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# Biomaterial-Based Scaffolds as Carriers of Topical Antimicrobials for Bone Infection Prophylaxis

RAFAŁ B. LEWANDOWSKI*

Biomedical Engineering Centre, Institute of Optoelectronics, Military University of Technology, Warsaw, Poland

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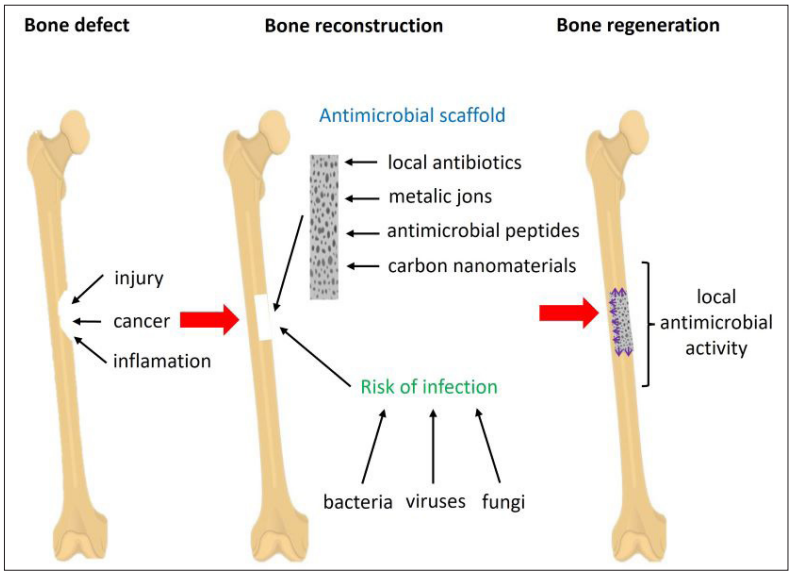
Abstract




In recent years, several modern therapies in orthopedics have been introduced, and these have been significantly influenced by the development of innovative medical devices made from various biomaterials. All orthopedic procedures involving the use of medical devices can lead to the occurrence of postoperative infectious complications, generally referred to as biomaterial-associated infections (BAIs). Currently, the classical antimicrobial treatment of BAIs consists mainly of systemic antibiotic therapy, which does not provide adequate clinical efficacy and is associated with the risk of many adverse effects. Therefore, numerous studies have been conducted to develop various methods to limit BAIs locally. Most of them involve the development of bioactive coatings or modified surfaces of biomaterials capable of releasing various antimicrobial substances. Applying such solutions in bone surgery is primarily related to the anti-infective protection of bone scaffolds, which is currently one of the most advanced and promising techniques in regenerative medicine. Using scaffolds in the damaged tissue provides an artificial structure that supports cell growth in the appropriate spatial configuration and restores the mechanical properties of the damaged bone in a short time. Therefore, the long-term protection of bone scaffolds against infection is crucial for achieving complete therapeutic success and currently represents one of the most significant challenges in bone surgery. This article presents selected strategies for modifying bone scaffolds that have been developed to reduce the risk of BAI.

Keywords: *antimicrobial scaffolds, bone infection, orthopedic device*

## Introduction

Over the past few decades, the use of medical devices has increased significantly in diagnostic and therapeutic applications in various areas of modern medicine. This is mainly due to modern technologies that allow the production of increasingly advanced biomaterials tailored to different functional applications. In addition, with the observed progressive aging of societies in various parts of the world, the demand for

* Corresponding author: R. B. Lewandowski, Biomedical Engineering Centre, Institute of Optoelectronics, Military University of Technology, Warsaw, Poland; **e-mail: rafal.lewandowski@wat.edu.pl**

medical devices that enable the rapid restoration of lost tissue or organ functions and contribute to a significant improvement in the quality of life has increased significantly and will continue to do so (Litowczenko et al. 2021; Biomaterials Market 2023). Of all the medical devices currently in use, orthopedic devices are the most widely used (Ratner et al. 2020). In recent years, several modern therapies have been introduced in orthopedics, thanks in part to the development of innovative biomaterials (Liang et al. 2024). The group of orthopedic medical devices includes fracture treatment instruments (wires, pins, screws, plates, spinal stabilization devices, and artificial ligaments), joint replacement instruments (for hip, knee, ankle, shoulder, elbow, wrist, and finger arthroplasty procedures), and other medical devices (reconstructive implants, arthroscopy products, and electrical stimulation products).

All orthopedic procedures involving the use of medical devices can lead to postoperative infectious complications. The highest risk of such primary complications is associated with procedures using biomaterials for stable osteosynthesis performed for urgent indications, especially for open fractures (Fang et al. 2017). Despite systemic antibiotic prophylaxis, the incidence of infection associated with osteosynthesis ranges from 1% to 7% in closed fracture treatment, and as high as 30% in open fracture treatment (Fang et al. 2017; Renz et al. 2017). However, in the case of primary implantation of prosthetic joints (hip, knee, elbow, and ankle prostheses), although these surgical procedures are performed under aseptic conditions, the frequency of periprosthetic infections does not fall below 1–2% (Schwarz et al. 2019). In addition, the recurrence rate of infections is very high, ranging from 33% to 35%, when revision surgery is required after implant failure (Rosas et al. 2017; Schwarz et al. 2019). This fact is related to the tissues' significantly worse anatomical and physiological condition, longer surgical time, and more extensive tissue damage caused during surgery (Momodu and Savalija 2023). In addition, the cost of revision surgery increases significantly, being approximately five times higher than primary arthroplasty and ranging from 80,000 to 95,000 euros (Kapadia et al. 2016).

The etiology of biomaterial-associated infections (BAIs) in orthopedics depends on the type of surgery (emergency or elective), the surgical technique, the operative conditions, the coexistence of inflammation in the surrounding tissues, and the overall condition of the patient (Cuérel et al. 2017; Pirisi et al. 2020). The most common etiologic factors of these types of infections are Gram-positive bacteria, mainly *Staphylococcus aureus* (33–43%) and *Staphylococcus epidermidis* (17–21%). In contrast, Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, or *Proteus mirabilis* are etiologic factors in only about 6% of BAI cases (Barros et al. 2022). However, antimicrobial treatment of implant infections caused by Gram-negative bacteria is more difficult and often results in worse outcomes than those caused by Gram-positive bacteria, especially when a two-stage implant exchange is required (Kalbian et al. 2020). In addition, specific etiological factors of BAIs have been shown to be related to the time elapsed since the primary implantation procedure. Early infections, with symptoms appearing less than three weeks after surgery, are dominated by highly virulent microorganisms such as *S. aureus*, *E. coli*, and *P. aeruginosa*. In contrast, delayed infections, which occur between 3 and 10 weeks after surgery, are caused by less virulent microorganisms, mainly coagulase-negative staphylococci such as *S. epidermidis* and anaerobes (e.g., *Propionibacterium acnes*). Late infections occurring more than 10 weeks after implantation result from hematogenous spread of microorganisms or may be due to improper treatment of early BAIs (Zimmerli 2014).

An important phenomenon in the course of BAIs from the perspective of the effectiveness of antimicrobial therapy is the ability of bacteria to form biofilms on the surfaces of implants (Rozis et al. 2021; Pietrocola et al. 2022). A biofilm is a well-specialized, multilayered structure reinforced by an extracellular matrix that protects bacteria from the influence of adverse external factors, including protection from the action of antimicrobial drugs (Ahmed et al. 2019; Rather et al. 2021). The susceptibility of bacteria within a biofilm to antimicrobial agents is usually significantly reduced compared to the same bacteria in planktonic form, which clinically leads to treatment failure and chronic infection and is a direct cause of higher mortality in patients with such infections (Høiby et al. 2011). In the case of BAI, the implantation of foreign material into tissues also induces a strong local immune response in the tissues in direct contact with the biomaterial and the surrounding tissues. Within a short period, a local inflammatory response occurs that leads to accelerated degradation of the implant surfaces, facilitating the adhesion of planktonic bacteria to the biomaterials and ultimately leading to biofilm formation (Ahmed et al. 2019).

The classical treatment of early BAIs in orthopedics currently includes surgical debridement of the infected tissue, systemic antibiotic therapy, and prosthesis stabilization. In contrast, in the case of late BAIs, implant removal is performed to eliminate the mature biofilm from the body in combination with systemic antibiotic therapy (Osmon et al. 2013; Shah et al. 2020). In both clinical situations, the systemic use of antibiotics as BAI therapy does not provide sufficient clinical efficacy because most antibiotics, despite high doses, do not reach the minimum inhibitory concentration in the tissues directly surrounding the implant (Valour et al. 2014). This is mainly due to the limited blood supply to the site of infection and, later, numerous areas of necrotic bone tissue. In addition, long-term systemic antibiotic therapy using high doses of drugs to achieve effective concentrations at the site of infection often leads to serious side effects, which can affect as many as 15% of all treated patients (Valour et al. 2014; Lau et al. 2018).

In order to reduce the risk of infection associated with the implantation of medical devices in orthopedics and to avoid the side effects of systemic antibiotic administration, numerous studies have been conducted over many years, developing various methods to limit biofilm formation on orthopedic implants. Most of them involve modifying biomaterial surfaces to achieve passive bacterial repulsion or obtain surfaces with antimicrobial activity (Kennedy et al. 2022). Modification of the surface of biomaterials to achieve inhibition or reduction of the early stages of bacterial adhesion and disruption of biofilm formation can be carried out by a variety of physical (e.g., plasma treatment) or chemical (e.g., adsorption, cross-linking, or chemical linkage) methods that allow alteration of the surface topography or its chemical properties while maintaining high biocompatibility rates. Active BAIs control strategies, on the other hand, primarily involve the presentation of antimicrobial agents embedded on the surface of biomaterials, in a coating or carrier, whereby these agents may exhibit action only directly at the biomaterial or be released into the implant environment (Olmo et al. 2020). Currently, the most commonly used antimicrobial agents for this purpose are antibiotics, metal ions, antimicrobial peptides (AMPs), carbon nanomaterials, chitosan, quaternary ammonium salts, ε-polylysine and others.

In recent years, various microbial products have increasingly been indicated in the context of effective anti-biofilm agents, among which, in addition to AMPs, biosurfactants, enzymes, and bioactive compounds are mentioned. These products are chemically diverse and have a broad spectrum of activity against biofilms. They can now be produced as synthetic products based on natural templates, which allows for products with good stability and chemical purity and enables large-scale production at much lower manufacturing costs than natural products (Al-Madboly et al. 2024; Hussaini et al. 2024). For this reason, shortly, the use of, among other things, the enzyme Dispersin B (DspB) as a potential antimicrobial agent, which can efficiently hydrolyze poly-β(1,6)-*N*-acetylglucosamine (PNAG), which is a polysaccharide of the biofilm matrix that plays an important role in bacterial attachment to surfaces and biofilm formation by many Gram-negative and Gram-positive pathogens, is being considered for modification of implanted medical device coatings (Kaplan et al. 2024).

Another interesting solution in the development of biomaterials with antimicrobial activity is the use of polymers that mimic host defense peptides to modify surfaces made of plastics (e.g., thermoplastic polyurethane), whose killing mechanism is based on disruption of the bacterial membrane upon contact with the surface (Lu et al. 2021). The simultaneous use of chemicals possessing bactericidal activity, which is further enhanced by physical factors, provides excellent opportunities for the antimicrobial activity of biomaterials. For example, reduced polydopamine nanoparticles (PDA NPs) are added to hydrogels, which have redox activity to transfer electrons to oxygen to produce reactive oxygen species (ROS). At the same time, near-infrared irradiation accelerates the release of ROS due to the photothermal effect of PDA NPs, which in effect increases the bactericidal effect (Sun et al. 2020).

In addition, innovative composite biomaterials with self-activating antimicrobial activity regulated by external or internal stimuli are increasingly being developed. One such example is composite hydrogels that respond to various changes in the metabolic microenvironment of bacteria, resulting in self-activation of a cascade of nitric oxide release capacity combined with chemodynamic therapy enhanced by nanozyme activity to achieve on-demand elimination of bacteria and biofilm (Yang et al. 2024).

However, due to the complexity and breadth of the work's thematic scope, this mini-review presents only selected active BAIs control strategies based on the presentation of antimicrobial agents embedded on the surface of biomaterials, in coatings or carriers.

**Orthopedic scaffolds with antimicrobial activity**

For many years, there has been a systematic increase in the demand for transplantation of damaged tissues and organs in various fields of surgical medicine, mainly due to advances in surgical techniques and greater accessibility to regenerative medicine procedures. The response to this demand has been the emergence of a new scientific field called tissue engineering, which has become an important adjunct to interventional medicine by, among other things, enabling the regeneration of living tissues and organs through cell culture (Ashammakhi et al. 2022). Tissue engineering methods are also used for the needs of orthopedic and trauma surgery, which primarily involves providing an appropriate environment for cell growth, protection from microorganisms, providing additional structures that support cell growth in the correct spatial configuration, and restoring the mechanical properties of the damaged bone in a short period (Sarigol-Calamak and Hascicek 2018). Cells must grow in three dimensions to form an organ or tissue, so porous spatial structures, called scaffolds, that simulate the extracellular matrix of tissues are used in tissue engineering. Due to their high biocompatibility and in combination with various therapeutic agents, these structures can facilitate the attachment of cells from surrounding tissues to their surface, stimulate cell proliferation and differentiation, and protect the growing cells from infection (Dorati et al. 2017). Using orthopedic scaffolds can significantly reduce the regeneration time of damaged bones and restore lost tissue functionality.

Studies conducted by many authors to date have shown that scaffolds that locally release various antimicrobial substances can significantly reduce the occurrence of infectious complications in orthopedic procedures. The use of appropriately selected amounts of antimicrobial agents along with the selection of the appropriate release rate from the scaffolds can help reduce the frequency of recurrent infections (Dorati et al. 2017; Sarigol-Calamak and Hascicek 2018). The results of many previous studies indicate that one of the more effective ways to regulate the dosing of antimicrobial substances to prevent or combat bone infections is their local release at the site of damage from a nanomaterial embedded in a scaffold, which serves as a carrier that regulates the release profile of the active substance (Afewerki et al. 2020). Therefore, it should be noted that any newly developed scaffolds with antimicrobial activity will require extensive microbiological studies, both *in vitro* and *in vivo* in experimental animals, before being approved for use in patients.

Scaffolds that have been designed and fabricated as carriers of local antimicrobial agents for orthopedic applications have been made from polymers (Lee et al. 2020; Aslam Khan et al. 2021a), ceramics (Zhao et al. 2022; Atkinson et al. 2024), metals (Kiselevskiy et al. 2023), or composites (Cui et al. 2022). Current techniques used to prepare scaffolds for bone tissue engineering include traditional preparative techniques such as electrospinning, freeze drying, solvent casting/particle leaching, and additive manufacturing techniques such as fused deposition modeling, selective laser sintering, stereolithography, 3D and 4D bioprinting (Qin et al. 2024). However, the techniques currently used to apply bone scaffolds include implantation of a prefabricated scaffold created prior to the surgical procedure (Shen et al. 2022; Lu et al. 2024), *in situ* scaffold formation using an injectable system (Zhang et al. 2023; Ghiasi Tabari et al. 2024), and 3D printing directly at the site of injury in clinical conditions to create or repair living tissues or organs (*in situ in vivo* bioprinting) (Li et al. 2020; Mahmoudi et al. 2023).

**Scaffolds with antibiotics.** Controlled release of antimicrobial drugs from scaffolds designed for bone tissue engineering is one of the methods used to reduce the risk of infectious complications following medical device implantation procedures. This method makes it possible to achieve high antimicrobial efficacy at the site of medical device implantation using relatively low concentrations of antibiotics, which significantly reduces the occurrence of microbial resistance. To date, most studies on the antimicrobial activity of antibiotic-releasing scaffolds have been conducted with glycopeptide antibiotics, mainly vancomycin (VAN) and aminoglycosides e.g. gentamicin (GEN) (Hassani Besheli et al. 2018; Zhou et al. 2018b; Budiatin et al. 2021).

In studies using a biodegradable 3D-printed scaffold made of polylactic acid (PLA) combined with hydroxyapatite (HA) with VAN, the scaffold was found to have sufficient antibacterial and antibiofilm activity against *S. aureus*, and the antibiotic was released for more than 7 days as the polymer degraded (Pérez-Davila et al. 2023). In the case of scaffolds made of mesoporous bioactive glass combined with poly(lactic-co-glycolic acid) (PLGA) with VAN, the antibiotic was shown to be released from the scaffold for more than 8 weeks, resulting in inhibition of *S. aureus* growth and biofilm formation *in vitro* (Cheng et al. 2018). In other studies of 3D polycaprolactone (PCL) composite scaffolds coated with polydopamine, VAN-loaded PLGA microspheres were adsorbed

(Zhou et al. 2018b). The antibiotic was shown to be released from the scaffolds *in vitro* for more than 4 weeks, and its effective bactericidal activity against *S. aureus* was observed throughout this period. In the *in vivo* studies on laboratory animals conducted by Zhou et al. (2018a), the efficacy of scaffolds made of gelatin and β-tricalcium phosphate (β-TCP) filled with vancomycin in clearing infections and repairing bone defects in the course of osteomyelitis was investigated. The results showed that the antibiotic was released from the scaffolds over 8 weeks, resulting in the elimination of the bone infection and the rapid repair of bone defects without complications. Other studies have also used gelatin and β-TCP scaffolds, combined with chitosan microspheres containing GEN (Liu et al. 2022). In this case, the antibiotic released from the scaffolds initially rapidly and effectively inhibited bacterial proliferation. Then, it was released at lower concentrations in the later phase to provide anti-infective protection during bone formation.

In some studies, scaffolds of different biomaterials were loaded with selected fluoroquinolone antibiotics such as ciprofloxacin (CPFX) and levofloxacin (LFX). It was observed that under *in vitro* conditions, approximately 70% of the antibiotic was released from the scaffold within 14 days, ensuring high antibacterial efficacy against *S. aureus* and *E. coli*, and the scaffold underwent spontaneous biomineralization leading to osteoinduction. In other studies, the *in vitro* antibacterial activity of monticellite (calcium-magnesium silicate ceramic) bone scaffolds containing different doses of CPFX was determined (Bakhsheshi-Rad et al. 2019). In the case of a scaffold containing 6% CPFX, 100% inhibition of *S. aureus* and *E. coli* growth was demonstrated after 48 hours of incubation, and the antibiotic was released from the scaffold in effective concentrations for at least 16 hours. In the studies conducted by Wei et al. (2019), the antibacterial properties of a bone scaffold made of a bioactive nanohydroxyapatite/polyurethane composite with LFX-loaded mesoporous silica microspheres immobilized on its surface were investigated. The release of the antibiotic from the scaffold *in vitro* was maintained for 42 days at concentrations that caused the death of 99.99% of *S. aureus* and *E. coli* cells.

Most studies evaluating the antimicrobial efficacy of antibiotic-releasing biomaterials have shown that these substances released at the implant site effectively kill bacteria and prevent biofilm formation. This therapeutic approach has made it possible to reduce the extent of systemic antibiotic use in most orthopedic procedures, which in turn has slowed the buildup of antibiotic resistance associated with this type of clinical situation. However, optimizing antibiotic release from biomaterials to minimize the risk of drug resistance remains the most serious problem in this area. On the one hand, too rapid release of antibiotics from the surface of biomaterials can result in toxic effects. On the other hand, gradual, continuous release of antibiotics over too long a period can result in antibiotic resistance in specific clinical situations, even though in most cases it allows better reduction of bacterial proliferation. Therefore, in recent years, many researchers have indicated that shortly, precise control of antibiotic release from biomaterials may be possible through the implementation of innovative reactive coatings that are designed to release antibiotics in response to detecting changes in the microenvironments infected with bacteria (the drug release profile depends on changes in the pH of the surrounding environment, for example).

**Antimicrobial scaffolds with metal ions.** Another way to achieve a local antimicrobial effect at the site of bone scaffold implantation is to incorporate into its structure ions of selected metals or chemical compounds of these metals that have previously been shown to have antimicrobial activity *in vitro*. There are many potential options in this area. Currently, they are most commonly based on the use of silver ions ($Ag^{+}$), zinc ($Zn^{2+}$), magnesium ($Mg^{2+}$), as well as zinc oxide (ZnO) and titanium dioxide ($TiO_2$). Less commonly, selenium (Se) and strontium ($Sr^{2+}$) ions and copper oxide (CuO) are used. It should be noted, however, that the incorporation of metal ions or their compounds into the structure of bone scaffolds requires exact control of the released concentrations of these chemicals, since exceeding specific doses can cause local toxic effects (e.g., cell necrosis) or systemic effects (damage to the brain, spleen, liver, and other organs), as presented in the review by Gulati et al. (2021). Therefore, for many years, the majority of research in this area has focused on the use of metals or their oxides in the form of nanoparticles (NPs), which are more effective at low concentrations due to their higher surface-to-volume ratio, and also possess other specific properties such as high reactivity and greater diffusivity in tissues. In addition, the use of nanocarriers loaded with antimicrobial agents allows for prolonged release of antimicrobial agents and precise regulation of their concentrations, which in the case of metals or their oxides, allows for effective antimicrobial action while avoiding toxic effects, as discussed in the review by Agnihotri and Dhiman (2017).

Among all metal nanostructures, silver nanoparti-

cles combined with various organic or inorganic carriers are most commonly used to create scaffolds for bone surgery with antimicrobial activity. For example, Zhang et al. (2017) used silver nanoparticles (AgNPs) uniformly dispersed on graphene oxide (GO) to create a homogeneous nanocomposite, which was successfully modified on 3D-printed bioceramic scaffolds with β-TCP. The resulting bone scaffolds were not only effective in killing bacteria, but also had a positive effect on osteogenesis by promoting the expression of an osteoblast-related gene in bone marrow stem cells. In another study, a nanocomposite of cellulose nanowhiskers decorated with AgNPs was used to create bone scaffolds containing chitosan and carboxymethylcellulose, achieving high antimicrobial activity of the scaffold against Gram-positive and Gram-negative bacteria (Hasan et al. 2018). Chitosan is often used to fabricate bone scaffolds with metals due to its biodegradability, excellent biocompatibility and bioactivity, and very good antimicrobial properties. Cao et al. (2018) developed a composite scaffold of double hydroxide/chitosan with a MgSrFe layer, which was uniformly loaded with AgNPs on the surface. It was demonstrated that the scaffold effectively prevented the formation of *S. aureus* biofilm due to the released Sr ions and AgNPs, and also exhibited good osteogenic properties. In other studies, the synergistic antimicrobial effect of chitosan, HA, and silver nanowires was exploited to create a composite bone scaffold that could not only inhibit the growth of bacteria in suspension, but also completely prevent the formation of biofilm by methicillin-resistant *S. aureus* (MRSA) strains (De Mori et al. 2019).

Many studies to date have confirmed that Zn-based biomaterials support bone repair by promoting cell proliferation, osteogenic activity, angiogenesis, and inhibiting osteoclast differentiation. They also have very good antimicrobial properties, as discussed in an extensive review by Wen et al. (2023). Zn-doped bone tissue scaffolds are fabricated using various biomaterials. Felice et al. (2018) developed nanofibrous scaffolds made of PCL combined with HA and different concentrations of ZnO, and demonstrated that these scaffolds accelerated bone tissue regeneration and exerted an effective antibacterial effect on *S. aureus*. In other studies, zinc oxide nanoparticles (ZnONPs) were incorporated into a porous poly(3-hydroxybutyrate-co-3-hydroxyvalerate) scaffold (Shuai et al. 2020). The addition of ZnONPs to the biomaterial used increased its crystallinity, thereby improving its mechanical properties, and the $Zn^{2+}$ released from the scaffold effectively inhibited the growth of *E. coli*. In addition, the scaffold promoted cell behavior regarding proliferation and differentiation toward bone tissue. Sehgal et al. (2016) proposed rigid nanocomposite scaffolds for functional bone regeneration, which were prepared from biodegradable gellan and xanthan polymers reinforced with bioactive glass nanoparticles and additionally crosslinked with zinc sulfate ions to enhance their osteoconductive and antimicrobial properties. The scaffolds were shown to significantly inhibit the growth of Gram-positive bacteria, *Bacillus subtilis* (70% reduction), and Gram-negative bacteria, *E. coli* (81% reduction), and caused a 62% increase in alkaline phosphatase (ALP) activity expression and a 150% increase in calcium deposition. Another interesting solution was the development of 3D-printed antibacterial bone scaffolds consisting of PLA and halloysite nanotubes (HNT) filled with zinc nanoparticles and additionally coated with an outer layer containing gentamicin (Luo et al. 2020). Again, in addition to high osteoinductive potential, *S. aureus* growth was inhibited even when the scaffolds were stored at 37°C for 3 weeks.

In addition to the metals mentioned above, it is worth mentioning the increasing use of Mg as a component of bone scaffolds. One example is the porous forsterite scaffolds with antibacterial properties produced by combining 3D printing and Mg-containing polymeric ceramics, which, in addition to a uniform macroporous structure and high compressive strength, strongly inhibited the growth of *S. aureus* and *E. coli in vitro* (Zhu et al. 2020).

Antimicrobial coatings of biomaterials containing metal ions have been an alternative to antibiotic-releasing coatings for many years, as they reduce the risk of developing drug-resistant bacteria and exhibit long-term antibacterial activity. In particular, combinations of different metal ions make achieving a broad antimicrobial and antifungal spectrum possible. In addition, these coatings show osteointegration-promoting effects and usually exhibit excellent biocompatibility. The disadvantage of this type of coating in the case of long-term release of metal ions is the risk of excessive accumulation of these particles in the surrounding tissues, which, once specific concentrations are exceeded, can lead to somatic cell damage. In addition, metal nanoparticles released from biomaterial coatings can travel with the blood to tissues and organs throughout the body, where they can cause oxidative damage when they reach certain concentrations.

**Scaffolds with antimicrobial peptides.** AMPs are bioactive small proteins composed of 10 to 50 amino

acids, characterized by a unique antimicrobial mechanism of action that is highly effective against various species of bacteria, viruses, and fungi, but different from the mechanism of action of classical antibiotics, which has been described in detail in numerous reviews by, for example, Caplin et al. (2019), Moretta et al. (2021), Li et al. (2025). AMPs are used, among other things, to create antimicrobial scaffolds intended for bone surgery, dentistry, and as an additive to modern dressings that protect wounds from infection, which was very well presented in their review by Min et al. (2024).

Biomaterials with antimicrobial activity containing AMPs have many advantages, as these substances exhibit many different mechanisms of antimicrobial action, act synergistically with many antibiotics, can be readily incorporated into various biomaterials, and can be released into surrounding tissues in a controlled manner and provide effective local concentrations. AMP molecules can be incorporated into bone scaffolds by attaching them to the surface or embedding them within the scaffold structure. For example, Chen et al. (2019) modified the surface of PLGA-based bone scaffolds by incorporating bone morphogenetic protein-2 and ponericin-G. The authors demonstrated better adhesion of mouse preosteoblast cells (MC3T3-E1) to the scaffold surface, and an increased proliferation rate and faster calcium deposition in these cells. In addition, the scaffold exhibited long-lasting antibacterial activity against *E. coli* and *S. aureus*.

On the other hand, Tian et al. (2020) developed 3D-printed polycaprolactone/hydroxyapatite (PCL/HA) composite scaffolds whose surface was modified with ε-poly-L-lysine, achieving not only excellent biocompatibility and osteoconductivity, but also very good antibacterial activity against *S. aureus*, *E. coli*, and *Streptococcus mutans*. However, among the studies in which AMPs were incorporated into the interior of bone scaffold structures, the research of Karamat-Ullah et al. (2021) can be cited, who developed a series of antibacterial and biocompatible 3D scaffolds combining antibacterial silk fibroin modified with AMPs and silica, and demonstrated that this hybrid scaffold exhibited bactericidal activity against both Gram-positive and Gram-negative bacteria and was biocompatible with MC3T3-E1 cells. In other studies, a hybrid scaffold based on intrafibrillar mineralized collagen coated with AMP derived from human salivary protein was developed (Ye et al. 2021). The scaffolds exhibited high antibacterial activity against *E. coli* and *Streptococcus gordonii* bacteria over an 8-day incubation period *in vitro* and were also not cytotoxic to human bone marrow-derived mesenchymal stromal cells (hBM-MSCs). Another interesting idea in this regard was the development of a porous bone scaffold made of mineralized collagen containing embedded PLGA microspheres loaded with two synthetic antibacterial peptides: Pac-525 or KSL-W (He et al. 2020). The scaffolds benefited MC-3T3 cell proliferation and osteogenic differentiation, and exhibited excellent antibacterial activity against *E. coli* and *S. aureus* for 2 weeks.

Although many versions of AMP-containing bone scaffolds have been developed in recent years and some of their beneficial properties have been demonstrated in treating infectious bone defects, it should be noted that there are still many limitations that prevent the widespread use of such solutions *in vivo*. The antimicrobial activity of AMPs *in vivo* can be interfered with by various host factors such as proteases, apolipoproteins, DNA, host proteins, and glycosaminoglycans, as presented in the review by Mookherje et al. (2020). In addition, the relatively high production costs and technological difficulties in producing large quantities of AMPs by chemical or recombinant methods remain a significant limitation to the widespread use of AMPs in therapy (Gao et al. 2021; Chaudhary et al. 2023).

**Antimicrobial scaffolds with carbon nanomaterials.** Research on bone scaffolds developed based on different forms of carbon nanomaterials has been carried out for many years because these materials have a very high osteoconductive potential, can significantly improve the mechanical properties of other biomaterials, do not show cytotoxic effects on osteoblasts, and in addition, many of them have intrinsic antimicrobial activity which was very well presented in their review by Eivazzadeh-Keihan et al. (2019). In this brief review, only single studies in this field can be cited, referring to the classification of carbon nanomaterials according to their dimensions, i.e., 0D, 1D, 2D, and 3D.

As an example of the use of 0D carbon nanomaterials to develop antimicrobial bone scaffolds, Xu et al. (2021) fabricated osteoconductive, antibacterial porous membranes with high mechanical strength from PLA by direct electrospinning of microfibers impregnated with carbon quantum dots (CQDs). The authors demonstrated that when the CQD content was 1.5% relative to the weight of the PLA matrix, the reduction in the number of *S. aureus* and *E. coli* bacteria after 24 hours of incubation *in vitro* was 90.4% and 99.4%, respectively. This effect was probably caused by the synergism of membrane stress and oxidative stress induced by CQD. However, among the 1D carbon nano-

materials used for the development of bone scaffolds, the most research has been devoted to carbon nanotubes (CNT), which, in addition to very good antimicrobial activity, are also characterized by exceptional mechanical strength. These unique properties of CNTs have been exploited to fabricate composite bone scaffolds from various polymers and CNTs, resulting in a scaffold with better mechanical properties, which additionally facilitates the adhesion of BM-MSCs to the scaffold and accelerates their proliferation, growth, and differentiation into bone cells (Kandhola et al. 2023). For example, Shrestha et al. (2017) developed bioactive bone scaffolds made of nanotopographic polyurethane containing uniformly dispersed multi-walled CNTs and ZnONPs. The obtained scaffolds had, among others, very good mechanical strength, thermal stability, biodegradability, biomineralization, biocompatibility, and CNTs exposed on the surface of nanofibers ensured effective destruction of *S. aureus* and *E. coli* cells, most likely due to piercing the microbial cell membranes/walls and disrupting their nuclei.

However, most studies on the use of carbon nanomaterials for the development of antimicrobial bone scaffolds focus on using 2D nanomaterials, primarily GO and reduced graphene oxide (rGO), which exhibit different mechanical, electrical, and chemical properties. For example, Melo et al. (2020) developed 3D-printed fibrous bone scaffolds from PCL containing GO at various concentrations. They demonstrated an approximately 80% increase in *S. epidermidis* and *E. coli* mortality after 24 hours of contact with scaffolds containing 7.5% GO. In addition, PCL/GO composite scaffolds allowed adhesion, spreading, and colonization of human fibroblasts within 14 days of culture. Other studies have also confirmed that the addition of GO to various nanocomposite scaffolds at an appropriate concentration has a significant impact on achieving high antibacterial activity against Gram-positive and Gram-negative bacteria, promotes the proliferation of pre-osteoblasts, and has no cytotoxic effect (Aslam Khan et al. 2021a; 2021b). However, in the case of rGO, some researchers have exploited the excellent electrical conductivity of rGO to enhance antibacterial activity and increase cell viability through electrostimulation. Angulo-Pineda et al. (2020) developed 3D-printed composite bone scaffolds from PCL and rGO and applied an electrical stimulus at 30 V for 3 hours, achieving complete elimination of *S. aureus* on the scaffold surface and a 4-fold increase in the viability of hBM-MSCs attached to the conductive PCL/TrGO 3D scaffold compared to the pure PCL scaffold. The above studies certainly need to be repeated under *in vivo* conditions, considering the conductivity of the surrounding tissues and body fluids, to evaluate the possible adverse effects of direct electric current on tissues and organs, especially with exposures of several hours. In other studies, a mixture of tricalcium phosphate, gelatin, chitosan, and GO was used to create antimicrobial 3D bone scaffolds in which GO was subjected to *in situ* reduction (Cabral et al. 2019). The scaffolds functionalized *in situ* with rGO exhibited increased wettability and better mechanical properties, and enhanced calcium deposition on their surface and increased ALP activity over a 21-day incubation period. In addition, these scaffolds also exhibited very good antimicrobial activity without affecting osteoblast viability and proliferation.

Although many of these studies have confirmed the good antimicrobial activity of biomaterials containing various forms of carbon, their clinical applications remain severely limited due to the poorly understood mechanisms of antimicrobial action of these materials and the potential for cytotoxic effects over time. In addition, the vast majority of studies are *in vitro* only. Therefore, more extensive *in vivo* experiments are needed to evaluate the long-term adverse effects of carbon nanomaterial scaffolds, especially regarding changes in the stability of various somatic cells and their functionality. Some researchers indicate that one way to address the cytotoxicity of these materials may be to use effective methods to target them to specific tissues or cells to maximize the desired therapeutic effect and minimize side effects (Jayaprakash et al. 2024). An analogous approach should be used for biomaterials with embedded carbon nanomaterials with antimicrobial activity, using ligands specific to certain microorganisms.

## Conclusions

This review presents selected strategies for developing scaffolds with local antimicrobial activity for bone surgery. These solutions may significantly reduce the risk of BAI in the future. However, it should be noted that in most of these studies, the antimicrobial activity of the developed scaffolds was evaluated only *in vitro* under simple experimental models using classical microbiological methods. There is an urgent need to verify the results of these studies *in vivo* in experimental animals to consider the influence of various host-related factors on the antimicrobial activity of the substanc-

es used and, in the next step, to conduct clinical trials. In addition, some technologies used to produce the presented antimicrobial scaffolds are currently too expensive, which may be a significant barrier to the widespread use of these scaffolds as a therapeutic method accessible to all patients.

**ORCID**
Rafał B. Lewandowski https://orcid.org/0000-0002-7134-0809

**Conflict of interest**

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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