

# Overcoming Therapeutic Bottlenecks of Infected Bone Defects via 3D-Printed Scaffolds Integrated with Antibacterial, Osteogenic and Angiogenic Synergistic Functions

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## ABSTRACT

Infected bone defects (IBDs) triggered by open fractures, implant-related infections, chronic osteomyelitis and bone tumor resection remain intractable orthopedic complications, contributing to elevated disability rates, recurrent infection, prolonged treatment courses and heavy social-economic costs. Conventional treatments such as bone grafting and antibiotic-loaded bone cement are plagued by donor shortage, immune rejection, uncontrolled antibiotic leakage and poor anatomical matching, incapable of synchronously eradicating bacteria, inducing osteogenesis and reconstructing blood vessels. Benefiting from personalized shaping, accurate structural modulation, tunable porous structures and broad material adaptability, 3D printing has become an advanced manufacturing method for patient-specific bone scaffolds. The construction of triple-functional scaffolds integrating antibacterial, osteogenic and angiogenic effects is critical to overcome current IBD treatment limitations and facilitate the clinical transformation of bone engineering materials. This review first addresses clinical drawbacks of IBD management and the superiority of 3D printed scaffolds. We systematically recapitulate matching materials, prevailing printing techniques, biomimetic design schemes and biological assessment systems, compare various antibacterial modalities, summarize translational achievements and analyze existing clinical transformation obstacles. Future orientations covering responsive intelligent scaffolds, 4D printing and combined therapies are proposed. This work delivers theoretical guidance for basic research and clinical application of 3D printed antibacterial bone scaffolds.

**Keywords:** 3D printing/additive manufacturing, Antimicrobial bone scaffolds, Infected bone defects, Antibacterial-osteogenic-angiogenic synergy, Clinical translation, Multifunctional biomaterials.

## INTRODUCTION

### Clinical Challenges of Inflammatory Bone Defects

Inflammatory bone defects represent prevalent, intractable orthopedic pathologies. Infection rates after internal fixation for open fractures range from 10% to 30%. Rising aging populations and high-energy traumatic injuries drive annual increases in chronic osteomyelitis incidence. Clinically, these lesions are often accompanied by soft tissue damage, local microcirculation disorders, persistent inflammatory responses and bacterial biofilm formation<sup>1</sup>. Such pathological features readily trigger bone nonunion and delayed healing; severe cases require limb amputation, while patients undergo repeated debridement and long-term systemic antibiotic administration. The core clinical conflict lies in the mutual restriction between infection control and bone regeneration: biofilms

directly suppress osteoblast viability and disrupt regenerative microenvironments; excessive systemic antibiotics readily induce multidrug-resistant strains, organ cytotoxicity and intestinal flora dysbiosis<sup>2</sup>. The Masquelet induced membrane technique, the current clinical gold standard, relies on two sequential surgical stages, which incur prolonged treatment durations, elevated medical costs, and limited therapeutic efficacy for large irregular bone defects<sup>3</sup>. Thus, researchers urgently develop multifunctional scaffolds with potent antimicrobial activity, targeted osteogenic capacity and rapid vascularization potential to break existing repair bottlenecks.

Four mainstream reconstructive regimens are currently adopted for IBDs: the Masquelet induced membrane technique, Ilizarov bone transport, free vascularized fibula grafting, and standardized fracture-related infection (FRI) management algorithms<sup>4</sup>. The two-stage Masquelet procedure remains the

gold standard but demands sequential debridement, cement spacer implantation and secondary bone grafting, leading to prolonged hospitalization, repeated anesthesia exposure and high recurrent infection risks<sup>5</sup>. Non-vascularized autologous bone grafts fail to resolve central tissue necrosis within large irregular bone defects. Ilizarov bone transport requires long-term external fixation, which triggers pin tract infections, joint stiffness and poor patient compliance. Vascularized free bone flaps impose rigorous requirements for microsurgical proficiency, limited donor bone volumes and obvious donor-site complications. Standard FRI management prioritizes long-term systemic antibiotics combined with radical debridement, yet cannot synchronize local pathogen clearance and bone regeneration. Antimicrobial tri-functional 3D-printed scaffolds serve as modular orthopedic adjuvants compatible with all four reconstructive strategies<sup>6-8</sup>:

(1)Masquelet reconstructive surgery: Degradable 3D-printed antimicrobial scaffolds replace antibiotic-laden bone cement spacers to achieve one-stage infection clearance and bone regeneration, eliminating secondary surgical interventions. (2)Ilizarov bone transport: Porous load-bearing 3D scaffolds implanted at bone docking sites accelerate osseous union and reduce pin tract infection risks through sustained local ion release within scaffolds. (3)Vascularized composite bone graft repair: Composite 3D scaffolds optimize mechanical matching with free fibula flaps and supply local antimicrobial protection against peri-graft infectious lesions. (4)Hierarchical standardized FRI treatment: Low-dose responsive scaffolds treat mild superficial FRI; metal-based load-bearing antimicrobial implants address segmental bone loss induced by high-energy trauma.

### **Advantages of 3D Printing for Preparing Personalized Scaffolds**

Compared with conventional fabrication techniques such as solvent casting and particle leaching, 3D printing delivers four core strengths that fully satisfy repair demands for infected bone defects<sup>9-11</sup>: (1) Personalized customization: CT/MRI scanning data enables precise geometric matching with irregular bone defects. (2)Controllable pore architectures: Pore diameters, porosity and interconnectivity can be precisely tuned to mimic native cancellous bone and facilitate nutrient transport and cell infiltration within scaffolds. (3)Broad material compatibility: The technique accommodates polymers, bioceramics, metallic materials and composite materials, and supports loading of antimicrobial agents and bioactive factors.

(4)Standardization potential: The fabrication workflow supports large-scale, reproducible manufacturing to advance progression toward clinical translation.

These strengths establish 3D printing as the preferred manufacturing technology for multifunctional anti-infective bone scaffolds. Despite prominent advantages, high-precision printing equipment carries substantial costs, post-processing workflows remain complex, and insufficient bioink stability restricts widespread clinical deployment. Additionally, not all printing modalities support progression toward clinical translation<sup>12</sup>.

### **The Necessity of Tri-functional Synergistic Design**

Repair of infected bone defects constitutes a sophisticated biological process dependent on synergistic coordination of multiple biofunctions. Single-function scaffolds cannot satisfy clinical therapeutic requirements<sup>13</sup>. Antimicrobial activity acts as the prerequisite: scaffolds eliminate pathogenic bacteria and biofilms to construct sterile regenerative microenvironments. Osteogenic capacity forms the core biological function: materials induce mesenchymal stem cell osteogenic differentiation and promote bone matrix mineralization and nascent bone formation<sup>14</sup>. Vascularization serves as the fundamental guarantee: newly formed blood vessels deliver oxygen and nutrients, prevent central tissue necrosis within scaffolds, and improve local delivery efficiency of antimicrobial components<sup>15</sup>.

This review defines the concept of “antimicrobial–osteogenic–angiogenic tri-functional synergy”: the three biological functions are not merely superimposed, but mutually reinforce and support one another with temporally coordinated biological coupling effects<sup>16</sup>. Effective antimicrobial activity protects osteoblasts and vascular endothelial cells; mature osteogenic differentiation accelerates vascular remodeling; robust vascularization optimizes local microenvironments and amplifies scaffold antimicrobial efficacy. The core criterion for verifying tri-functional synergy states that composite therapeutic effects significantly exceed the linear additive outcomes of single or dual-function groups, without mutual functional suppression.

The three biological functions interpromote and interdepend on one another, rendering tri-functional synergistic design an indispensable development orientation of targeted scaffolds for IBD repair. To date, most published studies only achieve simultaneous expression of antimicrobial, osteogenic and angiogenic capacities without rigorous experimental validation of

authentic synergistic biological crosstalk<sup>17</sup>. Researchers frequently confuse concurrent multi-function expression with synergistic therapeutic effects, while quantitative synergy quantification and mechanistic exploration remain largely absent, forming a pervasive methodological flaw across this research field.

Clear conceptual boundaries must be drawn between independently coexisting bioactivities and authentic tri-functional biological synergy, a widespread misconception throughout current literature<sup>18</sup>. Coexisting biofunctions only indicate scaffolds simultaneously possess antimicrobial, osteogenic and angiogenic capacities without interactive promotion between functional modules. In contrast, tripartite synergy requires spatiotemporal coupling and bidirectional promotional biological crosstalk, with composite therapeutic efficacy markedly surpassing linear additive effects of single or dual-function scaffolds, and no inhibitory interactions between functional modules. Regrettably, over 90% of published relevant studies merely report simultaneous detection of three functional readouts. These works fail to establish hierarchical control groups (antimicrobial-only groups, osteogenic-only groups, angiogenic-only groups, dual-function composite groups) or conduct two-way statistical interaction analysis, and thus cannot provide convincing evidence of authentic synergistic effects. Most manuscripts incorrectly equate coexistence of multiple biofunctions with synergistic performance, leading to exaggerated biological efficacy without rigorous quantitative verification<sup>19</sup>.

## **MATERIAL SYSTEMS AND ANTI-INFECTION STRATEGIES OF 3D-PRINTED ANTI-INFECTION BONE SCAFFOLDS**

Ideal scaffolds possess favorable printability, robust biocompatibility, tailored mechanical strength, and degradation rates matched with bone regeneration kinetics. Material modification endows scaffolds with coordinated antimicrobial, osteogenic and angiogenic capacities<sup>20</sup>. This review categorizes scaffold materials into four primary classes, summarizes material modification strategies centered on tri-functional synergy, and systematically compares diverse antimicrobial approaches to clarify their applicable clinical scenarios.

### **Classification and Functional Characteristics of Scaffold Materials**

#### *Synthetic Polymer Materials*

Representative materials include PLA, PLGA, PCL

and PEEK. Among these polymers, PEEK remains non-degradable, while PLA, PLGA and PCL exhibit biodegradable properties suitable for printing. Single polymers lack intrinsic antimicrobial and osteogenic bioactivities, yet composite incorporation with bioactive additives (e.g., growth factors) enhances osteogenic and angiogenic performance. Loading antibiotics or antimicrobial peptides (AMPs) achieves robust antimicrobial effects, while incorporation of angiogenic factors drives vascularization within scaffolds<sup>21</sup>. PCL/HA and PLGA/ $\beta$ -TCP composites represent the most widely modified polymer systems for laboratory scaffold fabrication, featuring low manufacturing costs and straightforward molding workflows. However, these composites suffer from insufficient mechanical strength and unregulated degradation kinetics. In terms of progression toward clinical translation, only PEEK and medical-grade PLA/PLGA have obtained regulatory approval<sup>22</sup>.

#### *Bioceramic Materials*

Primary bioceramic matrices consist of HA,  $\beta$ -TCP and bioactive glass. Their chemical compositions mimic native bone tissues, conferring favorable osteogenic capacity and biocompatibility. Doping with  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  imparts broad-spectrum antimicrobial activity to scaffolds;  $\text{Zn}^{2+}$  and  $\text{Mg}^{2+}$  ions simultaneously facilitate angiogenesis<sup>23</sup>. Composite blending with polymers mitigates inherent high brittleness and low toughness of ceramics, generating hybrid scaffolds integrating osteogenic and antimicrobial functions. Nevertheless, bioceramics exhibit limited load-bearing capacity, and high ion concentrations may trigger cytotoxicity. Large-sample long-term in vivo biosafety data for ion-doped ceramic scaffolds remain insufficient<sup>24</sup>.

#### *Metallic Materials*

Metallic scaffolds target load-bearing bone defects, including titanium alloys, magnesium alloys and zinc alloys. Titanium alloys display outstanding mechanical matching with cortical bone yet lack intrinsic antimicrobial activity, requiring surface functional coatings for anti-infective performance<sup>25</sup>. Degradable Mg/Zn alloy scaffolds release bioactive metal ions to achieve antimicrobial effects, while metallic substrates deliver coordinated osteogenic and angiogenic capacities, marking them ideal candidates for load-bearing scaffold construction. Alloy modification regulates magnesium degradation to avoid rapid hydrogen evolution and local alkalization within scaffolds. Novel Mg-Zn and Mg-Ca alloy systems

remain confined to preclinical animal experiments. For clinical translation, porous titanium alloys have entered routine clinical application, while degradable magnesium alloys still require long-term safety validation and formal medical device registration<sup>26</sup>.

### Composite Materials

Composite scaffolds integrate complementary advantages of single-component materials, resolve inherent performance conflicts, and realize coordinated tri-functional bioactivities. Typical composite formulations include polymer/ceramic/antimicrobial agent ternary systems (PLGA/ $\beta$ -TCP/AgNPs), metal/ceramic hybrids (Mg-Zn/HA), and multi-component matrices loaded with vascular endothelial growth factor (VEGF) or desferrioxamine (DFO). Composite formulations balance mechanical properties, degradation kinetics and biological functions, making them the most promising candidates for progression toward clinical translation. However, multi-material blending readily induces phase separation, uncontrolled

biofactor release, complex manufacturing workflows and poor batch reproducibility, which constitute major barriers to clinical deployment<sup>27</sup>.

### Classification and Comparison of Antimicrobial Strategies

Antimicrobial modification represents the core module for infectious lesion control, divided into five distinct categories with divergent action mechanisms, strengths and limitations. Table I systematically compares these strategies to guide targeted selection for varied infectious pathologies<sup>28-30</sup>.

### Material Design Logic for Tri-Functional Synergy

Material hybridization, biofactor loading and topological structural optimization jointly integrate and coordinate the three core biofunctions of scaffolds<sup>31-33</sup>:

- (1) Composite antimicrobial additives (AgNPs, Zn<sup>2+</sup>) with osteogenic bioceramics (HA,  $\beta$ -TCP)

**Table I.** — Comparative analysis of diverse antimicrobial modification strategies for 3D-printed bone scaffolds.

| Treatment Route                                     | Core Functional Additives                                        | Inhibitory Mechanism Against Pathogens                                                                                  | Distinct Advantages                                                                                                | Existing Shortcomings                                                                                                   | Suitable Orthopedic Lesions                                                                                                  |
|-----------------------------------------------------|------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| Antibiotic-loaded composite modification            | gentamicin hydrochloride                                         | Interfere with bacterial wall biosynthesis and block intracellular protein translation processes                        | Rapid germicidal efficacy, abundant clinical trial data support                                                    | High probability of generating drug-resistant strains, abrupt local drug release, cellular toxic response at overdosage | Acute traumatic bone infection triggered by antibiotic-susceptible bacteria                                                  |
| Trace metal ion doping modification                 | Ag, Cu, Zn, Mg elemental ions                                    | Disrupt bacterial membrane lipid layers, trigger intracellular oxidative stress, deactivate essential metabolic enzymes | Broad-spectrum anti-pathogen capacity, simultaneously facilitate bone cell differentiation and capillary formation | Overdosed metal ions induce cellular damage, hard to stabilize sustained ion release rate                               | Long-term mixed bacterial osteomyelitis, bone cavities colonized by multi-drug resistant microbes                            |
| Natural bioactive macromolecule blending            | Antibacterial peptides, chitosan, lytic enzymes                  | Puncture bacterial lipid membranes, suppress the formation and adhesion of microbial biofilms                           | Excellent in-vivo biocompatibility, negligible drug resistance risk, fully degradable inside organisms             | High raw material expenditure, short in-vivo functional retention time, bioactivity loss under thermal molding          | Shallow mild bone infection, juvenile bone-soft tissue combined injuries, postoperative infection precaution                 |
| Light-responsive inorganic filler modification      | Graphene nanosheets, black phosphorus, photoactive nanoparticles | Generate thermal ablation or oxidative free radicals under light exposure to physically destroy bacterial structures    | Zero risk of antibiotic resistance, remote controllable activation, dual anti-infection and osteogenic effects     | Dependent on external light source, limited light penetration inside biological tissues                                 | Superficial bone cavity defects, biofilm-mediated recurrent osteomyelitis, shallow surgical wound infection                  |
| Lesion microenvironment responsive delivery coating | pH-sensitive, enzyme-activated bactericidal carriers             | Targeted release of antibacterial substances only in acidic, enzyme-abundant infectious microregions                    | Site-specific localized therapy, minimal systemic drug intake, lowered cytotoxicity and resistance risks           | Complicated multi-step manufacturing workflow, high production cost, insufficient clinical transformation verification  | Refractory recurrent osteomyelitis, diabetic foot-related infected bone loss, personalized precise orthopedic reconstruction |

and angiogenic factors (VEGF, DFO) to construct single-scaffold multi-functional integration systems.

(2) Adopt intrinsically tri-functional metallic substrates such as Mg-Zn alloys that simultaneously deliver antimicrobial, osteogenic and angiogenic capacities.

(3) Fabricate core-shell or gradient porous architectures to achieve spatial functional separation: outer layers undertake antimicrobial defense, while inner layers support osteogenic differentiation and vascular ingrowth within scaffolds.

(4) Deploy controlled-release technology to realize temporally staged functional expression: scaffolds first exert potent antimicrobial effects to eliminate infectious lesions, then activate osteogenic and angiogenic cascades to accelerate tissue regeneration, maximizing tri-functional synergistic therapeutic efficacy.

### **Experimental Verification Systems for Tri-Functional Synergy**

#### *In Vitro Tri-Functional Synergy Verification*

In vitro synergistic validation requires systematic quantitative evaluation of three core biofunctions<sup>34</sup>.

(1) Antimicrobial performance: Quantify scaffold bacteriostatic rates against common clinical pathogens, biofilm inhibitory capacity, and clearance efficacy for multidrug-resistant bacterial strains.

(2) Osteogenic capacity: Detect alkaline phosphatase (ALP) enzymatic activity, extracellular matrix mineralization levels, and expression profiles of osteogenic marker genes Runx2 and osteocalcin (OCN).

(3) Angiogenic potential: Assess endothelial cell tubular formation capacity, cellular migration efficiency, and expression levels of vascular marker genes VEGF and cluster of differentiation 31 (CD31).

The quantitative criterion for authentic tri-functional synergy specifies that composite therapeutic effects of tri-functional scaffold groups significantly exceed linear additive outcomes of single-function groups, without obvious elevation of cytotoxicity within co-culture systems.

#### *In Vivo Tri-Functional Synergy Verification*

In vivo synergistic validation requires simultaneous quantitative assessment of multiple readouts within

pathogen-induced bone defect animal models. Three primary indicators must improve concurrently: complete infectious lesion clearance, elevated nascent bone volume, and increased intrascaffold vascular density<sup>35</sup>. Histological staining reveals mild local inflammatory infiltration and favorable scaffold-bone tissue integration within lesions. Statistical validation relies on two-way analysis of variance (ANOVA) and multi-factor interaction effect modeling to confirm synergistic biological interactions at the statistical level. Currently, most published studies merely simultaneously detect three functional readouts without rigorous quantitative measurement and statistical validation of synergistic effects, nor exclude independent bioactivity from single active components. Thus, existing literature lacks sufficient evidence to confirm authentic biological tri-functional synergy<sup>36</sup>.

#### *Critical Analysis of Methodological Deficiencies*

Four pervasive experimental flaws prevent reliable verification of tri-functional synergistic effects:

(1) Absence of hierarchical single/dual-function control groups to calculate additive therapeutic baseline effects.

(2) Insufficient statistical interaction modeling (two-way ANOVA, response surface methodology) to quantify synergistic enhancement magnitudes.

(3) Neglect of cytotoxic interference induced by high-concentration antimicrobial ions, which masks beneficial osteogenic and angiogenic bioactivities.

(4) Simplified in vitro culture microenvironments fail to recapitulate infectious inflammatory feedback loops that mediate synergistic biological crosstalk within living organisms.

Accordingly, simultaneous multi-function expression observed in vitro cannot be extrapolated to authentic in vivo tri-functional synergy without standardized hierarchical control groups and statistical interaction testing<sup>37</sup>.

### **3D PRINTING TECHNOLOGIES AND BIOMIMETIC STRUCTURAL DESIGN**

Printing modalities and topological structural design directly determine scaffold mechanical properties, pore interconnectivity and functional bioactivity exertion. Rational technology selection and structural optimization amplify tri-functional synergistic effects. This section summarizes mainstream printing techniques and biomimetic design strategies.

### Mainstream 3D Printing Technologies and Application Scenarios

Printing technology selection depends on raw material properties, fabrication precision requirements and target clinical indications. Five widely adopted printing modalities are systematically compared in Table II<sup>38-40</sup>.

### Biomimetic Structural Design for Tri-Functional Synergy

Biomimetic structural design replicates native cancellous bone microarchitectures, optimizing pore dimensions and topological configurations to balance mechanical performance and biological functions. Ideal pore parameters for bone repair scaffolds include

pore sizes of 300–800  $\mu\text{m}$ , overall porosity of 60%–85%, and pore interconnectivity exceeding 90%, which facilitate cell infiltration and vascular ingrowth within scaffolds<sup>41</sup>. Gradient pore architectures are recommended for IBD treatment: narrow outer pores (200–400  $\mu\text{m}$ ) strengthen antimicrobial defense and block external bacterial invasion, while enlarged inner pores (500–800  $\mu\text{m}$ ) promote osteogenic differentiation and vascular network formation. Triply periodic minimal surface (TPMS) structures (e.g., Gyroid, Diamond) serve as preferred topological designs, featuring uniform stress distribution, superior pore interconnectivity and enhanced bioactive factor loading capacity compared with conventional grid architectures. For clinical personalized matching,

**Table II.** — Comparative summary of mainstream additive manufacturing technologies for bone tissue engineering scaffolds.

| Full Technical Denomination                       | Processable Raw Material Categories                                                           | Fabrication Accuracy Range        | Manufacturing Surroundings                              | Unique Technical Superiorities                                                                            | Primary Technical Limitations                                                                                   | Lab Research & Clinical Deployment Directions                                                                              |
|---------------------------------------------------|-----------------------------------------------------------------------------------------------|-----------------------------------|---------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| Fused Deposition Modeling                         | Heat-meltable polymer composites including PLA and PCL                                        | Low dimensional accuracy          | Ordinary open-air setting                               | Affordable production hardware, efficient mass batch output, streamlined post-fabrication polishing steps | Rough surface texture, inadequate dimensional precision for delicate microstructures                            | Primary material performance screening, scaffold preparation for small rodent bone injury animal assays                    |
| Selective Laser Sintering                         | Fine PEEK granules, titanium alloy powder, micron-level bioceramic particles                  | Moderate forming precision        | Closed airtight chamber filled with inert shielding gas | Robust mechanical bearing capacity, compact forming without adhesive binders                              | Expensive laser processing hardware, substantial power consumption, narrow range of compatible biomaterials     | Reconstruction of load-bearing segmental bone defects, bulk manufacturing of tailor-made orthopedic implantation devices   |
| Stereolithography / Digital Light Projection      | UV-crosslinking biocompatible resin, ceramic suspension, photo-crosslinkable composite bioink | Ultra-precise forming             | Sealed isolated UV curing compartment                   | Enables production of intricate TPMS biomimetic porous microstructures with ultra-fine resolution         | Limited variety of printable raw materials, compulsory degassing and secondary curing post-processing workflows | High-accuracy custom scaffolds for minor bone voids, minimally invasive craniofacial reconstructive prostheses             |
| Electron Beam Melting                             | Titanium alloy powders, degradable Mg-Zn alloy microgranules                                  | Medium-to-high forming resolution | High-vacuum enclosed forming cavity                     | High-density metallic component molding, mechanical parameters consistent with natural cortical bone      | Exorbitant hardware procurement and routine maintenance expenses                                                | Clinical manufacturing of large weight-bearing metallic bone implants, pelvic and tibial reconstructive prosthetic devices |
| Extrusion-based biological additive manufacturing | Cell-laden hydrogel matrices, collagen hybrid bioinks, degradable polymer slurries            | Medium forming precision          | Mild aqueous room-temperature environment               | Low-temperature molding preserves viability of seed cells and bioactive growth factors                    | Weak compressive mechanical properties, high risk of porous structural collapse during molding                  | Fabrication of cell-loaded tissue engineering scaffolds, exploratory research on pre-vascularized bone graft substitutes   |

load-bearing lesion regions adopt high-strength metal or PEEK scaffolds with dense outer surfaces and porous inner layers, while non-load-bearing defects utilize biodegradable polymer-bioceramic composite matrices. Despite prominent *in vitro* and small-animal therapeutic advantages, biomimetic scaffolds commonly suffer from insufficient intrascaffold vascularization and central tissue necrosis within large-animal and human bone defects<sup>42</sup>.

### IN VITRO AND IN VIVO EVALUATION SYSTEMS

Standardized performance evaluation pipelines are critical for validating scaffold bioactivity and advancing progression toward clinical translation, covering physicochemical characterization, *in vitro* tri-functional quantitative testing and *in vivo* preclinical validation. Core evaluation readouts are summarized in Table III<sup>43–45</sup>.

#### *In Vitro Tri-Functional Synergy Verification*

*In vitro* evaluation pipelines focus on coordinated quantitative validation of three core biofunctions.

Antimicrobial assays test common clinical pathogens and methicillin-resistant *Staphylococcus aureus* (MRSA) to verify scaffold broad-spectrum bactericidal and resistant-strain clearance efficacy. Osteogenic experiments utilize bone marrow mesenchymal stem cells to characterize scaffold biocompatibility and osteogenic differentiation induction capacity. Angiogenic assessments adopt human umbilical vein endothelial cells to quantify tubular formation potential. Core verification criteria include two key points: antimicrobial components do not suppress viability of osteoblasts and vascular endothelial cells, and angiogenic factors enhance scaffold anti-infective performance by optimizing local microcirculation within scaffolds<sup>46</sup>. Nevertheless, simplified *in vitro* culture environments cannot recapitulate complex infection-inflammation-regeneration pathological microenvironments, so synergistic effects observed *in vitro* cannot reliably predict *in vivo* therapeutic performance.

**Table III.** — Standardized evaluation indicators and detection workflows for multifunctional antimicrobial bone scaffolds.

| Evaluation Module                                    | Core Measurable Indicators                                                                                                                                                      | Test Samples                                                             | Common Analytical Instruments & Assays                                                                                                 | Qualified Performance Standards                                                                                                                                                                       |
|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Physical and chemical property profiling             | Compressive stiffness, total void proportion, pore interconnection rate, <i>in-vitro</i> degradation tendency, sustained release profiles of metal ions or antibacterial agents | Monolithic scaffold specimens                                            | Universal mechanical tester, micro-computed tomography, scanning electron microscopy, inductively coupled plasma mass spectrometry     | Mechanical stiffness matches cancellous or cortical bone; overall porosity maintained at 62%–83%; degradation speed coordinates with neo-bone formation rate                                          |
| Ex vivo pathogen suppression profiling               | Total bacteriostatic efficiency, suppression capacity against microbial biofilms, clearance effect on multidrug-resistant pathogenic strains                                    | Co-cultured mixtures of scaffolds and bacteria                           | Colony counting assay, inhibition zone measurement, crystal violet staining for biofilm quantification                                 | Bacteriostatic efficiency reaches over 92% for common clinical pathogens; obvious suppression on adhesion and proliferation of methicillin-resistant <i>Staphylococcus aureus</i>                     |
| Osteogenic differentiation profiling in cell culture | Survival rate of mesenchymal stem cells, alkaline phosphatase activity, extracellular mineral deposition, expression abundance of osteogenic marker genes (Runx2, osteocalcin)  | Co-cultures of bone marrow mesenchymal stem cells and scaffold materials | CCK-8 cell viability assay, ALP cytochemical staining, alizarin red mineral staining, real-time quantitative polymerase chain reaction | No obvious cytotoxic response; osteogenic marker genes show significant upregulation after 7–14 days of induced culture                                                                               |
| Endothelial angiogenic potential profiling           | Tube-forming capacity of endothelial cells, cellular migration ability, expression levels of vascular marker genes (VEGF, CD31)                                                 | Co-cultures of human umbilical vein endothelial cells and scaffolds      | Endothelial tubule formation test, Transwell migration experiment, immunofluorescent labeling staining                                 | Complete interconnected tubular networks form within 6 hours of cultivation; angiogenic functional genes exhibit prominent upregulation                                                               |
| <i>In vivo</i> animal model therapeutic validation   | Elimination degree of local infectious lesions, newly formed bone volume ratio, microvascular density inside scaffolds, infiltration level of inflammatory cells                | Animal tissue slices collected from pathogen-induced bone defect models  | 3D reconstruction via micro-CT, histological slice staining, histomorphometric quantitative analysis                                   | Complete removal of local bacterial infection; abundant neo-bone tissues and microvessels grow into scaffolds; only mild short-term inflammatory infiltration without persistent chronic inflammation |

### ***In Vivo Preclinical Research and Translational Progress***

Pathogen-induced bone defect models constructed in New Zealand rabbits and SD rats exhibit the highest clinical correlation. Jin et al. performed multi-timepoint evaluation at postoperative weeks 4, 8 and 12, focusing on infectious lesion clearance, nascent bone volume and intrascaffold vascular density. This field has achieved preliminary translational breakthroughs: antibiotic-loaded 3D-printed porous titanium alloy scaffolds are applied to treat chronic osteomyelitis, realizing simultaneous infection control and bone healing within single-stage surgical interventions. Personalized PEEK/HA composite scaffolds are deployed for infectious maxillofacial bone defect reconstruction with favorable anatomical matching. Zinc-doped degradable magnesium alloy scaffolds treat load-bearing bone defects, achieving synchronized scaffold degradation and nascent bone formation. Several scaffold formulations have entered small-sample clinical trials, yet absent standardized evaluation pipelines and insufficient long-term in vivo safety data restrict large-scale clinical deployment<sup>47</sup>. Clinicians combine porous 3D-printed titanium scaffolds with modified Masquelet protocols for chronic tibial osteomyelitis treatment. After radical debridement, personalized Ag-ion-doped porous titanium implants replace PMMA cement spacers for direct implantation, delivering sustained local antimicrobial release and bone ingrowth without secondary grafting procedures. For maxillofacial infectious bone defect reconstruction, PEEK/HA composite scaffolds are combined with vascularized mucosal flaps to reduce postoperative biofilm formation risks. For pediatric calcaneal infectious lesions, biodegradable 3D bioplotting scaffolds coordinate with Ilizarov bone transport to shorten external fixation treatment durations.

#### ***Summary of Published Human Clinical Outcomes***

To clarify real-world translational performance, this review systematically summarizes peer-reviewed human clinical applications of antimicrobial 3D-printed bone scaffolds. Current human clinical evidence relies predominantly on small-sample retrospective case series, with three mainstream scaffold types entering routine orthopedic practice<sup>48</sup>:

(1) Antibiotic-loaded porous 3D-printed titanium alloy implants: Indicated for long-bone chronic osteomyelitis and post-traumatic FRI. Reported single-stage infection clearance rates range from

82%–94% across 12–24 month follow-up periods. Compared with conventional two-stage Masquelet therapy, these scaffolds reduce hospitalization durations by 40% without elevating clinical multidrug resistance incidence.

(2) Personalized chitosan-coated PEEK/HA composite scaffolds: Primarily applied to maxillofacial and cranial infectious bone defects, featuring superior anatomical matching and low soft tissue irritation. Medium-term follow-up (<18 months) records zero recurrent infectious lesions.

(3) Trace metal ion-doped degradable Mg/Zn porous scaffolds: Confined to early-phase small clinical trials for low-load extremity bone defects. Preliminary data demonstrate synchronized scaffold degradation and nascent bone formation, yet long-term ( $\geq 3$  years) human in vivo metal ion toxicology datasets remain absent.

An ongoing registered clinical trial (ClinicalTrials.gov ID: NCT06010433) investigates antibiotic sustained-release bioceramic bone filling materials for diabetic foot osteomyelitis treatment, representing a translational prototype of microenvironment-responsive ceramic antimicrobial scaffolds.

### **CORE BARRIERS RESTRICTING PROGRESSION TOWARD CLINICAL TRANSLATION**

(1) Performance coordination dilemmas: Conflicting trade-offs exist between mechanical strength and porosity, antimicrobial activity and biocompatibility, and degradation kinetics and bone regeneration velocity, which are particularly prominent for load-bearing IBD lesions.

(2) Unregulated biofactor release profiles: Burst or retarded release of antimicrobial and osteogenic factors disrupts tri-functional synergistic coordination. Antibiotic-laden scaffolds carry multidrug resistance risks, while long-term in vivo biosafety of inorganic metal ions requires comprehensive validation<sup>49</sup>.

(3) Absence of unified industrial standards: No globally standardized evaluation pipelines exist; animal model construction and detection readouts remain inconsistent across research teams. Large-scale multi-center clinical trials remain scarce, follow-up durations are limited, and long-term therapeutic efficacy remains unclear.

(4) Elevated manufacturing costs and technical thresholds: High-cost printing equipment and raw biomaterials increase production expenses. Multi-functional composite scaffold fabrication demands

cross-disciplinary collaborative research, hindering industrialized mass production<sup>50</sup>.

Additional orthopedic surgical compatibility bottlenecks limit widespread clinical deployment: Current 3D scaffold design workflows rarely consider intraoperative trimming convenience, external fixation pin matching, and postoperative weight-bearing rehabilitation requirements. Few biomaterial research projects stratify scaffold performance in accordance with clinical FRI grading standards, creating disconnection between laboratory-engineered products and routine orthopedic reconstructive workflows.

Beyond material performance conflicts, five human-specific practical barriers impede large-scale clinical popularization<sup>51,52</sup>: (1) Regulatory restrictions: Antimicrobial functional modification of 3D-printed orthopedic implants falls under Class III medical device classification, requiring rigorous multi-center clinical trial validation with lengthy approval cycles and substantial research funding thresholds. (2) Intraoperative processing limitations: Most laboratory-grade bioinks and composite scaffolds lack standardized terminal sterilization protocols, leading to partial loss of scaffold bioactivity after high-temperature ethylene oxide sterilization. (3) Economic barriers: Personalized CT-guided 3D printing manufacturing costs reach 2–3 times those of conventional autologous bone grafts,

restricting clinical promotion within primary medical institutions. (4) Clinical data gaps: All existing human clinical studies adopt short-term follow-up durations (<2 years), lacking large-cohort long-term safety data regarding metal ion tissue accumulation, residual polymer degradation fragments and delayed inflammatory reactions.

Clinical operation standardization gaps: Orthopedic surgeons lack unified intraoperative implantation protocols for multifunctional 3D scaffolds, without standardized guidelines for scaffold dimensional adjustment, supplementary local antimicrobial agent delivery and postoperative systemic anti-infection medication regimens.

## PROSPECTIVE FUTURE RESEARCH DIRECTIONS

Future research prioritizes scaffold intelligentization, standardized industrialization and accelerated clinical translation. Core developmental trends and corresponding clinical value are summarized in Table IV<sup>53-55</sup>.

## CONCLUSION

3D-printed scaffolds integrating coordinated antimicrobial, osteogenic and angiogenic capacities deliver an all-in-one regenerative therapeutic strategy for IBD treatment, which effectively resolves the core

**Table IV.** — Prospective research trends and translational clinical value of multifunctional anti-infective 3D-printed bone scaffolds.

| Future-Focused Research Initiatives                                                                                               | Core Technical Connotation                                                                                                                                                                                                                                                | Translational Clinical & Industrial Value                                                                                                                                                                                                      |
|-----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Stimulus-Responsive Intelligent Bone Implants Targeting Infectious Lesion Microenvironments                                       | Design implantable carriers that autonomously release bactericidal agents triggered by lesion-specific acidic or enzymatic stimuli; integrate photothermal and photocatalytic antibacterial modules for remote on-demand sterilization                                    | Enable precise localized therapeutic intervention, reduce systemic drug administration dosage, lower the risks of bacterial drug resistance and cellular toxicity; suitable for personalized management of intractable recurrent osteomyelitis |
| 4D Morphing Additive Manufacturing for Irregular Bone Defect Reconstruction                                                       | Develop bone repair scaffolds with shape memory properties; the constructs can automatically deform to perfectly fit irregular bone cavities under physiological temperature and pH stimulation during surgical implantation                                              | Simplify intraoperative trimming and shaping procedures, strengthen the integration between implants and native tissues, reduce surgical trauma and shorten operation duration                                                                 |
| Macrophage Polarization-Regulated Multi-Functional Bone Grafts with Synergistic Anti-Infective, Osteogenic and Angiogenic Effects | Equip scaffolds with macrophage phenotype modulation capacity on the basis of antibacterial, osteogenic and vascularization functions; induce macrophages to polarize into the anti-inflammatory M2 subtype to reconstruct a favorable bone regenerative microenvironment | Mitigate excessive inflammatory response at bone injury sites, accelerate the formation of vascularized new bone tissue, and reduce the recurrence risk of postoperative bone infection                                                        |
| Industrialized Standardized Manufacturing System for Cost-Effective Clinical Bone Grafts                                          | Formulate unified global testing specifications for in vitro and in vivo functional evaluation; develop high-throughput low-cost printing equipment; complete full medical device registration and certification procedures                                               | Facilitate the large-scale clinical application of customized bone implants in primary medical institutions, cut raw material and manufacturing costs, and form standardized commercial orthopedic repair products                             |

clinical conflict between infectious lesion elimination and bone tissue reconstruction. This review systematically elaborates scaffold material systems, mainstream additive manufacturing modalities, biomimetic topological design schemes and standardized in vitro/in vivo evaluation frameworks. It distinguishes conceptual laboratory prototype technologies from clinically translatable fabrication pipelines, clarifies standardized definitions and quantitative verification workflows of tri-functional synergy, conducts critical methodological discussion, summarizes core translational bottlenecks, and outlines promising future research avenues. Current fundamental research has yielded abundant achievements, yet coordinated performance balancing, precise spatiotemporal functional regulation, unified evaluation standards and full clinical translation remain dominant restrictive factors. Moving forward, advances in intelligent responsive biomaterials, 4D printing technology and multidisciplinary cross-integration will drive 3D-printed anti-infective bone scaffolds toward standardized clinical application and industrial mass production, delivering innovative therapeutic regimens to improve patient clinical prognoses and long-term quality of life.

A pervasive methodological limitation persists across the entire research field: insufficient rigorous quantitative validation of authentic tri-functional biological synergy. The vast majority of published reports merely demonstrate simultaneous existence of antimicrobial, osteogenic and angiogenic bioactivities rather than formally verified bidirectional synergistic biological crosstalk. Future research must establish unified standardized experimental evaluation frameworks equipped with hierarchical control groups and statistical interaction analysis pipelines to objectively quantify synergistic therapeutic magnitudes and eliminate the widespread conflation of concurrent multi-function expression and authentic biological synergy.

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