POMaC particles exhibited stronger resistance to deformation under the complex stresses exerted by

host tissues, while also stimulating local angiogenesis and attracting macrophage infiltration with a pro-repair phenotype.[21]

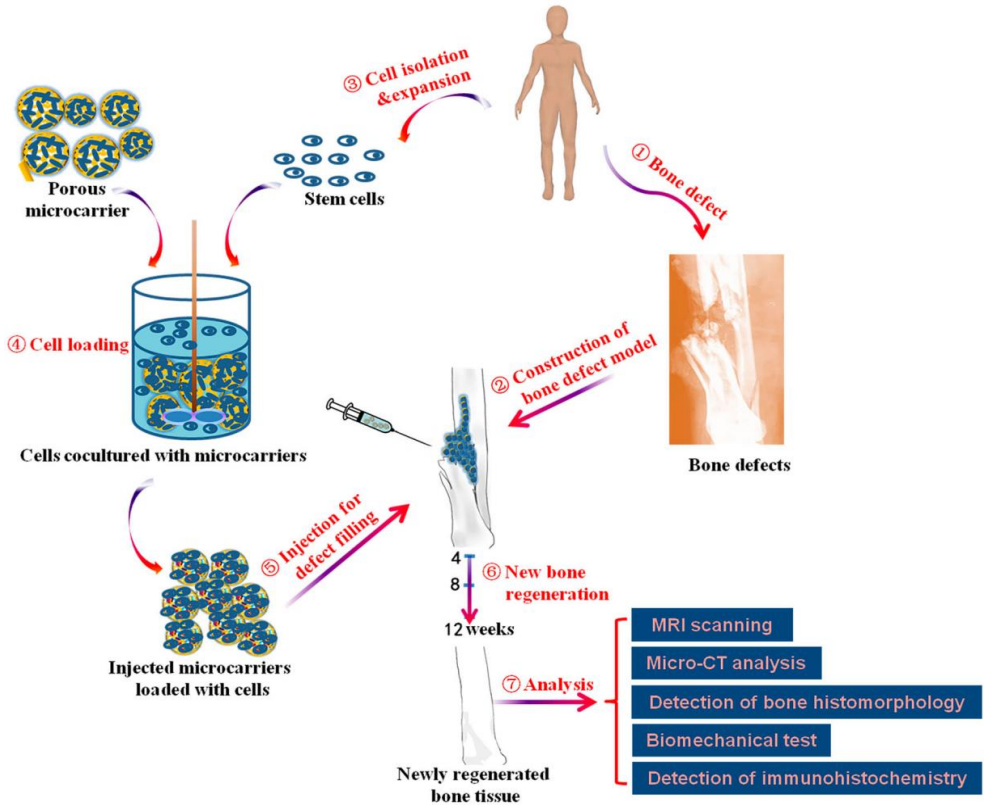

Figure 2. Schematic illustration of preparation methods for injectable microcarriers in bone tissue engineering applications. The advantage of porous microcarriers lies in their internally controlled porous structure, which provides sufficient attachment sites and nutrient channels during the cell growth phase, thereby more effectively promoting cell proliferation and differentiation. This allows for the delivery of a substantial number of seeded cells in one go for the subsequent new bone regeneration stage. Meanwhile, such injectable porous microcarriers can spontaneously aggregate into a gel-like network with good fluidity within the body, enabling smooth injection and tight filling of defect areas regardless of complex geometric contours. The aggregated cell-microcarrier construct facilitates regeneration.

Figure 2 presents a schematic of the application of injectable microcarriers in bone tissue engineering. Typically, the first step involves taking a small biopsy from the patient to isolate stem cells, followed by in vitro expansion. Subsequently, the harvested cells are mixed with microcarriers to form cell-laden microbeads, which are then injected to fill the bone defect area. Finally, over several weeks post-implantation, new bone gradually forms as the cells proliferate and differentiate and the microcarriers undergo biodegradation.[22]

5. Summary, Challenges, and Future Perspectives

5.1. Summary

The emergence of 3D printing technology has opened an unprecedented avenue for transformation in tissue engineering and regenerative medicine. Through point-by-point control over material spatial arrangement, micro-scale channels, and bioactive components, printers can produce highly biomimetic scaffolds with complex mechanical gradients, tailored to patient anatomical data, within hours. This article takes a broad view, systematically reviewing the major categories of "inks" currently loadable into printheads—from natural polymers like collagen and gelatin, to biodegradable synthetic polymers like PLA and PEG, to dynamic hydrogels that deform with temperature or pH,

and inorganic non-metallic particles like hydroxyapatite and bioglass. It then summarizes specific methods using physical-chemical means like plasma treatment, photocrosslinking, layer-by-layer self-assembly, or surface mineralization to "add a layer of functionality" to the printed framework. Through composite 3D printing approaches, dynamic designs are achieved, leading to innovative improvements in materials' mechanical properties, bioactivity, antibacterial capability, and smart responsiveness, driving tissue engineering from static scaffolds towards dynamic, interactive, life-like constructs.

5.2. Current Major Challenges

Despite considerable progress in the laboratory, 3D printed tissue engineering faces a series of constraints in real-world clinical translation. First, on the manufacturing side, improving resolution requires slowing printhead speed, directly prolonging cell exposure within the ink, while the dose of curing light or crosslinkers may conversely harm viability. Thus, precision, efficiency, and biocompatibility form a challenging triangle, difficult to optimize simultaneously. Second, from a scientific perspective, replicating the intricate intertwining of microvessels, nerve bundles, and ECM regeneration of natural tissues remains a significant hurdle. Currently printed scaffolds still appear "rudimentary" at the micro-scale, with functional maturity lagging noticeably behind native tissue. For the materials themselves, degradation rates must match the growth rate of new tissue; simultaneously, interface stability must be maintained over observation periods of months or even years, as any chronic inflammatory or toxic signals can become magnified into failure cases. Furthermore, during industrialization, unified standards are still lacking for aspects like bioink composition limits, temperature/humidity windows for printing chambers, and acceptable batch-to-batch variations, creating regulatory approval barriers and hindering large-scale production. These interrelated challenges collectively constitute multidimensional barriers that must be overcome for the technology to advance towards widespread clinical application.

5.3. Future Perspectives

Future research directions should revolve around the following areas: Four-Dimensional Bioprinting: Integrating stimulus-responsive materials directly into ink formulations, enabling scaffolds to "transform on a timer" post-implantation—changing shape or function according to preset programs triggered by signals like temperature, pH, or magnetic fields. These better fits the "spatially constrained, dynamically changing" reality of minimally invasive surgery and provides room for tissue regeneration to unfold over time. Intelligent and High-Throughput Biofabrication: Connecting artificial intelligence and machine learning to bioprinting pipelines, letting algorithms optimize ink formulations and process parameters, and performing in-line signal acquisition and trajectory correction; establishing high-throughput platforms to process hundreds of samples in parallel, using printed micro-tissues for drug screening, disease modeling, or personalized "tissue chips." Multi-Scale, Multi-Material Integrated Printing: Achieving single-print fabrication spanning nanoscale ECM fibers, microscale cell patterning, and centimeter-scale organ contours, while seamlessly integrating materials with vastly different properties like soft gels, hard ceramics, and elastomers. This would ultimately enable one-step printing of composites like bone-tendon-muscle junctions, avoiding interfacial weaknesses from segmented assembly. In Vivo Bioprinting and In Situ Regeneration: Moving the printer from the lab to the operating room, using lightweight printheads to directly deposit cells and hydrogels onto wounds, bypassing the old path of in vitro culture and re-implantation, allowing tissue to regenerate in situ at the defect site, shortening procedures and reducing secondary injury.

Overall, 3D-printed functional materials are acting as a bridge spanning materials science, biology, and clinical medicine, integrating technologies and theories originally scattered across laboratories, engineering workshops, and operating tables. They continuously push tissue engineering towards being "more like native tissue, more functionally complete, and more clinically viable." As interdisciplinary teams continue to tackle technical hurdles one by one, it may become possible in the

future to "print" artificial tissues or even organs in hospitals on-demand for patients—tissues that can both survive and integrate seamlessly with the body—bringing direct benefits to a wider range of patients.

References

[1] Tan Z, Liu T, Zhong J, Yang Y, Tan W. 2017. Control of cell growth on 3D-printed cell culture platforms for tissue engineering. J Biomed Mater Res Part A 2017: 105A: 3281–3292.

[2] Jeon O, Park H, Leach K J, et al.Biofabrication of engineered tissues by 3D bioprinting of tissue specific high cell-density bioinks [J]. Materials Today, 2025, 86172-182.

[3] Li Liang, Yang Han, Suo Hairui, Guan Lu, Wang Zhenlin. 3D printed methacrylated gelatin/chitosan scaffolds: evaluation of antibacterial, mechanical properties and cytocompatibility[J]. Chinese Journal of Tissue Engineering Research, 2026, 30(14): 3636-3642.

[4] Sabahi N, Roohani I, Wang H C, et al. Material extrusion 3D printing of bioactive smart scaffolds for bone tissue engineering [J]. Additive Manufacturing, 2025, 98104636-104636.

[5] Ma D, Liu J, Lu W W, et al. Dynamic bioinks for tissue/organ bioprinting: Principle, challenge, and perspective [J]. Progress in Materials Science, 2026, 155101527-101527. DOI:10.1016/J.PMATSCI.2025.101527.

[6] Chen J, Wu Y, Huang J, et al. Microscopically Adaptable Bioink Guide Cell Compartmentalization toward Morphogenesis of a Functional Vasculature-Like System. [J]. Advanced healthcare materials, 2025, 14(29): e02347.DOI:10.1002/ADHM.202502347.

[7] Wang J, Zhou D, Li R, Sheng S, Li G, Sun Y, Wang P, Mo Y, Liu H, Chen X, Geng Z, Zhang Q, Jing Y, Bai L, Xu K, Su J. Protocol for engineering bone organoids from mesenchymal stem cells. Bioact Mater 2024, 45: 388-400.

[8] MIN shuyuan, TIAN yun. Biocompatibility of 3D printed biodegradable WE43 magnesium alloy scaffolds and treatment of bone defects, 2025, 57(02): 309-316.

[9] J J C, Jin Y, T S B S, et al.3D Printed Porous Methacrylate/Silica Hybrid Scaffold for Bone Substitution. [J]. Advanced healthcare materials, 2021, 10(12): e2100117-e2100117.DOI:10.1002/ADHM.202100117.

[10] Maling G, Xin Q, Wei Z, et al.Bio-inspired detoxification using 3D-printed hydrogel nanocomposites.[J]. Nature communications, 2014, 5(May): 3774

[11] Yildirimer L, Thanh T N, Seifalian M A. Skin regeneration scaffolds: a multimodal bottom-up approach [J]. Trends in Biotechnology, 2012, 30(12): 638-648.

[12] JI Dongqing, SUN Dandan, HE Huanxiang, TIAN Xiaomeng, GUO Qingmei, et al. Research Progress on Antibacterial Activity of Chitosan [J]. Journal of Liaoning University of Traditional Chinese Medicine 2018, 20(03): 82-85. DOI:10.13194/j.issn.1673-842x.2018.03.024.

[13] Ya S, Yaqian L, Xueyan H, et al. Caffeic acid-grafted chitosan/sodium alginate/nanoclay-based multifunctional 3D-printed hybrid scaffolds for local drug release therapy after breast cancer surgery[J]. Carbohydrate Polymers, 2024, 324121441-121441.

[14] Hui I, Pasquier E, Solberg A, Agrenius K, Håkansson J, Chinga-Carrasco G. Biocomposites containing poly (lactic acid) and chitosan for 3D printing - Assessment of mechanical, antibacterial and in vitro biodegradability properties. J Mech Behav Biomed Mater. 2023 Nov; 147:106136.

[15] Patil V T, Jin H, Dutta D S, et al.Zn@TA assisted dual cross-linked 3D printable glycol grafted chitosan hydrogels for robust antibiofilm and wound healing[J].CarbohydratePolymers,2024,344122522-122522.

[16] Li, Zeqing, Chen, Long, Wu, Jialin, Chen, Yikang, Zhu, Yizhun, Li, Gang, Xie, Guoxi, Tang, Guosheng and Xie, Maobin. "A review of 3D bioprinting for organoids" Medical Review, vol. 5, no. 4, 2025, pp. 318-338.

[17] Sousa, A.C.; Alvites, R.; Lopes, B.; Sousa, P.; Moreira, A.; Coelho, A.; Santos, J.D.; Atayde, L.; Alves, N.; Maurício, A.C. Three-Dimensional Printing/Bioprinting and Cellular Therapies for Regenerative Medicine: Current Advances. J. Funct. Biomater. 2025, 16, 28.

[18] K. Yao, P. Xia, W. Kong, et al. “3D Printing of Gradient Biomimetic Scaffold via Electrochemical Molecular Lock for Tissue Regeneration.” Adv. Mater. (2025): e13484.

[19] Zhang K, Fang H, Cheng X, et al. Biomimetic Fibrous Bone Substitute Manufacture Through Non-Solvent-Assisted 3D Printing [J]. Advanced Functional Materials, 2025, 35(19): 2419464-2419464.

[20] Sabahi N, Roohani I, Wang H C, et al. Material extrusion 3D printing of bioactive smart scaffolds for bone tissue engineering [J]. Additive Manufacturing, 2025, 98104636-104636.

[21] Landau S, Kieda J, Khosravi R, Okhovatian S, Ramsay K, Liu C, Shakeri A, Zhao Y, Shen K, Bar-Am O, Levenberg S, Tsai S, Radisic M. Cell driven elastomeric particle packing in composite bioinks for engineering and implantation of stable 3D printed structures. Bioact Mater. 2024 Oct 29; 44: 411-427.

[22] Zhihua Zhou, Wei Wu, Jianjun Fang & Jingbo Yin (2020): Polymer-based

[23] Porous microcarriers as cell delivery systems for applications in bone and cartilage tissue engineering, International Materials Reviews