Nanomaterial-integrated injectable hydrogels for craniofacial bone reconstruction

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Abstract

The complex anatomy and biology of craniofacial bones pose difficulties in their effective and precise reconstruction. Injectable hydrogels (IHs) with water-swollen networks are emerging as a shape-adaptive alternative for noninvasively rebuilding craniofacial bones. The advent of versatile nanomaterials (NMs) customizes IHs with strengthened mechanical properties and therapeutically favorable performance, presenting excellent contenders over traditional substitutes. Structurally, NM-reinforced IHs are energy dissipative and covalently crosslinked, providing the mechanics necessary to support craniofacial structures and physiological functions. Biofunctionally, incorporating unique NMs into IH expands a plethora of biological activities, including immunomodulatory, osteogenic, angiogenic, and antibacterial effects, further favoring controllable dynamic tissue regeneration. Mechanistically, NM-engineered IHs optimize the physical traits to direct cell responses, regulate intracellular signaling pathways, and control the release of biomolecules, collectively bestowing structure-induced features and multifunctionality. By encompassing state-of-the-art advances in NM-integrated IHs, this review offers a foundation for future clinical translation of craniofacial bone reconstruction.

Keywords Craniofacial bones, Injectable hydrogels, Nanomaterials, Bone tissue regeneration

Introduction

Craniofacial bones protect the brain, support facial structure, and drive mastication, speech, and aesthetics. Craniofacial bone defects, secondary to trauma, infection, congenital deformities, and tumor resection, often require surgical intervention using autografts and allografts [1, 2]. Clinically, a myriad of limitations, such as insufficient availability of bone, difficulty in shape matching, and the risk of inflammation, still hinder their optimal utilization [3]. These findings provide an incentive for developing alternative interventions to repair craniofacial bone defects anatomically and functionally.

In the context of regenerative medicine, hydrogels represent very attractive ready-to-use biomaterials with highly hydrophilic 3D network structures. These versatile bioplatforms, formed via crosslinked polymer chains, possess desirable features, such as reliable biocompatibility, safe biodegradability, and structural similarity to extracellular matrix (ECM) [4]. Notably, in-situ gelation and shear-thinning/self-healing strategies yield hydrogels with injectability [5]. Compared to other counterparts (i.e., autografts, metals, and ceramics), injectable hydrogels (IHs) allow for minimally invasive intervention and adaptive matching of irregular lesions due to their viscoelastic and diffusive behavior [6]. Such porous and permeable structures also offer the distinct advantages of flexible modification and payload encapsulation [7]. Currently, increasing efforts have focused on functionalizing biosynthetic hydrogels to tackle the multifaceted paucity of craniofacial bone defects.

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Nanotechnology innovations among interdisciplinary communities have offered immense potential for assembling types of bioactive particles, drugs, cells, and molecules into a platform [8, 9]. Scholars have exploited nanoengineered IHs to achieve improved and customizable tissue regeneration [10, 11]. Nanomaterials (NMs) possess a wealth of physical and chemical properties (i.e., larger specific surface areas, better mechanical properties, and chemical reactivities) to simultaneously optimize the mechanical properties and biological functions of IHs during craniofacial bone reconstruction (Fig. 1). For example, nanohydroxyapatite (nHA) acted as a high-energy phase to toughen the hydrogel matrix, which better satisfied the load-bearing and chewing stress requirements for maxillofacial bone repair [12]. Apart from mechanical enhancement, NM-integrated IHs combine additive biofunctionalized and stimuli-responsive features that govern the behavior of native tissues and permit ingenious bone formation as never before. To compensate for the deficient bioreactivity of IHs, mesoporous bioactive glass nanoparticles (MBGNs) were incorporated to stimulate cell types of interest, providing an advantageous bone microenvironment in craniofacial deformity applications [13]. Furthermore, deploying NMs facilely tunes the IHs' properties by applying "smart" stimuli, especially under damaged conditions (poor bone quality/quantity). As an example, the inclusion of copper-based nanozymes in IHs was shown to respond to the increasing levels of matrix metalloproteinases in periodontitis, realizing on-demand and precise alveolar bone regeneration [14].

Successful bone tissue engineering requires an understanding of bone biology and structure, as well as appropriate selections of material types and combinations [15]. Previous reviews have discussed advances in hydrogel-based bone tissue regeneration according to the diversities of material selections and fabrication approaches [16, 17]. Nevertheless, rebuilding craniofacial bone based on NM-incorporated IHs has not been systematically summarized, mainly due to the complex structural specifications and physiological characteristics of craniofacial bones and the underappreciated super-additive effects of NMs. To identify relevant publications, we conducted a meticulous search strategy on Web of Science and PubMed. Search queries were defined as follows: (nano*) AND (injectable hydrogel) AND (craniofacial or skull or cranial or calvarial or maxillofacial or alveolar or maxillary or mandibular). First, the clinical challenges and drawbacks of current treatments for craniofacial bone defects are presented. Following this, the mechanical and biological properties of NM-enhanced IHs are elucidated, revealing the potential mechanisms. Design considerations and translational perspectives are also included. The insights presented on these topics provide rational information for further designing and optimizing multifunctional nanoengineered IHs within the realm of craniofacial bones.

Current challenges of craniofacial bone regeneration

Craniofacial bones display fundamental differences and more complicated biological conditions compared with long bones [18, 19]. In this context, successful and predictable reconstructions require considerations of skeletal tissue characteristics, sensory organ presence, high vascular density, and bacterial contamination. First, craniofacial bones involve interfaces with multiple tissue types, and their deficiencies typically present irregular and fissure-like features. Thus, malleable repairs must adapt to the three-dimensional geometries to ideally
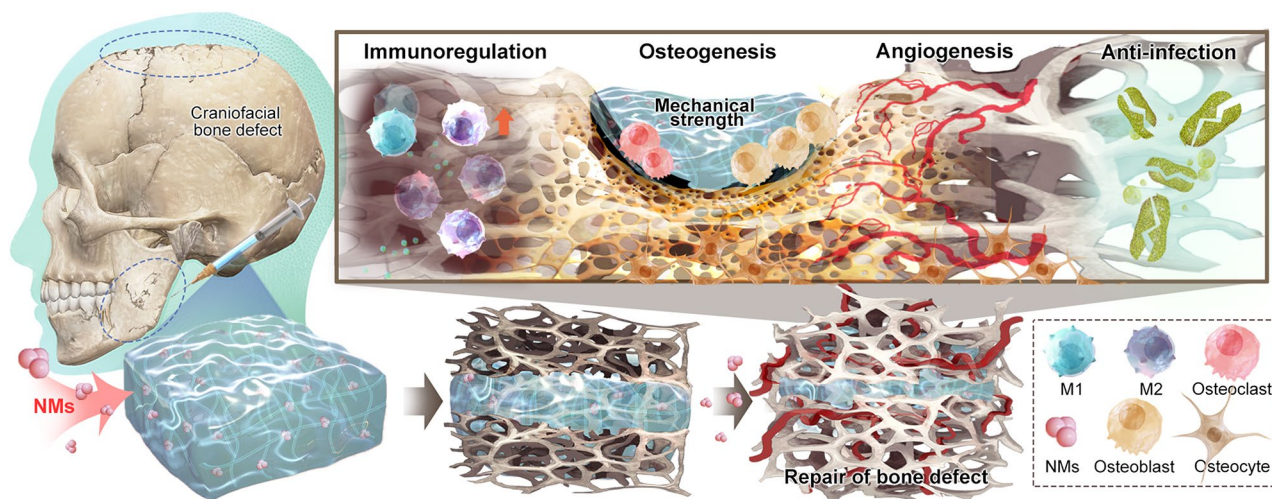

Fig. 1 NM-incorporated IHs with charming mechanical properties and versatile biofunctional characteristics are promising candidates for craniofacial bone reconstruction

precisely match the contours and continuity [20]. Second, craniofacial bones, especially the jawbone, exhibit a greater remodeling rate and lower mass density than the femur. Of note, physiological conditions, such as the relatively thin nature of the periosteum and the comparative lack of marrow space, lead to limited osteogenesis [21, 22]. Third, the reconstruction mode of craniofacial bones (mainly intramembranous ossification) often leads to avascular necrosis and degradation in central regions due to insufficient angiogenesis and poor nutrient perfusion, particularly in harsh microenvironments [23]. Fourth, although calvarial bones are typically considered non-load-bearing, they are not absent of biomechanical function, as they displace forces of mastication and must withstand high acceleration and impact forces to protect the underlying head and face [1]. Last but not least, frequent exposure to bacterial environments (e.g., oral cavity) and external bacterial infections may compromise ongoing repair processes [24]. In summary, regenerative repair of craniofacial bone defects to improve patients' quality of life faces a formidable challenge.

To address this paucity, a myriad of clinically available strategies, such as autologous bone grafts, scaffolds, and hydrogels, have been proposed to yield efficient outcomes. Among these therapies, bone grafts have been hailed as the gold standard, but they are associated with several drawbacks, including limited availability, donor site breakage, morphology mismatch, and the risk of postoperative complications [3]. Modern bone implant materials develop from bioinert metals (e.g., Ti alloys) or nondegradable ceramics (e.g., HA) to bioactive materials. The implanted metal scaffolds are still plagued by releasing toxic metal ions through corrosion or wear, which may lead to inflammatory cascades and allergic reactions [25]. Inorganic materials, including glass and ceramic scaffolds, are limited by their inherent brittleness and cannot integrate well with the host bone [26]. Although solid porous scaffolds have good strength and are easy to shape, most osteoblasts only attach and extend on the surface of the scaffold pores, forming a monolayer of cells, which is different from the morphology, quantity, and distribution of cells in natural bone [27]. Recent studies point toward a promising role of hydrogel-based strategies in craniofacial bone regeneration (Fig. 2). IHs could feasibly realize fit-to-shape filling and effectively induce new bone formation in a noninvasive manner,
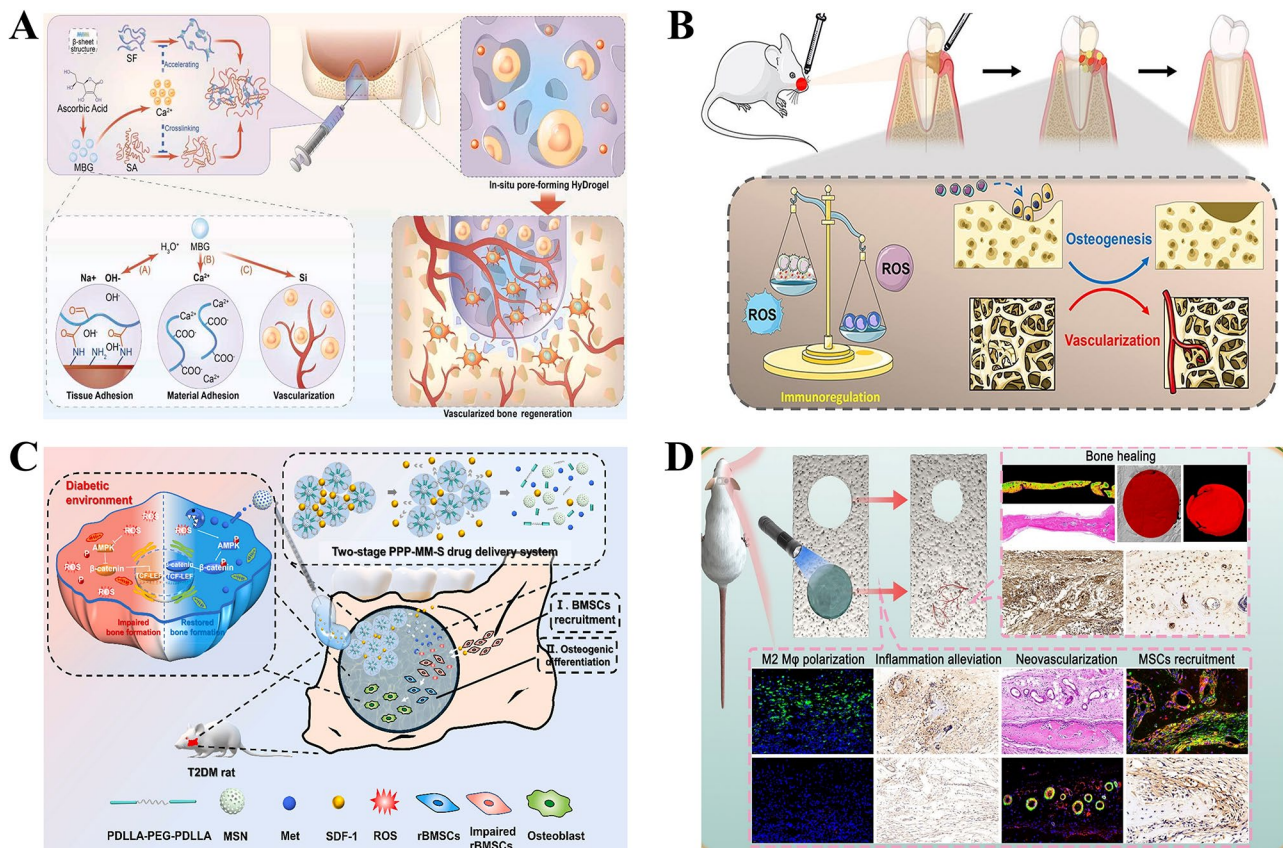
Fig. 2 Application of nanocomposite IHs in different craniofacial bone scenes. (**A**) Maxillary lacunar bone deficiency. Reproduced with permission [31]. Copyright 2022, Elsevier B.V. (**B**) Alveolar bone destruction. Reproduced with permission [32]. Copyright 2022, Elsevier Ltd. (**C**) Mandibular bone defects. Reproduced with permission [33]. Copyright 2022, Elsevier B.V. (**D**) Calvarial bone injury. Reproduced with permission [34]. Copyright 2023, Elsevier Ltd

depending on their fascinating multifunctionality and customizability [28–30]. These advances provide ever-growing possibilities to address the drawbacks of conventional materials for treating craniofacial bone defects.

Advantages of NM-integrated IHs in reconstructing craniofacial bones

Most single-network hydrogels are static and biologically inactive, sparingly adaptable to cell-mediated changes with generally linear-elastic mechanical properties. In this scenario, doping versatile NMs into IHs will contribute towards achieving appropriate mechanical properties and desired biological activities. At the early implantation stage, NM-enhanced IHs tightly manipulate the local immune response, forming a microenvironment conducive to tissue regeneration and functional restoration. During the regenerative stage, nanomodified IHs boost new bone by mediating the differentiation and ECM secretion of osteogenic-related cells. More importantly, the inclusion of NMs within IHs demonstrates unique revascularization abilities by regulating the behavior of endothelial cells (ECs) and their interactions with osteogenic-related cells. Additionally, introducing NMs into IHs exhibits better bacteriostatic effects during craniofacial bone healing. Below, we systematically summarize the advantages of NM-integrated IHs in treating craniofacial bone defects.

Mechanical properties

Implanted scaffolds should exhibit mechanical properties that match those of tissues at craniofacial bone sites to provide adequate mechanical support, withstand masticatory forces, and fit mechanical needs. However, IHs, water-based systems, possess an inherent mechanical softness. Although strategies such as adjusting the polymer concentration, initiator dosage, and crosslinking conditions have been conducted to produce mechanically reinforced IHs, these strategies may lead to property and compatibility capriciousness [35]. NM incorporation is another attractive method for reinforcing IHs through different/combined mechanisms, such as strengthening crosslinks, homogenized potential, stress distribution, and energy dissipation [36].

Ceramic-based NMs have been routinely embedded into soft polymer matrices with noncovalent and/or covalent interactions to achieve more optimized mechanical strength for craniofacial purposes. For example, nHA was incorporated into chitin-poly(ε-caprolactone), resulting in an improved elastic modulus without changing the viscoelastic nature of the matrix [37]. To reduce nHA agglomeration, bisphosphonates were utilized to chelate with nHA, which favored the mechanical stability and strength of the dually crosslinked IHs [38]. MBGNs constitute another highly practical example. They possess

a high specific surface area and porosity to selectively interact with the polymer matrix and thus display significant mechanical integrity via ion complexation and hydrogen bonding. MBGN-hybrid IHs showed tunable degradation behavior and tough mechanical strength in terms of enhanced storage modulus and compressive strength for craniofacial bone regeneration applications (Fig. 3A) [39].

Silicon-based NMs, including silica nanoparticles (NPs) [40], mesoporous silica nanoparticles (MSNs) [41], nanoclays [42, 43], and xonotlite [44], commonly retain favorable mechanical rigidity and make a distinctive contribution to toughening the IH networks. Dou et al. [42] developed a self-healing and osteogenic nanocomposite IH through the electrostatic assembly of nanoclay and gelatin NPs. This nanostructured IH gained remarkable mechanical profiles for realizing proper bone-material interface connections, as reflected by the maximal elastic modulus reaching ~150 kPa. In another example, the inclusion of Sr-substituted xonotlite (Sr-CSH) nanowires endowed the gelatin methacryloyl (GelMA) hydrogel system with a higher elastic modulus and compression modulus in a dose-dependent manner (Fig. 3B) [44]. Moreover, carbon-based NMs (graphene oxide (GO) [45] and nanodiamond [46]), metal NMs (MgO [47] and Au [48]), and nanocrystals [49] have been employed as nanofillers to create robust mechanical properties and stability for IH-based craniofacial tissue regeneration. For example, rod-shaped cellulose nanocrystals act as a reinforcement agent to form a percolating ternary system with low nanocrystal concentrations [49].

Substantial strategies have provided in-depth insights into mechanically reinforced IHs based on experimental gelation conditions. The mechanical strength and other hydrogel attributes of in situ products may be variable due to morphological changes (i.e., degradation-induced swelling), less efficient crosslinking, or cell/tissue cross-reactivity [7]. Solutions to these issues still need to be carefully characterized and investigated. Furthermore, delving into the pertinent principles governing the complex interactions among different formulations would contribute to a deeper understanding of tough NM-incorporated IHs.

Immune regulation

Implantation of a foreign body inevitably elicits inflammatory responses, hallmarked by the recruitment of immune cells and the accumulation of cytokines and reactive oxygen species (ROS). Unterminated inflammatory cascades can induce fibrous encapsulation and nonunion, which may partially explain why some hydrogels exhibit osteoinductive activity in vitro but drive undesired bone regeneration when applied in vivo [50]. Effective immunomodulation not only resolves acute
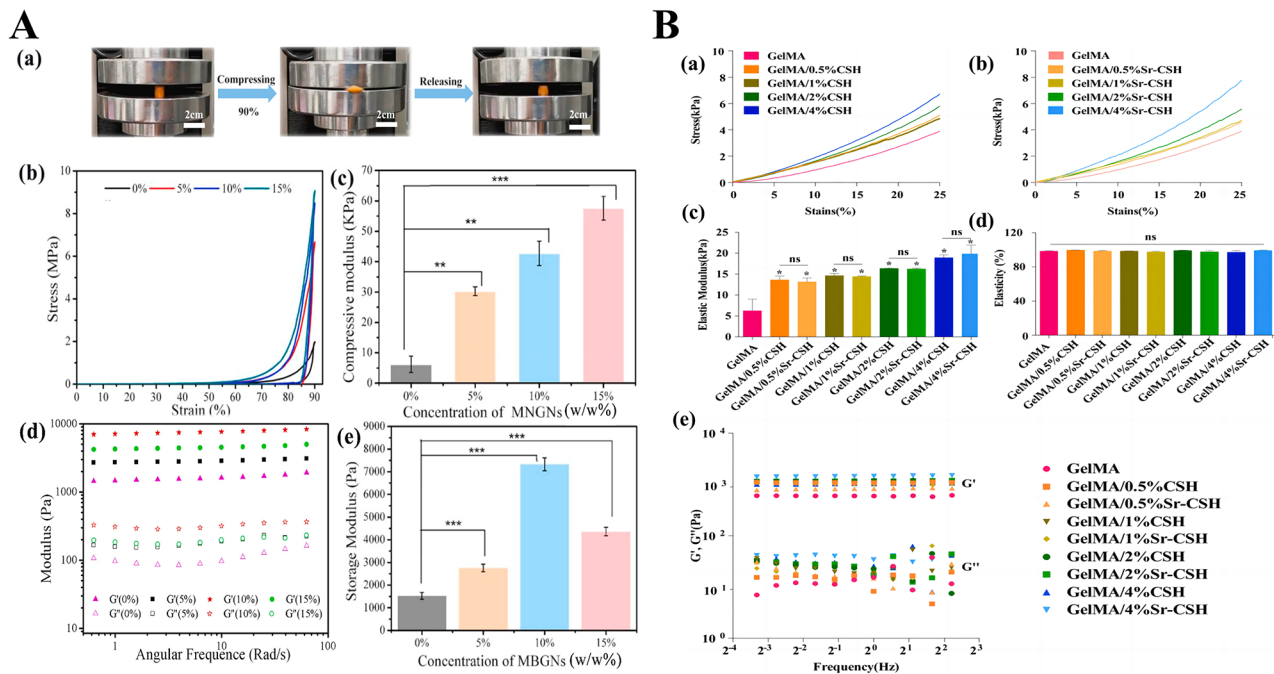
Fig. 3 The incorporation of NMs enhanced the mechanical properties of IHs. (**A**) MBGNs boosted storage modulus and compressive strength in the hydrogel matrices. Reproduced with permission [39]. Copyright 2021, Elsevier B.V. (**B**) With the integration of different nanowire contents, the IH systems exhibited remarkable mechanical strength. Reproduced with permission [44]. Copyright 2022, Elsevier B.V

inflammation reactions but also remedies disease-associated chronic inflammation, such as diabetes and periodontitis [51, 52]. Strikingly, the inclusion of eligible NMs in IHs could appropriately manipulate the interactions between implants and the immune system, mainly through modulating macrophage polarization and improving the osteoimmune microenvironment.

Inflammatory tissue-resident macrophages are indispensable drivers of the local immune response, taking on a spectrum of inflammatory and reparative roles [53]. Well-designed NMs containing functional groups and bioactive ions can modulate macrophage polarization from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype, facilitating necessary crosstalk between bone-related cells. Zheng et al. [31] developed an MBGN-incorporated IH to repair lacunar bone deficiency. The nanocomposite IHs promoted the proinflammatory response of macrophages at the early implantation stage. At the late stage, MBGNs actively tuned the reaction of the host immune system by steering the long-term M2 polarization through sensing inflammatory pH and Ca^{2+} concentration. A similar in-time and effective switch from M1 to M2 macrophages has been leveraged with ceria NP-enhanced IHs, which performed electron transfer between Ce^{3+} and Ce^{4+} and accordingly led to proregenerative effects in irregular periodontal regions [32]. In addition to bioactive ions, NMs employ functional groups to transition toward a proreparative macrophage phenotype. A particular focus

was the polydopamine-functionalized laponite (Lap@PDA) nanosheets, which directed hydrogel-derived macrophages toward bone-stimulating behavior, increased macrophage-bone mesenchymal stem cell (BMSC) crosstalk, and correspondingly promoted personalized craniofacial bone regeneration without any other drugs or molecules (Fig. 4A) [34]. Osteoimmunology is multifactorial and involves multiple immune cells. It remains elusive how NM-enhanced IH interventions interact with other immune cells (i.e., neutrophils, monocytes, or T cells) and how the transition between proinflammatory and reparative phenotypes occurs.

Another thriving strategy involves tethering NMs to suppress oxidative stress and enhance anti-inflammatory effects against pathological environments for IH-based craniofacial bone regeneration. Based on multi-ion complexation, nanoassembled flower micelles allow nitric oxide radicals to act as specific ROS scavengers in inflamed alveolar bone sites, rescuing the impaired proliferation, differentiation, and mineralization of osteoblasts [54]. Another study introduced a bioactive cyclodextrin-derived nanotherapy into hydrogels, which dose-dependently reduced the levels of proinflammatory and oxidative mediators, thereby protecting ectomesenchymal stem cells from ROS-induced cell apoptosis and promoting the healing of alveolar bone defects (Fig. 4B) [55]. In coordination with their intrinsic anti-inflammatory properties, NMs can also serve as nanocarriers for loading inflammation-modulating drugs. For example,
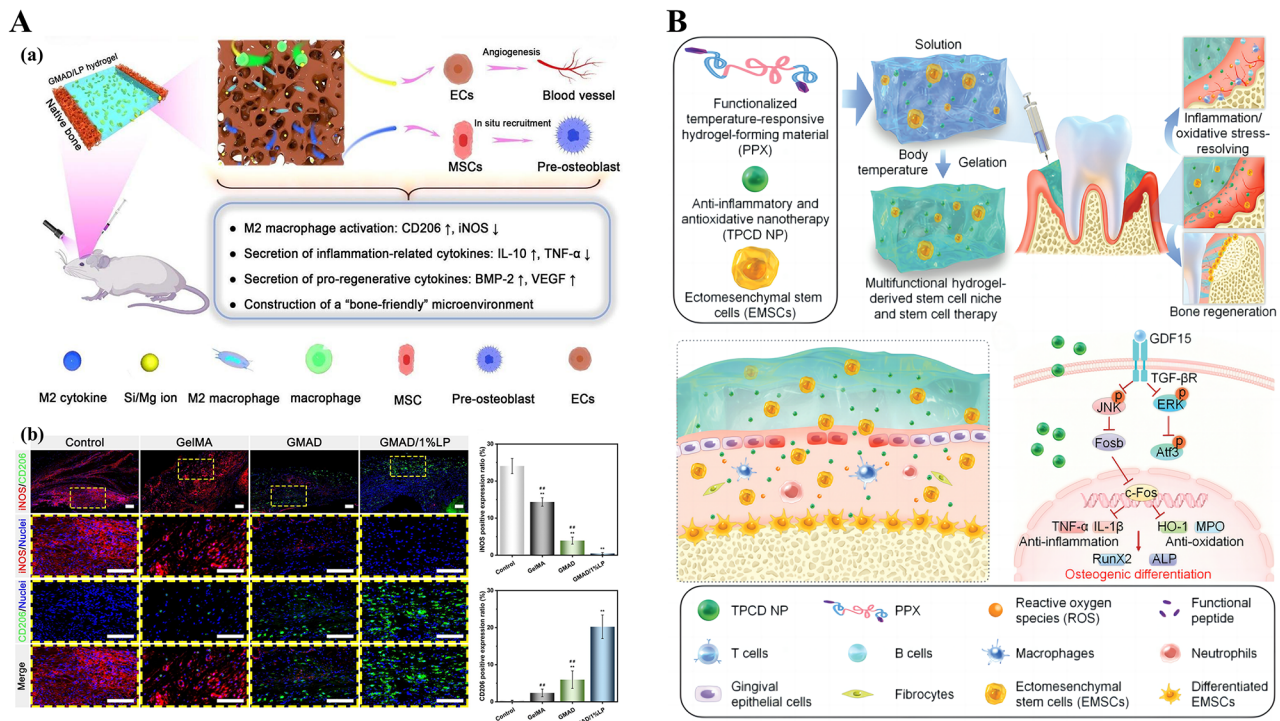
Fig. 4 NM-encapsuled IHs orchestrated the immune environment and resolved inflammation for augmented bone reconstruction. (**A**) The integration of Lap@PDA nanosheets into IH induced pro-regenerative M2 macrophage polarization and influential macrophage-BMSC crosstalk. Reproduced with permission [34]. Copyright 2023, Elsevier Ltd. (**B**) Schematic illustration of improved alveolar bone regeneration by an advanced stem-cell niche based on inflammation-resolving nanocomposite IHs. Reproduced with permission [55]. Copyright 2022, Wiley-VCH

MSNs allowed IHs to continuously release metformin during degradation, significantly clearing excessive ROS production and achieving craniofacial bone regeneration in a diabetic state [33]. Insights from these studies above suggest that NMs could circumvent possible inflammatory responses and optimize the regenerative process in the multitherapy of bone defects.

Osteogenesis promotion

The flat bones of the skull and jaw mainly involve the osteogenic process of intramembranous ossification. Typically, osteoclasts migrate and attach to defect sites. Simultaneously, osteoblasts, which are differentiated from BMSCs, deposit mineralized ECM and reconstruct the Haversian system [56]. Researchers have explored NM-enhanced IHs that promote osteogenesis by synergistically regulating osteoblastic differentiation/biomineralization and maintaining the osteoblast/osteoclast balance.

One of the key contributions of nanoengineered IHs in promoting osteogenesis lies in enhancing biomineralization ability and osteoinductive activity. Encapsulating mineral NMs (mainly CaP-based NMs [5]) benefit from their similarity in composition to natural bone and inherent bone conductivity. However, nHA has high crystallinity and slow absorption kinetics, leading to a poor hydrogel-tissue interface. As a result, doping with metal ions (such as Zn [57] and Sr [58]) or surface modification could reduce HA crystal size and crystallinity, enabling more uniform mineralization and mature bone formation in biomaterial substrates. Other bone-seeking NMs assembled with Ca and P, such as MBGNs [39], MSNs [41], and whitlockite bioceramic NPs [59], accelerated ECM deposition by providing higher concentrations of mineralizing components, effectively stimulating craniofacial bone healing. In addition, NMs rich in active functional groups, such as black phosphorus (BP) [60], GO [45], MgO [47], nanoclays [61], and chitosan-based NMs (nCSs) [62], created effective nucleation sites, pH environments, and high affinity to HA for better biomineralization (Fig. 5A). For example, MgO NMs raised surface pH to provide attachment points and amorphous hydroxyapatite layers for Ca and P deposition via a high release rate of Mg^{2+} [47]. Apart from mineralization-derived osteogenic effects, osteogenesis could be modulated by cellular and molecular mechanisms, as discussed in the next section.

Another integral role of NMs is to resume and maintain the balance and function of osteoblasts/osteoclasts. CaP NP-coordinated IH stimulated the orderly generation of osteoblasts and osteoclasts by the controlled release of parathyroid hormone, leading to effective cranial bone
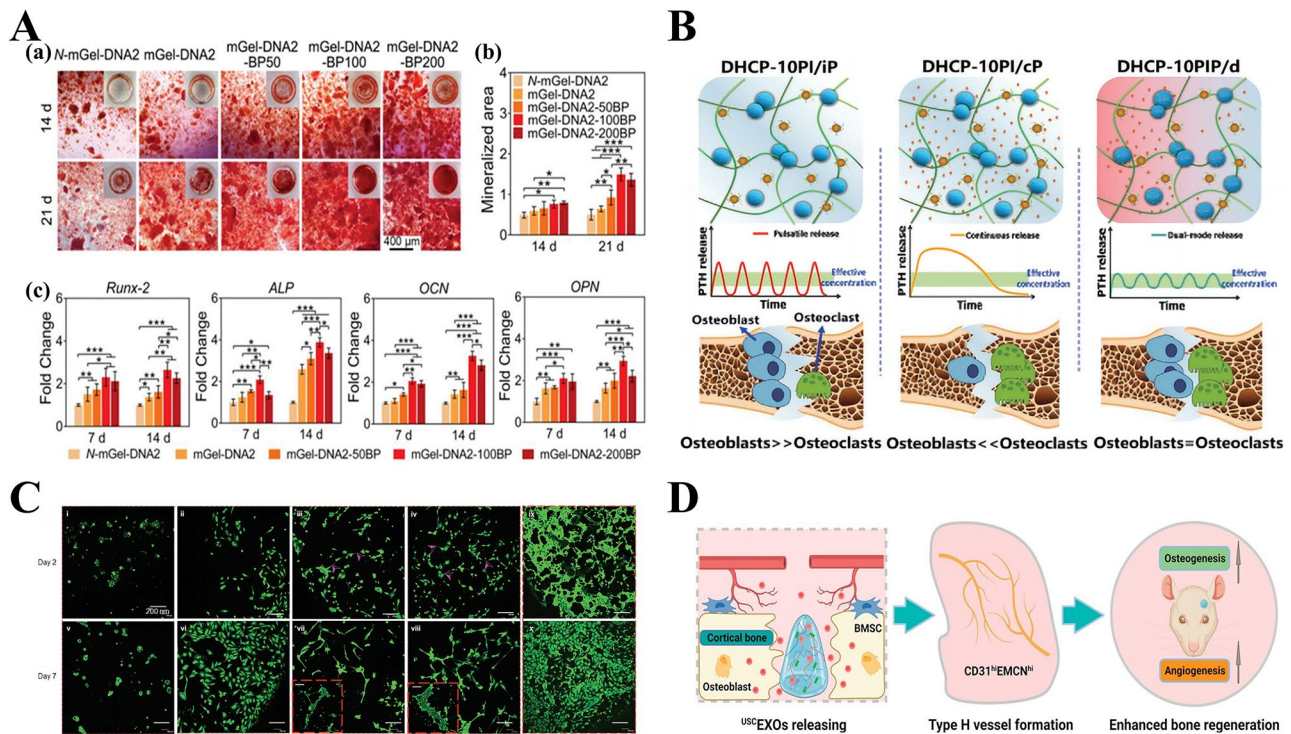
Fig. 5 NM-incorporated IHs exerted pro-osteogenesis and pro-angiogenesis effects. (**A**) Adapting BP nanosheets to IH improved calvarial bone healing with promising matrix mineralization and osteogenic differentiation. Reproduced with permission [60]. Copyright 2023, Wiley-VCH. (**B**) CaP NP-coordinated IH controlled the PTH release mode to restore osteoblast/osteoclast balance for osteoregeneration. Reproduced with permission [63]. Copyright 2021, Wiley-VCH. (**C**) GHK-Cu^{2+}-containing IHs promoted cell elongation and angiogenic sprouting of hUVECs. Reproduced with permission [68]. Copyright 2020, Wiley-VCH. (**D**) USCEXOs/GelMA-HAMA/nHAP IHs stimulated type-H vessels to augment angiogenic-osteogenic coupling. Reproduced with permission [69]. Copyright 2023, Elsevier Ltd

repair in ovariectomized rats (Fig. 5B) [63]. Compared to mounting osteogenesis-related studies, research on how NM-incorporated IHs affect osteoclast behavior and the underlying intracellular signaling during craniofacial bone reconstruction is currently limited.

Angiogenesis effects

Regeneration of craniofacial bone entails a highly perfusable and mature vascular network to support cells, oxygen, and nutrients at the newly formed site [64]. The establishment of stable blood vessels is initiated by biomolecular factors such as HIF1-α and vascular endothelial growth factors (VEGFs), undergoes degradation of vascular basement membrane, proliferation, migration, and branching (sprouting) of endothelial cells (ECs), and organization and maturation of a new capillary network [65]. Functionally, NMs could invite opportunities for pro-angiogenesis at different stages, primarily by altering the properties of IHs to regulate EC behavior and angio-osteogenesis coupling, ultimately developing well-vascularized bones.

Nanocomposite IHs favorably interact with ECs and promote the inward growth of mature blood vessels. For instance, Cu-containing BG-integrated chitosan-based IHs led to a highly porous structure and administered

bioactive ions in a sustained and controlled manner, which dramatically promoted the migration and proliferation of human umbilical vein ECs (hUVECs). In this process, the released Cu ions triggered the upregulation of HIF-1a expression first, and subsequently, the released Si^{2+}, Ca^{2+}, and Cu^{2+} cooperatively induced the cascade upregulated expression of VEGF and other angiogenic genes [66, 67]. Similarly, the inclusion of MgO NPs within IHs exerted proangiogenic effects by stimulating CD31-mediated migration of ECs and increasing branching points and capillary length [47]. Consistently, injecting this IH into the critical-sized cranial defect area effectively formed vascularized bones and mineralized collagen deposition. Incorporating bioactive peptide amphiphile (GHK-Cu^{2+}) nanofibers into IHs triggered cell elongation and angiogenic sprouting of ECs with microvascular-like structures on day one. After five days, the multicomponent IHs formed more complex vascular luminal structures similar to those in the positive control group, which may be related to enhanced integrin binding ability (Fig. 5C) [68].

Angiogenesis and osteogenesis are mutually dependent during craniofacial bone remodeling. Vascular systems, such as H-type vessels, function as integral factors in regulating the secretion of osteogenic signals. Recent

studies have emphasized the implantation of nanocomposite hydrogels (USCEXOS/GelMA-HAMA/nHA) to accelerate cranial bone regeneration through coupling angiogenesis, primarily manifested as HIF-1a-mediated induction of H-type vessel formation (Fig. 5D) [69]. On the other hand, osteoblasts boost angiogenesis by releasing paracrine factors, reducing the necrosis of repair cells [70]. Despite promising results at the histological level, the underlying angiogenesis mechanisms of nanocomposite IHs have rarely been revealed. Analogous to vessels, nerve fibers are increasingly understood to exert critical effects on bone remodeling and should not be overlooked [71]. On top of this, it is intriguing to explore whether NM-integrated IHs can be exploited to stimulate reinnervation during craniofacial bone healing.

Antibacterial activities

Craniofacial bone defects may be further complicated by the regular presence of microbiota in areas such as the oral and nasal sinus cavities [72]. Once local infection occurs or persists, invading bacteria consume local nutrients and oxygen and release acidic metabolites, threatening the adherence of bone cells and devastating the healing phase [73, 74]. As such, the use of IHs with antibacterial activity at contaminated sites is a multipronged approach for successfully implementing IH-based bone reconstruction.

Nanoengineered IHs have been readily adopted to combat difficult-to-treat bacterial infections while minimizing potential systemic side effects. NMs offer eye-catching advantages in the antimicrobial characteristics of IHs, which could be outlined in the following aspects. (1) The combination of nanodopants and antibiotics synergistically increases the antibacterial efficiency and prevents the emergence of resistant bacteria compared to a single strategy [75]. Doping MBGN into hydrogel system was proved to be antibacterial, which might be attributed to the pH change (Fig. 6A) [31]. Based on the synergetic effect of MBGN and vancomycin, higher antibacterial performance was achieved with an obvious decrease in bacterial abundance (Fig. 6B) [76]. (2) Inhibiting and killing bacteria through multiple mechanisms prevents the development of resistance to multiple targets. For instance, GelMA-based hydrogels reinforced with ZIF-8 NPs intrinsically inhibited the viability and colonization of various periodontal pathogens, further promoting alveolar bone regeneration. More specifically, ZIF-8 nanostructure aggregated or penetrated bacterial cell membranes, affecting proton transport and disrupting DNA replication processes. Meanwhile, ROS generated from Zn^{2+} disrupted bacterial structures through oxidative stress and membrane lipid peroxidation, leading to bacterial death [77]. (3) Well-targeted and responsive antimicrobial systems serve as alternatives to
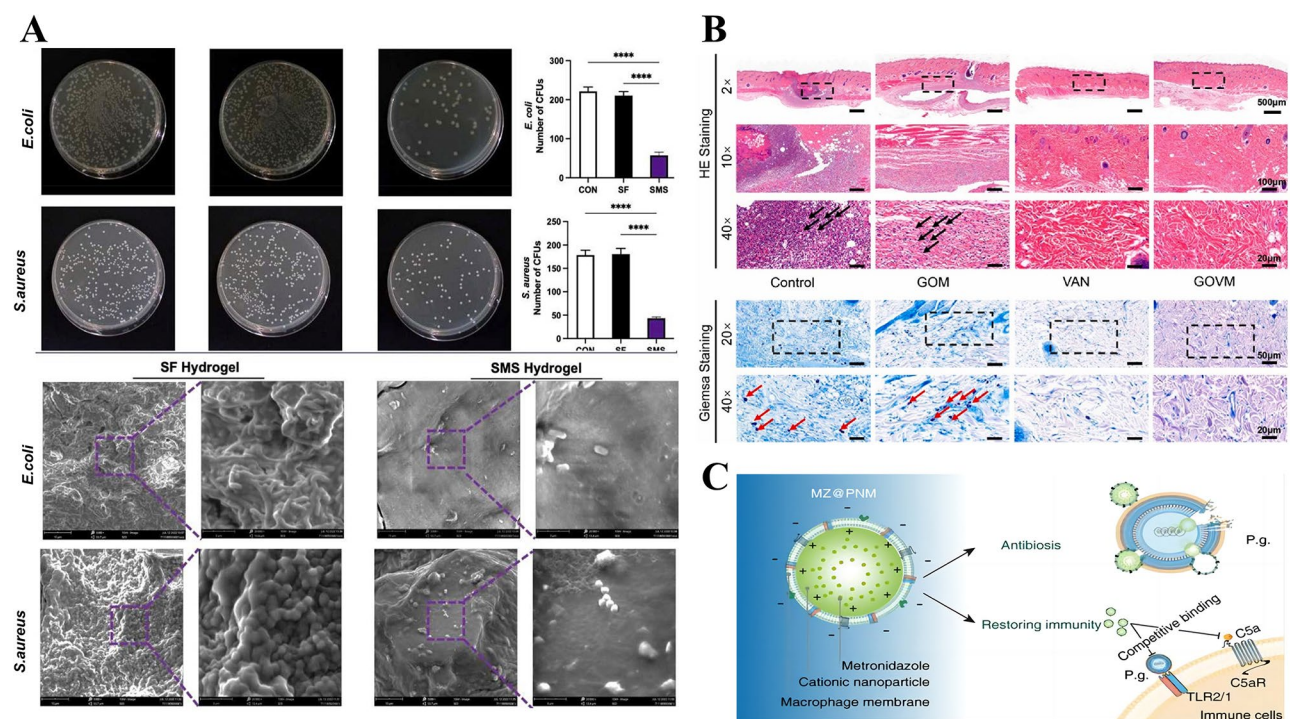
Fig. 6 Antibacterial performance of nanocomposite IHs. (**A**) Silk fibroin/MBGN/sodium alginate IH inhibited bacterial and biofilm formation. Reproduced with permission [31]. Copyright 2022, Elsevier B.V. (**B**) Gel-OS/VAN@MBGNs exhibited decreased bacterial abundance in vivo. Reproduced with permission [76]. Copyright 2023, Elsevier B.V. (**C**) Schematic illustration of the MZ@PNM@GCP hydrogel for killing periodontal pathogenic bacteria. Reproduced with permission [78]. Copyright 2022, American Chemical Society

antibiotics and revolutionize the field of drug-resistant bacterial therapy. Yan et al. [78] developed a penetrating nanoformulation-encapsulating IH that targeted *Porphyromonas gingivalis* via Toll-like receptors on its macrophage-mimicking membrane. It effectively disrupted the bacterial structural integrity while maintaining favorable biocompatibility (Fig. 6C). Additionally, the embedded photo-responsive T8IC NMs into hydrogels effectively destroyed bacterial biomacromolecules under the action of near-infrared (NIR) radiation [79]. Through dual-light regulation, IHs doped with Cu_2O and dopamine-modified TiO_2 NPs switch between antibacterial and osteogenic effects to realize customized alveolar bone repair [80].

Overall, the antibacterial activities of NM-integrated IHs make them highly valuable for craniofacial bone reconstruction. Future research will continue to focus on establishing broad-spectrum, long-lasting, precise, and safe nanocomposite hydrogels based on advanced antibacterial methods and mechanisms.

Mechanisms of craniofacial bone reconstruction by nanocomposite IHs

NM-integrated IHs exhibit eye-catching advantages for addressing the multifaceted challenges and promoting better therapeutic outcomes in the treatment of craniofacial bone defects, making them promising contenders over traditional materials. This section discusses the underlying mechanism of tailoring functionalities to better harness NM-integrated IHs.

Optimization of physical cues to direct cell responses

Typically, NM-integrated IHs are designed by coordinating bioreactive NMs with polymer chains, thus bolstering structural integrity and allowing on-demand tailoring of in situ physical cues [81]. Vital pro-healing cell behaviors could be facilitated by tuning the biomechanics of

hydrogels including mechanical response of stiffness, pore structure, topographical features, viscoelasticity, and degradation (Fig. 7) [82].

Biomaterial stiffness, an essential physical cue, can dictate cellular phenotypes, behaviors, and functions [83]. NM-integrated IHs can mechanostimulate local cells by precisely controlling microenvironmental stiffness. Rigid inorganic nanofillers have been demonstrated to stiffen hydrogels and modulate cellular responses. For instance, GO could act as a domain to form polymer bridges in IH to increase its mechanical stiffness, leading to enhanced osteogenic differentiation and bone tissue regeneration as compared to pristine IH [45]. The surface reactivity of MBGNs has been found to be relatively high, resulting in the creation of hybrid hydrogels with tunable stiffness and favorable osteogenic activity through dynamic ionic crosslinking [39]. Yu et al. [44] demonstrated that Sr-CSH nanocomposite IHs were suitable for MSC growth and induced MSCs toward osteogenic differentiation with a change in the order of magnitude of stiffness (13-20 kPa), promoting cranial bone reconstruction. Together, these works highlight the need to carefully assess and design mechanical stiffness in multimodal matrices to provide conditions favorable for craniofacial bone healing.

Appropriate pore sizes can profoundly enhance cell infiltration and proliferation, as well as the diffusion of nutrients and waste exchange [84]. The introduction of NMs into IHs provides a facile approach for altering pore architecture. Specially, the intrinsic porosity of MBGNs elevated the average pore sizes of IHs to over 100 μm but did not affect the mechanical strength. MSCs were prone to adhering to surfaces and prolifing with a particular range of higher porosities due to the increasing surface areas, resulting in more rapid and efficient healing of bone defects [39]. Similarly, the pore size of aminated-MBGN-enhanced hydrogel was 150±20 μm, allowing for cell-cell interactions and the expression of
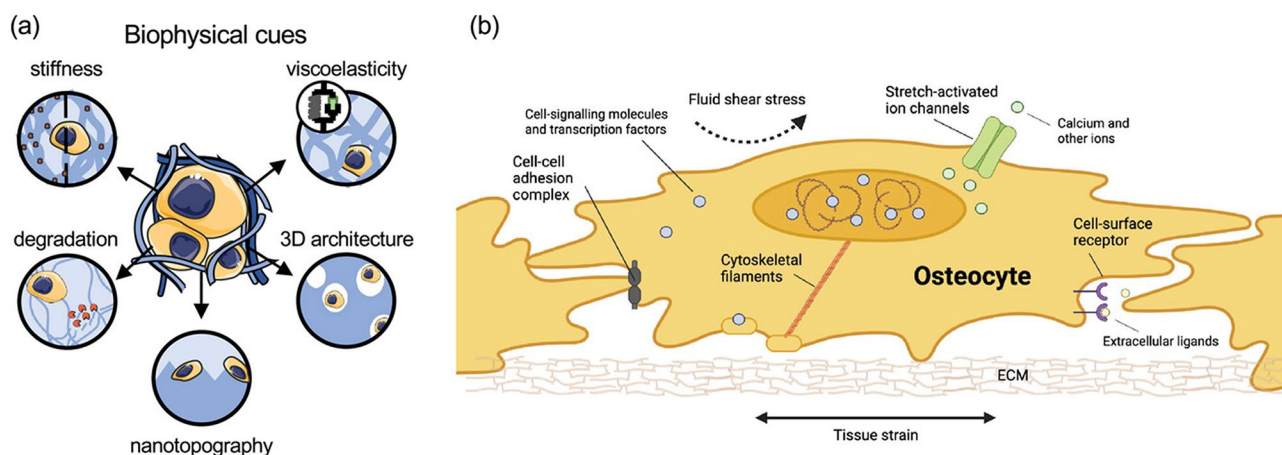

Fig. 7 Schematic representation of physical cues for cell behaviors. Reproduced with permission [82]. Copyright 2023, Wiley-VCH

signal pathways generating cascade osteogenesis [85]. This contradicts the results of a previous study in which IHs doped with 30% nHA formed the smallest pore size (15 μm) with a uniform pore distribution. It acted as an interactive osteogenic platform for communication and interplay between BMSCs and macrophages during the reconstruction process of maxillofacial bones [12]. Certainly, there is an ongoing debate regarding the optimal pore size for enabling cell migration, tissue ingrowth, and angiogenesis, which might be related to differences in hydrogel dimensionality and cell type.

Cells have demonstrated sensitivity to other mechanical characteristics of hydrogels [86]. Work has been focused on surface topography and stress relaxation. Miao et al. [60] observed that the double-network hydrogels with BPs exhibited a macroporous structure, providing sufficient space for BMSC proliferation, migration, and directional osteogenic differentiation. Interestingly, nanoclay-coordinated IHs showed a highly porous microstructure, thereby favoring osteogenesis, whereas a smooth nonporous surface and limited osteoinductive capability were observed without nanoclay [61]. Moreover, nanoclay-engineered IHs exhibited rapid stress relaxation compared to others, which synergistically stimulated MSC proliferation and osteoblastic differentiation [68]. Other interdependent biophysical properties of hydrogels also influence cell behaviors, but they are not focused on craniofacial bone reconstruction and can be referred to some excellent reviews for details [82].

Overall, NM-incorporated IHs are devised to generate multicomponent and coordinated biophysical parameters to interact with cells at the bone defect sites. Even if current insights are established on different nanocomposite IH-mediated physical traits, future discovery and optimization of biofabrication may be practically advanced through continuous high-throughput screening of cellular responses.

Regulation of intracellular signaling pathways

In addition to the impact on the physical traits, the NM-incorporated IHs push forward new bone by mediating regulatory effects on specific intracellular signal pathways, including MAPK [44], Wnt [87], bone morphogenetic protein (BMP) [62, 88], and PI3K/AKT signaling pathways [89, 90]. They can stimulate the expression of osteogenesis-related proteins through RUNX2-specific serine residue phosphorylation, thus motivating certain therapeutically favorable behaviors and bone repair. The NM-incorporated IHs adopted in craniofacial bone reconstruction are outlined, alongside their respective signaling pathways, in Table 1.

BMSCs, bone marrow mesenchymal stem cells; BP, black phosphorus; Gel, gelatin; GelMA, gelatin methacryloyl; GQDs, graphene quantum dots; hUVECs, human umbilical vein endothelial cells; Lap, laponite; MBGNs, mesoporous bioactive glass nanoparticles; Met, metformin; MSNs, mesoporous silica nanoparticles; nCS, carboxymethyl chitosan/sodium alginate nanoparticles; nHA, nanohydroxyapatite; PDA, polydopamine; PDLLA, poly(DL-lactide); SA, sodium alginate; SAG, smoothened agonist; SF, silk fibroin; SIM, simvastatin.

MAPK signaling pathways, including the ERK, p38, and JNK subfamilies, are potent guides for osteogenic differentiation and inflammation responses. In recent research, a bioactive IH was developed by integrating Sr-CSH, demonstrating its capability to induce in situ bone regeneration by activating the ERK/p38 signaling pathway. Mechanistically, Si and Sr ions synergistically promoted FAK phosphorylation via transmembrane receptors, followed by phosphorylated ERK and p38, which were translocated into the nucleus to promote the transcription of osteogenic and angiogenic genes (Fig. 8A) [44]. Similarly, bioactive ions released from the imparted MBG facilitate the phosphorylation levels of ERK and JNK, activating MAPK signaling pathway to promote osteogenic differentiation and anti-inflammatory macrophage polarization [31]. Interestingly, NMs also indirectly increased osteogenic differentiation by attenuating MAPK-mediated inflammation/oxidative stress. TPCD NPs downregulated GDF15 and downstream genes (Atf3, Fosb, and c-Fos) to reverse ROS-mediated inflammation and regulate p38/MAPK-dependent osteogenesis, thus resulting in alveolar bone defect healing [55].

Wnt proteins govern the transcription of genes associated with cellular proliferation and differentiation, which are closely related to bone regeneration and vessel remodeling. Typically, Wnt binding to the Fzd receptor and low-density lipoprotein (LRP)-5/6 coreceptors leads to β-catenin accumulation and activation of the canonical Wnt pathway. A study verified that blocking the Wnt/β-catenin signaling pathway impaired the osteoinductive activity of BP-incorporated hydrogels (Fig. 8B) [60]. Notably, nanocomposite hydrogels can simultaneously regulate Wnt and other signaling networks to exert synergistic or multifunctional effects. As proof, nanoclay-derived byproducts (lithium, magnesium, and orthosilicic acid) showcased efficient upregulation of Wnt signaling pathway, while Hedgehog pathway was synchronously activated with a smoothened agonist (SAG) to increase osteogenic differentiation for IH-based cranial defect regeneration [61]. Additionally, the bidirectional regulatory (osseointegration and lipid-lowering) abilities of nanoSIM@ZIF-8 modified IHs in bone defects were reported to be closely related to mutual regulation of the Wnt/β-catenin and PPARγ pathways [91]. Furthermore, the osteogenic and immunomodulatory functions of laponite nanosheets on IHs were realized via modulation

Table 1 NM-incorporated IHs promote craniofacial bone reconstruction and their mechanistic pathways

| Matrix	Nano composition	Crosslinking	Ratio/ concentrations	Shape/size	Model	Main outcome	Mechanism	Study
GelMA	Sr-Xonotlite	Photopoly	0.5–4% w/v	Nanowire 20 nm	BMSCs, rat cranial defect	Promoted cell adhesion, proliferation, differentiation, and bone formation	ERK/p38 signaling pathway	[44]
SF/SA	MBGNs	Ionic interactions	0.5%, 1%, 1.5%, 2% w/v	Mesoporous	BMSCs, hUVECs, rabbit maxillary sinus elevation	Promoted osteogenic differentiation, hUVEC migration, tube formation, and macrophage M2-phenotype polarization	MAPK signaling pathway	[31]
Poloxamer 407	TPCD	Thermogel	0.1, 1, 10 mg/ml	Spherical 89±1 nm	MSCs, rat with periodontitis	Attenuated oxidative stress and inflammation and promoted cell adhesion, survival, osteogenic differentiation, and alveolar bone regeneration	GDF15/Atf3/c-Fos axis of MAPK signaling pathway	[55]
PEGDA/SA	SIM@ZIF-8	Physical blending	0.4, 1, 1.6 mg/ml	100–200nm	BMSCs, rat premaxillary defect with hyperlipidemic	Stimulated osteogenic differentiation and inhibited adipogenic differentiation	PPARγ and Wnt/β-catenin pathways	[91]
modified CS	SAG@Lap	Covalent and noncovalent interactions	1.0% w/v	Nanosheet	MSCs, mouse calvarial defect	Supported cell growth, proliferation and promoted osteogenic differentiation	Wnt/β-catenin and Hedgehog pathways	[61]
GelMA-Alg	Lap@PDA	Physical bonds	0.5, 1, 3 wt%	Nanosheet	BMSCs, rat cranial bone defect	Promoted adhesion, proliferation, spreading, osteogenic differentiation, and macrophage M2 phenotype polarization	Wnt/β-catenin and PI3K/AKT pathways	[34]
Gel-DNA	BP	Double network	0.05, 0.1, 0.2 mg/ml	Nanosheet	BMSCs, rat cranial defect	Enhanced recruitment, osteogenic differentiation, matrix mineralization, and new bone formation	Wnt/β-catenin signaling pathway	[60]
PDLLA-PEG-PDLLA	Met@MSN	Hydrophilic–hydrophobic interactions	11 mg/ml	Mesoporous	BMSCs, rat diabetic periodontal bone defect	Scavenged overproduced ROS, reversed impaired osteogenic differentiation, and promoted periodontal bone regeneration	ROS/AMPK/β-catenin signaling pathway	[33]
GelMA	GQDs	Photopoly	300 μg/mL	3.8±1.4 nm	hMSCs, mouse cranial defect	Enhanced osteogenic differentiation	BMP/Smad signaling pathway	[88]
nCS	Pluronic F-127/nHA	Thermogel	5, 10, 15 wt%	-	MC3T3-E1, rat cranial defect	Promoted osteal formation and maturation	BMP/Smad signaling pathway	[62]
SF	Lap	Physical blending	0, 1, 3, 5% w/w	Disc-shaped, 25*1 nm	BMSCs, rat cranial defect	Promoted osteogenic differentiation	PI3K/AKT signaling pathway	[90]
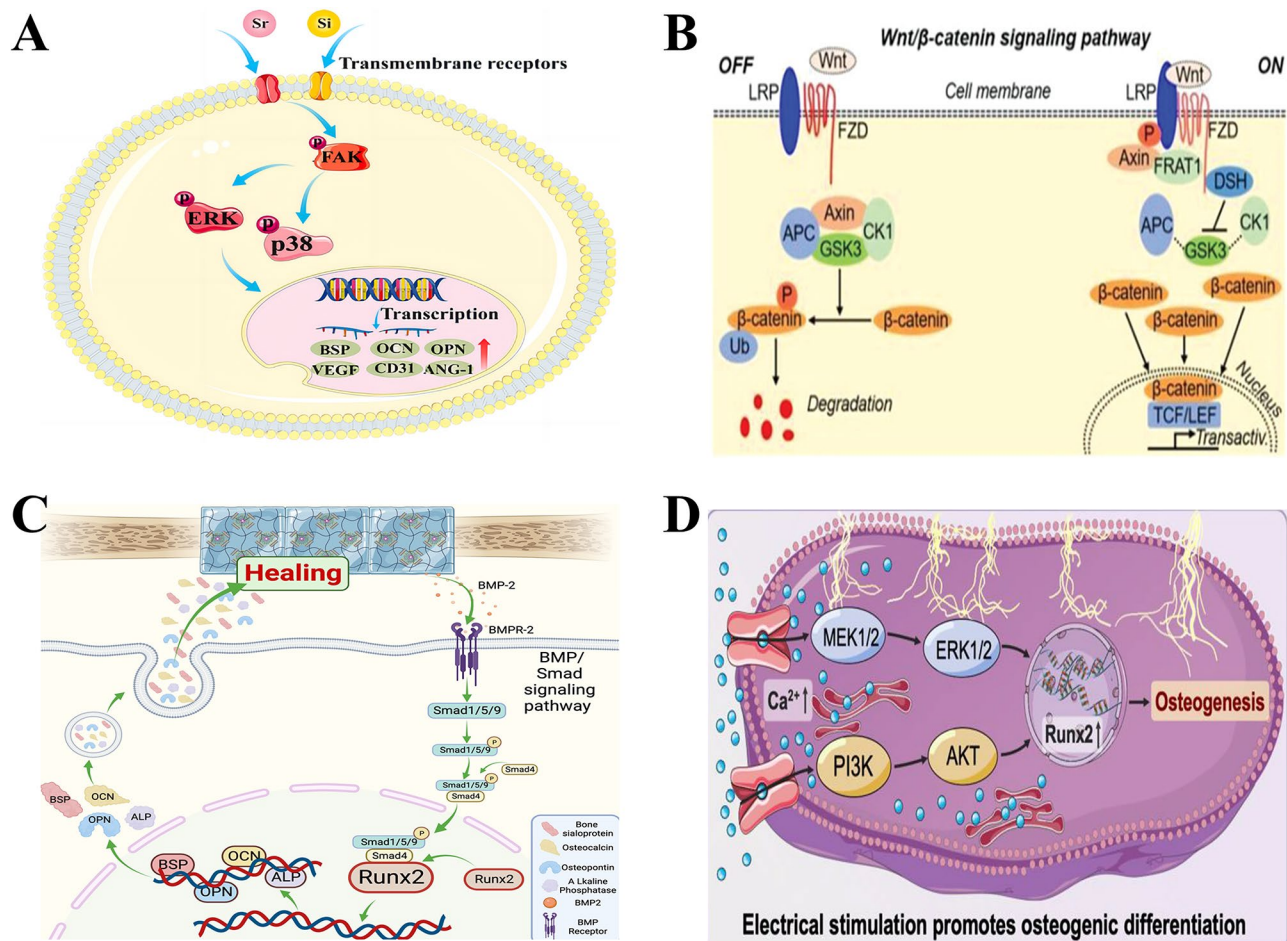
Fig. 8 Nanocomposite IHs regulate intracellular signaling pathways. (**A**) GelMA/Sr-CSH IHs induced BMSC osteogenic differentiation by activating the ERK/p38 pathway. Reproduced with permission [44]. Copyright 2022, Elsevier B.V. (**B**) mGel-DNA2-BP100 macroporous IHs promoted BMSC osteogenic differentiation by activating the Wnt/β-catenin signaling pathway. Reproduced with permission [60]. Copyright 2023, Wiley-VCH. (**C**) IHs containing nCS promoted osteal wound healing through BMP/Smad signaling pathway. Reproduced with permission [62]. Copyright 2022, Elsevier B.V. (**D**) A nanocomposite IH electrically accelerated bone healing by activating the PI3K/AKT and MEK/ERK signaling pathways. Reproduced with permission [89]. Copyright 2024, The Author(s)

of Wnt and PI3K/AKT signaling pathways, respectively [34].

BMPs emerge paramount roles in the osteoinduction process and facilitate stem cell differentiation. Employing NMs (graphene quantum dots (GQDs) [88], nCSs, and nHA [62]) for IHs accelerates eventual new osteal formation and maturation through BMP/Smad signaling pathway. Upregulated BMP induced by NMs promoted Smad1/5 phosphorylation and allowed the activated isoforms to interplay with Smad4, which translocated into the nucleus to activate gene Runx-2 to start transcription (Fig. 8C). Extensive research has attempted to modify hydrogels to carry BMP. In this context, nanohybrid hydrogels upregulating endogenous BMPs are attractive targets for accelerating craniofacial bone formation at the injection site while avoiding the need for additional BMPs.

Therefore, miscellaneous and interwoven signaling networks are involved in complex but precise regulation of optimally nanostructured IHs (Fig. 8D). However, the specific mechanisms that trigger this process are still uncertain, and the fine-tuned multistep regulation or relationships between these pathways deserve more attention.

Controlled release of bioactive molecules

Bioactive molecule-loaded IH strategies open up possibilities for remarkable craniofacial bone repair. Rapid release and aggregation of hydrophobic drugs often occur due to the hydrophilic nature of hydrogels [92]. IHs containing compatible NMs have approached advantageous regiospecific biodistribution and high efficacy of payloads through one or a combination of diffusion, degradation, or changes in polymer-drug interactions [93–95]. These NM-encapsuled hydrogel systems with

better control and orchestrated codelivery abilities are characterized according to their functional mechanisms, including sequential stacking or optimized affinity, intricate spatial vectors, and selective release reactions under certain stimuli.

NM-integrated IHs minimize burst release and achieve long-acting release by sequential stacking or optimized affinity to drugs and bioactive factors for preferable craniofacial bone regeneration. For instance, deferoxamine (DFO) and BMP-2 were sequentially carried by silk nanofibers and nHA, forming IHs with angiogenic and osteogenic cues. Degradation of the drug-loaded hydrogel meets the needs of skull regeneration in an orderly manner [96]. Besides, particular NMs (whitlockite NPs [95], nanoclays [61], and nCSs [97]) in hydrogel systems electrostatically bound to biomolecules to adjust their kinetic properties by creating specific affinity. Mi et al. [97] encapsulated chemokine stromal cell-derived factor-1 (SDF-1) in the negatively charged nCSs within hydrogels, which showed only a 40% accumulated release over four weeks and led to favorable bone formation in a calvarial defect model. Another similar study intercalated pro-osteogenic agonist SAG into nanoclay-incorporated IHs to realize continuous and enhanced release. The ion exchange of cations by nanoclay with the loaded SAG enables a steady pharmacokinetic profile to activate Hedgehog pathway [61]. These engineered IHs are endowed with sequential stacking or affinity between nanotherapeutics and drugs, enabling precise control over the rate and timing of release.

The second approach is to prepare IHs with intricate spatial structures, such as tubular, porous, or core-shell nanocarriers, to achieve high-dose drug delivery and reduce adverse diffusion with proper spatiotemporal kinetics. Double-layered halloysite clay nanotube-modified hydrogels with anisotropic charges enable the prolonged release of dexamethasone (Dex) for seven days and enhance osteogenic differentiation of MSCs under inflammatory stimulation [98]. In another Dex depot, silicate nanodisks reduced pore diameter with a more interconnected porous structure of DNA-based IHs, which exhibited long-acting release [40]. Specially, MSNs provide stable shells to incorporate and manipulate considerable drug cores, synergistically enhancing the osteogenic performance of in situ-forming IHs [41]. Wang et al. [33] performed a stepwise-cargo-release IH by first encapsulating metformin in MSNs and then coassembling them with an SDF-1-mixed matrix. Differential release of codelivery drugs (relatively quick for SDF-1 and slow release for metformin) emulated the "recruitment-osteogenesis" cascade of MSCs for diabetic periodontal bone regeneration. Although shape-controlling NMs in IHs enable steric hindrance during drug release, further optimization regarding the dosage, crosslinking degree, and assembly steps of possible multicompartment nanocarriers should be investigated.

Artificially programmed nanosystems involve sudden changes in physicochemical properties and biomolecule release under external or internal stimuli. (1) Noninvasive external triggers (e.g., magnetic field, ultrasound, and light) have triggered the on-demand release at desired sites. Light-absorbing gold nanocages were incorporated into hydrogel matrix to enhance systemic slow release of encapsulated antibiotics in periodontitis models owing to the unique light-to-heat conversion properties induced by NIR [99]. Another NIR-activatable IH based on CaP NPs and poly(dimethylaminoethyl methacrylate-co-2-hydroxyethyl methacrylate) was constructed for the precise controlled release of parathyroid hormone, which successfully boosted cranial defect repair in osteoporotic rats [63]. More recently, a smart thermoresponsive IH has been designed for highly efficient bone regeneration by combining BMP-2 with MgFe-layered double hydroxide (LDH) nanosheets and entrapping platelet-derived growth factor-BB into hydrogels [100]. Although encapsulation of responsive NMs in IHs achieved craniofacial bone healing under other external (e.g., magnetic field and ultrasound irradiation) regulation, these nanoplatforms have not been applied to control release of therapeutic agents with possible amplified therapeutic efficacy. (2) The utilization of internal physiological/pathological conditions (e.g., pH and enzymatic reactions) enables drug release in nanohydrogel platforms. For example, a pH-responsive IH encapsulated within a penetrating macrophage-based nanoformulation gradually degraded as the pH decreased, ensuring the sustained release of metronidazole at the site of periodontitis microenvironment [78]. Enzymes overexpressed in specific pathological states can serve as triggering factors for nanodrug delivery, with particular interest in MMPs. Xu et al. [14] developed a TM/BHT/CuTA hydrogel that can release CuTA NMs as needed with the increase of MMPs in periodontitis, which cleared ROS, upregulated anti-inflammatory factors and osteogenic gene expression to accelerate periodontal tissue regeneration.

Collectively, NM-reinforced IHs typically possess special affinity, spatial organization, and stimulus responsiveness with tailored, sustained, and continuous drug release and reduced side effects during functional tissue regeneration. Undoubtedly, more effort is necessary for the clinical translation of such IHs in clinics, especially regarding the multimodal therapy modalities (cells/genes), long-term safety, and suitable production technologies.

Efficient design considerations of tailored therapy from nanocomposite IHs

Reasonable selection of NM compositions, physicochemical properties, and related synthesis techniques are issues worthy of attention during the design process. These considerations can better tailor a set of derivative properties and biomedical applications that promote future regulatory approval and commercialization potential.

NM compositions

Different types of NM, including inorganic/ceramic, carbon-based, metal-based, and other NMs, can be incorporated into the polymeric network to form nanocomposite IHs. Inorganic/ceramic NMs, such as nHA [101, 102], MBGN [103, 104], MSN [105], and nanoclays [10, 106], prolonged structural support and guided osteogenesis due to good biocompatibility, osteoconductivity, facile modification and lower degradation rate than hydrogels [107]. Excellent craniofacial bone can be assisted by inorganic/ceramic NM-incorporated IHs with released bioactive ions and plentiful nuclear sites for in-situ mineralization. Carbon-based NMs have a specific surface area, well-organized structure, and high mechanical strength, which can transport proteins and drugs while preserving their bioactivity for addressing bone defects [108, 109]. However, the possibility of aggregation should be considered [110]. Metal-based NMs were employed as a versatile additive to formulate dynamic self-healing and stimuli-responsive IHs for craniofacial bone reconstruction owing to their antioxidant effects, magnetic behavior, and electrical /thermal conductivity [111–113]. Future studies are needed to fully elucidate the effectiveness and long-term consequences of these innovative concepts [114]. Therefore, the careful selection of NM compositions allows engineers to bolster the IHs with desired attributes that cater to the unique environment of craniofacial bones.

NM physicochemical properties

The physicochemical characteristics of NMs, such as changing their size, shape, distribution, charge density, and ratio, influence the structure-induced biological behaviors, which consequently affect craniofacial bone repair. First, the size of NMs can affect the mechanical properties of the nanoengineered hydrogels. In a crosslinked matrix, NMs with a diameter of 8 nm represent the optimal size for mechanical reinforcement due to their maximum interfacial surface area and minimal aggregation, as opposed to those with diameters of 4–12 nm [115]. Second, tailoring special shapes of NMs (tubular, porous, or spherical) to serve as drug storage warehouses and prolong drug action time is being investigated. Porous ZIF-8 NMs encapsulate hydrophobic

simvastatin to modify IH, resulting in dual regulation of osteogenesis and antiadipogenesis for complex bone regeneration [91]. Third, the homogeneous distribution of NMs in hydrogel networks determines their ostentatious biocompatibility and therapeutic performance. Uniformly dispersed Lap@PDA nanosheets interweave in the GelMA matrix, achieving a biohydrogel for reconstructing bone defects through integrated design with diverse functions [34]. Particularly, surface modifications (ligands or functional groups) [38, 48] and functionalization (growth factors or cell-binding peptides) [116] endow NMs with reduced agglomeration and enhanced cell-material interactions during the regenerative process. Fourth, the NM surface charges can be exploited to form crosslinks via electrostatic attraction or to adsorb ECM proteins, hence improving the hydrogel mechanical and biological properties. Shen et al. [88] achieved favored BMP interactions by incorporating negatively charged GQDs into hydrogel system, thus promoting in situ cranial bone regeneration. Last but not least, the ratio of NMs should be adapted in proportion to their intended applications. Elevating certain concentrations of NMs reinforced mechanical strength and stalled the degradation of IHs, but they may confound the interior pore architectures and affect cell responses to different parameters [10]. For instance, the Young's modulus of the nanocomposite IHs was enhanced with increasing amounts of nanosilicates (0.5-4% w/v). Notably, IHs mixed with proportional nanosilicates (1.5% w/v) led to the formation of interconnected microporous structures, which effectively promoted cell infiltration, proliferation, and differentiation in the absence of any growth factors [117]. Consequently, a suitable NM ratio should strike the rigidity—degradability—permeability balance into a structurally stable as well as multifunctional IH to optimize craniofacial bone tissue regeneration.

Taken together, multiple NM parameters should be carefully tailored for desired morphological, mechanical, and biological properties. As research advances, versatile NMs can mimic both the structural and physicochemical cues of native craniofacial bones at a nanoscale. ECM-like nanoarchitectures (for instance, nHA [118] and PLLA nanofibers [119]) in IH have been employed to construct an excellent osteogenic microenvironment. Moreover, NM-incorporated IHs, inspired by the natural bone healing cascade, are advantageous for emulating the MSC "recruitment-osteogenesis" cascade for targeted bone regeneration [33]. Further ahead, the selected NMs into biomimetic hierarchical IHs that are synchronously degraded with the mineralized tissue deposition, may afford critical insight to fulfil the verge of most satisfactory craniofacial bone regeneration.

Synthesis strategies and techniques

Well-suited strategies and techniques have been explored to fabricate nanoengineered IHs for craniofacial bone reconstruction, which primarily realized the feasibility and precision of these systems. Physical/noncovalent, chemical/covalent and dual crosslinking methods are applied during the embedding process of NMs. Physically crosslinked hydrogels utilize the abundant groups, unique surface charges or metal ions of NMs through various noncovalent interactions, such as hydrogen bonds, electrostatic interactions, or metal coordination. These bonds can dynamically dissociate and reassociate to exhibit self-healing and stimulus-responsive properties but also bring issues such as instability and undesired mechanical integrity [120]. Compared with physical hydrogels, chemical crosslink schemes (e.g., oxidative coupling, enzymatic and Schiff base reactions) give rise to more stable systems and tunable properties, which might comprise complex and toxic methodologies in worse-case scenarios [121]. Another option is to combine chemical linkages with physical interactions [38, 85]. In this way, IHs are apparently robust from the densely covalent crosslinks, but also facilitate loading and retention of drugs due to noncovalent interactions.

Beyond design strategies, a range of techniques combining emerging theories precisely achieve diverse structures and physicochemical features tailored for craniofacial bone defect repair. Indeed, no one-size-fits-all design exists for nanocomposite IH systems. To improve the preparation efficiency, practical technology requires interrelated considerations on multiple controllable NMs, polymer components, intended applications and synthesis scalability.

Self-assembly

The self-assembly approach organizes NMs into a 3D network through external attraction forces, such as temperature, pH changes and charge distribution [122]. For example, a nanoparticle-converted IH was self-assembled at 37 °C under physiological salt concentrations, scavenging ROS at peri-implantitis. In addition to temperature, pH changes facilitate the controlled assembly of polymer-stabilized ACP NPs to form elastic IHs. Consequently, the as-fabricated systems revealed effective osteogenic-osteoclastogenic regulation in vivo [123]. Okesola et al. [68] fabricated a multicomponent system (HA-Tyr-Lap-GHK-Cu^{2+}) to facilitate osteogenic signaling in a charge-triggered self-assembly manner. Similarly, assembling negatively charged TM/BHT/CuTA IH was proposed with excellent anti-inflammatory properties [14]. Self-assembling is employed to design IHs with multiple building-blocks and functionalities, but it typically provides limited structural integrity and might need combinations with other advanced techniques.

Microfluidics

Microfluidics leverages laminar solvent flow and length scale to effectively manipulate NM size, morphology, and distribution in a more reproducible manner. An example of such a system is homogeneous microspheres and highly dispersed fullerol nanocrystals in GelMA hydrogels. Subsequently, these fullerol-hydrogel microfluidic spheres exhibited excellent antioxidant activity to quench ROS via microfluidics, effectively inducing new bone formation in rat calvarial defects [124]. Another micro/nano microsphere system designed for macrophage-targeted engineered reprogramming was recently carried out. Directly acting as a local injectable carrier, the hydrogel regulated macrophage-related inflammation and ultimately promoted refractory bone healing [125]. However, there are remaining difficulties with this approach, including clogging of the microfluidic channels, batch production dilemmas and limited polymer materials.

3D printing

3D bioprinted hydrogel yields an oriented distribution of cells and/or bioactive factors, offering a distinctive solution for bone organoid construction [126]. Wang et al. [127] fabricated printed nanoparticle-enhanced cryogels, allowing shape fidelity during the injection to induce pro-osteogenesis. Moreover, 3D printing enables precise control of nanocomposite IHs with multiscale pore architectures. Using a 3D printer, an IH composed of GelMA/Alginate and covalent organic framework nanoparticles is specially designed with enhanced porosity and decreased pore size [128]. Despite advances in inject bioprinting, the major challenge is the balance of fast gelation and low viscosity to establish seamless fusion of structure–property–function.

Other fabrication techniques

In addition, more recent efforts have been evolved to include advanced strategies (e.g., nucleic acid nanotechnology, photoinitiated click chemistry and bio-orthogonal reactions) to endow hydrogels with rapid gelation kinetics and minimal cytotoxicity [129]. Nucleic acid nanotechnology, involving DNA or RNA, offers a viable avenue to program distinctive IHs with predetermined designs. Interestingly, a nanostructure-modified IH with framework nucleic acid gelled in a biocompatible environment and demonstrated rapid mineralization and revascularization in a convenient and cost-effective way [130]. Furthermore, nucleic acid nanotechnology could design and manipulate molecules with predictable responses, which may be one of the prospective areas of research [131]. Click chemistry and bio-orthogonal reactions in designing IHs have yet to be widely tested for osteogenesis and bone regeneration, and specific parameters are also desirous to establish.

Clinical applications and trials

To the best of our knowledge, no nanocomposite IHs have been commercially available for craniofacial bone reconstruction. While various reasons can be cited for the difference between scientific discoveries and clinical applications, one persistent challenge is the time-consuming and labor-intensive regulatory approvals concerning the biosafety evaluation and batch-to-batch consistency of nanocomposite IHs (Fig. 9). In a recent clinical trial (ClinicalTrials.gov ID: NCT06373757), chitosan and nHA-incorporated IH revealed the promising potential for noninvasive treatment of periodontal defect. To expound substantial equivalence among hydrogel devices in regulatory studies, more analysis on in vivo bone formation and hydrogel degradation is critically necessary.

Conclusion and perspectives

Effective craniofacial bone regeneration remains a challenging medical intervention due to its unique biological and morphological characteristics. IHs with degradable networks not only possess structural similarities with endogenous ECM but also fill geometrically complex craniofacial regions with minimal invasiveness. Nanocomposite IHs creatively utilize their polyfunctionality to grapple with a spectrum of craniofacial defects. By consolidating ongoing advancements, this review systematically discussed the incorporation of NMs in IH-based craniofacial bone therapeutics, addressing the advantages and mechanisms to tackle a panoply of challenges. For mechanical optimization, NMs lend themselves to external stress dissipation or highly functional covalent crosslinking to provide adequate mechanical strengths within the craniofacial system. For biofunctionalization, NM-integrated IHs expand a host of biological performances (immunomodulation, osteogenesis, angiogenesis, and antibacterial activity) through optimizing the physical traits to direct cell responses, regulating intracellular signaling pathways and controlling release of therapeutic payloads. These satisfying outcomes lay foundation on clinical translation of NM-enhanced IHs, and represent a step toward tuning robust therapeutic schedules for personalized craniofacial bone engineering.

Although significant progress has been achieved, some limitations hinder the clinical applications of nanocomposite IHs.

(1) NM dopants in IH should prioritize excellent biocompatibility and biodegradability, as their long-term safety and degradation profiles have not been clearly determined. The selection, ratio, distribution, and stability of NMs as well as their interactions with polymer formulations warrant more elaborate assessments, not only cellular responses. Further advances are required to collect systemic in vivo data (toxicity, genotoxicity, and biodegradation) with long term follow-up in preclinical and clinical development.

(2) Delicately designed NMs and hydrogel structures responsible for craniofacial bone reconstruction may increase the complexity and cost of manufacturing process, which renders mass production difficult. Furthermore, evaluating the diverse intraoperative and postoperative scenarios of nanocomposite IHs under appropriate regulatory procedures might be lengthy and costly. Accordingly, it is advocated to consider these factors during the design process and slim down the IH platform while meeting the clinical requirements.
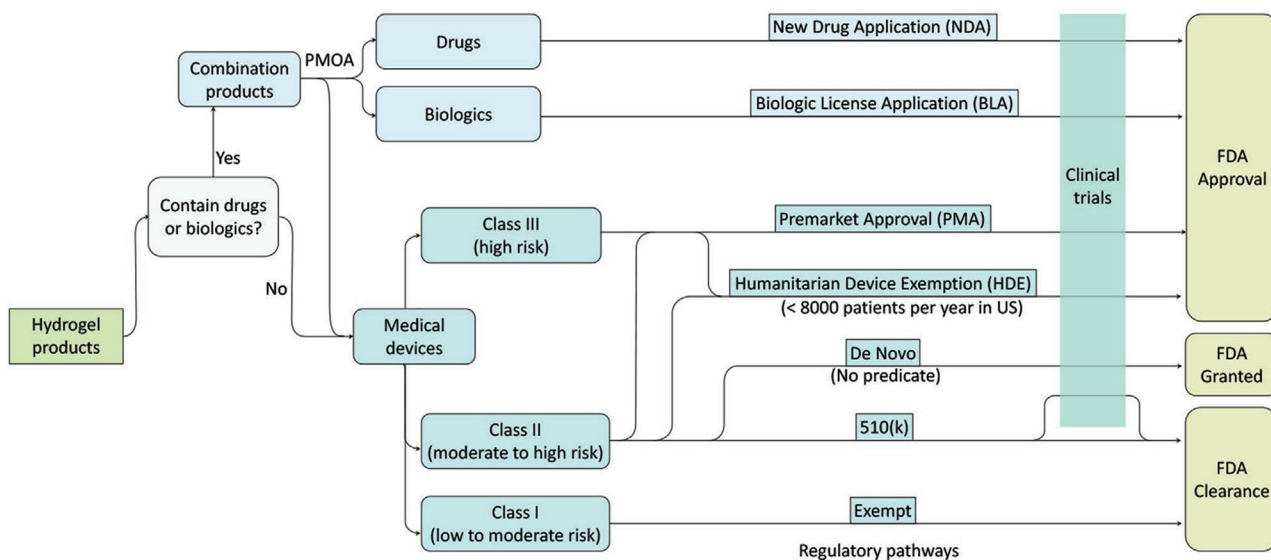

Fig. 9 The Food and Drug Administration (FDA) regulatory pathways of hydrogel products. Reproduced with permission [132]. Copyright 2021, Wiley-VCH

(3) The characterization and quantitative benchmarks of rheological and mechanical properties in nanocomposite IHs should be comprehensively addressed since current results are mainly obtained under idealized gelation conditions. Nonetheless, NMs provide additional directions to balance injectability and structural stabilization of hydrogels to avoid premature disintegration and adjust the self-healing kinetics to the specific time scale suitable for injection.

(4) The physicochemical properties of NM-integrated IHs are interactive. It is difficult to judge the impact of each parameter on hydrogel optimization. A multiscale, comprehensive evaluation system should be established to advance more promising IHs for craniofacial bone therapeutics.

(5) Bone regeneration is a multi-factorial process involving multiple cell populations. Although NMs inspired the synthesis of next-generation hydrogels with tailored physicochemical properties, some molecular mechanisms are inconsistent and need further studies. Besides, the crosstalk between two or more kinds of cells is worth studying. Future fabrication of nanocomposite IHs can be centered on dynamically in tune with cells over a time-scale mapping as well as multicue integration toward native-like tissue functionality during craniofacial bone remodeling.

(6) With burgeoning demands for diversified use, expanded therapeutic modalities (various biologics, cells, and responsive NMs) for IH-based bone regeneration should be delved to overcome the obstacles in undesired microenvironments.

In summary, nanocomposite IHs provide promising platforms for craniofacial bone reconstruction. Prospecting future development, continuous efforts will catapult the field of biomaterial development and combinatorial therapeutics to ultimately advance translational potential.

Author contributions
YX and YMC contributed to the conception of the manuscript. YX, ZHC, ZBZ, and HMC analyzed the articles, generated the figures, and drafted the manuscript, which was critically revised by YMC. All authors have read and approved the final manuscript.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
All authors are consent for publication.

Competing interests
The authors declare no competing interests.

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