ACCSCIENCE PUBLISHING

International Journal of Bioprinting

REVIEW ARTICLE

Macrophage-centered bone regeneration: A review of in vivo efficacy across tailored, hybrid, and 3D-printed biomaterial platforms

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(This article belongs to the *Special Issue: Intelligent bioprinting: From printable structures to functional systems*)

Abstract

Bone tissue engineering has evolved from the passive use of structural fillers to a sophisticated discipline that actively harnesses endogenous regenerative mechanisms. At the core of this paradigm shift lies the immune system, particularly macrophages, as dynamic regulators of repair. Rather than merely suppressing inflammation, contemporary biomaterials are designed to modulate its trajectory, orchestrating the timely transition from a pro-inflammatory (M1) phenotype toward a pro-resolutive (M2) state. This review synthesizes a decade of progress in macrophage-centered bioengineering, focusing on strategies validated in preclinical *in vivo* models to ensure biological relevance and translational potential. These approaches are categorized across three levels of increasing complexity: (i) tailored biomaterials, where intrinsic physical and chemical properties direct cell fate; (ii) hybrid scaffolds, integrating diverse material classes and advanced delivery systems; and (iii) 3D-printed bioactive constructs, combining structural precision with ions, drugs, or cellular components. Together, these strategies define the emerging field of osteoimmunomodulation, characterized by the design of immuno-instructive materials. By critically evaluating the evolution of these principles, including their translational barriers and potential pitfalls, this review provides key insights into the field's progression, identifying effective strategies to guide the development of next-generation bone therapies.

Keywords: Macrophages; Osteoimmunomodulation; Bone regeneration; Biomaterials; 3D printing; Tissue engineering

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Citation: Almeida PP, Dias RB, Leichtweis KS, Frade BB, Gemini-Piperni S, Bonfim DC. Macrophage-centered bone regeneration: A review of in vivo efficacy across tailored, hybrid, and 3D-printed biomaterial platforms. *Int J Bioprint.* 2026:12(2):026050033. doi: 10.36922/IJB026050033

Received: January 27, 2026

Revised: February 27, 2026

Accepted: March 3, 2026

Published online: April 23, 2026

1. Introduction

Bone regeneration is a highly coordinated and dynamic process that unfolds across three major phases—inflammation, tissue neoformation, and remodeling—involving the interaction of multiple cell populations and signaling pathways.[1] Upon injury, neutrophils, macrophages, platelets, and granulocytes rapidly infiltrate the fracture

site, forming a hematoma and initiating the inflammatory cascade through the release of cytokines and growth factors. As inflammation subsides, skeletal stem and progenitor cells (SSPCs) are recruited and activated, differentiating to drive bone neoformation either through direct ossification or via an endochondral pathway, in which a cartilaginous callus is first formed and subsequently replaced by bone.[2] Finally, the newly deposited bone undergoes extensive remodeling, thereby acquiring its characteristic micro- and macro-architecture and recovering its mechanical strength and original function.[3]

Within this well-orchestrated sequence, macrophages have emerged as central regulators at every stage of bone healing, with recent evidence dramatically expanding our understanding of their multifaceted roles.[4,5] Following injury, the inflammatory microenvironment within the hematoma promotes the activation of tissue-resident macrophages and the recruitment of additional populations from the circulation. Beyond merely clearing cell and tissue debris, these activated macrophages adopt a pro-inflammatory M1 profile, secreting cytokines such as interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and monocyte chemotactic protein-1, which amplify the inflammatory response and prime the injury site. This initial microenvironment is essential for osteogenesis, as it triggers the subsequent recruitment of SSPCs.[6] The transition toward a resolutive microenvironment is driven by a phenotypic shift of macrophages toward an anti-inflammatory M2 profile. In this stage, macrophages secrete factors such as IL-10, transforming growth factor-β (TGF-β), and bone morphogenetic protein-2 (BMP-2), which suppress excessive inflammation and directly promote the differentiation of SSPCs to drive tissue neoformation.[7]

Consequently, maintaining a balanced M1/M2 ratio throughout the regenerative process is essential for bone repair.[5] An aberrant increase in M1 signaling may lead to persistent tissue damage and chronic inflammation, whereas a deficiency in M2 polarization impairs osteogenesis and disrupts the transition to bone formation. Consistently, the depletion of macrophages, whether tissue-resident or recruited, or disruptions in their functional polarization, results in impaired ossification and delayed union.[5,8–11] Such imbalances underscore the pivotal role of macrophage plasticity in preventing fibrotic outcomes and ensuring successful fracture repair.

Although bone possesses a remarkable ability to regenerate, several pathological or clinical conditions, such as diabetes, immunodeficiencies, chronic inflammation, polytrauma, tumor resections, infections, and extensive bone loss (critical-sized defects), can disrupt macrophage-mediated repair mechanisms, leading to nonunion.[12–14] These non-healing fractures represent a major challenge in orthopedics, requiring invasive and resource-intensive treatments, often with prolonged hospitalization, repeated surgical interventions, and costly implants. The consequences extend beyond clinical management: patients experience long-term disability, reduced quality of life, psychological stress, and loss of income, placing substantial socioeconomic burdens on families and healthcare systems.[15,16] Thus, developing new therapies capable of restoring or enhancing the bone regeneration process remains an urgent clinical need.

Recognizing the central role of macrophages in both physiological bone repair and the integration of implanted materials, the field of bioengineering has shifted toward designing instructive biomaterials that actively modulate macrophage behavior. This narrative review synthesizes the progress made over the last decade, specifically focusing on studies that have validated immunomodulatory strategies in preclinical *in vivo* models, a fundamental inclusion criterion to ensure biological relevance and translational potential. These approaches are categorized into three levels of increasing complexity: (i) tailored biomaterials, where intrinsic physical and chemical properties direct cell fate; (ii) hybrid scaffolds, which integrate diverse material classes and advanced delivery systems for bioactive cues; and (iii) 3D-printed constructs, representing the integration of structural precision with bioactive and cellular components. By critically evaluating the evolution of these macrophage-centered strategies, this review aims to provide key insights into the field's current progression and identify the most effective, translatable principles that can guide the development of the next generation of bone regeneration therapies.

2. Methods

A literature search was conducted in the PubMed database to identify studies addressing macrophage-mediated immunomodulation in bone regeneration. The search strategy employed the following keywords combined with Boolean operators: ("macrophage" AND "bone regeneration" AND "tissue engineering") OR ("fracture healing" AND "macrophage polarization" AND "biomaterials") OR ("tissue engineering" AND "bone repair" AND "macrophages"). The search was restricted to articles published between 2015 and 2025. Only original research articles reporting evidence from preclinical *in vivo* models in the context of bone tissue engineering or regenerative biomaterials were included. Studies were required to explicitly evaluate macrophage involvement, such as polarization status, functional modulation, or immunoregulatory roles, and to report outcomes related to bone repair efficacy. The screening process was performed

in two stages: first, titles and abstracts were assessed for relevance; subsequently, the full texts of potentially eligible articles were evaluated to confirm adherence to the inclusion criteria. Only articles meeting all predefined criteria were included in the qualitative synthesis.

3. Bioengineering approaches to promote bone repair through macrophage modulation

3.1. Tailored biomaterials

Moving far beyond the original concept of "osteoconductive structural fillers," modern biomaterials are now recognized as active agents in immune regulation and bone tissue regeneration. In this context, diverse strategies have been developed to actively modulate macrophage behavior through material modification, including the fine-tuning of mechanical properties, the design of nanostructured systems, the application of chemical or ionic functionalization, and the engineering of surface topography. These approaches aim to guide the inflammatory response toward a pro-resolutive, M2-dominant profile that supports osteogenesis, angiogenesis, and successful osseointegration.

Regarding mechanical and structural modulation, physical cues have emerged as powerful determinants of macrophage behavior. For instance, reducing the stiffness of demineralized bone matrix (DBM) has been shown to promote M2 macrophage polarization, enhance anti-inflammatory cytokine expression, and stimulate the biosynthesis of pro-resolutive lipid mediators, ultimately improving bone regeneration in rat calvaria defects.[17] Similarly, increasing the porosity of polyetheretherketone (PEEK) through the application of a nanoscale porous film reduced acute macrophage-driven inflammation, thereby enabling increased osteogenesis and osseointegration in rat femurs.[18]

Nanotechnological strategies have further expanded the immunoregulatory potential of biomaterials. The application of 45 nm gold nanoparticles in a rat periodontal regeneration model reprogrammed macrophages toward an M2 phenotype, suppressing inflammatory cytokines while upregulating BMP-2 signaling.[19] In parallel, titanium (Ti)-based nanostructures were shown to exert strong immunomodulatory effects. In one study, 30 nm Ti nanotubes improved lamellar osteogenesis and osseointegration, while in another, strontium (Sr)-containing sodium titanate nanorods induced M2 macrophage differentiation and stimulated the formation of $CD31^{hi}$ $Emcn^{hi}$ vessels associated with enhanced bone regeneration.[20,21]

Complementing these structural changes, chemical and ionic functionalization adds another powerful layer of modulation. Coating porcine bone xenografts with magnesium (Mg)-doped nanohydroxyapatite (nHA) enhanced the expression of TGF-β and vascular endothelial growth factor (VEGF), promoting both osteogenesis and vascularization in calvaria defects.[22] Furthermore, the functionalization of mesoporous bioactive glass (MBG) with amino groups resulted in increased mineralization and a reduced number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells in a rabbit sinus lift procedure.[23]

More complex, dual-functional surface engineering approaches have also proven successful in challenging pathological environments. Sr–adenosine coatings on Ti scaffolds stimulated osteogenesis, promoted M2 polarization, and achieved robust osseointegration in diabetic rat femurs.[24] Similarly, functionalizing polyethylene terephthalate (PET) with polydopamine (PDA)–chondroitin sulfate shifted macrophages toward an M2 phenotype and enhanced graft-bone integration in a ligament reconstruction model.[25] Additionally, multifunctional designs obtained by coating Ti implants with a konjac gum–gelatin hydrogel embedded with tannic acid–*D*-tyrosine nanoparticles enabled antibacterial, antioxidant, and immunomodulatory activity, promoting M2 polarization, eliminating bacteria, and improving bone regeneration in infected rat femurs.[26]

Together, these findings underscore that the regenerative potential of biomaterials is closely tied to their capacity to modulate the immune microenvironment. The evidence demonstrates that, depending on the model, different substrates (soft/low-stiffness matrices, unmodified PEEK, conventional Ti materials, MBG scaffolds, and unmodified PET) or biological stimuli (lipopolysaccharide [LPS]-stimulated macrophages and uncoated xenografts) can polarize macrophages into an M1 state. Yet, this early inflammatory phase is not inherently pathological; it is essential for debris clearance, antimicrobial defense, and the initiation of downstream regenerative cascades. Meanwhile, advanced nanotechnological scaffolds (LPS + gold nanoparticles and nanostructured Ti), functionalized materials (Mg-doped nHA-coated grafts and amino-functionalized MBG), and dual-functionalized platforms (immunomodulatory modified Ti, PDA + chondroitin sulfate PET, and immunomodulatory hydrogel coatings) can direct macrophages toward a pro-resolutive and osteogenic stage. These materials do not simply convert macrophages from M1 to M2 but modulate the magnitude, duration, and kinetics of the inflammatory response. Materials that prematurely suppress inflammation may

impair host defense and early regenerative signaling, while those that prolong M1-dominant responses risk chronic inflammation and fibrotic encapsulation. Whether through adjustments in mechanical properties, nanoscale surface engineering, or ionic doping, these strategies consistently converge on promoting a reparative state by appropriately modulating macrophage plasticity (Figure 1).

3.2. Hybrid biomaterials

Building upon the fundamental modifications of material properties discussed previously, the field has evolved toward hybrid systems that integrate diverse material classes, including organic, synthetic, and bioactive components, to achieve sophisticated control over the healing environment.[27,28] The conceptual rationale behind modern hybrid designs focuses on creating instructive microenvironments through three main strategies: (i) biomimetic scaffolds engineered to replicate the hierarchical microarchitecture and mechanical cues of native bone; (ii) multifunctional surfaces combining antimicrobial properties with immunomodulatory potential; and (iii) advanced delivery systems designed for the spatiotemporal release of ions, signaling molecules, or drugs. By synergizing these approaches, hybrid materials actively orchestrate the crosstalk between the immune system and bone cells, facilitating the transition from an inflammatory state to a pro-regenerative milieu.

Among the diverse materials used in this setting, hydrogels remain a primary choice due to their water-rich network that closely mimics the native extracellular matrix (ECM), their capacity as reservoirs for sustained drug release, and their ability to incorporate multiple therapeutic agents with distinct release profiles.[29] For instance, a porous biodegradable hydrogel scaffold was designed to achieve the controlled release of luteolin through a reversible boronic acid ester bond, protecting the agent's bioactivity while ensuring its prolonged presence to continuously induce M2 macrophage polarization in a rat critical-sized calvarial bone defect model.[30]

Another strategy utilized an injectable thermosensitive hydrogel containing carbonated hydroxyapatite (CHA) microspheres co-loaded with resveratrol and dexamethasone (DEX). This system showed a synergistic effect in a bilateral ovariectomy rat osteoporotic bone defect model: resveratrol induced M2 polarization and scavenged reactive oxygen species (ROS), while DEX promoted the osteogenic differentiation of mesenchymal

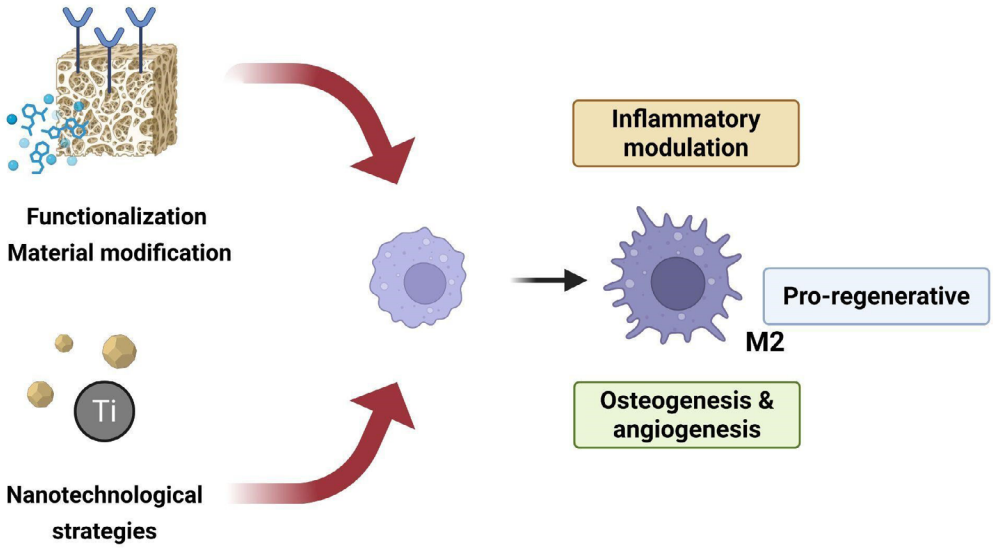

Figure 1. Tier I: Tailored biomaterials as intrinsic regulators of the osteoimmune microenvironment. Schematic representation of the first level of complexity described in this review (Section 3.1), whereby biomaterial-intrinsic properties, such as matrix stiffness, nanotopography, ionic functionalization, and surface chemistry, act as primary instructive cues to modulate macrophage behavior. These design parameters influence early post-implantation immune dynamics, promoting a controlled transition from pro-inflammatory M1-associated responses toward pro-resolutive M2-dominant phenotypes. Representative *in vivo* readouts supporting this tier include the modulation of macrophage markers, including the CD86/CD206 ratio and the expression of inducible nitric oxide synthase and arginase-1, shifts in cytokine profiles (tumor necrosis factor-α, interleukin [IL]-1β, and IL-10), enhanced angiogenesis (vascular endothelial growth factor expression and $CD31^+$ vessel formation), and increased bone formation assessed by micro-computed tomography and histomorphometry in preclinical defect models. This level establishes biomaterials not as passive osteoconductive fillers, but as active immunoregulatory platforms capable of directing osteogenesis and osseointegration through the modulation of macrophage plasticity. Created with BioRender.

stem/stromal cells (MSCs).[31] Similarly, the incorporation of immunomodulatory cytokines (e.g., IL-10) into hydrogel systems has proven effective in restoring the M1/M2 balance in both diabetic alveolar bone defects[32] and calvarial models.[29] Furthermore, the integration of inorganic elements, such as Sr, into these platforms has been shown to stimulate osteogenesis in air-pouch mice models of inflammation.[33]

Beyond drug delivery, hybrid hydrogels are also being designed to provide dynamic physical or biological cues. Studies have shown that piezoelectric hydrogels can generate electrical signals under mechanical loading, directing macrophage polarization toward a pro-resolutive state in a rat large-scale cranial injury model.[34] A similar effect was achieved with PDA-coated arrays responsive to photothermal heat in a bifemoral-condyle rat defect model.[35] Mimicking endogenous biological processes, hydrogels containing calcium-enriched beads were designed to self-mineralize and subsequently modulate macrophage activity.[36]

Furthermore, certain scaffolds are engineered to initiate a controlled inflammatory cascade that induces neutrophil apoptosis to trigger macrophage efferocytosis, a process that inherently promotes a switch to the M2 phenotype, as demonstrated in a diabetic cutaneous wound healing model.[37] Others, such as bisphosphonate-based hyaluronic acid hydrogels, specifically target the macrophage lineage to inhibit osteoclast differentiation while preserving pro-osteogenic preosteoclasts, which secrete anabolic factors essential for osteogenesis.[38] Finally, some designs combine angiogenic peptides with scaffolding materials to foster vascularization and immunomodulation in wound healing models.[39] Common to all these approaches is a significant shift in the local cytokine profile, characterized by the downregulation of pro-inflammatory factors, such as TNF-α, IL-1β, and inducible nitric oxide synthase (iNOS), and the upregulation of pro-resolutive markers, including IL-10, arginase-1 (Arg-1), and CD206.

A second major front in hybrid materials involves the surface engineering of metallic implants. The integration of Sr onto Ti surfaces is a recurring theme due to its dual capacity to influence both immune and bone cells. Sr-doped sodium titanate nanorods on Ti surfaces effectively induced M2 macrophage polarization, subsequently enhancing the secretion of platelet-derived growth factor-BB (PDGF-BB), which promotes the formation of vessel subtypes essential for osteogenesis.[40] Another design combined a Sr-doped nanoporous structure with wogonin nanoparticles, promoting an M1-to-M2 shift and increasing the expression of TGF-β1 and Arg-1, which favored osteoblast proliferation and differentiation in a combined osteoporosis and femur-defect model.[41] This approach is particularly relevant in compromised-healing scenarios; for instance, a dual-responsive hydrogel coating on Ti implants was developed to sequentially release adenosine—to promote M2 polarization and restore mitochondrial function—and Sr—to promote MSC differentiation in a rat diabetic model.[24]

Nanotechnology has also been harnessed within hybrid biomaterials to achieve precise immunomodulation. A shared strategy centers on utilizing functionalized nanomaterials to orchestrate the healing microenvironment. For example, the inherent immunomodulatory properties of nHA particles have been optimized by creating sericin–nHA/proanthocyanidins (Se–nHA/PC) composite microspheres [42], which scavenged ROS to improve the osteogenic differentiation of periodontal ligament stem cells in a rat periodontitis model.[43] Similarly, glycosylated nHA has been shown to effectively restore M2 macrophage polarization and enhance alveolar bone repair in periodontitis models.[44]

Moving forward, various nanodelivery systems leverage this scale for targeted immunomodulatory effects. Yeast–glucan-based nanoparticles loaded with methotrexate (MTX) were shown to induce an M1-to-M2 shift in macrophages via the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway while stimulating the osteogenic differentiation of dental pulp stem cells.[45] Similarly, injectable hyaluronic acid hydrogels co-delivering stem cells derived from human exfoliated deciduous-derived exosomes and copper ions (Cu^{2+}) effectively suppressed inflammation via the IL-6/janus kinase 2/signal transducer and activator of transcription 3 pathway and significantly upregulated the osteogenic markers runt-related transcription factor-2 (RUNX2) and osteocalcin in a mouse periodontitis model.[46] Furthermore, nanostructured polymer membranes doped with zinc (Zn^{2+}) or doxycycline promoted an M2 macrophage phenotype in a rabbit midline cranial bone defect model.[47]

Combining delivery with structural benefits, a core–shell nanofibrous scaffold integrating curcumin-loaded nanoparticles successfully repolarized M1 macrophages to an M2 state while releasing calcium (Ca^{2+}) and phosphate ions for hydroxyapatite (HA) biomineralization in a rat cranial defect model.[48] Similarly, an aspirin-loaded ZnO–SiO_2 aerogel scaffold assembled from nanofibers released silicon (Si^{4+}) and Zn^{2+} ions, promoting M2 polarization and the expression of RUNX2 and collagen-1 in MSCs.[49] Nanoparticles can also be immobilized onto metallic surfaces; for example, peptide LL-37-loaded silk fibroin nanoparticles anchored to Ti surfaces and enhanced M2 macrophage recruitment and osteogenesis in a rat femur

defect model.[50]

Moreover, structural designs that replicate or directly modulate the biological microenvironment by leveraging bioactive structural cues have shown significant promise. Scaffolds with a hierarchical bone-like nanointerface directly promoted M2 macrophage polarization and IL-4 secretion, enhancing MSC recruitment and differentiation in a critical-size rat mandible model.[51] An ECM-mimetic coating on a polymer graft similarly guided an M1-to-M2 shift in a rat bilateral extra-articular graft-bone healing model.[25]

The controlled release of bioactive ions and mediators remains a shared strategy[52], as seen in functionalized bioactive glass scaffolds, where amino functionalization promoted an anti-inflammatory milieu in a bilateral maxillary sinus floor elevation model.[23] Notably, the incorporation of pro-resolving mediators, such as maresin 1, into DBM scaffolds effectively polarized macrophages toward an M2 phenotype, creating a microenvironment that enhanced the recruitment and osteogenic differentiation of MSCs in a mouse skull defect model.[53]

Collectively, these studies suggest that a wide range of hydrogel-based and hybrid systems exert a profound influence on macrophage behavior during bone repair. While basic hydrogel matrices often lead to a more pro-inflammatory M1 response across diverse models, engineered platforms, including drug delivery hydrogels (e.g., luteolin-loaded, or resveratrol/DEX-loaded CHA), hybrid systems (piezoelectric, photothermal, or self-mineralizing), and functionalized metallic surfaces (Sr-doped nanorods or wogonin-loaded nanopores), are frequently reported to shift macrophage phenotypes toward a pro-resolutive M2-like profile. Furthermore, nanotechnology (including Se–nHA/PC microspheres and glycosylated nHA), nanodelivery systems (MTX-loaded nanoparticles, exosome/ion co-delivery, and ion-doped polymer membranes), and bio-instructive designs (such as maresin 1-functionalized DBM) effectively direct macrophages into an anti-inflammatory M2 cell state. Taken together, the literature demonstrates that many of the most effective hybrid and nanoengineered systems do not eliminate inflammation; rather, they precisely modulate its magnitude, duration, and trajectory, thereby establishing sophisticated avenues for next-generation biomaterials (Figure 2).

3.3. Advanced 3D-printed constructs from structural design to bioactive systems

Tissue engineering has advanced significantly with the development of 3D-printed scaffolds composed of bioactive materials capable of modulating the immune response and promoting tissue repair. While previous strategies focused primarily on material composition, 3D printing introduces a superior level of control over structural architecture, enabling the creation of scaffolds that can instruct the host tissue microenvironment through precise geometric and spatial arrangements.

For instance, 3D printing allowed the fabrication of polylactic acid (PLA) scaffolds with a fully interconnected and controlled porous network, ensuring the homogeneous distribution of bioactive bioglass. In an *in vitro* model, this architecture facilitated the steady release of bioglass degradation products, which actively promoted M2 polarization by inhibiting the expression of pro-inflammatory markers, such as IL-1 and iNOS, while upregulating Arg-1 and IL-10.[54] Furthermore, 3D-printed Ti scaffolds assembled with larger-diameter nanotubes (e.g., 134 nm) favored an M2-like macrophage activation in RAW264.7 cells, increasing the expression of IL-1 receptor antagonist, Arg-1, and CD206, while reducing pro-inflammatory cytokines.[55] Similarly, modified 3D-printed Ti-alloy scaffolds (Ti-24Nb-4Zr-8Sn, Ti2448), which synergistically combine a low elastic modulus to match native bone stiffness with a bioactive surface generated via microarc oxidation, successfully directed macrophages toward a reparative M2 state *in vitro*. This immunomodulatory effect was characterized by a significant downregulation of TNF-α and IL-1β, coupled with an increased secretion of IL-4 and BMP-2, thereby creating a pro-regenerative microenvironment.[56]

Three-dimensional printing has also enabled significant advances in the fabrication of bioceramic scaffolds with precisely controlled architectures. Ordered bredigite scaffolds were reported to upregulate the expression of CD206 and Arg-1 while downregulating M1-associated markers in *in vitro* studies using murine macrophage cells.[57] Likewise, silicate-based porous bioceramics fabricated by 3D printing consistently promoted the upregulation of M2-related markers, concomitant with the suppression of TNF-α and increased secretion of IL-10 and PDGF-BB, under *in vitro* conditions.[58] Similarly, 3D-printed scaffolds combining polycaprolactone (PCL) and biphasic calcium phosphate were shown to modulate macrophage responses and balance inflammation in a subcutaneous murine implantation model.[59]

However, a study comparing 3D-printed scaffolds composed of PCL, polyethylene glycol, and HA (PCL/PEG/HA) with sintered HA structures demonstrated distinct immunological outcomes in an *in vivo* calvarial defect model.[60] Immunohistochemical analysis of M1 and M2 macrophages at the implant site revealed that PCL/PEG/HA scaffolds elicited a stronger early inflammatory

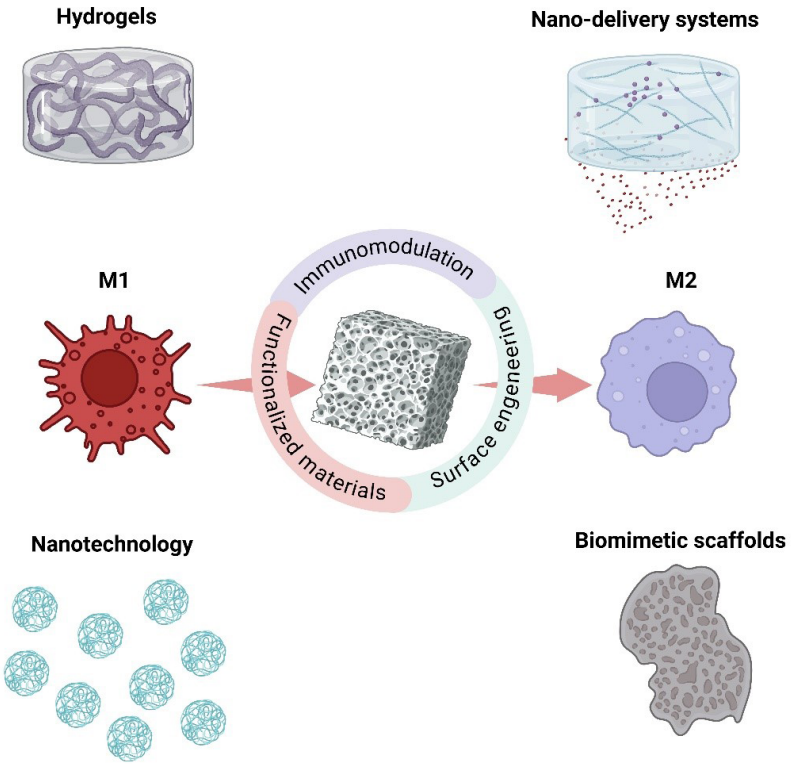

Figure 2. Tier II: Hybrid biomaterials integrating structural and bioactive strategies for macrophage-directed bone regeneration. Schematic representation of the second level of complexity described in this review (Section 3.2), whereby hybrid systems combine organic, inorganic, and bioactive components to orchestrate the osteoimmune microenvironment. These platforms integrate biomimetic scaffolds, multifunctional surface modifications, and advanced delivery systems to enable the sustained or sequential release of cytokines, drugs, or ions. By coupling structural guidance with spatiotemporal biochemical signaling, hybrid biomaterials promote a controlled M1-to-M2 macrophage transition and resolution of inflammation. Representative *in vivo* readouts include the modulation of tumor necrosis factor-α and interleukin-10, increased expression of arginase-1 and CD206, enhanced angiogenesis, and improved bone formation in preclinical defect models. Created with BioRender.

response, characterized by increased M1 macrophage infiltration and fibrotic tissue formation, which delayed osseointegration. In contrast, pure sintered HA scaffolds promoted macrophage polarization toward the M2 phenotype and were associated with improved bone integration.

The influence of 3D-printed geometry extends to complex composite systems, where the structural arrangement of these hybrid materials dictates the timing and type of the immune response. Illustrating this strategy, the 3D-printed architecture of a PCL composite containing carbon nanotubes and ceramics showed the capacity to induce the expression of M2 markers (IL-10 and CD163) while maintaining transient increases in M1 markers, such as C–C motif chemokine receptor 7 (CCR7) and CD86, in a murine calvarial critical-sized defect.[61] Similarly, the use of chitosan/silk fibroin/cellulose nanoparticle bioinks enabled the fabrication of stable, multi-layered structures that facilitated a significant shift toward an M2 macrophage profile *in vitro*, characterized by the enhanced expression of CD163 and the secretion of pro-healing cytokines, such as C–X–C motif chemokine ligand 1 (CXCL1).[62]

In line with this, scaffolds composed of xonotlite nanofibers, silk fibroin, and gelatin have been shown to promote M2 macrophage polarization, reducing the secretion of IL-1β and IL-6 while increasing IL-10 and TGF-β signaling to enhance MSC migration and osteo/angiogenic differentiation in both *in vitro* and *in vivo* models.[63] Moreover, the inherent surface roughness of PLA–bioglass scaffolds fabricated via fused deposition modeling was reported in an *in vivo* biocompatibility study to enhance cell attachment and accelerate the resolution of the acute immune response, thereby promoting the macrophage transition toward an ECM-producing

phenotype.[64]

Another strategy involves the incorporation of biologically derived ECM components into bioinks to orchestrate host immune regulation. A scaffold fabricated from porcine-treated dentin matrix combined with PCL and cerium oxide (CeO_2) nanoparticles, recognized for their redox-active and immunomodulatory properties, was evaluated using both *in vitro* assays with murine macrophage cell lines and *in vivo* craniofacial defect models.[65] The scaffold was reported to promote macrophage polarization toward an M2-like phenotype. In parallel, to address diabetic bone defects, a composite scaffold was 3D-printed using alginate, nHA, and a xenogeneic ECM derived from small intestine submucosa.[66] This study likewise combined *in vitro* experiments using murine macrophage models with an *in vivo* craniofacial defect model in diabetic animals; immunohistochemical evaluation of the regenerated tissue demonstrated enhanced M2 macrophage polarization, as evidenced by the elevated expression of the M2-associated marker CD206.

Expanding into more complex systems, hydrogels have been extensively explored in 3D printing, either as standalone functionalized constructs or as surface coatings, to incorporate a wide range of bioactive agents. For instance, the incorporation of platelet-rich plasma into a gelatin methacryloyl (GelMA) scaffold was evaluated *in vivo* in a rabbit osteochondral defect model, where immunohistochemical analyses revealed enhanced macrophage polarization toward an M2 phenotype, evidenced by the increased expression of Arg-1 and CD206 and a concomitant reduction in the M1-associated marker CCR7.[67]

Similarly, a strategy employing the small-molecule inhibitor SW033291 was investigated using *in vitro* assays with murine macrophage cell lines (RAW264.7 cells) and further validated *in vivo* following scaffold implantation.[68] This approach demonstrated a skewing of macrophage polarization toward an M2-like state through elevated prostaglandin E2 levels, accompanied by increased CD206 and IL-10 expression. In addition, GelMA scaffolds functionalized with laponite were assessed using both *in vitro* and *in vivo* models, in which macrophage-mediated immunomodulation promoted a transition from the M1 to the M2 phenotype, thereby facilitating MSC osteogenic differentiation and enhanced bone regeneration.[69]

In line with this functionalization strategy, a biomimetic glycopeptide hydrogel was used as a coating on a PCL/nHA scaffold and evaluated in both *in vitro* (RAW264.7 macrophages) and *in vivo* cranial defect models, where it promoted M2 macrophage polarization, evidenced by increased IL-10 and TGF-β expression, and supported enhanced cranial bone regeneration.[70]

Three-dimensional printing has also emerged as a platform for the controlled delivery of therapeutic ions, drugs, and biological components. The incorporation of ions such as Sr, Zn, and Fe^{2+} into 3D-printed bioceramics was reported to modulate the macrophage M1/M2 balance, reduce pro-inflammatory cytokines, and enhance the secretion of angiogenic factors, including VEGF and PDGF-BB, as demonstrated in both *in vitro* macrophage assays and *in vivo* bone defect models.[71] Several 3D-printed scaffolds have been engineered to deliver immunomodulatory agents and regulate macrophage polarization. DEX-loaded PLA/PEG/nHA scaffolds and methacrylate/silica hybrid scaffolds were evaluated primarily *in vitro* using murine macrophage models, where the suppression of pro-inflammatory markers and the promotion of M2 polarization were reported.[72] In contrast, Ti-based 3D-printed systems enabling controlled IL-4 delivery, either alone or combined with BMP-2, were investigated *in vivo* using bone defect models, with histological and immunohistochemical analyses revealing M2-enriched microenvironments and enhanced osteogenic and angiogenic responses.[73,74]

More complex platforms, including poly(caprolactone fumarate) scaffolds functionalized with 2D hetero-nanostructures and methacryloylated PCL scaffolds incorporating engineered adipose stem cell-derived nanovesicles, were assessed through both *in vitro* macrophage assays and *in vivo* bone regeneration models, demonstrating the establishment of pro-healing, M2-dominated niches that support angiogenesis and bone regeneration.[75,76]

Recent progress highlights integrative strategies that combine 3D printing with complementary fabrication techniques to generate multiscale hybrid systems. By integrating 3D-printed PCL scaffolds with electrospun poly(L-lactide) fibers, Liu *et al.*[77] demonstrated, using both *in vitro* macrophage assays and *in vivo* bone defect models, that the resulting microtopography directed macrophage polarization toward a pro-healing M2 phenotype, accompanied by increased VEGF expression and reduced TNF-α and IL-6 via activation of the phosphoinositide 3-kinase/AKT signaling pathway. Another example employed mussel-inspired PDA coatings on biodegradable elastomers composed of poly(glycerol sebacate) and PCL to create 4D-patterned membranes.[78,79] These systems were evaluated primarily *in vivo* in craniofacial defect models, where histological and immunohistochemical analyses revealed sustained recruitment of M2 macrophages and enhanced MSC infiltration, ultimately promoting osteogenesis.

At the most advanced level, cell-based strategies have been explored by encapsulating MSCs within a thermosensitive hydroxypropyl chitin hydrogel integrated with a 3D-printed PCL/nHA scaffold. This approach was assessed using both *in vitro* immunomodulation assays and *in vivo* bone defect models, showing MSC-mediated suppression of TNF-α and IL-6, upregulation of IL-10 and Arg-1, and subsequent stimulation of macrophage-derived BMP-2, TGF-β1, and VEGF, leading to significantly enhanced bone repair.[80]

Although many 3D-printed scaffolds are designed to promote an M2-dominant pro-regenerative environment, immunomodulation may fail depending on dose, timing, degradation kinetics, and biological context. While sintered HA scaffolds favored M2 polarization and improved osseointegration, PCL/PEG/nHA composites triggered stronger early M1-driven inflammation, fibrosis, and delayed integration. A transient M1 phase is physiologically necessary for debris clearance and antimicrobial defense; however, excessive early suppression may impair pathogen control or proper remodeling. Conversely, sustained M1 activation, driven by unfavorable surface chemistry, acidic polymer degradation products, or excessive ion/drug release, can lead to chronic inflammation and fibrotic encapsulation. Dose-dependent effects of bioactive ions and immunomodulatory agents further complicate outcomes, as optimal concentrations may promote repair while higher levels may induce cytotoxicity or persistent inflammation. Therefore, rather than enforcing a static M2 phenotype, effective scaffold design should support a balanced, time-resolved M1-to-M2 transition that reflects the natural dynamics of bone healing while minimizing the risks of fibrosis and infection.

Collectively, these studies underscore that 3D printing technology is not merely a structural fabrication tool but an integrative bio-instructive platform. The ability to precisely control macro-architecture, combined with the incorporation of chemical agents, ions, drugs, and cellular components, positions 3D-printed constructs as the next generation of therapies for bone regeneration, capable of actively shaping the biological environment for superior regenerative outcomes (Figure 3).

To facilitate a structured comparison across biomaterial strategies with different levels of technological complexity and translational readiness, a comparative framework is summarized in Table 1.

4. Challenges and limitations

Despite these promising advances, several critical barriers to clinical translation remain and demand a more pragmatic analysis. Approaches based on intrinsic material properties, such as surface-modified Ti implants, nanotopographical adjustments, and ion-doped bioceramics, currently present the highest potential for near-term clinical translation.[81,82] These strategies frequently rely on materials already approved for orthopedic use, require minimal incorporation of biologically active agents, and are compatible with established industrial manufacturing pipelines.[83] Consequently, they tend to exhibit lower batch-to-batch variability and a more straightforward regulatory classification, often remaining within the scope of medical devices.

In contrast, hybrid systems and advanced 3D-printed constructs pose significant challenges related to scalability and precise calibration of stimuli-responsive release. Minor variations in formulation parameters can significantly alter the immunomodulatory profile, thereby complicating standardization and quality control. Furthermore, devices incorporating pharmacological agents or cytokines are frequently regulated as combination products, which substantially increases development costs and extends approval timelines.[84]

Moreover, the heterogeneity of experimental models and the lack of standardized macrophage characterization criteria remain major hurdles that hinder cross-study comparability and slow regulatory progression.[4] Effective clinical translation will therefore require not only biological efficacy but also manufacturing reproducibility, regulatory clarity, and economic viability. In summary, the future of bone tissue engineering is inextricably linked to the development of materials capable of predictably and sustainably guiding the host immune system. Overcoming current barriers will require coordinated, interdisciplinary efforts to transform these "intelligent" platforms into safe, effective, and accessible regenerative therapies.

5. Conclusion

The evolution of bone bioengineering has driven a fundamental paradigm shift: successful regeneration requires more than structural support; it depends on the deliberate orchestration of the local immune microenvironment. The evidence reviewed here consistently identifies the coordinated transition from pro-inflammatory M1 phenotypes to pro-resolving M2 states as the central axis of osteoimmunology and a primary determinant of successful bone repair. Collectively, the diverse strategies discussed, spanning from subtle adjustments in matrix stiffness and nanotopography to advanced 3D-printed hybrid systems, converge on a unified biological principle: the ability to instruct macrophages toward regenerative profiles to promote angiogenesis and osteogenesis, even under challenging clinical conditions

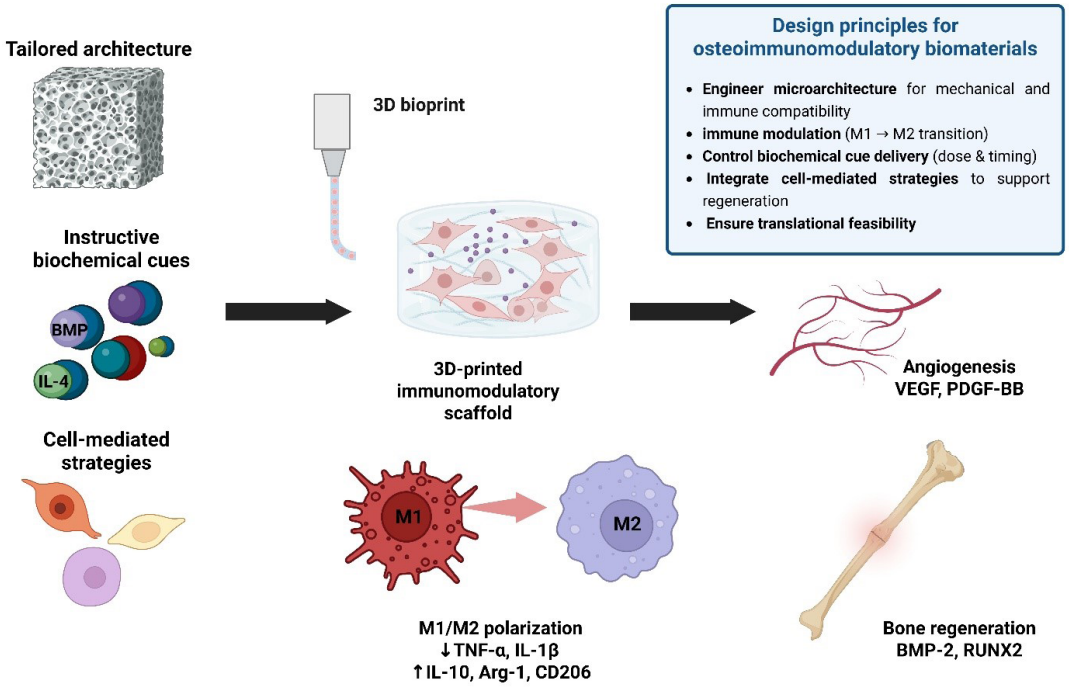

Figure 3. Tier III: Advanced 3D-printed immunomodulatory constructs integrating architectural control and bioactive programming. Schematic representation (Section 3.3) of how scaffold architecture, bioactive cues (e.g., ions and cytokines), and cellular strategies converge through 3D printing and bioprinting into programmable constructs capable of modulating the osteoimmune microenvironment. These integrated platforms regulate the immune trajectory, promoting the transition from early M1-associated inflammation (characterized by elevated tumor necrosis factor-α [TNF-α] and interleukin [IL]-1β) toward an M2-dominant phenotype (associated with increased IL-10, arginase-1 [Arg-1], and CD206). This controlled immune modulation enhances angiogenic signaling (including vascular endothelial growth factor [VEGF] and platelet-derived growth factor-BB [PDGF-BB] expression), osteogenesis, osseointegration, and critical-sized defect repair, including regeneration under disease-compromised conditions. The highlighted design rules summarize guiding principles for aligning biological precision with translational potential. Created with BioRender
Abbreviations: BMP-2: Bone morphogenetic protein-2; RUNX2: Runt-related transcription factor-2.

Table 1. Tiered overview of osteoimmunomodulatory biomaterials

Level of complexity (tier)	Main strategies	Key *in vivo* outcomes	Translational maturity	Key translational barriers
Tier I: Tailored materials	Matrix stiffness modulation; nanotopography; ion doping (Mg^{2+}, Sr^{2+})	Early M1-to-M2 transition; accelerated osseointegration	High	Limited control over temporal immunoregulation
Tier II: Hybrid materials	Stimuli-responsive hydrogels; sequential cytokine/ion delivery (e.g., IL-4/Ag^{+})	Modulation of complex inflammatory microenvironments; enhanced angiogenesis	Intermediate	Release kinetics reproducibility; scalability; complex combination product regulation
Tier III: 3D-printed constructs	Controlled porous architecture; biofabrication with exosomes or encapsulated cells	Repair of critical-sized defects; programmable regenerative niches	Proof-of-concept	Donor variability; dose standardization; GMP manufacturing constraints; high production costs

Abbreviations: GMP: Good Manufacturing Practice; IL: Interleukin.

such as diabetes and chronic inflammation.

Building upon this recognition, the field is transitioning away from conventional static systems toward "biocommunicative" and immuno-intelligent scaffolds. These next-generation materials are designed to sense and dynamically respond to pathophysiological

cues in real-time, such as local acidosis, elevated ROS levels, and proteolytic overexpression (e.g., matrix metalloproteinases). Representative systems, such as pH-responsive hydrogels that selectively release IL-4 in acidic inflammatory environments[29] and ROS-responsive nanoparticles[48], demonstrate the potential for spatially and temporally controlled immunomodulation. This "material intelligence" further extends to the programmed, sequential release of biological cues, utilizing multi-layered architectures or 4D bioprinting to recapitulate the temporal dynamics of physiological bone healing; for instance, by orchestrating the M1-to-M2 transition through the sequential release of interferon-γ and IL-4.[52]

In parallel, cell-free yet bio-instructive strategies are emerging through the functionalization of scaffolds with extracellular vesicles and exosomes. These nanoscale carriers, derived from MSCs or M2 macrophages, can reprogram the host immune response by transferring bioactive cargo, offering a powerful immunomodulatory alternative without the complex regulatory risks associated with live cell therapies.[75,85] Furthermore, the integration of computational modeling and artificial intelligence is increasingly being leveraged to analyze large datasets of immune-material interactions. These tools allow for the *in silico* prediction of macrophage responses, accelerating the rational design of scaffolds with optimized immunomodulatory profiles tailored to the patient's specific immune signature, age, and comorbidities.[52,86]

Acknowledgments

None.

Funding

This work was supported by the Ministry of Health of Brazil and the National Council for Scientific and Technological Development (grant number 445067/2023-3), the Brazilian National Program of Genomics and Precision Health (grant number 444206/2023-0), the Carlos Chagas Filho Support Foundation of the State of Rio de Janeiro (FAPERJ; grant number E-26/201.403/2022), and the Maria Emilia Foundation (grant number 02/2024). The sponsors had no role in study design, data collection and analysis, decision to publish, or manuscript preparation.

Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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