



# Dual-functional composite scaffolds for inhibiting infection and promoting bone regeneration



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## ABSTRACT

The treatment of infected bone defects is an intractable problem in orthopedics. It comprises two critical parts, namely that of infection control and bone defect repair. According to these two core tasks during treatment, the ideal approach of simultaneously controlling infection and repairing bone defects is promising treatment strategy. Several engineered biomaterials and drug delivery systems with dual functions of anti-bacterial action and osteogenesis-promotion have been developed and demonstrated excellent therapeutic effects. Compared with the conventional treatment method, the dual-functional composite scaffold can provide one-stage treatment avoiding multiple surgeries, thereby remarkably simplifying the treatment process and reducing the treatment time, overcoming the disadvantages of conventional bone transplantation. In this review, the impaired bone repair ability and its specific mechanisms in the microenvironment of pathogen infection and excessive inflammation were analyzed, providing a theoretical basis for the treatment of infectious bone defects. Furthermore, we discussed the composite dual-functional scaffold composed of a combination of antibacterial and osteogenic material. Finally, a series of advanced drug delivery systems with antibacterial and bone-promoting capabilities were summarized and discussed. This review provides a comprehensive understanding for the microenvironment of infectious bone defects and leading-edge design strategies for the antibacterial and bone-promoting dual-function scaffold, thus providing clinically significant treatment methods for infectious bone defects.

## 1. Introduction

Infectious bone defects have long posed a difficult problem in orthopedics, where infection can significantly impair local tissue and the regeneration ability of bones, making the defect difficult to heal [1-3]. Traditional standard treatments for infectious bone defects include two main missions, namely that of infection control and reconstruction of bone defects [4,5]. Infection control represents the primary part of treatment, and current clinical treatments include debridement of infected site, systemic application of antibiotics, and implantation of antibiotic-impregnated spacers. However, it is difficult to achieve effective concentrations locally with the systemic administration of antibiotics due to vascular destruction and osteonecrosis caused by infection. Antibiotic-impregnated spacers can deliver the antibiotic locally, and membrane induction can be performed to facilitate bone grafting, but a secondary surgery is required about 6 weeks after the first operation to remove spacers [6-9]. Only when the infection is controlled, the repair of

the bone defect commences, typically with bone grafting or bone transport technique. Although autogenous bone forms an effective bone graft, it faces problems such as insufficient donor bone mass and complications in the donor area [10,11]. The alternative treatment of allogeneic graft is also limited by its potential risk of immune rejection and infection [12, 13]. Bone transportation technology based on distraction osteogenesis is an effective method to bone reconstruction, but its main disadvantage is that the treatment time is too long, which can reach 10 months or more [14-16]. The traditional multi-stage treatment strategy has several shortcomings, and the treatment time is long, which imposes a burden on patients. Therefore, the development of bone substitutes with dual functions of anti-bacteria and bone promotion for treatment of infectious bone defects in one stage without subsequent surgery would be a treatment strategy of great clinical significance.

Several engineered scaffolds with dual functions have been developed by combining drugs or materials with osteogenic effects with biomaterials, metal ions and drugs with antibacterial properties through

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coating, adsorption, mixing, etc. [17-20]. The design of these materials is based on improving the microenvironment at the local infectious bone defect site. The antibacterial component rapidly inhibits the proliferation of pathogens and prevents excessive inflammatory response in the early stage. The osteogenesis inducing component improves the damaged osteoblast capacity and manages the bone metabolism disorder caused by infection to facilitate bone repair [21-23]. Therefore, a comprehensive understanding of the local microenvironment of infectious bone defects is the basis for the design of these scaffolds. However, previous studies only focused on the functionality of scaffolds, whereas there is no comprehensive and thorough analysis and discussion of the mechanism of infection on the repair process of bone defects.

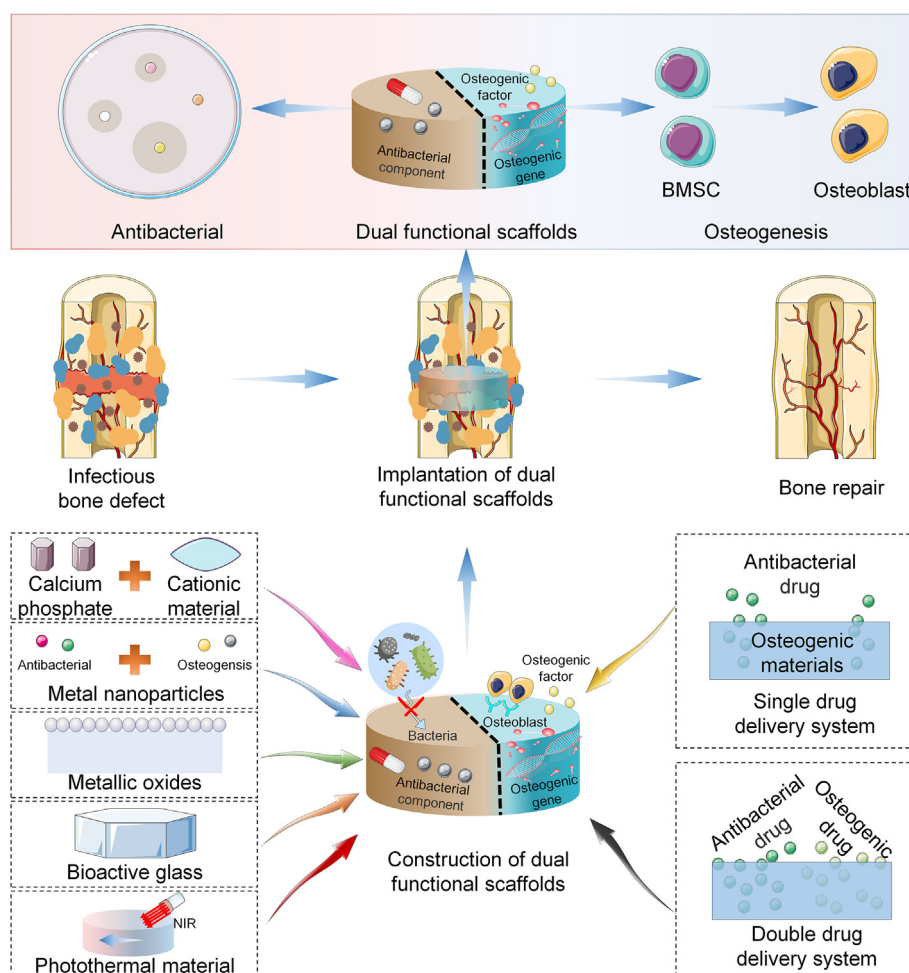
These dual-functional biomaterials include two major categories, which are composite scaffolds and drug delivery systems with functional materials and functional drugs as their respective cores. Functional materials can be directly transplanted to the bone defect to provide support for the bone repair process. For example, bone-promoting hydroxyapatite (HA) can promote osteogenesis by promoting the adhesion of endogenous osteoblasts and bone-forming related proteins, while chitosan with antibacterial property can inhibit the colonization of pathogenic bacteria in the bone defect site by dissolving their cell membranes [24,25]. Functional drugs combine with carriers to construct drug delivery systems, which exert effects by contacting with related cells or pathogenic bacteria after being released to local areas [26-28]. The functioning of these dual function drug delivery systems also requires appropriate carrier materials including polymeric materials as well as materials of natural origin. For example, silk fibroin is an excellent natural material due

to its biocompatibility and biodegradability. Hydrogel composites and nanocomposites designed with the combination of silk fibroin with antibacterial and osteogenic factors were confirmed to have good antibacterial and osteogenic effects [29,30]. The construction mode and action mechanism of these two strategies are different, but both form a complementary effect between the antibacterial and promoting bone.

In this review, we will analyze the effects of infection on osteogenesis and osteoclasts, and summarize the effects of different inflammatory factors on the formation of new bone, thus providing a theoretical basis for controlling infection and promoting osteogenesis therapy. Furthermore, based on the cornerstone of functional components, a series of materials with antibacterial and osteogenic functions will be summarized, and finally, the drug delivery system with dual functions will also be discussed (Scheme 1). This review will summarize the latest advances to provide a novel and more promising therapeutic perspective for the clinical treatment of infectious bone defects.

## 2. Damaged bone formation under infection

The local reconstruction of bone defects includes three overlapping stages, namely, that of inflammation response, bone regeneration and bone remodeling [31,32]. The coordination of these three processes is indispensable for bone repair. The dysregulated inflammation caused by infection is detrimental to the process of bone regeneration [33,34]. Furthermore, pathogenic bacteria can also directly damage bone repair. Osteoclast-mediated bone resorption can be regulated by immune factors and osteoblasts. The chronic inflammatory state and osteogenesis



**Scheme 1.** Various material-based and drug delivery system-based dual-functional scaffolds promote the repair of infectious bone defects by simultaneously inhibiting bacteria and promoting osteogenesis.

damage caused by infection will cause excessive bone resorption. Understanding the mechanisms of the effect of infection on bone repair is of paramount importance for treatment of infectious bone defects, such as providing options for antimicrobial and osteogenic therapy at the molecular level. In this section, we discuss the relationship between the local inflammation and bone regeneration. Then, the effect of the infection on the bone extracellular matrix, osteoblasts and osteoclasts, is analyzed.

2.1. Excessive inflammatory microenvironment caused by infection

At bone injury site, the tightly regulated inflammatory process can be divided into two stages, which are essential for bone repair. In the early stages, the activated neutrophils locally remove necrotic substances through the release of various factors, such as neutrophil-derived serine, and recruit macrophages into the injury site by releasing chemokines, such as interleukin-6 (IL-6) [35,36]. Furthermore, neutrophils are involved in the process of osteogenesis and angiogenesis. The depletion of neutrophils in fractured areas will lead to delayed bone regeneration and poor fracture healing [37]. Moreover, neutrophils promote the formation of fibrin in the early stage of inflammation, thereby supporting the attachment and migration of cells as a temporary extracellular matrix [38].

In the next stage, macrophages are essential for bone repair. They clear the fibrin matrix and necrotic cells by phagocytosis, secrete various

inflammatory factors, chemokines, and growth factors to regulate the local inflammatory response and cell activities [39]. Macrophages exhibit different phenotypes that play different roles in the process of bone repair. M1 macrophages secrete a variety of pro-inflammatory factors, such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and chemokines such as monocyte chemoattractant protein-1, which can lead to local tissue damage and recruitment of inflammatory cells [40]. Moreover, M1 macrophages promote angiogenesis by regulating the expression of vascular endothelial growth factor (VEGF) [41,42]. M2 macrophages are marked by the increase of anti-inflammatory factors, such as interleukin-10 (IL-10) and arginase 1 [43]. Necrotic tissue and cell debris will polarize macrophages into M2 phenotypes. More importantly, M2 macrophages promote the migration and differentiation of mesenchymal stem cells (MSCs) and regulate local bone formation by secreting a variety of osteogenic growth factors, such as bone morphogenetic protein-2 (BMP-2) and transforming growth factor- $\beta$  [44,45]. In addition to its effect on osteoblasts, macrophages also regulate osteoclastogenesis. Osteoclasts are differentiated from monocyte/macrophage cell lineages and are mainly regulated by macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor-kappa B (RANK) ligand (RANKL) [46]. Macrophages promote the expression of RANKL through pro-inflammatory factors, such as TNF- $\alpha$ , interleukin-1 (IL-1), and IL-6, to induce osteoclastogenesis, while they can also inhibit the activation of intracellular downstream signaling molecules of RANKL through

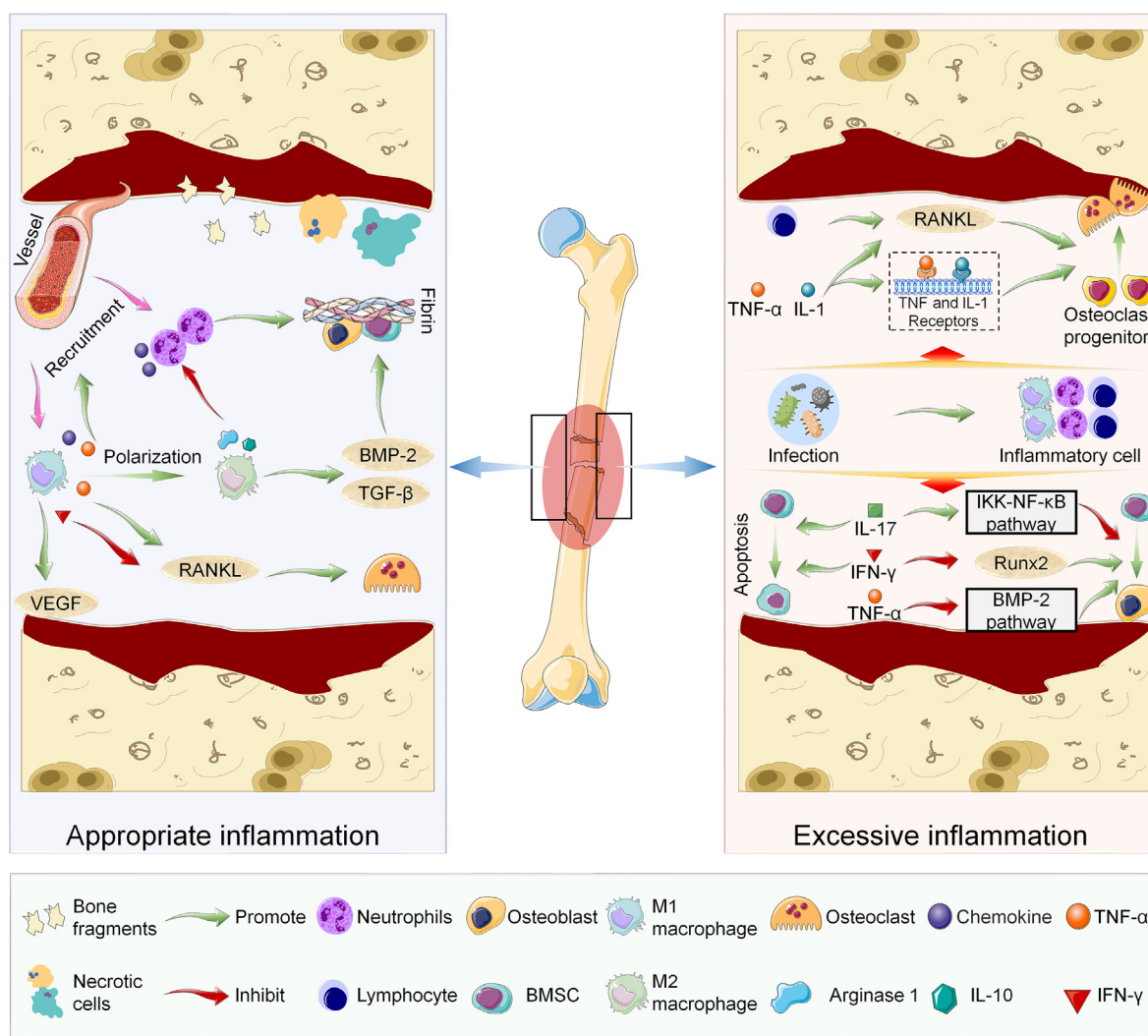


Fig. 1. Effect of local inflammation on osteogenesis: Osteogenesis is promoted by an appropriate inflammatory response due to local trauma (left); Chronic and excessive inflammation due to infection inhibits bone repair (right).



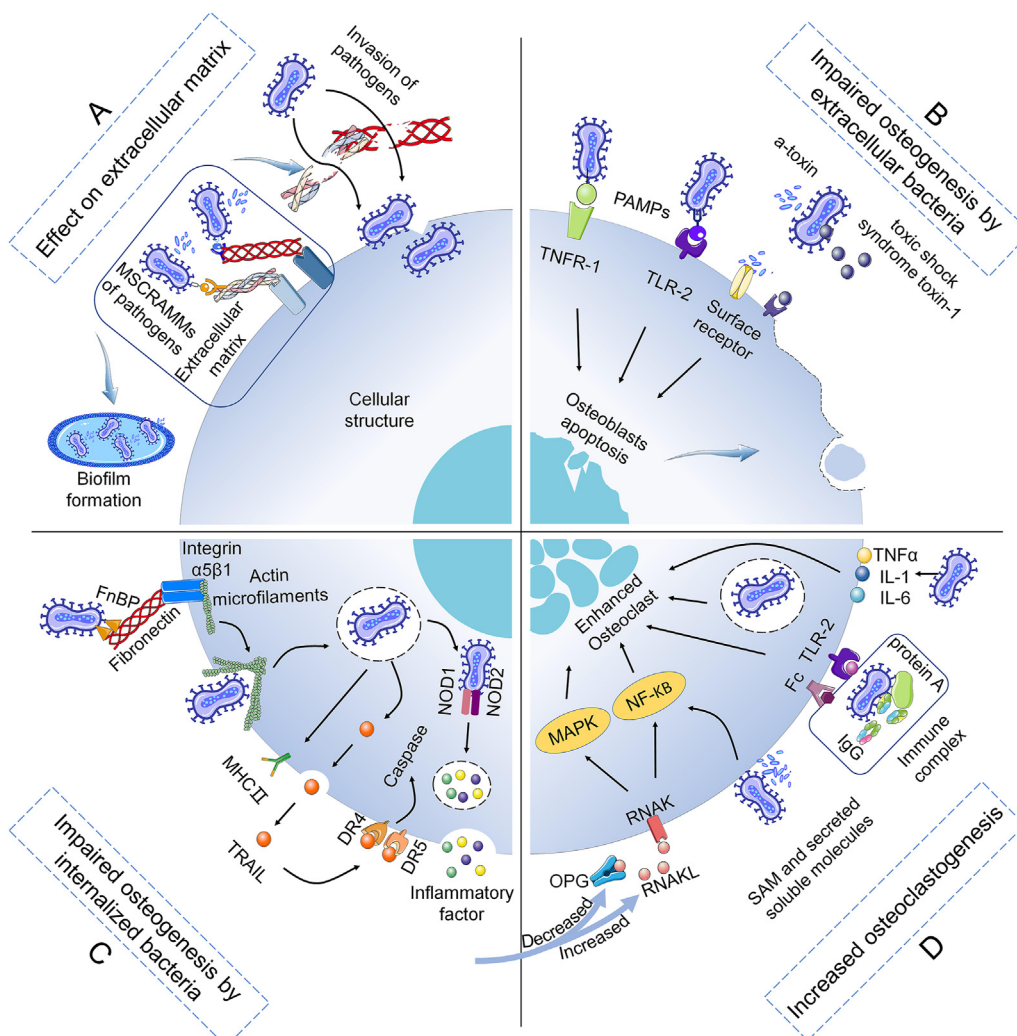
interferon  $\gamma$  (IFN- $\gamma$ ) to inhibit bone resorption [36,47] (Fig. 1).

Bone repair is initiated by an inflammatory response, but a controllable and appropriate level of inflammatory response is crucial for subsequent bone reconstruction. However, infection is a persistent stimulus that keeps inflammation at a persistent excessive level at the site of the bone injury, leading to complications such as fibrosis and hindering bone repair [44]. Compared with aseptic bone injury, infectious bone defects exhibit elevated levels of inflammatory cells such as helper T cells and cytotoxic T cells and a variety of pro-inflammatory factors such as IL-1 $\beta$  significantly increased [48]. Activated T cells and B cells have been shown to release abundant RANKL *in vivo* to induce bone resorption and bone loss [49]. In addition, a large number of pro-inflammatory cytokines and mediators secreted by inflammatory cells such as macrophages and T cells also promote osteoclastogenesis through the RANKL-dependent and RANKL-independent pathways. TNF- $\alpha$  and IL-1 promote bone resorption by increasing the production of RANKL [50, 51]. On the other hand, they also bind to TNF receptor and IL-1 receptor respectively to activate nuclear factor- $\kappa$ B(NF- $\kappa$ B), or NF- $\kappa$ B and JUN molecules to promote osteoclastogenesis [52]. Excessive bone resorption causes the imbalance of osteoblasts and osteoclasts, thus impairing the bone repair. Furthermore, several pro-inflammatory factors have conflicting effects due to factors such as their concentration and exposure time to affect bone formation. For example, transient TNF- $\alpha$  recruit MSCs to the defect site for bone regeneration, and the absence of TNF- $\alpha$  will impair fracture healing process and delay bone regeneration process [53, 54]. However, persistently high levels of TNF- $\alpha$  will inhibit BMP-2

signaling pathway by promoting ubiquitination and proteasome degradation of Smad1 and runt-related transcription factor 2 (Runx-2), thus inhibiting the process of bone regeneration [55,56]. Moreover, some pro-inflammatory factors directly inhibit the osteogenic differentiation process. IL-1 $\beta$  suppressed the proliferation of MSCs, the expression of osteogenic genes Runx2 and alkaline phosphatase (ALP) and the mineralization of extracellular matrix *in vitro* [57]. Interleukin-17 (IL-17) inhibit the osteogenic differentiation of MSCs by activating the inhibitor of  $\kappa$ B kinase (IKK)-NF- $\kappa$ B pathway [58]. Liu et al. [59] demonstrated that IFN- $\gamma$  down-regulated the expression of the osteogenic gene Runx-2. IFN- $\gamma$  and IL-17 can promote apoptosis of BMSC by up-regulating the expression levels of Fas and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and activating the caspase cascade [33] (Fig. 1).

### 2.2. Infection on bone extracellular matrix

After entering the bone defect site, the surface adhesion molecules of the pathogens facilitate their binding to the bone extracellular matrix. The multiple surface adhesion molecules of pathogens involved in this colonization process are termed as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [60,61]. Various proteins and polysaccharides in bone extracellular matrix, such as type I collagen, bone sialoprotein, osteopontin, and fibronectin, can specifically recognize and interact with MSCRAMMs to become their binding sites [62]. These extracellular matrix components, such as fibronectin, are also



**Fig. 2.** Effect of infection on bone repair: A. Pathogens indirectly bind to cells through colonization onto the ECM, which can destroy ECM components and lead to the biofilm formation; B. Pathogens and their secreted soluble factors promote osteoblast apoptosis by binding to cell surface receptors; C. Pathogens infect osteoblasts with the participation of fibronectin and cytoskeletal components, and the internalized pathogens inhibit osteogenesis and maintain excessive inflammation; D. Infected osteoblasts, excessive inflammation, surface components, and secreted soluble factors of pathogens as well as immune complexes formed by pathogens promote osteoclastogenesis.

a bridge between bacteria and bone cells, which can both bind to MSCRAMMs and the cell surface [63]. After binding and colonizing the bone matrix, bacteria can produce a variety of virulence factors, such as acidic substances and proteases, resulting in the destruction of the extracellular matrix (Fig. 2A). The destruction of bone extracellular matrix can further promote the invasion of bacteria [60,64]. Moreover, the interaction between extracellular matrix components and osteoblast surface receptors, such as integrin, is also an important regulatory signal pathway for osteoblast differentiation, maturation, and mineralization [65,66]. Therefore, the damage of the extracellular matrix will reduce the local bone repair ability.

The chronic development of pathogens on the surface of the scaffold and bone matrix will form biofilms, which are surface attached colonies composed of single or multiple bacteria, and their extracellular polymer matrix (Fig. 2A). The biofilm matrix is mainly composed of various secreted pathogen products, such as DNA, protein, lipid, and lipopolysaccharide [67,68]. The bacteria in the biofilm are a metabolically diverse populations due to the gradients of available nutrients and oxygen. Within the biofilm, the virulence of some bacteria is enhanced, and the selection of small colony variants and persister cell populations are also significantly facilitated, which increase the infection recurrence rate [69-71]. The presence of these biofilms allows bacteria to escape the antibiotics and the immune system, leading to difficulties in antibacterial treatment [72]. Biofilm is also a reservoir of bacteria, and the bacteria can disperse from the mature biofilm to aggravate the infection [73]. Therefore, the development of materials and drugs that inhibit and remove biofilm will have important clinical significance.

### 2.3. Impaired osteogenesis

Pathogens can directly damage the extracellular matrix components of bone by releasing acidic substances and proteases to reduce the bone repair ability, but more importantly, they inhibit bone repair by directly inhibiting osteoblasts and promoting the formation of osteoclasts. There are a variety of pathogen-associated molecular patterns (PAMPs) on the surface of pathogens such as lipopolysaccharide, flagellin, etc., which interact with osteoblasts to promote their apoptosis and inhibit osteogenic differentiation [74]. PAMPs bind to various extracellular and intracellular pattern recognition receptors (PRRs) of osteoblasts, such as Toll-like receptors (TLR), the tumor necrosis factor receptor 1 (TNFR-1), and nucleotide-binding oligomerization domains (NOD) like receptors [75]. TLR-2 and TNFR-1 are extracellular receptors that can recognize and bind PAMPs of *Staphylococcus aureus* (*S. aureus*), thereby inducing the apoptosis of osteoblasts [76]. In addition to PAMPs, soluble factors secreted by pathogens are also damaging factors for bone repair. *Mycobacterium tuberculosis* heat shock protein 10 is a soluble protein secreted by *Mycobacterium tuberculosis*, which inhibits the proliferation of osteoblasts and alkaline phosphatase activity, damages calcium deposition, and promotes the formation of osteoclasts to affect bone metabolism [77]. *S. aureus* secretes a variety of soluble factors such as  $\alpha$ -toxin, toxic shock syndrome toxin-1, etc., which induce osteoblast apoptosis [78] (Fig. 2B). In addition to planktonic bacteria, the soluble factors produced by *S. aureus* biofilm promote the development of osteomyelitis by reducing the activity and osteogenic capacity of osteoblasts and promoting bone resorption [79]. However, the exact mechanism of the inhibitory effect of soluble factors on osteogenesis is not clear to date, and requires further study.

The intracellular pathogens after invading osteoblasts are the also the important cause of damage to bone regeneration [78]. Infection of osteoblasts begins by bridging through extracellular matrix components, such as fibronectin. During the infection of *S. aureus*, on the one hand, fibronectin binds to the fibronectin binding protein (FnBP) on the bacterial surface, and on the other hand, it is connected to the surface of osteoblasts through  $\alpha 5\beta 1$  integrin, such that *S. aureus* is indirectly combined with osteoblasts [63,80]. The collagen adhesion and bone sialoprotein binding protein on the surface of *S. aureus* also participate and

support the internalization process of bacteria [75]. The internalization of bacteria is an active process of osteoblasts, which requires the participation of cytoskeletal components, such as actin microfilaments and microtubules [81]. As osteoblasts are not immune cells, *S. aureus* membrane-damaging virulence factors facilitate its escape from the intracellular immunity. Moreover, *S. aureus* will change its characteristics after internalization and mutate into a low sensitivity subtype. This allows *S. aureus* to survive for a long time in osteoblasts, thus escaping clearance by the immune system, and making antibiotic treatment more difficult. Thus, the infected osteoblasts become a reservoir of bacteria, from which bacteria can be released to infect other osteoblasts [75,81]. Internalized *S. aureus* can interact with intracellular PRRs such as NOD1 and NOD2 to promote the release of inflammatory factors [82]. Furthermore, the infected osteoblasts will release the TRAIL that can combine with death receptor (DR) 4 and DR5 expressed on the surface of infected osteoblasts to induce cell apoptosis [83,84] (Fig. 2C). Moreover, *S. aureus* also releases membrane-damaging virulence factors, such as phenol soluble modulins, to cause damage to the cell membrane of osteoblasts, thereby causing cell necrosis [85].

Infected osteoblasts also contribute to maintain local chronic inflammation [75]. Studies have shown that infection can promote osteoblasts to secrete chemokines, such as IL-6 and MCP-1, which can promote the recruitment of macrophages [86]. Infected osteoblasts also produce a variety of pro-inflammatory factors such as IL-1, TNF- $\alpha$ , interleukin-12 (IL-12), etc. [64]. Further, the expression of growth factors, such as the granulocyte-macrophage colony stimulating factor and granulocyte colony stimulating factor, was upregulated [87]. Dapunt et al. [88] showed that osteoblasts also respond to components in bacterial biofilms, especially heat shock protein GroEL, and thus participate in the chronic inflammatory response process. The infected osteoblasts express MHC class II molecules and co-stimulatory receptors, and thus have antigen-presenting properties to activate infiltrating T cells (Fig. 2C) [64,88].

Although a large number of *in vitro* studies have confirmed that pathogenic bacteria can invade into osteoblast-related cells, it is still unclear how many osteoblast-related cells are infected and how long pathogenic bacteria can live in these cells due to the lack of *in vivo* experiments. These questions deserve further clarification by establishing stable animal models in the follow-up study.

Osteoblasts also produce antimicrobial peptides themselves to resist the invasion of pathogens. The application of these antimicrobial peptides will be an effective treatment method for osteomyelitis. Synthetic antimicrobial peptides have been produced and used for the treatment of infectious diseases [89,90].

### 2.4. Increased osteoclastogenesis

A balanced process of bone regeneration and bone resorption is essential to maintain local bone mass and reconstruct the normal bone tissue structure in bone defects [91]. The increase in osteoclastogenesis induced by pathogen infection is also very important at the microenvironment of infectious bone defects. Osteoclasts are differentiated from hematopoietic monocytes/macrophages regulated by M-CSF and RANKL. M-CSF is critical for the proliferation and the RANK expression of osteoclast progenitor cells. RANKL mainly secreted by osteoblasts and lymphocytes bind to RANK on the surface of osteoclast progenitor cells to activate mitogen-activated protein kinase (MAPK) pathways and NF- $\kappa$ B signaling pathways, to promote the maturation and activation of osteoclasts [92]. Osteoprotegerin (OPG) secreted by osteoblasts, dendritic cells, B cells, and MSCs serves as a decoy receptor for RANKL, which negatively regulates the differentiation of osteoclasts [36,93]. The infection of osteoblasts by pathogenic bacteria directly promotes the secretion of RANKL and downregulates the expression of OPG, thereby promoting the production of osteoclasts [84,94]. The infected osteoblasts also secrete prostaglandin E2, which can upregulate the expression of RANKL via the EP4 receptor in osteoblasts [95]. The soluble factors

released by *S. aureus* biofilms promote the expression of RANKL in osteoblasts and increase the ratio of RANKL/OPG, thereby significantly promoting bone resorption [79]. The release of pro-inflammatory factors, such as TNF- $\alpha$ , IL-1, and IL-6, caused by pathogenic bacteria in the local area of bone defects likewise promote the production of osteoclasts [96, 97].

In addition to indirectly acting on osteoclast differentiation through osteoblasts and inflammatory factors, surface-associated materials (SAM) and secreted soluble molecules of bacteria can directly promote osteoclast differentiation. Studies have shown that the SAM of *S. aureus* promoted osteoclast formation via a RANKL-independent pathway [98,99]. Ren et al. [100] showed that the effect of cell wall components and secreted soluble factors of *S. aureus* on promoting the formation of osteoclasts and bone resorption was related to the NF- $\kappa$ B signaling pathways in the absence of RANKL, in which inhibitor of  $\kappa$ B- $\alpha$  was degraded, NF- $\kappa$ B was phosphorylated, and the expression of nuclear factor of activated T cells 1, which is a key regulator to promote osteoclast differentiation, was increased. Moreover, *S. aureus* forms an immune complex through the combination of protein A and IgG, which promotes osteoclastogenesis of osteoclast progenitor cells by stimulating Fc receptors and TLR-2 [101]. *S. aureus* also had the ability to directly infect osteoclasts, and the internalization process differs from the FnBP- $\alpha$ 5 $\beta$ 1 integrin interaction pathway of osteoblasts, which is more likely to be achieved by phagocytosis of osteoclasts. Unlike their progenitors, osteoclasts cannot confine internalized *S. aureus* and provide a replicative niche for bacteria, and this entire process depends on the RANKL signaling ways. Internalized *S. aureus* promotes the fusion of osteoclasts and enhances their bone resorption capacity [102,103] (Fig. 2D).

### 3. Materials based strategy for dual-functional composite scaffolds

The risk of infection of large segmental bone defects caused by severe open wounds, tumors, etc. increases significantly [104]. In these defect areas, the surface of implants not only facilitates the migration of bone cells, but also becomes a promotive surface for bacterial adhesion. The concept of “race to the surface” demonstrates the balance between bone integration and bacterial colonization on the surface of the implant. Successful bone repair depends on the attachment of osteoblast-related cells to the implant surface and their subsequent mineralization and maturation, thereby inhibiting bacterial colonization and biofilm formation. However, if bacterial colonization occurs first, implant-related infection will begin and the bone repair process will be hindered [105]. Therefore, it is of great clinical significance to develop a dual-functional implant that can promote bone regeneration while inhibiting bacterial colonization.

#### 3.1. Cationic antibacterial material and calcium phosphate ceramic material containing biomaterials

Cationic polymer material is a kind of functional biomaterials with broad-spectrum antibacterial ability, which mainly functions through the membrane-lysis mechanism [106]. The cationic moieties of the cationic polymer bind to the negatively charged bacterial cell membrane, and the hydrophobic fragment can be inserted into the bacterial cell membrane to cause leakage of the cytoplasmic components. In addition, recent studies have shown that the exchange of cationic polymers with cations in bacterial cell membranes to destroy charge balance is also one of its mechanisms for destroying bacterial cell membrane [106,107]. Calcium phosphate material is the most commonly used material for bone replacement and regeneration in bone tissue engineering due to its excellent biological activity and similarity to normal bone tissue components [108]. Therefore, the implants prepared by combining the cationic antibacterial material with the calcium phosphate material can play the dual functions of promoting bone repair and inhibiting bacteria after being implanted into the bone defect. Further, this

organic-inorganic combination can solve the problems of limited mechanical properties of cationic antibacterial material and the fragility of calcium phosphate material to make the composite material have more similar mechanical properties to normal bone tissue [109].

While effective, several cationic polymers exhibit poor biocompatibility [110]. Chitosan is a natural cationic antibacterial material, which is often used for the treatment of infectious bone defects because of its good biocompatibility, biodegradability, and antibacterial properties [111]. HA, the main component of bone matrix, exhibits satisfactory biocompatibility and the performance of promoting bone regeneration, and is one of the most commonly used calcium phosphate materials [112]. As a result, a large number of studies have been conducted on chitosan-HA composite scaffolds, which exhibit good antibacterial and bone-promoting effects [113-115]. Furthermore, the derivative materials obtained by further modification on the basis of chitosan/HA also have good application efficacy.

The introduction of quaternary ammonium groups on the free amino or hydroxyl groups of chitosan forms quaternized chitosan (HACC) [17]. HACC has a stronger antibacterial effect and even kills resistant bacteria, such as methicillin-resistant *S. aureus*. The antibacterial effect of quaternized chitosan is positively correlated with the degree of quaternized substitution, but too highly quaternized chitosan will also cause damage to osteoblasts [116]. Yang et al. [117] fabricated a HA/PLGA scaffold via 3D printing technology and grafted HACC with a substitution degree of 26% through covalent bonding for the repair of infectious femoral shaft defects in rats and rabbit femoral condyle defects. The composite scaffold containing HACC with a substitution degree of 26% showed good biocompatibility and evident enhanced antibacterial and bone regeneration. HA was crucial for this composite scaffold, as it improved bone regeneration and slowed down the degradation rate of the scaffold and thus prolonged the treatment effect (Fig. 3).

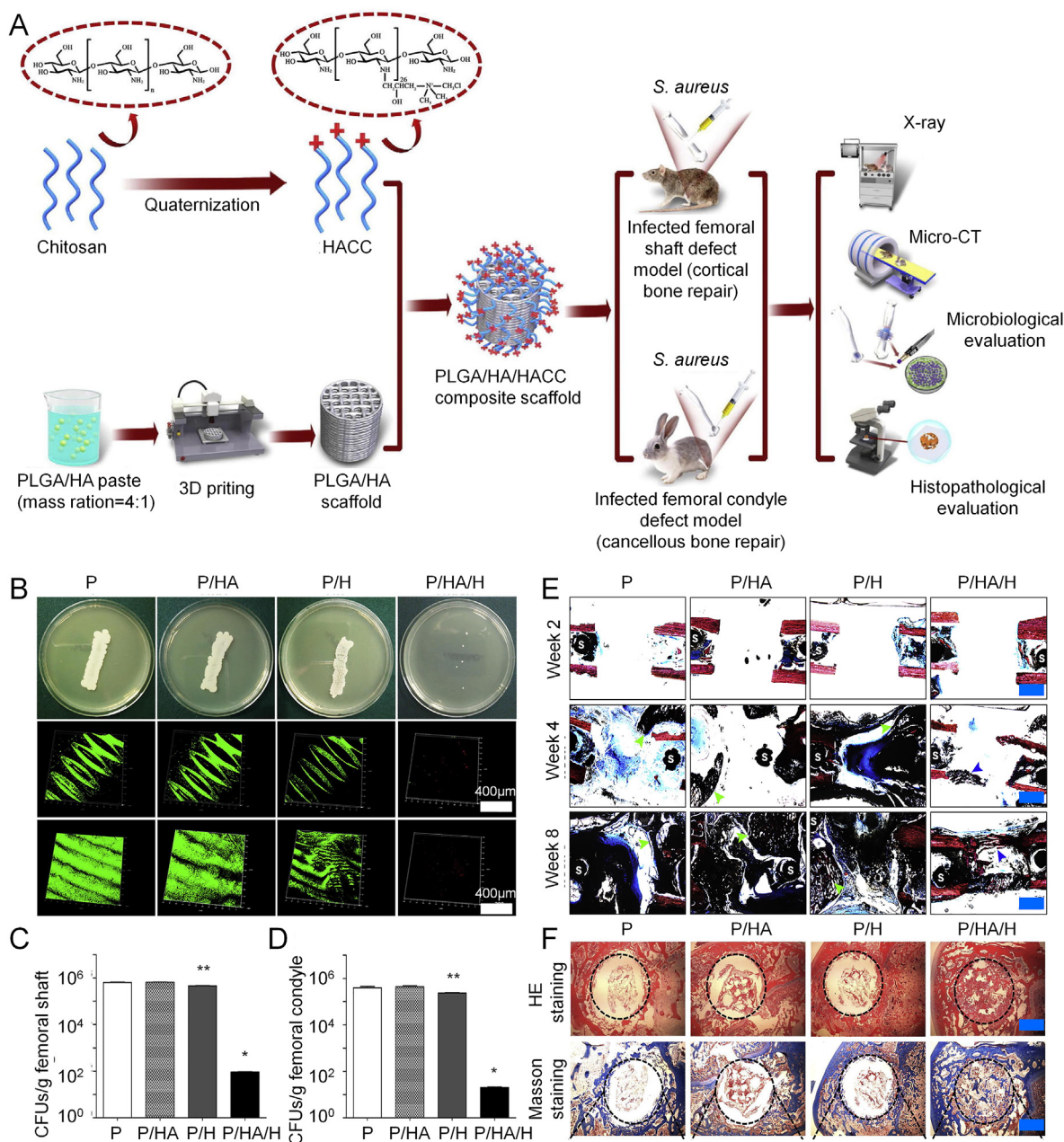
Integrating other materials into the chitosan matrix can also enhance its antibacterial and even bone-promoting ability. Zero-dimensional carbon dots (CD) are a carbon-based nanomaterial with photothermal effects. The composite scaffold made by CD-doped chitosan and HA had good effects of promoting bone formation, antibacterial and antitumor activity. The addition of CD enhances the abilities of the composite scaffold of MSCs adhesion and osteogenesis by upregulating the expression of related genes. The photothermal effect of CD also enhance the antibacterial properties of chitosan [118]. For the improvement of HA, metal particles with antibacterial or bone-promoting ability, such as silver (Ag), strontium (Sr), etc., can be incorporated into the HA manufacturing procedures [119,120]. Xu et al. [109] prepared porous scaffolds of Sr-doped HA and chitosan by freeze-drying, and then deposited Ag on the composite scaffolds via a silver mirror reaction for repairing bone defects and avoiding infection. The incorporation of Sr increased the activity of ALP and promoted mineralization of the extracellular matrix. Ag improved the inhibition of the adhesion and proliferation of *S. aureus*.

In addition to HA,  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) is also one of the most commonly used calcium phosphate materials. The chitosan/ $\beta$ -TCP composite scaffold was also confirmed to promote the migration, adhesion and mineralization of osteoblasts, and inhibit *S. aureus* growth [121]. Moreover, the combinations of other cationic antibacterial materials and osteogenic materials are also appropriate design strategies for antibacterial and bone-promoting dual-functional materials, but these need to be confirmed and optimized by further research.

#### 3.2. Functional metallic nanoparticles doped biomaterials

A variety of metallic nanoparticles have been shown to exhibit antibacterial effects, such as Ag, zinc (Zn), and bone-promoting effects such as Sr, and copper (Cu). These can be doped into biomaterials to fabricate dual-functional scaffolds with antibacterial and osteoinductive abilities. Ag nanoparticles (AgNPs) are commonly used metallic nanoparticles with excellent antibacterial properties, which are achieved by inducing





**Fig. 3.** HACC grafted HA/PLGA scaffold for the repair of infectious defects [117]: A. Schematic diagram of HACC grafted HA/PLGA scaffold (P/HA/H) and its experimental protocol; B. Bacterial culture of the screws and confocal microscope images of the biofilm on the surfaces of screws and the polyethylene plates after eight weeks of implantation of PLGA (P), PLGA/HA (P/HA), PLGA grafted with HACC (P/H), and P/HA/H into infected rat femoral defects fixed with polyethylene plates and stainless steel screws; C-D: Colony counts of infectious defects in rats and rabbits in each group after eight weeks of implantation; E-F: Histological analysis of infected bone defect in rats (E) and infected femoral condyle defect in rabbits (F) after scaffold implantation in each group (scale bar = 2 mm). Reprinted with permission from Ref. [117]. Copyright 2018, Elsevier Ltd.

the destruction of bacterial cell membrane, hindering the replication of bacterial DNA and protein synthesis, and affecting cell respiration [122, 123]. AgNPs can be synthesized in situ to form functional coating on the surface of the scaffold via mussel-inspired polydopamine (PDA) technology. PDA has good biocompatibility and hydrophilicity, it can undergo oxidative self-polymerization under specific conditions, and form a coating by combining covalently and non-covalently with various materials surfaces. The surface-coated PDA acts as a reducing agent to immobilize AgNPs to the composite scaffold [124,125]. Studies have shown that the PDA coating itself enhances the expression of ALP and promotes osteogenesis due to the natural active sites of catecholamine moieties contained in PDA [126,127]. Therefore, AgNPs/PDA coated scaffolds promote the expression of osteogenic related genes and inhibit

the adhesion and growth of Gram-positive bacteria, such as *S. aureus* and Gram-negative bacteria such as *Escherichia coli* (*E. coli*) [124,125,127]. Moreover, there are studies showing that AgNPs directly promoted osteoblast differentiation by activating the integrin  $\alpha 5$  orchestrated MAPK/ERK signaling cascade, which further confirmed the potential of AgNPs/PDA coated scaffolds as antibacterial and bone-promoting dual-functional scaffolds [128,129].

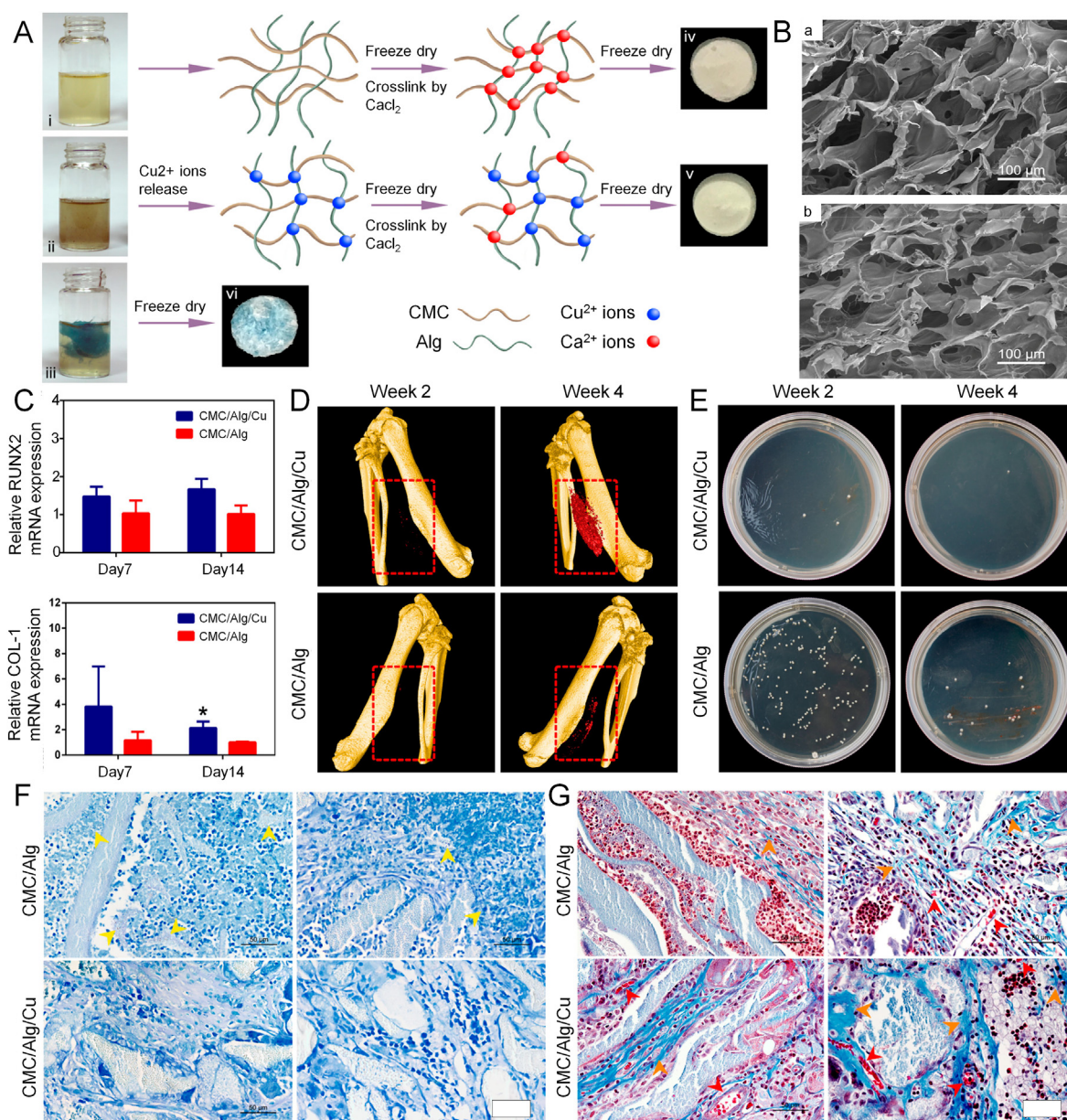
The combined application of antibacterial nanoparticles and bone-promoting metallic nanoparticles is an alternative strategy for dual-function composite scaffolds. Cheng et al. [130] used hydrothermal treatment to load nanotubular containing Sr and Ag on the surface of a titanium (Ti) scaffold. The presence of Ag nanotubules was confirmed to inhibit the adhesion and colonization of methicillin-resistant *S. aureus*

and *E. coli*, while Sr nanotubes upregulated the expression of osteogenic genes, such as ALP, osteocalcin (OCN), and inhibited osteoclastogenesis, resulting in promoted normal and osteoporotic bone integration. Another metallic nanoparticle with antibacterial effects, Zn, works by altering the permeability of bacterial cell membranes and inhibiting the respiratory process of cells [131]. The porous HA scaffold doped with Zn nanoparticles and Sr nanoparticles showed good biocompatibility, osteoinductivity, and antibacterial properties [132]. There are also biological metals that have the dual ability to both resist bacteria and promote bone formation. Cu had been proven to inhibit bacterial growth by generating reactive oxygen species (ROS) and inhibiting proliferation bacterial DNA and RNA. Further, Cu has the ability to promote osteogenic differentiation and angiogenesis [133,134]. Lu et al. [135] added Cu nanoparticles to the mixture of carboxymethyl chitosan and alginate,

and further prepared a composite scaffold by freeze-drying. The scaffold significantly promoted the adhesion of MC3T3-E1 cells, upregulated the expression of osteogenic genes and extracellular calcium deposition. It also exhibited good antibacterial effects, and promoted bone regeneration and angiogenesis within four weeks in rats with local infection of *S. aureus* (Fig. 4).

### 3.3. Metallic oxides containing biomaterials

Metallic oxides, including magnesium oxide (MgO), zinc oxide (ZnO), and titanium dioxide (TiO<sub>2</sub>) are the cornerstones of the dual functionalized scaffold. Nano-MgO is an antibacterial material, which can damage the cell membrane, DNA, RNA and protein of bacteria by generating ROS, and it causes mechanical damage to the cell wall and membrane of



**Fig. 4.** Carboxymethyl chitosan/sodium alginate mixed scaffold (CMC/Alg) containing Cu nanoparticles (CMC/Alg/Cu) for antibiosis and osteogenesis [135]: A. Fabrication process of CMC/Alg/Cu; B. SEM images of CMC/Alg/Cu (a) and CMC/Alg (b); C. Expression of osteogenesis-related genes after 14-day culture of MC3T3-E1 cells with composite scaffolds; D. Micro-CT reconstruction image of new bone after implantation of MC3T3-E1 cell-loaded composite scaffold into infected muscle capsule of rat gluteus maximus. E. Colonies of *S. aureus* on the implant at two and four weeks after implantation; F-G: After implantation of two and four weeks, the result of (F) Giemsa staining for residual bacteria and (G) Masson's trichrome staining for new bone formation (scale bar = 50  $\mu$ m). Reprinted with permission from Ref. [135]. Copyright 2018, American Chemical Society.



bacteria by direct contact. In addition, the enrichment of nMgO changes the membrane permeability of bacteria [136,137]. Similar to nano-MgO, nano-ZnO has a broad-spectrum antibacterial effect, it can destroy the cell wall of bacteria through direct contact, and it can also produce ROS. In addition, ZnO can release Zn ion in aqueous medium, which can destroy the cell membrane of bacteria and inactivate a variety of proteases in the cells [138,139]. In addition to antibacterial effect, the MgO and ZnO also have the function of promoting bone regeneration. Nano-MgO promoted cell adhesion and proliferation as well as the expression of osteogenic marker ALP by releasing magnesium ions in aqueous medium, and nano-ZnO was confirmed to promote osteogenic differentiation by up-regulating the expression of osteogenic genes Runx-2 and OCN and promoting collagen secretion and mineralization of extracellular matrix of osteoblasts [136,140,141].

TiO<sub>2</sub> is generated in situ on the surface of the Ti scaffold, which changes the structural properties of the surface of the Ti scaffold and therefore enhances the cell adhesion, tissue ingrowth, and bone integration [141]. Jia et al. [142] in situ fabricated a TiO<sub>2</sub> coating with sub-micron pores on the surface of the porous Ti6Al4V scaffold through micro-arc oxidation, and then further functionalized the scaffold with AgNPs via PDA technology. The TiO<sub>2</sub> coating conferred the original flat

Ti6Al4V scaffold a defined micro- and ultra-topographies, which stimulated the differentiation of MG-63 cells into osteoblasts and enhanced the production of ALP and the extracellular matrix such as collagen. The integration of AgNPs made the composite scaffold capable of inhibiting bacteria (Fig. 5).

### 3.4. Bioactive glass-based biomaterials

Bioactive glass (BG) is a kind of biocompatible, biodegradable biomaterial usually formed from network materials of silicon dioxide and further combined with other oxides, such as calcium oxide, sodium oxide, phosphate, or modifiers such as Sr oxide [143]. During the degradation of BG, a variety of ions is released into the surrounding medium, making it capable of stimulating diverse biological processes. The calcium ions in BG combine with the phosphate in the body fluid to form an amorphous calcium phosphate (ACP) layer deposited on its surface. Subsequently, with the release of other ions, the increase of surrounding pH and the addition of carbonate, the ACP layer gradually grows into a carbonated hydroxyl-apatite layer, which promotes the adhesion and proliferation of osteoblasts and fuels osteogenic differentiation [144]. The silicon in BG promotes the expression of osteoblast-related genes in osteoblasts,

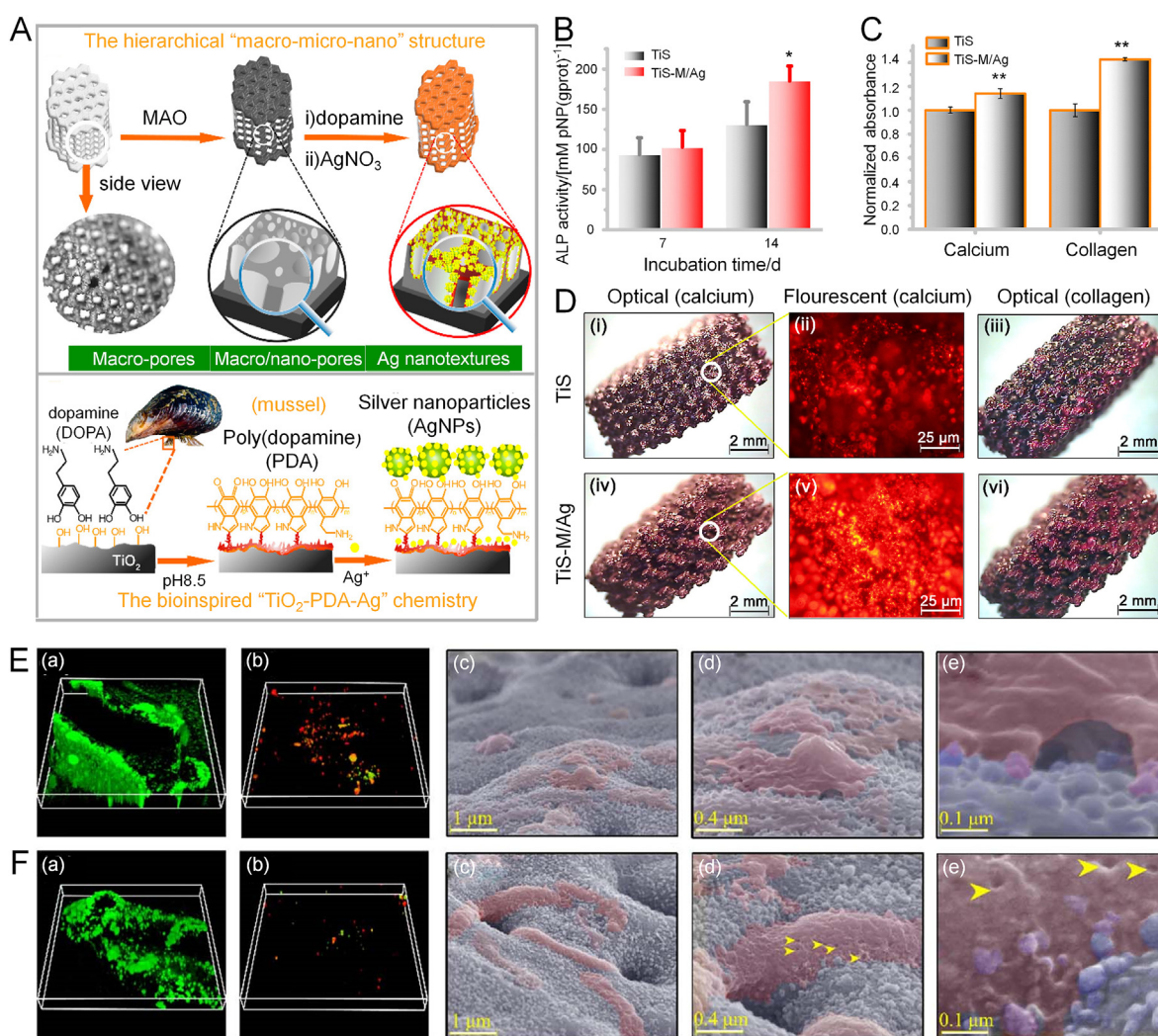


Fig. 5. TiO<sub>2</sub> and dopamine-AgNP co-modified the Ti6Al4V scaffold with antibacterial and osteogenic functions [142]: A. Manufacturing process of composite coating scaffold; B. Effects of titanium alloy scaffold without surface treatment (TiS) and composite coated scaffold (TiS-M/Ag) on ALP activity of MG63 cells; C, D: Quantitative analysis (C) and staining (D) of calcium and collagen deposits by alizarin red and Sirius red staining after 28 days of coculture of MG63 cells with TiS and TiS-M/Ag scaffolds; E-F: Inhibition of TiS and TiS-M/Ag on *S. aureus* (E) and *E. coli* (F): live and dead staining of bacteria (a-b), the interaction between AgNP and bacteria (c-e) (carmine represented bacteria, purple represented AgNP, and yellow arrow represented pores on membrane). Reprinted with permission from Ref. [142]. Copyright 2016, American Chemical Society.

synthesis of extracellular matrix proteins, and new bone formation. Therefore, BG promotes the proliferation of MC3T3-E1 osteoblastic cells and increases the activity of ALP and the production of type I collagen and bone nodules [145]. Furthermore, BG also has certain antibacterial properties, which is owing to the increase in local pH and osmotic pressure caused by the release of various ions during its degradation. These local microenvironment changes will alter the integrity of the bacterial cell membrane and affect the activity of key enzymes for cellular metabolism, resulting in the inhibition of bacteria [146]. Although pure BG has certain bone-promoting and antibacterial properties, these capabilities are limited [144,147]. Integrating materials with bone-promoting or antibacterial activity into BG is a popular strategy to improve their capabilities. Ryan et al. [143] integrated copper-doped BG into a collagen scaffold for the study of osteomyelitis treatment. Compared with copper-free BG, copper-containing BG possessed enhanced osteogenic and angiogenic properties. It also exhibited better antibacterial activity against *S. aureus* (Fig. 6). Therefore, BG is an advantageous candidate for fabricating scaffolds with dual functions of bone formation and antibacterial.

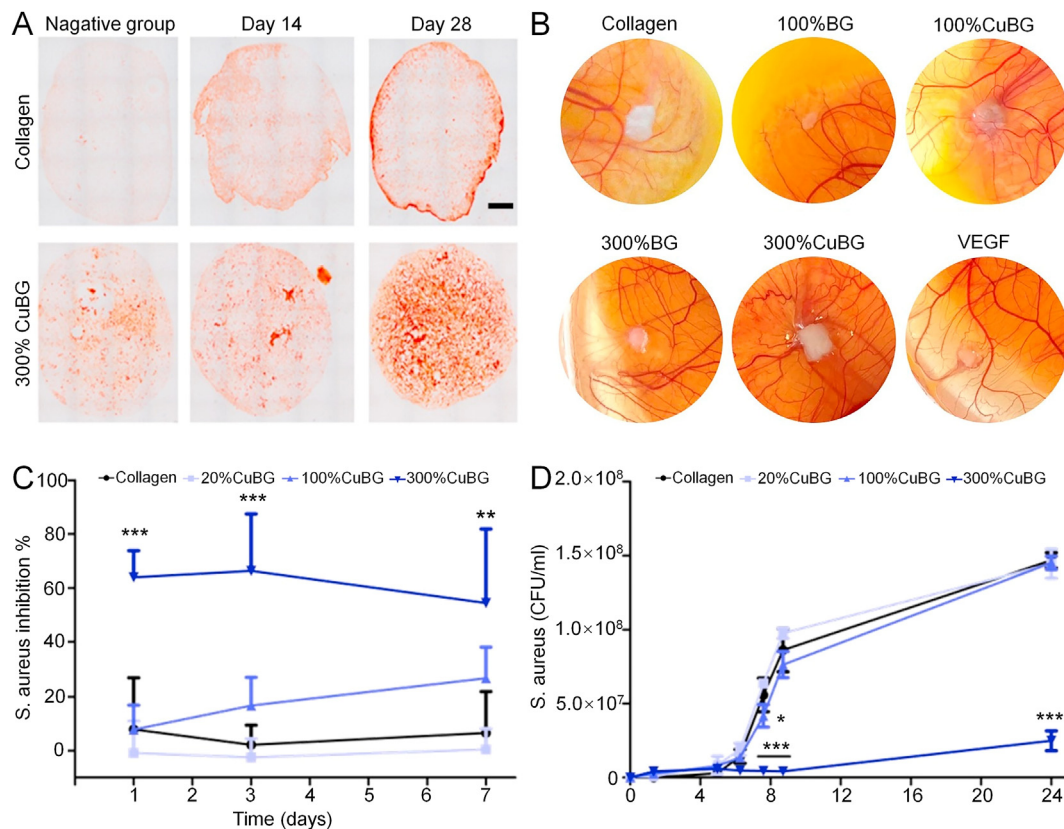
### 3.5. Photothermal agent containing biomaterials

Photothermal therapy is a promising strategy for inhibiting bacteria and promoting bone formation. Photothermal agent can convert light energy into thermal energy under the irradiation of near-infrared (NIR) light to exert biological effects [148]. The temperature generated can be changed by adjusting the proportion of photothermal agent, the time and the intensity of NIR irradiation [149]. The different biological functions can be performed by producing different amounts of heat. Mild localized heat at 40–43 °C can promote cell proliferation, osteogenic

differentiation and mineralization, thus promoting bone repair. The mechanism is mainly related to the fact that mild heat can activate the Wnt signaling pathway and promote the expression of osteogenic genes such as ALP, BMP-2, Runx-2, OCN [150,151]. Hyperthermia over 50 °C effectively exert the antibacterial effect by destroying the cell membrane structure of bacteria and damaging a variety of protein and enzymes in the cytoplasm [152,153]. Inevitably, hyperthermia also poses a threat to the normal tissues in the bone defect site. However, few clear *in vivo* evidence of tissue damage has been found in the current photothermal materials for infectious bone defects, which may prove that the damage caused by hyperthermia is limited. Studies have shown that even if excessive temperature (60–65 °C) caused some damage to cells *in vitro*, these damages can be recovered in a short time [154]. In view of the fact that most current studies only focus on the biocompatibility of photothermal agents without irradiation, future studies should clarify the effects of photothermal agents on the activity of osteoblast-related cells under NIR irradiation.

Besides photothermal effect, some photothermal materials can also exert antibacterial and bone-promoting functions through other mechanisms. The black phosphorus nanosheets (BPs) is a new two-dimensional nanomaterials material with good photothermal conversion effect and biocompatibility. In addition to photothermal treatment, the phosphate generated by the degradation of BPs also contribute to promote osteogenic differentiation. Moreover, the BPs inhibit bacteria by destroying bacterial cell membranes with directly contacting, and generating ROS [155,156]. The research by Li et al. confirmed that the mixed scaffold of BPs and PLGA could promote the osteogenic differentiation of cells and the healing of rat calvarial defects, and showed good antibacterial effect both *in vitro* and *in vivo* [156].

MXenes are a class of transition metal carbide/nitride/carbonitride



**Fig. 6.** Dual-function collagen scaffold incorporated with Cu-doped bioactive glass [143]: A. Alizarin red staining of MC3T3-E1 culturing with collagen scaffolds (Collagen) and collagen scaffolds containing 300%(w/w) bioactive glass (300% CuBG) (scale bar = 1 mm); B. Promotion of collagen scaffolds containing different concentrations of BG (w/w) for the angiogenesis of chicken embryo model, in which VEGF was used as the control group; C-D: Inhibition of collagen scaffolds containing different concentrations of BG (w/w) for *S. aureus*. Reprinted with permission from Ref. [143]. Copyright 2019, Elsevier Ltd.

with excellent physicochemical properties, strong NIR absorption capacity, photothermal conversion efficiency and biocompatibility. Many studies have demonstrated the effective antibacterial activity of  $\text{Ti}_3\text{C}_2$  MXene under NIR irradiation [157,158]. Studies have shown that  $\text{Ti}_3\text{C}_2$  MXene also promoted osteogenic differentiation by combining with the cell surface and activating osteogenic-related pathways such as Wnt pathway [159]. On the other hand, Ti-based substances produced by the interaction of  $\text{Ti}_3\text{C}_2$  MXene with water and oxygen accelerated bone regeneration [160]. Therefore, it is highly feasible to utilize the antibacterial and osteogenic functions of  $\text{Ti}_3\text{C}_2$  MXene to construct dual-function scaffolds. Nie et al. [161] prepared GelMA/ $\beta$ -TCP/sodium alginate/ $\text{Ti}_3\text{C}_2$  MXene composite scaffold by 3D printing for the treatment of infectious bone defects. The excellent photothermal effect of  $\text{Ti}_3\text{C}_2$  MXene allowed the temperature of composite scaffold to rise to about 56°C to kill Gram-positive and Gram-negative bacteria under NIR irradiation. Furthermore,  $\text{Ti}_3\text{C}_2$  MXene, together with  $\beta$ -TCP and sodium alginate, promoted the osteogenic differentiation of cells and accelerated the repair of infected bone defects.

#### 4. Dual-functional drug delivery system

Clinically, antibiotics are the most common used agents for controlling local infections of bone defects. With the development of bone tissue engineering, the local sustained release system loaded with various antibacterial agents has shown good antibacterial effects in the treatment of infectious bone defects [162,163]. To meet the needs of local antibacterial activity and bone formation in infectious bone defects, adding osteogenic factors to the local antibacterial drug delivery system yields excellent improvements. In this section, we discuss dual-functional scaffolds based on various drug delivery systems.

##### 4.1. Single drug delivery system with dual functions

###### 4.1.1. Appropriate drug carrier

At present, complete debridement to remove infected and necrotic tissue and the implantation of an antibiotics-loaded drug delivery system to the infectious bone defect is considered to be an effective method of infection control [164,165]. Combining antibiotics with carrier materials capable of promoting bone repair to construct a dual-functional drug delivery system is an appropriate strategy with promising clinical transformation prospects. The characteristic of initial burst release of the drug delivery system can rapidly achieve a high level of antibiotic concentration at the site of infection to control the proliferation of pathogens, and steady release thereafter can provide a sustained inhibitory concentration without affecting bone regeneration. Meanwhile, the slow degradation of the bone-promoting carrier material provides osteogenic induction over a long period of time, such that the ideal antibiotic-loaded bone-promoting composite material can be matched with the process of infectious bone defect repair [166,167]. Therefore, it is critical to select a suitable carrier material that exhibits biocompatibility, osteogenic induction ability, and appropriate biodegradability [168]. Nano-hydroxyapatite (nHA) is the most studied antibiotic carrier. Previous studies confirmed that nHA stimulates new bone formation and promotes the repair of bone defects, and it has a higher solubility and calcium release profile [169]. Furthermore, nHA is a better drug delivery carrier material than conventional HA due to its high surface area, nanopore structure, and degradability [170]. Multiple studies were performed by loading antibiotics, such as vancomycin, ciprofloxacin, metronidazole, etc., into materials containing the nHA carrier for anti-infection effects while promoting bone repair [171-173]. Jiang et al. [173] mixed vancomycin with nHA to prepare nHA pellets for the treatment of chronic osteomyelitis in rabbits. The pellets maintained an effective antibiotic concentration *in vivo* for 12 weeks, and their implantation into the infected bone defect after debridement effectively prevented the recurrence of infection and promoted the repair of the defect. BG is also an alternative carrier for antibiotics owing to its

degradability and pore structure. As mentioned previously, BG is a good osteoinductive and osteoconductive biomaterial. Liu et al. [174] fabricated the vancomycin-loaded BG composite scaffold and confirmed that the scaffold had good degradability *in vitro*, and could form crystalline HA *in vitro* and maintain drug release for four days. Its implantation into the site of osteomyelitis in rabbits confirmed its efficacy in resisting bacteria and promoting the ingrowth of new bone and blood vessels.

##### 4.1.2. Alternative antibacterial agents

Although antibiotics are commonly used in clinical practice, high local concentrations of antibiotics can produce cytotoxicity, and the frequent use of antibiotics will also lead to the generation of drug-resistant bacteria [175]. Interest has been established to study alternative antibacterial therapies. Extracts of various natural substances, such as chlorogenic acid, and *in vivo* antibacterial extracts, such as antimicrobial peptides and lysostaphin, have been confirmed to exert antibacterial activity and exhibit better biocompatibility [176-178]. These antibacterial substance-loaded drug delivery systems expand the arsenal for antibacterial activity with excellent biocompatibility, thus reducing the incidence of drug-resistant bacteria and cytotoxicity caused by single antibiotic application. Zhang et al. [179] loaded natural-derived antimicrobial agent of hinokitiol onto macroporous-microporous nBG/PEEK and constructed a dual-functional scaffold. The macroporous-microporous nBG in the scaffold promoted apatite mineralization, the proliferation of MC3T3 cells and the expression of osteogenic related genes ALP and BMP-2, accelerating the healing of rabbit bone defects. Simultaneously, hinokitiol released from the scaffold displayed an inhibitory effect on *S. aureus* (Fig. 7). However, these antibacterial substances also have some shortcomings, such as antibacterial effects not being as strong as antibiotics, and poor stability *in vivo*. Therefore, simultaneously improving their stability and antibacterial ability while maintaining cell compatibility deserves further research.

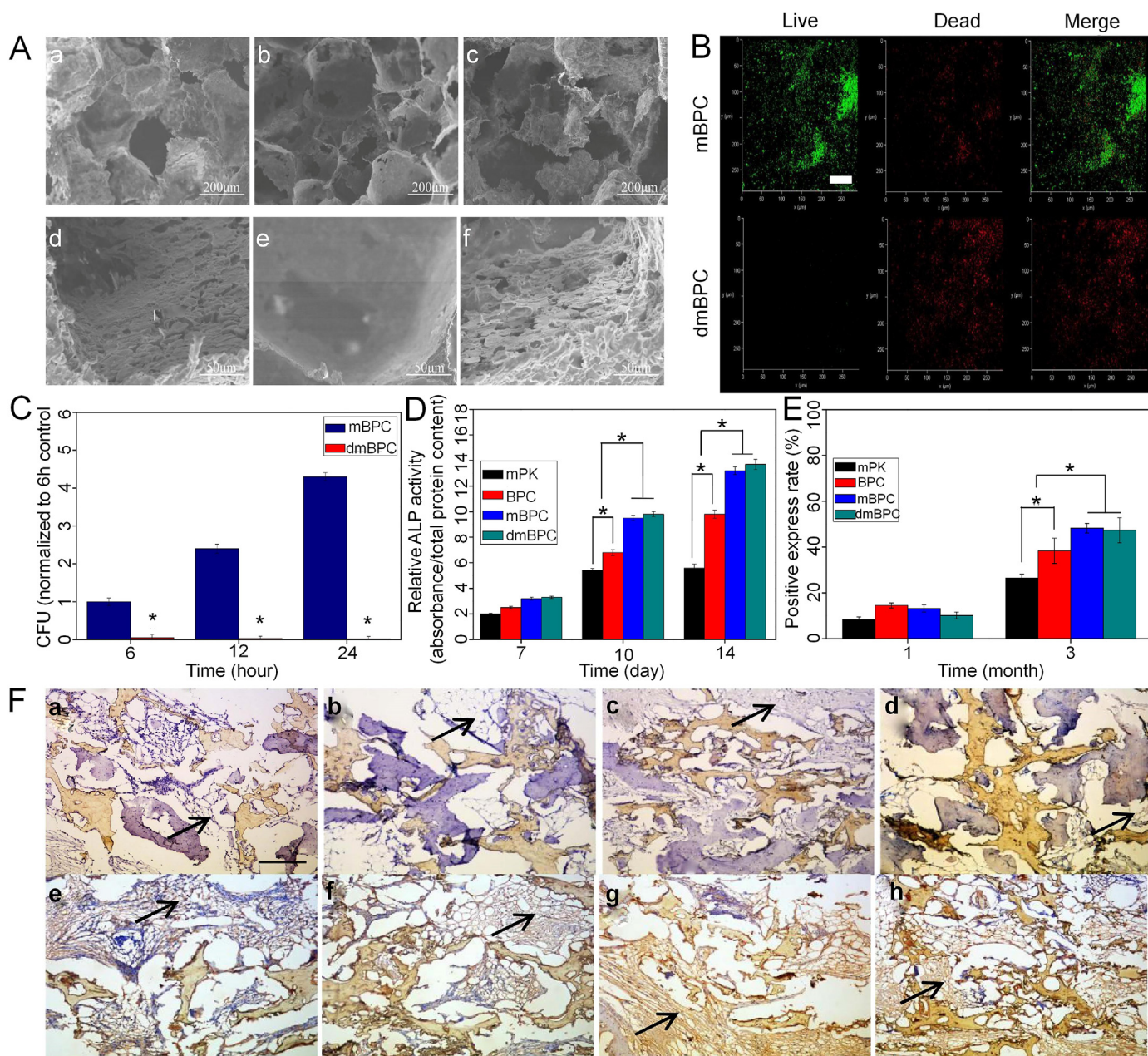
Apart from their antibacterial effects, some antibacterial agents have been confirmed the ability to regulate osteogenesis and osteoclastogenesis. Minocycline is a semisynthetic tetracycline with a broad antibacterial spectrum that acts by inhibiting the synthesis of the bacterial protein, which has also been confirmed to increase the activity of osteoblasts, upregulate the expression of osteogenic genes Runx-2, OCN, osteopontin, and type I collagen, and inhibit osteoclast resorption to promote bone regeneration and mineralization [180]. Clindamycin is also an antibiotic drug that inhibits bacterial protein synthesis. Local delivery of clindamycin promote OPG, BMP-5 expression, and inhibit the expression of inflammatory factors IL1, IL6, and TNF- $\alpha$ , thereby promoting osteogenesis and inhibiting osteoclasts [181]. Human cathelicidins exert the antibacterial effect by destroying the cell membrane of bacteria, and are capable of neutralizing microbial pathogens such as lipopolysaccharide and flagellin. In addition to antimicrobial functionality, human cathelicidins regulate the local microenvironment by inhibiting RANKL-dependent osteoclastogenesis activated by infection [175]. Local delivery of these antimicrobial agents therefore has the effect of simultaneously inhibiting bacteria and regulating bone repair. However, the effects and specific mechanisms of these drugs in regulating cell activity still need to be confirmed and clarified in future studies.

##### 4.2. Dual drug delivery system with dual functions

###### 4.2.1. Interaction between loaded drugs

By loading the antimicrobial and osteogenic agents into one device, the two phases of treatment can be combined to establish codelivery systems. An ideal dual drug delivery system must locally release the two drugs at the appropriate biological time point needed for osteogenic repair to provide a bacteria-free and osteogenic-inducing microenvironment [182,183]. However, the codelivery system reveals problems, in that the two drugs may interact with each other, and in particular, antimicrobial agents with an appropriate therapeutic concentration may

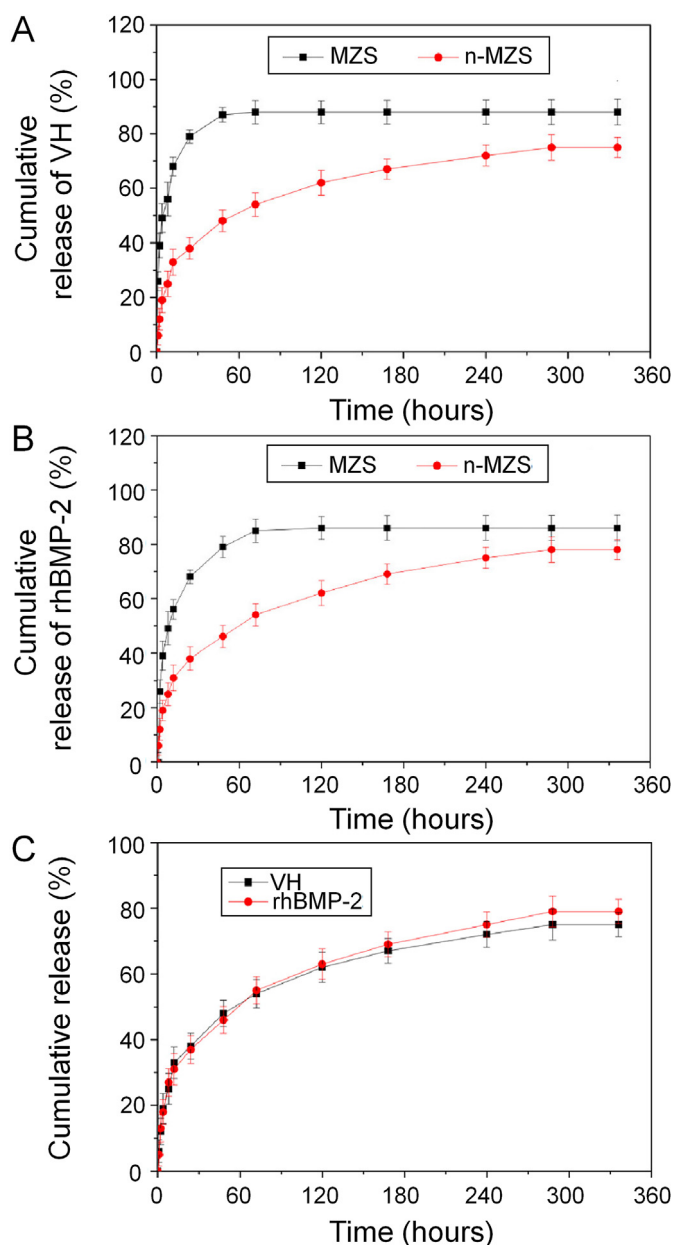




**Fig. 7.** Hinokitiol loaded nano-BG(nBG)/PEEK scaffold (dmBPC) [179]: A. SEM images of the macroporous PEEK (mPK) (a, d), macroporous nBG/PEEK (BPC) (b, e) and macro-microporous/nBG/PEEK (mBPC) (c, f); B. Confocal laser scanning microscope images of mBPC and dmBPC cultured with *S. aureus* for 24 h; C. Colony number of viable bacteria on the surface of mBPC and dmBPC after culturing with *S. aureus*; D. Effect of composite scaffolds on ALP enzyme activity of MC3T3-E1 cells; E-F: Statistical analysis (E) and images (F) of positive areas of BMP-2 immunohistochemical staining at 1 (a-d) and 3 (e-h) months after implantation of scaffold (a, e represent mPK; b, f represent BPC; c, g represent mBPC and d, h represent dmBPC) into the femur of a rabbit (scale bar = 200  $\mu$ m). Reprinted with permission from Ref. [179]. Copyright 2018, Elsevier Ltd.

reduce the bone induction ability of bone-promoting growth factors [184]. For example, HACC with 95–98% degree of substitution has a good antibacterial effect, and its minimum inhibitory concentration has been proved to be 40  $\mu$ g/ml. The study of the dual drug delivery system constructed by combining HACC with BMP-2 confirmed that after 40  $\mu$ g/ml HACC co-cultured with MC3T3-E1 for more than three days, the osteoinductive effect of BMP-2 would be significantly reduced [185]. This reason might be that the antibacterial substances directly affect the biological activity of BMP-2 or change the related receptors of BMP-2 after interacting with cells. Therefore, the selection of drug types is the cornerstone for the construction of a dual drug delivery system. A combination of vancomycin and BMP-2 has been demonstrated in numerous studies to be an appropriate choice for a dual drug delivery system.

Vancomycin is a first-line agent in the clinical treatment of osteomyelitis, and BMP-2 has been shown to be a potent osteogenic growth factor [186, 187]. In the drug delivery system with nanoporous magnesium–zinc–silicon as the carrier, the co-release profile of BMP-2 and vancomycin is not significantly different from that of the two drugs alone, which proves that vancomycin and BMP-2 have no mutual influence on drug diffusion and release (Fig. 8) [188]. Moreover, vancomycin and the BMP-2 double-drug-loaded silicon-imprinted calcium phosphate scaffold and BMP-2 single-drug-loaded silicon-imprinted calcium phosphate scaffold both significantly promote new bone formation of rat tibial defects. The amount of new bone formation between the two is comparable, suggesting that vancomycin with appropriate dose would not damage the osteogenic induction ability of BMP-2 [189]. Similarly,



**Fig. 8.** Individual and co-release profiles of vancomycin and BMP-2 [188]: A. Release profiles of vancomycin from magnesium–zinc–silicon carriers without pores (MZS) and nanoporous magnesium–zinc–silicon carriers (n-MZS); B. Release profiles of BMP-2 from MZS and n-MZS; C. Co-release profiles of vancomycin and BMP-2 from n-MZS. Reprinted with permission from Ref. [188]. Copyright, the authors, published by Dove Medical Press Ltd.

vancomycin can also play a good antibacterial role in this dual drug loading system. Lu et al. [190] uniformly coated graphene on the Ti scaffold by poly-dopamine coating, and then anchored the vancomycin and BMP-2-loaded gelatin microspheres on the coating through functional groups of graphene to form a dual drug-delivery system. This dual drug delivery system promoted the proliferation of BMSC and the expression of ALP, and inhibited the activity of *Staphylococcus epidermidis*, thus promoting the formation of ectopic bone under the skin of rats.

Besides growth factors, peptides derived from various growth factors, such as BMP-2 peptides and peptides derived from extracellular matrix, also show good osteogenic activity with better stability, and thus can be used in combination with antibacterial drugs to construct the dual-function drug delivery system [191,192]. The technique of biopanning

by phage is an effective method to find tissue-specific peptides, so it will be of great clinical significance to screen peptides with stronger stability and bone inducing effect via this method [193]. Furthermore, the use of alendronate, simvastatin, icariin, and other osteogenic inducers with better biological stability than growth factors combined with antimicrobial agents is likewise an option [194–197]. However, the osteogenic induction effect of these drugs is not as strong as that of the growth factors, and high concentrations of these drugs inhibits osteogenesis.

#### 4.2.2. Optimal release modes of loaded drugs

After the appropriate drug is selected, the release modes of drugs will be another determining factor in the efficacy of the dual drug delivery system. The release mode of the dual drug delivery system is mainly dependent on the drug loading strategy. The loading strategy of directly and uniformly blending the two drugs with the carrier enables the two drugs to be released through free diffusion and material degradation [198]. Although these composite materials have been confirmed to exert therapeutic effects, this makes the release patterns of the two drugs identical, which renders impossible their individual regulation. For the treatment of infectious bone defects, initial treatment must focus on regulating the harsh microenvironment created by infectious and hyperinflammatory processes, followed by continuous osteogenesis promotion [183]. Therefore, the optimal release pattern of the dual drug-loaded system requires different release patterns, in which the antibacterial drugs are first appropriately and rapidly released to inhibit bacterial proliferation and regulate excessive inflammation, while osteogenic drugs have a delayed release profile, which is also consistent with the clinical treatment of resisting the infection prior to healing the bone [185,199]. Such a release profile can be achieved by loading the antimicrobial and osteogenic drugs in a different strategy with the carrier.

For this mission, the drug carrier material with core-shell structure marks one of the appropriate strategies. The antibacterial agent is loaded on the shell part of the material, while the osteogenic drug is loaded on the core part. The shell part first contacts the external environment such that the antibacterial agent can be released rapidly at the initial stage. The release of the osteogenic drug positioned on the core is blocked and delayed by the shell part, and continuous release is obtained after the shell part is degraded [194,195].

The layer-by-layer (LbL) self-assembled film is another alternative strategy. LbL have been demonstrated as a versatile drug delivery system for co-depositing multiple drugs on the surface of a substrate material at different levels [200]. Meanwhile, the fabrication processes, such as electrostatic self-assembly of LbL coatings, are generally conducted under mild conditions, which avoid severe reduction of biopharmaceutical activity due to harsh manufacturing conditions. The LbL film can achieve high drug loading on the nano scale, and the resulting nano-to-micron coatings can achieve independently adjusted multi-drug release kinetics [201,202]. Thus, coating the scaffold material with antibacterial and osteogenic drugs in a LbL self-assembly manner achieves the desired release mode. Min et al. [203] fabricated a dual drug-loaded multilayer delivery system comprising an underlying BMP-2 releasing part and an outer layer gentamicin releasing part on the PEEK surface by LbL deposition. The underlying BMP-2 layer was prepared by alternately soaking the scaffold material in 20 kDa of poly ( $\beta$ -amino ester), poly(acrylic acid) (PAA), and BMP2 solution, while the outer gentamicin layer was prepared by alternately soaking the scaffold material in 15 kDa of poly ( $\beta$ -amino ester), PAA, and gentamicin solution. The degradation rate of 15 kDa poly (beta-amino ester) is significantly faster than that of 20 kDa, and the release kinetics of the two drugs can be adjusted by simply changing the number of deposition layers or adjusting the physical properties of the structural components. The outer gentamicin layer also acts as a barrier to delay the release of BMP-2. Its drug release kinetics also confirmed a slight burst of gentamicin on the first day followed by a sustained release that remained above the minimum inhibitory concentration until complete elution. BMP-2 exhibited a relatively slow and continuous two-phase release, with early diffusion controlled release and



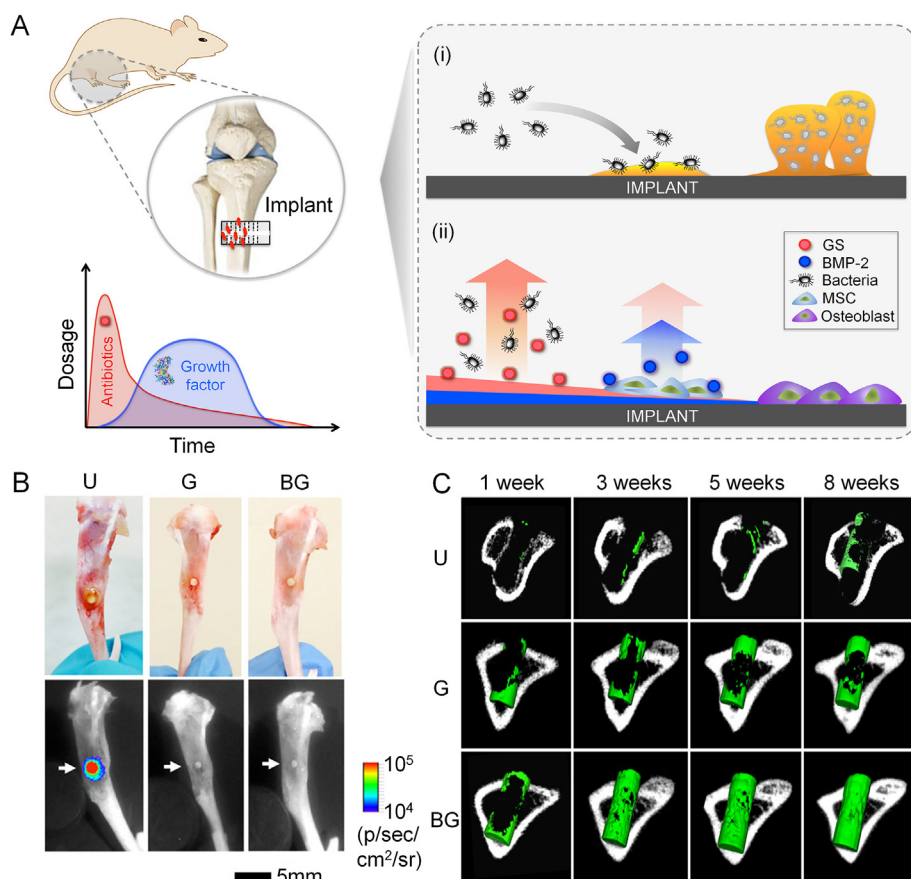
subsequent degradation controlled release until complete elution at 40 days. The composite scaffold inhibited the proliferation of *S. aureus* Xen 29 *in vitro*, and promoted the osteogenic differentiation of MC3T3 cells, playing an efficient antibacterial role *in vivo*, and promoting the repair of rabbit tibial osteomyelitis (Fig. 9).

## 5. Conclusion and perspectives

The treatment of contaminated or infectious bone defects has long been an intractable issue in orthopedic clinical practice. In the local area of an infectious bone defect, the excessive inflammatory reaction hinders bone regeneration. Moreover, bacteria can damage the extracellular matrix, inhibit osteogenic differentiation and the function of osteoblasts, and promote the osteoclastogenesis through a variety of extracellular and intracellular pathways, resulting in excessive bone resorption and damaged osteogenic ability. The rapid progress in the research spectra of bone tissue-engineered antibacterial and bone-promoting dual-functional materials is promising to simplify and resolve the treatment of infectious bone defects. Biomaterials with dual functions of anti-bacterial action and osteogenesis-promotion include functional biomaterials based composite scaffold and drug delivery systems that function through bioactive drug release. Material-based strategies involve mainly the combination of antibacterial and bone-promoting biomaterials, such as cationic antibacterial materials and calcium phosphate ceramic material, as well as functional metal nanoparticles, metal oxides, bioactive glass materials and photothermal materials with dual functions. These materials provide anti-infection activity and bone-promoting matrix at the defect site to inhibit the colonization and proliferation of bacteria, and promote the adhesion and proliferation of osteoblasts as well as extracellular matrix mineralization. The drug delivery system can be designed as a single antibacterial agent loaded on osteogenic materials, or the antibacterial agent itself has the ability to promote bone formation. This

can also be designed as a drug delivery system loaded with dual drugs. In dual drug delivery systems, it is necessary to consider selecting drugs that will not affect each other's activity and release profile, and design its release mode as rapid antibacterial substance release at an initial stage and delayed and sustained osteogenic drug release.

Although significant progress has been made in the research of materials with the dual functions of anti-bacterial activity and bone-promotion, there are several existing problems that must be addressed. As for the specific mechanism of infection on bone repair, most current studies focus on a certain aspect, which leads to a lack of a comprehensive understanding of infection in the complex bone repair. In the following studies, an overall interconnected model must be established to study the effect of infection on multiple aspects and their interaction of bone repair. Due to the generation of biofilm or the colony variation, the complete elimination of local pathogenic bacteria will become difficult and the recurrence rate of bone infection increase. Phage and its related agents are promising strategies to address these problems and construct dual-functional materials. Phages can play an antibacterial role by binding with bacterial receptors, so they can be designed according to that characteristics of biofilm and drug-resistant colony to exert specific and efficient antibacterial effect. In addition, novel antibacterial peptides can be developed by phage biopanning techniques to enhance the diagnosis and treatment of infection. At present, *in vivo* animal studies of dual-function scaffolds lack dynamic studies on the effects of materials for antibacterial activity and bone repair. By implanting fluorescence-labeled bacteria into the body, the antibacterial effect of materials *in vivo* can be studied through dynamic fluorescence imaging, and matching the antibacterial effect with the image of bone repair can provide evidence for designing the release profile of the dual-function scaffold. In addition, most studies have neglected the regulatory effect of materials on the excessive inflammatory response. Therefore, subsequent studies on the regulatory effects of materials on inflammation and the



**Fig. 9.** LbL-coated PEEK composite scaffold loaded with BMP-2 and gentamicin for treatment of infected bone defect [203]: A. Schematic representation of antibacterial and osteogenic properties of composite scaffold *in vivo* and ideal dual drug release profile; B. External image and bacterial bioluminescence image at eight weeks after the implantation of uncoated scaffold (U), gentamicin-coated scaffold (G), and gentamicin-and BMP-2-coated scaffold (BG) into the rat tibial infected by bioluminescent *S. aureus*; C.  $\mu$ CT reconstruction images of infected tibial defects implanted with U, G, BG. Reprinted with permission from Ref. [203]. Copyright 2016, American Chemical Society.



relationship between inflammation regulation and bone repair are desired. We believe that future in-depth research on current deficiencies will promote the dual-functioning scaffold of anti-infection and promoting bone regeneration from the bench to the bedside.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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