

Hydroxyapatite-Based Materials for Potential Use in Bone Tissue Infections

Katarzyna Szurkowska, Aleksandra Laskus and Joanna Kolmas

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.71604>

Abstract

Hydroxyapatite materials, due to their high biocompatibility, play a crucial role in orthopaedics and bone surgery as alternatives to autologous bone grafts. It was also found that coatings of metallic implants with hydroxyapatite layer improve significantly their osseointegration. Due to its bioactivity, osteoconductivity and non-toxicity, hydroxyapatite is also widely used as a component of hybrid biomaterials. The implantation of "foreign" materials brings one major concern that is the risk of potential bone tissue infections or chronic osteomyelitis. In turn, the main problem concerning bacterial infection treatment is to obtain an adequate, bactericidal drug concentration maintained for a sufficient period of time in the bone tissue. Therefore, recent developments of materials engineering are focused on delivery antibiotics directly into the affected bone. To achieve this goal, hydroxyapatite-based materials are frequently studied as carriers for antibacterial drugs. For effective support of antibiotic therapy, the antibacterial activity of certain ions (including silver, zinc or copper) may be applied. In our work, we present recent developments on ceramic materials for bacterial bone infections: hydroxyapatite-based carriers for antibiotics and modifications of hydroxyapatite with antibacterial ions. In this review, state-of-the-art and current applications of such materials are presented and discussed. We want to also present our recent results.

Keywords: hydroxyapatite, drug delivery, antibiotics, ionic substitution, antibacterial properties

1. Introduction

Hydroxyapatite (HA) is a material widely used in regenerative medicine, bone and dental surgery, conservative dentistry as well as implantology [1, 2]. HA resembles the main

inorganic component of mineralized tissues (biological apatite), which in combination with its non-toxic and, most importantly, osseointegrative properties makes it an asset for biomaterial engineering [3]. HA is considered to be the gold standard in bone tissue regeneration. In clinical practice, it is used in the form of powders or granules as filler for bone replacement or for repair of post-resection defects [4, 5]. HA is also successfully used as a coating material for metallic implants due to its bioactivity and favourable effects on the osseointegration process [6]. Porous structures may be used as temporary scaffolds for newly formed osseous tissue. In dentistry, HA is a component of dental materials such as dental cements and toothpastes [7]. Moreover, it has further uses in polymer/ceramic bone composite materials, not only as a bioactive material but also as a provider of desirable mechanical properties [8, 9]. Current research on HA bioceramics is conducted with a view to achieve two main goals: (1) to improve the biocompatibility of synthetic HA and (2) to provide synthetic HA with additional biological properties. The first goal can be achieved using partial ionic modification of synthetic HA. It should be stressed at this point that biological apatite is not pure hydroxyapatite, it is carbonated hydroxyapatite with a considerably reduced content of calcium and structural hydroxyl groups [10]. It also contains a number of various ions, primarily magnesium (Mg^{2+}), but also sodium (Na^+), potassium (K^+), zinc (Zn^{2+}), manganese (Mn^{2+}), silicate (SiO_4^{4-}) and hydrogen phosphate (HPO_4^{2-}). The "foreign ions" incorporated into the structure of HA contribute significantly to its properties such as the size of single crystals, agglomeration tendency and solubility.

New biological properties of HA may also lead to its enrichment with additional ions. For example, the introduction of strontium ions (Sr^{2+}) provides HA with antiresorptive properties, as the strontium ions have an inhibiting effect on the activity of osteoclasts, while also stimulating osteoblasts [11]. HA material containing selenites (SeO_3^{2-}) may be used in turn in bone tumour therapy [12]. Commercially available apatite material enriched with silicon ions (Actifuse[®]) contributes positively to osteogenesis by promoting the formation of bone and its natural remodelling [13].

Upgrading HA materials may be achieved using physical or chemical binding of drugs. Therefore, recent research on HA bioceramics focused on producing multifunctional materials, which, in addition to being used as scaffolds for growing tissue, could also release drugs directly into the bone in the affected area [14]. The literature describes research on HA as a delivery system for antiresorptive (e.g., bisphosphonates) and anticancer drugs (e.g., doxorubicin and cisplatin), as well as antibiotics mainly against perioperative and intraoperative infections [15–17].

This chapter presents so far achievements in the field of HA materials for bone tissue infections (see **Figure 1**). In addition to antibiotic delivery systems, herein the focus will be put on HA modified by ions with proved antibacterial activity. Further on, opportunities for developing multifunctional HA-based materials for applications related to prevention and treatment of bone infections will be discussed.

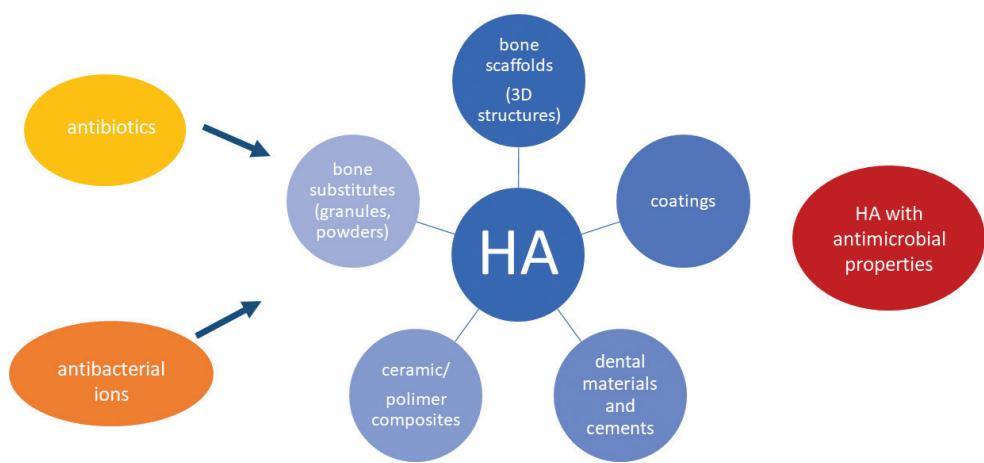


Figure 1. Scheme illustrating the main applications of HA and the ways to obtain antibacterial properties.

2. Hydroxyapatite-based antibiotic delivery systems

Bone tissue infections are one of the most frequently occurring side effects of bone surgeries. Such a complication may lead to severe bone loss, implant failure or even amputation [14]. Osteomyelitis, periodontitis and spondylodiscitis are important bone tissue infections [18, 19]. They are most commonly caused by infectious isolates of G-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus* spp.; G-negative bacteria: *Salmonella* spp., *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*; and fungi: *Candida* spp. The treatment of bone infections meets several serious clinical problems. Usually, antibiotic therapy involves 3 weeks of oral treatment followed by 3 weeks of intravenous therapy [19]. Bone tissue is poorly vascularized; thus, the antibiotic doses must be high enough to reach prolonged antibacterial concentration at the infected site. This high dosage of antibiotics may cause systemic toxic effects like nephrotoxicity, ototoxicity, hepatotoxicity, allergy or gastrointestinal syndromes [14].

Despite long, high-dose therapies, standard treatments of bone infections are still not effective enough. Due to the problems mentioned above, drug delivery systems targeting bones have been developed. The material frequently chosen as the system matrix is hydroxyapatite (HA).

Due to its porosity, HA may provide proper loading and long-term release of antibacterial agents, which is crucial for the antibacterial effectiveness of such a system. However, its poor mechanical properties (brittleness) have led scientists to combine pure HA with natural or synthetic polymers. Gelatine [20, 21], alginates [22–25], chitosan [25–27], collagen [28–30], polyvinyl alcohol (PVA) [31–33], polyacids [34–40] and cyclodextrins [41–43] are frequently

used to improve not only the properties mentioned above but also the stickiness of fabricated composite scaffolds, microspheres, etc. Thus, investigations into drug delivery systems loaded with antibiotics include the use of HA alone [44–63] and HA accompanied by other substances [20–40, 42, 43, 64–66].

The most frequently used antibiotics in local drug delivery systems are vancomycin (VAN) [18, 20, 21, 27, 33, 42, 48, 53, 60–63, 65] and gentamicin (GT) [18, 23, 25, 27, 35, 46, 57–59, 65]. These are also the most ubiquitously applied antibacterial agents in systemic therapy of bone tissue infections. Herein, the examples of antibiotic delivery systems based on HA and loaded with VAN or GT will be presented.

2.1. Vancomycin

Vancomycin (VAN) is used to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections in bone. The drug is administered parenterally; however, poor vascularization of bone tissues may cause insufficient local concentration of the antibiotic. Furthermore, severe side effects, such as ototoxicity and nephrotoxicity, are driving investigations into local delivery systems for VAN.

In one study [62], different materials characterized by various pHs were used to incorporate VAN. Namely, the investigations were focused on brushite cement ($\text{pH} = 2.4$), HA cement ($\text{pH} = 9.4$) and apatite xerogel ($\text{pH} = 7.4$). The influence of pH on the antibiotic release mode was analysed. The outcomes of the experiment revealed that pH affected the release kinetics. Despite the fact that the eluent from apatite cement exceeded the minimum inhibitory concentration (MIC), the system based on this material was ineffective against *S. aureus*. Yang et al. [27] covered metallic implants of bone with a chitosan/vancomycin composite. The composite's components were interconnected with hydrogen bonding. The electrochemical deposition technique was employed to cover the implant with a layer of composite. Next, the additional, external HA layer was placed on the implant. The kinetics of the antibiotic release from both type coatings were then compared. The kinetics showed that chitosan coating resulted in an impressive initial burst of a drug compared with the chitosan/HA composite. It may be concluded that the addition of HA has a significant impact on the prolonged release of the antibiotic.

The antibacterial activity of HA-based VAN-loaded delivery systems is usually examined *in vitro*. However, some studies involve *in vivo* tests to investigate the antibacterial effectiveness of fabricated systems. Joosten et al. [61] tested the antibacterial activity of VAN-loaded HA cement in *S. aureus*-induced chronic osteomyelitis. The infection was induced in the tibia of New Zealand white rabbits. The HA cement was an effective VAN carrier even for the treatment of MRSA.

Lian et al. [31] tested HA/collagen/calcium sulphate composites loaded with VAN also in rabbits. Bone infection was induced in the condyle lateralis femoris. After 12 weeks of implantation, micro-CT graphs have shown an excellent bone reconstruction with implants containing VAN (see **Figure 2**).

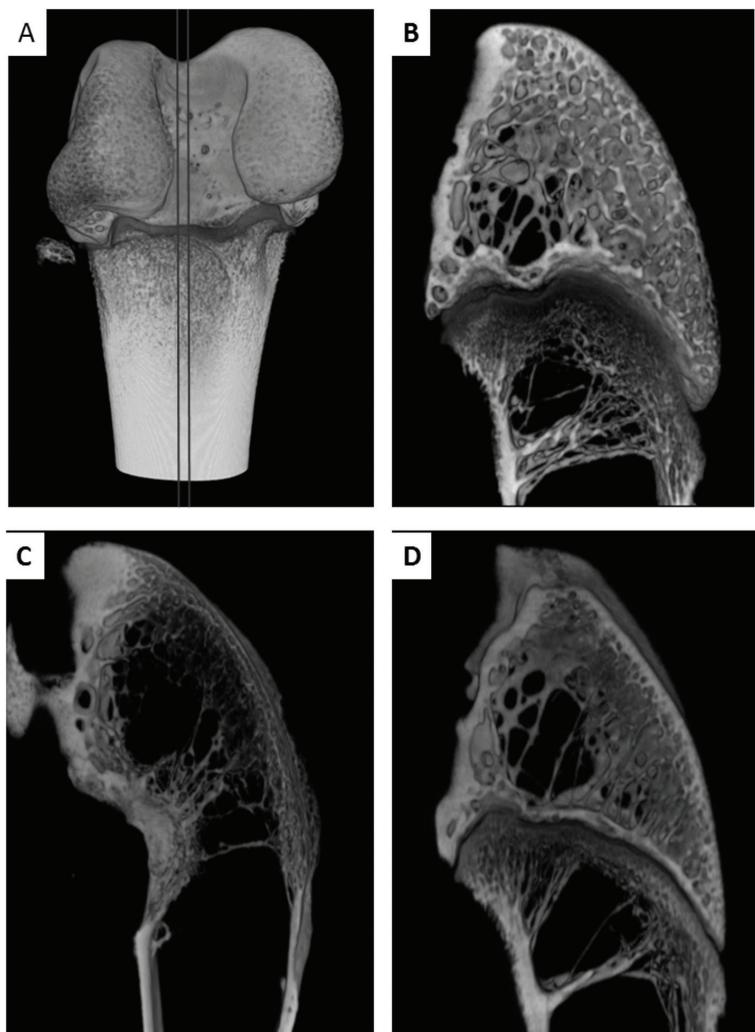


Figure 2. Micro-CT graphs taken 12 weeks after focal debridement. (a) Cross-section position (red line), (b) normal bone, (c) nHAC/CSH group, and (d) VCM/nHAC/CSH group. Abbreviations: nHAC/CSH – nanohydroxyapatite/collagen/ calcium sulphate composite VCM/nHAC/CSH – nanohydroxyapatite/collagen/calcium sulphate composite loaded with vancomycin. Reprinted from Ref. [31], the open access article distributed under the Creative Commons Attribution License.

Some commercial materials were also tested for their effectiveness as the matrices of antibiotic drug delivery systems [18, 53, 65]. Interesting outcomes were found by Rauschmann et al. [65] who compared PerOssal® and calcium sulphate (CS) as drug loading matrices. PerOssal® is a biodegradable composite consisting of nano-sized HA and CS. The pellets synthesized from

both materials were soaked in two antibiotics: VAN and gentamicin. The release of the drugs from the materials was studied. Surprisingly, PerOssal® demonstrated a higher initial release and a lower release of VAN after approximately 5 days, while in the case of gentamicin, the release mode from the materials exhibited no significant difference.

2.2. Gentamicin

Gentamicin (GT) is a broad-spectrum antibiotic from the group of aminoglycosides. It is mainly used in infections involving Gram-negative bacteria (i.e. *Pseudomonas* and *Enterobacter* spp.). Due to poor oral absorption, GT is commonly administered by injection. GT is frequently used as a model, antibacterial agent in HA-based drug delivery systems. Guo et al. [57] examined the influence of the HA's porosity on GT's loading. Mesoporous, carbonated HA microspheres exhibited a higher drug loading efficiency of 70–75% more than the conventional HA particles. It is important to note that the hierarchical nanostructure with developed meso- and microporosity allowed for an efficient loading of drug and, at the same time, a slow and sustained release of GT.

The association between porosity and drug loading was also studied by other researchers. To synthesize porous HA microspheres, the ice-template spray drying (ITSD) technique was applied by Yu et al. [34]. Drug loading efficacy increased with the increase of the porosity of the HA microspheres. Additionally, the transformation of the structure of the pores from cellular and independent ones to three-dimensional interconnected pore networks had a significant impact on the initial burst of the drug.

A hybrid material containing HA and covalently coated, hardly degradable keratin was described in Ref. [66] as an innovative system for GT delivery. The presence of keratin resulted in a greater immobilization of the antibiotic compared with HA/gelatine material. Moreover, the hybrid was non-toxic and stimulated osteoblast proliferation. It is important to note that the sustainable, prolonged GT release provided efficient antibacterial activity for at least 120 days.

2.3. Other antibiotics used in HA-based delivery systems

Among the other antibiotics applied as the model drugs in the systems targeting bones, penicillins, mainly amoxicillin [22, 37, 38, 44–46], cephalosporins [44, 46, 47], fluoroquinolones [33, 42, 43, 48, 49], including ciprofloxacin [33, 42, 43, 48, 49] and tetracyclines [28, 36, 51, 52], should be mentioned. In some studies, aminoglycosides (tobramycin and amikacin) [44, 46, 54], erythromycin (macrolides) [22, 32], tigecycline (glycylcyclines) [55, 56], linezolid (oxazolidinones) [64], rifapentine (ansamycin-like antibiotic) [41], clindamycin (lincosamides) [39, 40, 50], chloramphenicol [45] or chlorhexidine [67] (a bactericidal and bacteriostatic agent, not classified as an antibiotic) were used. The most interesting investigations concern loading more than one antibiotic into the same material [22, 42, 44–46, 48, 49].

Stigter et al. [44] compared the efficacy of the incorporation of different antibiotics into carbonated HA coatings on titanium implants. The outcomes showed that the incorporation rate

depends on the chemical structure of the drug. Antibiotics that contained a carboxylic group, such as cefalotin, carbenicillin or cefamandole, were better incorporated than the others. In addition, these drugs exhibited a slower release from HA coatings.

In turn, Ferraz et al. [22] loaded nanohydroxyapatite microspheres with amoxicillin, amoxicillin + clavulanic acid and erythromycin. Two types of microspheres, with varied porosity, were tested. The release profile from both types of microspheres consisted of a fast initial release followed by long-term sustained release. The microspheres with higher porosity and a greater surface area released more antibiotic during the first days. The antibacterial activity was tested against *S. aureus* and *Escherichia coli*. The obtained results have shown that the materials exhibited good, long-term antimicrobial activity.

Detailed study focused on HAs with controlled porosity and loaded with three antimicrobial agents (vancomycin, ciprofloxacin and gentamicin) were described in Ref. [48]. It was concluded that the adsorption of antibiotics was significantly higher in microporous HA than in crude dense discs. Moreover, the amount of adsorbed VAN was significantly higher than ciprofloxacin and gentamicin. Exposure to different bacteria species such as *S. aureus*, *Staphylococcus epidermidis* and *E. coli* demonstrated efficient antibacterial activity for all the materials. However, the microporosity of HA disc significantly prolonged the release of antibacterial agents.

A very interesting research was presented by Ghosh et al. [49]. HA cements were prepared with two types of nanohydroxyapatites and loaded with ciprofloxacin or VAN. Self-setting time reactions were controlled using the different weight ratios of the nanohydroxyapatites and had an impact on the release rate of antibiotics. The results have shown that, with modification of cement components, tuneable antibiotic release rates may be obtained. The biological tests presented good biocompatibility and non-toxicity to osteoblastic and osteoclastic cells.

The possibility of efficient fast loading of antibiotics in HA was studied by Brohede et al. [46]. The HA coatings on titanium implants were loaded with tobramycin, gentamicin, amoxicillin or cefalotin via soaking for varying periods of time (15 mins to 24 h). The results of antibacterial tests have shown that even the shortest loading time was sufficient to release enough drug for the next 24 h and inhibit bacterial growth.

3. Hydroxyapatite materials doped with antibacterial ions

The antibiotic resistance demonstrated by many bacterial species has stimulated attempts to produce new materials with efficient antibacterial properties. It is also important to note that implant-related/bone infections are caused by bacterial adhesion and biofilm formation. Biofilms are difficult to treat with standard antibiotic therapy. Thus, searching for new antibacterial strategies seems to be justifiable. As was mentioned above, HA doped with functional ions (i.e. Ag^+ , Zn^{2+} , Cu^{2+} , SeO_3^{2-}) may be applied for perioperative and intraoperative prevention and treatment of bone infections.

3.1. Silver-substituted hydroxyapatite

Silver exhibits a wide spectrum of actions against bacteria, viruses and fungi with a relatively low risk of resistance developing [68]. Silver compounds are effective against some common pathogens such as *E. coli*, *S. aureus* and *S. epidermidis* and, more importantly, methicillin- and vancomycin-resistant *S. aureus* (MRSA and VRSA) [69–72]. Other susceptible microorganisms include *Klebsiella pneumoniae*, *Providencia stuartii*, *Citrobacter freundii*, *Micrococcus luteus*, *P. aeruginosa*, *Pneumococcus* spp., *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* as well as yeasts *Issatchenkia orientalis* and *C. albicans* [73–76].

The main mechanism of action consists of the inactivation of microbial proteins through interactions with thiol groups ($-SH$) and the formation of inactive S-Ag bonding. Silver also affects bacterial DNA, precluding its replication. Another mechanism includes increased reactive oxygen species (ROS) production, leading to abnormally high permeability of microbial cells [68].

Silver-substituted HA (Ag-HA) can be obtained using several main synthesis methods, such as wet precipitation (using salts [74] or the neutralization reaction [77]), sol-gel technique [71, 78], hydrothermal method [79], electrochemical deposition [80] and magnetron sputtering [73]. Additional treatment includes sintering [81] or microwave assistance [72]. A wide range of silver substitutions have been investigated—from ultra-trace amounts such as 0.04 ppm [79] or 0.002 mole Ag per 1 mole HA [77] up to 10 wt.% [82, 83]. To better evaluate the relationship between silver concentration and physicochemical properties and the biological activity of Ag-HA samples, studies usually include a series of samples with various Ag contents.

The antibacterial activity of silver is dose-dependent and increases with higher silver concentrations. However, higher doses of silver increase the risk of severe cytotoxic effects to mammalian cells. HA with 10 wt% of silver was synthesized by Nath et al. [82] via the sintering of mechanically mixed powders at 1200°C. Biocompatibility was confirmed on mouse fibroblast (L929) and human osteosarcoma (MG-63) cells. Rajendran et al. [83] also confirmed >80% viability of NIH3T3 cells cultured on HA with 10 wt% Ag, but even 3 wt% Ag was sufficiently effective against *S. aureus*. However, Ag-HA nanocomposite coatings on Ti implants with 5 wt% content of metallic Ag exhibited cytotoxic effect on mice osteoblasts, while 2 wt% of Ag was both cytocompatible and inhibited growth of *S. aureus* [84]. These results are consistent with research by Yan et al. [80], where Ag⁺-substituted HA coatings with 2.03 wt% of silver exhibited optimal osteogenic and antimicrobial properties. According to Shi et al. [79], the optimal doping concentration of Ag ranges from 0.27 to 2.2 ppm. Lu et al. [85] also emphasized the importance of incorporating an adequate amount of the element to balance antibacterial activity and biocompatibility. Interestingly, heat treatment enhanced biocompatibility without decreasing antimicrobial properties. Another study indicated improved antibacterial activity against *S. aureus*, *K. pneumoniae* and *C. albicans* after thermal treatment at 600 and 1000°C [81].

Lee et al. [86] prepared nanocomposite fibres composed of Ag-doped HA and polyamide 6. Ag⁺ ions were loaded through the ion-exchange mechanism. HA was synthesized in agarose and ethanol medium to obtain the desired properties. Such composites exhibited excellent

antimicrobial activity against *K. pneumoniae* and *E. coli* while being slightly less effective against *S. aureus*. Further modification of the antibacterial fibre could extend the application field of Ag-HA, so far predominantly used in hard tissue injuries, to the treatment of skin diseases.

In Ref. [75], HA powders enriched in silver ions were used as coatings on a silicon previously covered with an elastomer, polydimethylsiloxane (PDMS). The antimicrobial activity was measured against *E. coli*, *S. aureus* and *C. albicans* strains. The obtained layers successfully inhibited microbial growth after 24 h of test (see Figure 3). Other polymer-based composites with polyvinyl alcohol [71], polyethylene glycol [78] and chitosan [87] were also examined.

Novel nanoscaffold biomaterials, based on porous HA, polyamide 66, titanium dioxide (TiO_2) and various concentrations of Ag^+ ions, were developed and thoroughly examined by Lu et al. [88]. Therapeutic effects of the biomaterial were tested *in vivo* on a large cohort of rabbits with osteomyelitis for 12 weeks. The treatment was successful, scaffolds exhibited both antimicrobial and anti-inflammatory effects and, in addition, stimulation of osteogenesis was observed. *In vivo* silver concentrations following implantation were under toxic levels and no failure of liver or kidney functions occurred.

Titanium discs coated with thermal sprayed Ag-HA (0.5–3.0 wt%) were tested *in vitro*, revealing a reduced ability of biofilm formation by a methicillin-resistant *S. aureus* strain. The effect was confirmed *in vivo* on rats with an MRSA-inoculated 3% Ag-HA disc implanted hypodermic for 7 days. No skin disorder (such as argyria) or wound healing complications were observed [89]. The reduction of viable MRSA by Ag-based coating on tibia implants was also

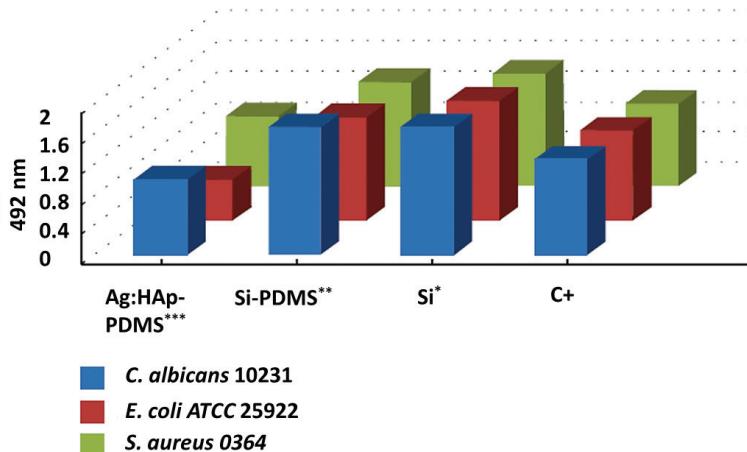


Figure 3. The graphic representation of the microbial activity of *S. aureus* 0364, *E. coli* ATCC 25922 and *C. albicans* 10,231 on Ag:HAp-PDMS layers on Si substrate, PDMS layers on Si substrate (Si-PDMS), and Si substrate (Si) at 48 h. *silicon substrate, **silicon substrate previously coated with PDMS, and ***Ag:HAp nanoparticles on a silicon substrate previously coated with a PDMS layer. Reprinted from Ref. [75], the open access article distributed under the Creative Commons Attribution License.

indicated *in vivo* on rat models [70]. An interesting study by Xie et al. [87] concerned the successful doping of bone morphogenetic protein 2 (BMP-2) into a nanosilver/hydroxyapatite/chitosan composite, which was then implanted into the femurs of rabbits. Favourable bone formation and antibacterial properties were demonstrated *in vivo*.

In 2016, the first clinical study was conducted on 20 human patients with total hip arthroplasty, in which a silver oxide (Ag_2O)-HA implant coating was used. The highest reported silver blood level following the surgery was far below the toxic level. For 1 year after surgery, no significant adverse reactions were observed and the coating prevented postoperative infection [90].

A popular strategy to further improve the properties of Ag-HA is to co-substitute additional ions. The most frequently studied combinations concern the addition of silicate SiO_4^{4-} ions (to improve osteogenic properties) [91] and strontium (Sr^{2+}) ions (to reduce silver cytotoxicity and boost antibacterial properties) [92]. Recently, Aksakal et al. [93] examined multiple HA substitutions with silver, zirconia and yttria, while Kolmas et al. [94] indicated that co-substitution of Ag-HA with carbonate (CO_3^{2-}) ions increased the solubility of samples, thus exhibiting greater antibacterial effect.

3.2. Zinc-substituted hydroxyapatite

Zinc (Zn^{2+}) ion substitution in biomaterials has been thoroughly investigated, for both its osteogenic [95] and antibacterial activities [96–104]. The mechanism of inhibition of microbial growth by zinc ions includes several aspects. Zn^{2+} ions cause damage to cell membranes by bonding with functional groups and increasing the permeability of cells. Moreover, zinc interacts with bacterial enzymes (such as ATPase, glycolytic enzymes or pyruvate kinase), disturbing their correct functionality [98, 103].

Recently, researchers have conducted in-depth investigations into the antibacterial action of zinc-substituted hydroxyapatites (Zn-HA). Samples with various levels of Zn^{2+} substitution were synthesized via the most common methods, namely co-precipitation [100, 102], ion exchange [99], sol-gel [104] and hydrothermal synthesis [101]. Anwar et al. [98] proposed a novel technique: continuous plastic flow synthesis (CPFS), which enables rapid production of HA nanocrystals with a high surface area. Electrospinning of fibres [96] and synthesis mediated by surfactant addition TritonX-100 [103] were also examined.

Common human pathogens used for testing antimicrobial activity were *S. aureus*, *E. coli* and *P. aeruginosa* [98, 100–102]. Individual works concerned the impact of Zn-HA on the growth of *Bacillus subtilis*, *Enterobacter aerogenes*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *S. mutans*, *Shigella flexneri*, *M. luteus*, *Bacillus cereus*, *Porphyromonas gingivalis* and yeast *C. albicans* [97–99, 101–104]. Biocompatibility of Zn-HA biomaterials was demonstrated *in vitro* on human osteoblast-like cells MG-63 [104], human adipose-derived mesenchymal stem cells (MSCs) [102], rat primary osteoprogenitor cells and fibroblast cells MRC-5 [101]. In some studies, the viability rate was better for Zn-containing samples than for pure HA [101, 102].

Thian et al. [102] proved that the addition of Zn^{2+} ions stimulated the bioactivity of HA, since the increased growth of MSC cells, as well as elevated expression of collagen type I and osteocalcin,

was observed in case of Zn-HA (1.6 wt% of Zn). Moreover, Tank et al. [103] indicated no significant haemolytic activity of Zn-HA on human blood. Bioactivity *in vitro* was proved by the ability of Zn-HA to form apatite crystals on samples soaked in simulated body fluid (SBF), which increased as the concentration of Zn raised.

Some research provides a comparison of antimicrobial activity against several pathogens. Radovanović et al. [101] investigated the inhibition of growth of *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans* caused by Zn-HA samples (0.2 and 0.4 mol%) and undoped HA. It was found that sintering the apatites at 1200°C, which led to partial decomposition to more soluble α -TCP, improved antibacterial activity of samples. All tested microorganisms were susceptible to Zn-HA and the degree of reduction increased with higher content of zinc ions.

Slightly different results were reported by Tank et al. [103] who focused on *P. aeruginosa*, *S. flexneri*, *M. luteus*, *S. aureus* and *B. cereus*. Zn substitution ranged from 1.3 wt% to 4.8 wt%. *S. aureus* was the most sensitive strain, even to undoped HA. *M. luteus* was also highly susceptible to Zn-HA samples, while both *B. cereus* and *S. flexneri* exhibited a moderate reduction in the number of colonies. In contrast, Zn-HA samples were ineffective against *P. aeruginosa*.

Several studies indicated that Zn-HA-based materials could also be suitable for the treatment of oral cavity bacterial infections. Zn-HA was effective in inhibiting the growth of common oral pathogenic strains, namely *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* and *S. mutans* [99]. Zn-HA coating on titanium implants demonstrated antibacterial properties against *Porphyromonas gingivalis*, a major cause of chronic periodontitis [104]. An additional advantage of Zn-HA used as an additive to toothpaste is protection against acid enamel erosion [105].

It should be noted that zinc is a popular dopant in multiple substituted HAs. Commonly examined combinations include Zn-HA with Ag⁺ [106] or Cu²⁺ [107] ions used to boost antibacterial activity, and Mg²⁺ [108], SiO₄⁴⁻ [109], Sr²⁺ [108] or F⁻ ions [110] for additional stimulation of the mineralization process.

3.3. Copper-substituted hydroxyapatite

The antimicrobial activity of copper is linked to its interaction with bacterial proteins, membranes and nucleic acids. An extensive review of antimicrobial applications of copper in the environment was provided by Vincent et al. [111].

Li et al. [112] synthesized copper-substituted HA (molar rate of Cu²⁺/Ca²⁺ up to 0.15) via ion exchange wet chemical reaction. Obtained materials exhibited a high antibacterial effect against *E. coli*, which increased with the concentration of Cu²⁺ ions. Unfortunately, all Cu-HA samples were cytotoxic to human foetal osteoblast (hFOB) cell lines.

Sahithi et al. [113] combined copper-soaked HA with polyethylene glycol 400 (PEG 400) to further extend its antimicrobial activity. Cu-HA exhibited antibacterial activity against *E. coli* and *S. aureus*, the effect of which increased against *S. aureus* after combination with PEG 400. MTT assay carried out on rat primary osteoprogenitor cells indicated cytocompatibility of the samples.

Antimicrobial activity of Cu-HA as well as Cu-FA (copper-substituted fluorapatite) was tested against *E. coli*, *S. aureus* and *C. albicans* [114]. The increase of copper substitution in hydroxyapatite enhanced activity against *S. aureus* and *C. albicans*, but Cu-HAs were not active enough against *E. coli*. Cu-FA was effective against all tested microorganisms with increasing activity in the following order: *C. albicans* < *S. aureus* < *E. coli*. Cu-FA may be more effective due to the release of fluoride ions. The same pathogen strains were used in Ref. [115], where the antibacterial activity of Cu-HA was compared with results for Zn-HA.

Radovanović et al. [116] compared Ag⁺- and Cu²⁺-substituted biphasic materials, based on ion-doped HA and α-TCP, obtained after annealing monophasic-substituted HA samples at 1200°C. For antimicrobial tests *in vitro*, *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* were used. Antimicrobial activity increased with the increase of ionic concentration. The activities of biphasic materials were very high and comparable in the case of Ag⁺ and Cu²⁺ substitution. The only difference was observed in monophasic Cu-HA against *C. albicans*, which was much less effective, especially with the smaller concentration of Cu²⁺ ions. *In vitro* biocompatibility was demonstrated on MRC-5 human fibroblast cells, but it should be noted that the addition of Cu²⁺ ions slightly reduced the viability of cells.

3.4. Selenium-substituted hydroxyapatite

Tran et al. [117] confirmed antibacterial properties of cellulose discs coated with organoselenium-methacrylate polymer against *P. aeruginosa* and *S. aureus*. 0.2 wt% of selenium completely inhibited bacterial attachment, growth and formation of a biofilm. Strong activity of selenium nanoparticles against *S. aureus* was confirmed by Tran and Webster [118]. These studies led to more research concerning the antimicrobial activity of selenium-based hydroxyapatite (Se-HA) [119–121]. Rodriguez-Valencia et al. [119] fabricated selenium-substituted carbonated HA coatings by the pulsed laser deposition method. Samples contained selenium in the form of selenite ion SeO₃²⁻. Coatings prevented the formation of biofilms by *P. aeruginosa* and *S. aureus* strains and reduced the number of colony-forming units (CFUs). Uskoković et al. [120] compared Se-HA obtained by co-precipitation and ion-exchange sorption methods. Selenite contents ranged from 0.3 to 3 wt% and the precipitation synthesis was about 10 times more effective in introducing selenium. Se-HA samples were strongly effective against *E. coli* and *S. aureus*, while being less effective against *Salmonella enteritidis* and ineffective against *P. aeruginosa*. Similar results were obtained by Kolmas et al. [121]. **Figure 4** illustrates significant bacterial growth inhibition caused by selenite anions. Moreover, selenium content was in correlation with the reduction of the viability of mouse osteosarcoma cells, and the induction of apoptosis was selective, without reducing the viability of fibroblast cells. Se-HA also exhibited osteoinductive effect by increasing the gene expression of pre-osteoblastic MC3T3-E1 cells. These promising results mean that selenium substitution in hydroxyapatite will probably get more popular in upcoming years.

In addition to the well-known elements with well-established antibacterial activity, some less popular elements for such a combination, like cerium, gallium, cobalt and strontium, should be mentioned [122].

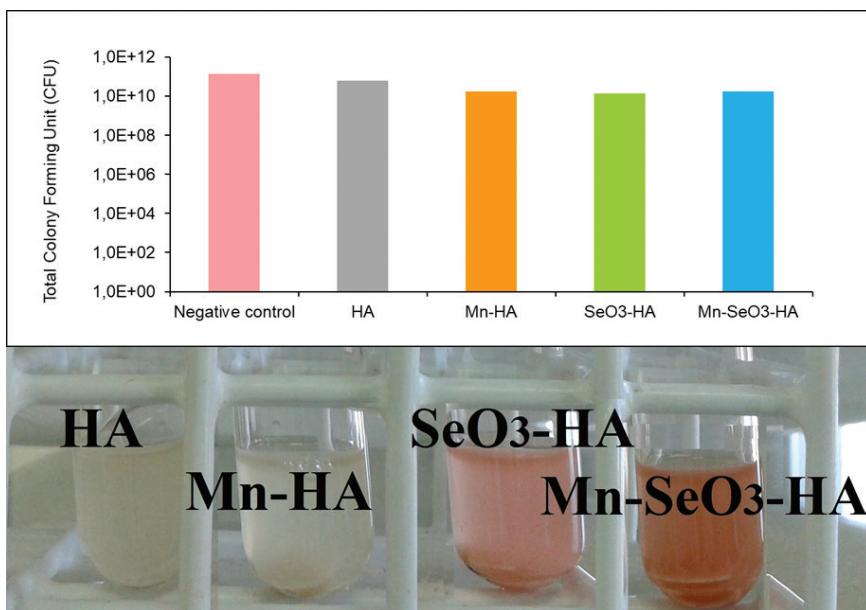


Figure 4. Antibacterials activity of the materials on *S. aureus*. Reprinted from Ref. [121], the open access article distributed under the Creative Commons Attribution License.

4. Hydroxyapatite with antibacterial ions and loaded antibiotics

Some hydroxyapatites enriched with antibacterial ions have been used to create systems containing antibiotics. Most of these systems have been developed using silver ions because of their strong antibacterial properties.

Ivashenko et al. [123] investigated the effect of silver ions in HA structure on the adsorption rates of ciprofloxacin. The research was carried out using commercially available materials such as Biomin G® (HA) and Biomin GIS® (HA enriched in an Ag⁺ amount of <0.1 wt%). Interestingly, the presence of silver ions in HA led to lowered specific surface area and significantly decreased adsorption rates of ciprofloxacin when compared with undoped material. Unfortunately, no research was done to test the antibacterial activity or release of silver ions or ciprofloxacin.

Another work [124] proposes long HA nanowires enriched with Ag⁺ ions and ciprofloxacin. The material performed high and long-term effectiveness against *E. coli* and *S. aureus*.

Ciprofloxacin and tetracycline were also adsorbed on a thin film made of Ag-HA [125]. *In vitro* microbiological tests have shown that thin films containing Ag-HA and selected antibiotics may become an effective solution in the prevention and treatment of bone infections (see **Figure 5**).

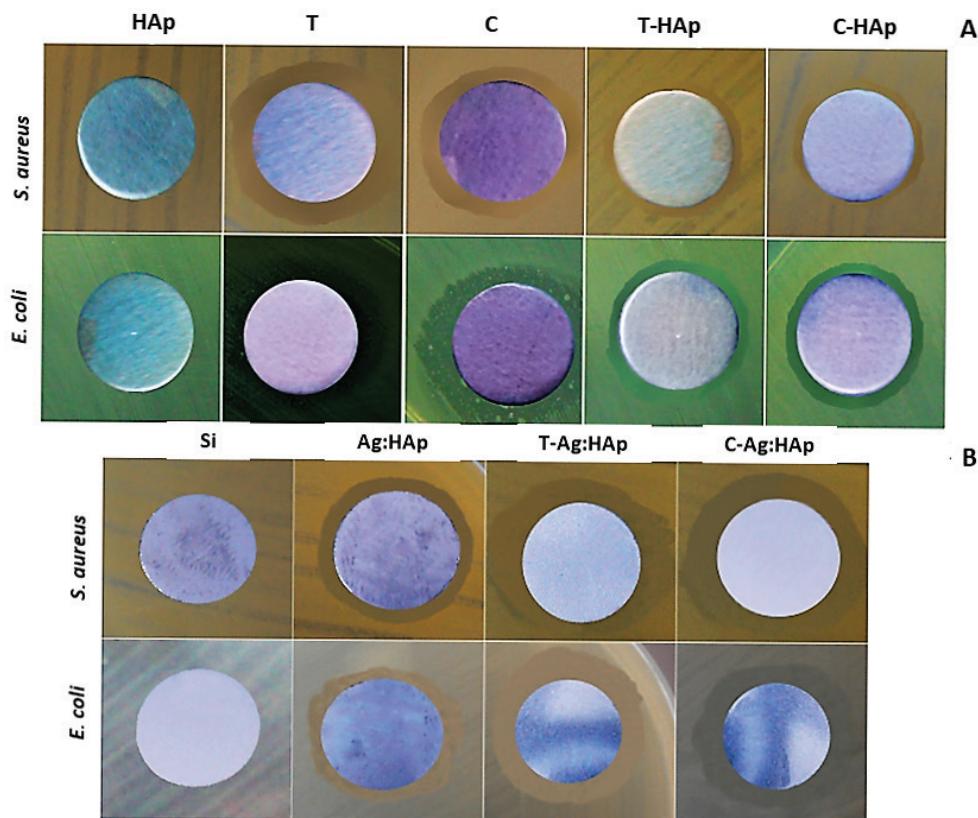


Figure 5. Antibacterial activity against *S. aureus* 0364 and *E. coli* ATCC 25922 cultures of (A) HAp, T-HAp and C-HAp, ciprofloxacin, tetracycline thin films and (B) Ag:HAp, T-Ag:HAp, C-Ag:HAp thin films. Reprinted from Ref. [125], the open access article distributed under the Creative Commons Attribution License.

Hydroxyapatite with an additional phase of sphere-shaped silver phosphate molecules and enriched with vancomycin or gentamicin has been developed by Suvannapruk et al. [126]. The authors have proved that such a combination of silver phosphate nanoparticles and antibiotic prolongs the antibacterial activity and increases the efficiency of the material.

An interesting experiment was proposed by Sampath Kumar [127], resulting in the creation of HA enriched with Ag^+ , Sr^{2+} or Zn^{2+} ions. These materials were used as doxycycline-releasing media. Of all the materials under investigation, Ag-HA had the lowest doxycycline loading. The most optimal system was the Zn-HA, because it produced a sufficiently effective level of antibacterial activity and, at the same time, contained an adequate quantity of loaded antibiotic.

Recently, Yu et al. [128] synthesized Cu-HA microspheres using a microwave-hydrothermal method. Interestingly, the phosphorous source for the synthesis was creatine phosphate – a substrate for ATP production. Chitosan-based scaffolds were created by freeze drying and

loaded with doxorubicin to examine drug loading and release. Osteogenic and angiogenic properties were evaluated both *in vitro* and *in vivo*. Samples were bioactive and non-toxic. The authors claim that the release of Cu²⁺ ions, by stabilizing HIF-1 α , induced hypoxia in the bone tissue, which significantly stimulated neovascularization and improved bone regeneration.

5. Conclusions

Sophisticated porous hydroxyapatite structures and hydroxyapatite/polymer structures seem to offer potential as systems for the delivery of antibacterial agents directly into the bone. Thus, rather than delivering a single medicine, it would be possible to conduct combined therapy with various antibacterial agents with different dissolution profiles. The simultaneous application of antibiotics and HA modified by ions with antibacterial activity may contribute to development of the effective prevention and treatment methodology for post-surgical osseous inflammations. A therapy designed to directly target the affected area may significantly reduce general side effects of using antibiotics, improving therapeutic efficiency, while also allowing a reduction in dosage, which seems to be beneficial in both medical and economic terms.

Acknowledgements

This work was supported by the National Science Center (Poland) within project "Synthesis and physicochemical and biological analysis of crystalline calcium phosphates substituted with various ions"; UMO-2016/22/E/ST5/00564 and by Medical University of Warsaw (FW23/N/17).

Author details

Katarzyna Szurkowska, Aleksandra Laskus and Joanna Kolmas*

*Address all correspondence to: joanna.kolmas@wum.edu.pl

Faculty of Pharmacy with Laboratory Medicine Division, Department of Inorganic and Analytical Chemistry, Medical University of Warsaw, Warsaw, Poland

References

- [1] Haider A, Haider S, Han SS, Kang I-K. Recent advances in the synthesis, functionalization and biomedical applications of hydroxyapatite: A review. RSC Advances. 2017;7:7442-7458. DOI: 10.1039/c6ra26124h

- [2] Mucalo M, editor. *Hydroxyapatite (Hap) for Biomedical Applications*. 1st ed. Amsterdam: Elsevier; 2015. 404 p
- [3] Marković S, Vasilinović L, Lukić MJ, Karanović L, Bracko I, Ignjatović N, Uskoković D. Synthetical bone-like and biological hydroxyapatites: A comparative study of crystal structure and morphology. *Biomedical Materials*. 2011;6:045005. DOI: [org/10.1088/1748-6041/6/4/045005](https://doi.org/10.1088/1748-6041/6/4/045005)
- [4] Dalmonico GML, Franczak PF, Levandowski N, Camargo NHA, Dallabrida AL, da Costa BD, Garcia Gil O, Cambra-Moo O, Rodriguez MA, Canillas M. An in vivo study on bone formation behaviour of microporous granular calcium phosphate. *Biomaterials Science* 2017;5:1315-1325. DOI: [10.1039/C7BM00162B](https://doi.org/10.1039/C7BM00162B)
- [5] Kasir R, Vernekar VN, Laurencin CT. Inductive biomaterials for bone regeneration. *Materials Research*. 2017;32:1047-1060. DOI: <https://doi.org/10.1557/jmr.2017.39>
- [6] Koju N, Sikder P, Ren Y, Zhou H, Bhaduri SB. Biomimetic coating technology for orthopedic implants. *Current Opinion in Chemical Engineering*. 2017;15:49-55. DOI: <https://doi.org/10.1016/j.coche.2016.11.005>
- [7] Elkassas D, Arafa A. The innovative applications of therapeutic nanostructures in dentistry. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2017;13:1543-1562. DOI: <https://doi.org/10.1016/j.nano.2017.01.018>
- [8] Ridi F, Meazzini I, Catroflorio B, Bonini M, Berti D, Baglioni P. Functional calcium phosphate composites in nanomedicine. *Advance in Colloid and Interface Science*. 2017;244:281-295. DOI: <https://doi.org/10.1016/j.cis.2016.03.006>
- [9] Wang C, Wang Y, Meng H, Wang X, Zhu Y, Yu K, Yuan X, Wang A, Guo Q, Peng J. Research progress regarding nanohydroxyapatite and its composite biomaterials in bone defect repair. *International Journal of Polymeric Materials and Polymeric Biomaterials*. 2016;65:601-610
- [10] Liu Q, Pan H, Chen Z, Matlinlinna JP. Insight into bone-derived biological apatite: Ultrastructure and effect on thermal treatment. *Biomed Research International*. 2015;2015. Article ID: 601025. DOI: [10.1155/2015/601025](https://doi.org/10.1155/2015/601025)
- [11] Kolmas J, Velard F, Jaguszewska A, Lemaire F, Kerdjoudj H, Gangloff SC, Kaflak A. Substitution of strontium and boron into hydroxyapatite crystals: Effect on physicochemical properties and biocompatibility with human Wharton-Jelly stem cells. *Materials Science and Engineering C*. 2017;79:638-646. DOI: <https://doi.org/10.1016/j.msec.2017.05.066>
- [12] Kolmas J, Pajor K, Pajchel L, Przekora A, Ginalska G, Oledzka E, Sobczak M. Fabrication and physicochemical characterization of porous composite microgranules with selenium oxyanions and risedronate sodium for potential applications in bone tumors. *International Journal of Nanomedicine*. 2017;12:1-10

- [13] Lerner T, Liljenqvist U. Silicate-substituted calcium phosphate as a bone graft substitute in surgery for adolescent idiopathic scoliosis. European Spine Journal. 2013;**22**(S2): 185-194. DOI: <https://doi.org/10.1007/s00586-012-2485-7>
- [14] Kolmas J, Krukowski S, Laskus A, Jurkiewicz M. Synthetic hydroxyapatite in pharmaceutical applications. Ceramics International. 2016;**42**:2472-2487. DOI: <https://doi.org/10.1016/j.ceramint.2015.10.048>
- [15] Oledzka E, Sobczak M, Kolmas J, Nałęcz-Jawecki G. Selenium-substituted hydroxyapatite/biodegradable polymer/pamidronate combined scaffold for the therapy of bone tumor. International Journal of Molecular Sciences. 2015;**16**:22205-22222. DOI: 10.3390/ijms160922205
- [16] De Miguel L, Popa I, Noiray M, Caudron E, Arpinati L, Desmaele D, Cebrian-Torrejon G, Domenech-Carbo A, Ponchel G. Osteotropic polypeptide nanoparticles with dual hydroxyapatite binding properties and controlled cisplatin delivery. Pharmaceutical Research. 2015;**32**:1794-1803. DOI: <https://doi.org/10.1007/s11095-014-1576-z>
- [17] Uskoković V, Desai TA. Simultaneous bactericidal and osteogenic effect of nanoparticulate calcium phosphate powders loaded with clindamycin on osteoblasts infected with *Staphylococcus aureus*. Materials Science and Engineering C. 2014;**37**:210-222. DOI: <https://doi.org/10.1016/j.msec.2014.01.008>
- [18] von Stechov D, Rauschmann MA. Effectiveness of combination use of antibiotic-loaded PerOssal® with spinal surgery in patients with spondylodiscitis. European Surgical Research 2009;**43**:298-305. DOI: 10.1159/000233525
- [19] Nandi SK, Mukherjee P, Roy S, Kundu B, Kumar De D, Basu D. Local antibiotic delivery systems for the treatment of osteomyelitis – A review. Materials Science and Engineering C. 2009;**29**:2478-2485. DOI: <https://doi.org/10.1016/j.msec.2009.07.014>
- [20] Martínez-Vásquez F, Cabaños MV, Paris JL, Lozano D, Vallet-Regi M. Fabrication of novel Si-doped hydroxyapatite/gelatin scaffolds by rapid prototyping for drug delivery and bone regeneration. Acta Biomaterialia. 2015;**15**:200-209. DOI: 10.1016/j.actbio.2014.12.021
- [21] Hasegawa M, Sudo A, Komlev VS, Barinov ML, Uchida A. High release of antibiotic from a novel hydroxyapatite with bimodal pore size distribution. Journal of Biomedical Materials Research B: Applied Biomaterials. 2004;**70**(2):332-339. DOI: 10.1002/jbm.b.30047
- [22] Ferraz MP, Mateus AY, Sousa JC, Monteiro FJ. Nanohydroxyapatite microspheres as delivery system for antibiotics: Release kinetics, antimicrobial activity, and interaction with osteoblasts. Journal of Biomedical Materials Research A. 2007;**81**(4):994-1004. DOI: 10.1002/jbm.a.31151
- [23] Sivakumar M, Rao KP. Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite – alginate composite microspheres. Journal of Biomedical Materials Research A. 2003;**65**(2):222-228. DOI: 10.1002/jbm.a.10495

- [24] Hess U, Mikolajczyk G, Treccani L, Streckbein P, Heiss C, Odenbach S, Rezwan K. Multi-loaded ceramic beads/matrix scaffolds obtained by combining ionotropic and freeze gelation for sustained and tuneable vancomycin release. *Materials Science and Engineering C*. 2016;**67**:542-553. DOI: 10.1016/j.msec.2016.05.042
- [25] Yu J, Chu X, Cai Y, Ton P, Yao J. Preparation and characterization of antimicrobial nano-hydroxyapatite composites. *Materials Science and Engineering C*. 2014;**37**:54-59. DOI: 10.1016/j.msec.2013.12.038
- [26] Thomas MB, Metoki N, Mandler D, Eliaz N. In situ, potentiostatic deposition of calcium phosphate with gentamicin-loaded chitosan nanoparticles on titanium alloy surfaces. *Electrochimica Acta*. 2016;**222**:355-360. DOI: <https://doi.org/10.1016/j.electacta.2016.10.186>
- [27] Yang C-C, Lin C-C, Liao J-W, Yen S-K. Vancomycin-chitosan composite deposited on post porous hydroxyapatite coated Ti6Al4V implant for drug controlled release. *Materials Science and Engineering C*. 2013;**33**:2203-2212. DOI: <https://doi.org/10.1016/j.msec.2013.01.038>
- [28] Teng SH, Lee EJ, Wang P, Yun SH, Han CM, Kim HE. Functionally gradient chitosan/hydroxyapatite composite scaffolds for controlled drug release. *Journal of Biomedical Materials Research B*. 2009;**90**(1):275-282. DOI: 10.1002/jbm.b.31283
- [29] Ionita D, Bajenaru-Georgescu D, Totea G, Mazare A, Schmuki P, Demetrescu I. Activity of vancomycin release from bioinspired coatings of hydroxyapatite or TiO_2 nanotubes. *International Journal of Pharmaceutics*. 2017;**517**:296-302. DOI: 10.1016/j.ijpharm.2016.11.062
- [30] Lian X, Liu H, Wang X, Xu S, Cui F, Bai X. Antibacterial and biocompatible properties of vancomycin-loaded nanohydroxyapatite/collagen/poly(lactic-acid) bone substitute. *Progress in Natural Sciences: Materials International*. 2013;**23**(6):549-556. DOI: <https://doi.org/10.1016/j.pnsc.2013.11.003>
- [31] Lian X, Mao K, Liu X, Vang X, Cui F. In vivo osteogenesis of vancomycin loaded nano-hydroxyapatite/collagen/calcium sulphate composite for treating infectious bone defect induced by chronic osteomyelitis. *Journal of Nanomaterials*. 2015 Article ID: 261492. <http://dx.doi.org/10.1155/2015/261492>
- [32] Song W, Ren W, Wan C, Esquivel AO, Shi T, Blasier R, Marker DC. A novel strontium-doped calcium polyphosphate/erythromycin/poly(vinyl alcohol) composite for bone tissue engineering. *Journal of Biomedical Materials Research A*. 2011;**98**(3):359-371. DOI: 10.1002/jbm.a.33127
- [33] Sasikumar S. Effect of particle size of calcium phosphate based bioceramic drug delivery carrier on the release kinetics of ciprofloxacin hydrochloride: An in vitro study. *Frontiers in Materials Science*. 2013;**7**(3):261-268. DOI: 10.1007/s11706-013-0216-6
- [34] Yu M, Zhou K, Li Z. Preparation, characterization and in vitro gentamicin release of porous HA microspheres. *Materials Science and Engineering C*. 2014;**45**:306-312. DOI: 10.1016/j.msec.2014.08.075

- [35] Schnieders J, Gbureck U, Thull R, Kissel T. Controlled release of gentamicin from calcium phosphate-poly(lactic acid-co-glycolic acid) composite bone cement. *Biomaterials*. 2006;7:4239-4249. DOI: 10.1016/j.biomaterials.2006.03.032
- [36] Wang X, Xu H, Zhao Y. Poly(lactide-co-glycolide) encapsulated hydroxyapatite microspheres for sustained release of doxycycline. *Materials Science and Engineering B*. 2012;177:367-372. DOI: <https://doi.org/10.1016/j.mseb.2011.12.030>
- [37] Tang Y, Chen L, Zhao K, Wu Z, Wang Y, Tan Q. Fabrication of PLGA/HA (core)-collagen/amoxicillin (shell) nanofiber membranes through coaxial electrospinning for guided tissue regeneration. *Composites Science and Technology*. 2016;125:100-107
- [38] Zheng F, Wang S, Wen S, Shen M, Zhu M, Shi X. Characterization and antibacterial activity of amoxicillin-loaded electrospun nano-hydroxyapatite/poly(lactic-co-glycolic acid) composite nanofibers. *Biomaterials*. 2013;34:1402-1412. DOI: 10.1016/j.biomaterials.2012.10.071
- [39] Vukomanovic M, Skapin SD, Poljansec I, Zagar E, Kralj B, Ignjatovic N, Uskoković D. Poly(D,L-lactide-co-glycolide)/hydroxyapatite core-shell nanosphere. Part 2: Simultaneous release of a drug and a prodrug (clindamycin and clindamycin phosphate). *Colloids and Surface B: Biointerfaces*. 2011;82(2):414-421. DOI: <https://doi.org/10.1016/j.colsurfb.2010.09.012>
- [40] Uskokovic V, Hoover C, Vukomanovic M, Uskoković DP, Desai TA. Osteogenic and antimicrobial nanoparticulate calcium phosphate and poly-(D,L-lactide-co-glycolide) powders for the treatment of osteomyelitis. *Materials Science and Engineering C*. 2013;33:3362-3373. DOI: 10.1016/j.msec.2013.04.023
- [41] Yan L, Jiang DM, Cao Z-D, Wu J, Wang X, Wang ZL, Li YJ, Yi YF. Treatment of *Staphylococcus aureus*-induced chronic osteomyelitis with bone-like hydroxyapatite/poly amino acid loaded with rifapentine microspheres. *Drug Design, Development and Therapy*. 2015;9:3665-3676. DOI: <https://doi.org/10.2147/DDDT.S84486>
- [42] Leprêtre S, Chai F, Hornez J-C, Vermet G, Neut C, Descamps M, Hildebrand HF, Martel B. Prolonged local antibiotics delivery from hydroxyapatite functionalized with cyclodextrin polymers. *Biomaterials*. 2009;30:6086-6093. DOI: 10.1016/j.biomaterials.2009.07.045
- [43] Selvakumar M, Kumar PS, Das B, Dhara S, Chattopadhyay S. Structurally tuned antimicrobial mesoporous hydroxyapatite nanorods by cyclic oligosaccharides regulation to release a drug for osteomyelitis. *Crystal Growth and Design*. 2017;17:433-445. DOI: 10.1021/acs.cgd.6b01190
- [44] Stigter M, Bezemer J, de Groot K, Layrolle P. Incorporation of different antibiotics into carbonated hydroxyapatite coatings on titanium implants, release and antibiotic efficacy. *Journal of Controlled Release*. 2004;99(1):127-137
- [45] Mir M, Siddiqi SA, Hussain T, et al. Synthesis and characterization of calcium deficient apatite granules for drug eluting bone graft applications. *Ceramics International*. 2014;40:10719-10725. DOI: <https://doi.org/10.1016/j.ceramint.2014.03.059>

- [46] Brohede U, Forsgren J, Roos S, Mihranyan A, Engqvist H, Stromme M. Multifunctional implant coatings providing possibilities for fast antibiotics loading with subsequent slow release. *Journal of Materials Science: Materials in Medicine*. 2009;20:1859-1867. DOI: 10.1007/s10856-009-3749-6
- [47] Bhattacharya R, Kundu B, Nandi SK, Basu D. Systematic approach to treat chronic osteomyelitis through localized drug delivery system: Bench to bed side. *Materials Science and Engineering C*. 2013;33:3986-3993. DOI: 10.1016/j.msec.2013.05.036
- [48] Chai F, Hornez J-C, Blanchemain N, Neut C, Descamps M, Hildebrandt HF. Antibacterial activation of hydroxyapatite (HA) with controlled porosity by different antibiotics. *Biomolecular Engineering*. 2007;24(5):510-514. DOI: 10.1016/j.bioeng.2007.08.001
- [49] Ghosh S, Wu V, Pernal S, Uskoković V. Self-setting calcium phosphate cements with tunable antibiotic release rates for advanced antimicrobial applications. *ACS Applied Materials Interfaces*. 2016;8:7691-7708. DOI: 10.1021/acsami.6b01160
- [50] Uskokovic V, Desai TA. Simultaneous bactericidal and osteogenic effect of nanoparticulate calcium phosphate powders loaded with clindamycin on osteoblasts infected with *Staphylococcus aureus*. *Materials Science and Engineering C*. 2014;37:210-222. DOI: 10.1016/j.msec.2014.01.008
- [51] Victor SP, Sharma CP, Sreenivasan K. Use of quartz crystal nanobalance to study the binding and stabilization of albumin and doxycycline on a thin layer of hydroxyapatite. *Applied Surface Science*. 2011;258:1666-1669. DOI: <https://doi.org/10.1016/j.apusc.2011.09.119>
- [52] Canal C, Pastorino D, Mestres G, Schuler P, Ginebra MP. Relevance of microstructure for the early antibiotic release of fresh and pre-set calcium phosphate cements. *Acta Biomaterialia*. 2013;9:8403-8412
- [53] Gomes PS, Santos JD, Fernandes MH. Cell-induced response by tetracyclines on human bone marrow colonized hydroxyapatite and Bonelike®. *Acta Biomaterialia*. 2008;4:630-637
- [54] Lilja M, Sørensen JH, Brohede U, Astrand M, Procter P, Arnoldi J, Steckel H, Stromme M. Drug loading and release of tobramycin from hydroxyapatite coated fixation pins. *Journal of Materials Science: Materials in Medicine*. 2013;24:2265-2274. DOI: 10.1007/s10856-013-4979-1
- [55] Kaya M, Simsek-Kaya G, Gürsan N, Girecci E, Dayi E, Gundogdu B. Local treatment of chronic osteomyelitis with surgical debridement and tigecycline-impregnated calcium hydroxyapatite: An experimental study Oral Surgery Oral Medicine Oral Pathology Oral Radiology. 2012;113(3):340-347. DOI:10.1016/j.tripleo.2011.03.032
- [56] Colovic A, Pasalic S, Jokanovic V. Influence of hydroxyapatite pore geometry on tigecycline release kinetics. *Ceramics International*. 2012;38:6181-6189. DOI: <https://doi.org/10.1016/j.ceramint.2012.04.069>

- [57] Guo Y-J, Long T, Chen W, Ning C-Q, Zhu Z-A, Guo Y-P. Bactericidal property and biocompatibility of gentamicin-loaded mesoporous carbonated hydroxyapatite microspheres. *Materials Science and Engineering C*. 2013;**33**:3583-3591. DOI: <https://doi.org/10.1016/j.msec.2013.04.021>
- [58] Joosten U, Joist A, Frebel T, Brandt B, Diederichs S, von Eiff C. Evaluation of an in situ setting injectable calcium phosphate as a new carrier material for gentamicin in the treatment of chronic osteomyelitis: Studies in vitro and in vivo. *Biomaterials* 2004;**25**:4287-4295. <https://doi.org/10.1016/j.biomaterials.2003.10.083>
- [59] Alt V, Bitschnau A, Österling J, Sewing A, Meyer C, Kraus R, Meissner SA, Wenisch S, Domann E, Schnettler R. The effects of combined gentamicin-hydroxyapatite coating for cementless joint prostheses on the reduction of infection rates in a rabbit infection prophylaxis model. *Biomaterials*. 2006;**27**:4627-4634. DOI: [10.1016/j.biomaterials.2006.04.035](https://doi.org/10.1016/j.biomaterials.2006.04.035)
- [60] Thanyaphoo S, Kaewsrichan J. Potential of bone scaffolds containing vancomycin and bone morphogenetic protein-2 in a rat model of osteomyelitis. *Asian Biomedicine*. 2014;**8**(5):651-657
- [61] Joosten U, Joist A, Gosheder G, Liljenqvist U, Brandt B, von Eiff C. Effectiveness of hydroxyapatite-vancomycin bone cement in the treatment of *Staphylococcus aureus* induced chronic osteomyelitis. *Biomaterials* 2005;**26**:5251-5258. <https://doi.org/10.1016/j.biomaterials.2005.01.001>
- [62] Jiang P-J, Patel S, Gbureck U, et al. Comparing the efficacy of three bioceramic matrices for the release of vancomycin hydrochloride. *Journal of Biomedical Materials Research B Applied Biomaterials*. 2010;**93**(1):58-61
- [63] Guo Y-P, Yao Y-B, Guo Y-J, Ning C-Q. Hydrothermal fabrication of mesoporous carbonated hydroxyapatite microspheres for a drug delivery system. *Microporous and Mesoporous Materials*. 2012;**155**:245-251. DOI: <https://doi.org/10.1016/j.micromeso.2012.01.037>
- [64] Perez LM, Lalueza P, Monzon M, Puertolas JA, Arruebo M, Santamaria J. Hollow porous implants filled with mesoporous silica particles as a two-stage antibiotic-eluting device. *International Journal of Pharmaceutics*. 2011;**409**:1-8. DOI: [10.1016/j.ijpharm.2011.02.015](https://doi.org/10.1016/j.ijpharm.2011.02.015)
- [65] Rauschmann MA, Wichelhaus TA, Stirnal V, Dingeldein E, Zichner L, Schnettler R, Alt V. Nanocrystalline hydroxyapatite and calcium sulphate as biodegradable composite carrier material for local delivery of antibiotics in bone infections. *Biomaterials*. 2005;**26**:2677-2684. DOI: [10.1016/j.biomaterials.2004.06.045](https://doi.org/10.1016/j.biomaterials.2004.06.045)
- [66] Belcarz A, Ginalska G, Zalewska J, Rzeski W, Slósarczyk A, Kowalcuk D, Godlewski P, Niedźwiadek J. Covalent coating of hydroxyapatite by keratin stabilizes gentamicin release. *Journal of Biomedical Materials Research B Applied Biomaterials*. 2009;**89**(1):102-113. DOI: [10.1002/jbm.b.31192](https://doi.org/10.1002/jbm.b.31192)

- [67] Garner S, Barbour ME. Nanoparticles for controlled delivery and sustained release of chlorhexidine in the oral environment. *Oral Diseases*. 2015;**21**(5):641-644. DOI: 10.1111/odi.12328
- [68] Lara HH, Garza-Trevino EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *Journal of Nanobiotechnology*. 2011;**9**:30. DOI: 10.1186/1477-3155-9-30
- [69] Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. *Journal of Applied Microbiology*. 2012;**112**:841-852. DOI: 10.1111/j.1365-2672.2012.05253.x
- [70] Akiyama T, Miyamoto H, Yonekura Y, Tsukamoto M, Ando Y, Noda I, Sonohata M, Mawatari M. silver oxide-containing hydroxyapatite coatings has in vivo antibacterial activity in the rat tibia. *Journal of Orthopaedic Research*. 2013;**31**:1195-1200. DOI: 10.1002/jor.22357
- [71] Anjaneyulu U, Priyadarshini B, Nirmala Grace A, Vijayalakshmi U. Fabrication and characterization of Ag doped hydroxyapatite-polyvinyl alcohol composite nanofibers and its in vitro biological evaluations for bone tissue engineering applications. *Journal of Sol-Gel Science and Technology*. 2017;**81**:750-761. DOI: 10.1007/s10971-016-4243-5
- [72] Ipekoglu M, Altintas S. Silver substituted nanosized calcium deficient hydroxyapatite. *Materials Technology*. 2010;**25**:295-301. DOI: 10.1179/175355510X12692596613648
- [73] Ciuca S, Badea M, Pozna E, Pana I, Kiss A, Floroian L, Semenescu A, Cotrut CM, Moga M, Vladescu A. Evaluation of Ag containing hydroxyapatite coatings to the *Candida albicans* infection. *Journal of Microbiological Methods*. 2016;**125**:12-18. DOI: 10.1016/j.mimet.2016.03.016
- [74] Ciobanu CS, Iconaru SL, Coustumer PL, Constantin LV, Predoi D. Antibacterial activity of silver-doped hydroxyapatite nanoparticles against gram-positive and gram-negative bacteria. *Nanoscale Research Letters*. 2012;**7**:324
- [75] Iconaru SL, Chifiriuc MC, Groza A. Structural and antimicrobial evaluation of silver doped hydroxyapatite-polydimethylsiloxane thin layers. *Journal of Nanomaterials*. 2017;**2017**:7492515. DOI: 10.1155/2017/7492515
- [76] Miranda M, Fernández A, Díaz M, Esteban-Tejeda L, López-Estebe S, Malpartida F, Torrecillas R, Moya JS. Silver-hydroxyapatite nanocomposites as bactericidal and fungicidal materials. *International Journal of Materials Research*. 2010;**101**:122-127. DOI: 10.3139/146.110256
- [77] Stanić V, Janaćković D, Dimitrijević S, Tanasković SB, Mitić M, Pavlović MS, Krstić A, Jovanović D, Raičević S. Synthesis of antimicrobial monophase silver-doped hydroxyapatite nanopowders for bone tissue engineering. *Applied Surface Science*. 2011;**257**:4510-4518. DOI: 10.1016/j.apsusc.2010.12.113

- [78] Jegatheeswaran S, Sundrarajan M. PEGylation of novel hydroxyapatite/PEG/Ag nano-composite particles to improve its antibacterial efficacy. *Material Science and Engineering C*. 2015;**51**:174-181. DOI: 10.1016/j.msec.2015.02.012
- [79] Shi C, Gao J, Wang M, Fu J, Wang D, Zhu Y. Ultra-trace silver-doped hydroxyapatite with non-cytotoxicity and effective antibacterial activity. *Materials Science and Engineering C*. 2015;**55**:497-505. DOI: 10.1016/j.msec.2015.05.078
- [80] Yan Y, Zhang X, Huang Y, Ding Q, Pang X. Antibacterial and bioactivity of silver substituted hydroxyapatite/TiO₂ nanotube composite coatings on titanium. *Applied Surface Science*. 2014;**314**:348-357. DOI: 10.1016/j.apsusc.2014.07.027
- [81] Popa CL, Ciobanu CS, Voicu G, Vasile E, Chifiriuc MC, Iconaru SL, Predoi D. Influence of thermal treatment on the antimicrobial activity of silver-doped biological apatite. *Nanoscale Research Letters*. 2015;**10**:502. DOI: 10.1186/s11671-015-1211-x
- [82] Nath S, Kalmodia S, Basu B. Densification, phase stability and in vitro biocompatibility property of hydroxyapatite-10 wt% silver composites. *Journal of Materials Science: Materials in Medicine*. 2010;**21**:1273-1287. DOI: 10.1007/s10856-009-3939-2
- [83] Rajendran A, Barik RC, Natarajan D, Kiran MS, Pattanayak DK. Synthesis, phase stability of hydroxyapatite-silver composite with antimicrobial activity and cytocompatibility. *Ceramics International*. 2014;**40**:10831-10838. DOI: 10.1016/j.ceramint.2014.03.075
- [84] Liu H, Man HC. Laser fabrication of Ag-HA nanocomposites on Ti6Al4V implant for enhancing bioactivity and antibacterial capability. *Materials Science and Engineering C*. 2017;**70**:1-8. DOI: 10.1016/j.msec.2016.08.059
- [85] Lu X, Zhang B, Wang Y, Zhou X, Weng J, Qu S, Feng B, Watari F, Ding Y, Leng Y. Nano-Ag-loaded hydroxyapatite coatings on titanium surfaces by electrochemical deposition. *Journal of Royal Society Interface*. 2011;**8**:529-539. DOI: 10.1098/rsif.2010.0366
- [86] Lee DH, Min BG. Preparation and antibacterial properties of nanocomposite fibers made of polyamide 6 and silver-doped hydroxyapatite. *Fibers and Polymers*. 2014;**15**:1921-1926. DOI: 10.1007/s12221-014-1921-1
- [87] Xie CM, Lu X, Wang KF, Meng FZ, Jiang O, Zhang HP, Zhi W, Fang LM. Silver nanoparticles and growth factors incorporated hydroxyapatite coatings on metallic implant surfaces for enhancement of osteoinductivity and antibacterial properties. *ACS Applied Materials Interfaces*. 2014;**6**:8580-8589. DOI: 10.1021/am501428e
- [88] Lu M, Liao J, Dong J, Wu J, Qiu H, Zhou X, Li J, Jiang D, He TC, Quan Z. An effective treatment of experimental osteomyelitis using the antimicrobial titanium/silver-containing nHP66 (nano-hydroxyapatite/polyamide-66) nanoscaffold biomaterials. *Scientific Reports*. 2016;**6**:39174. DOI: 10.1038/srep39174

- [89] Ueno M, Miyamoto H, Tsukamoto M, Eto S, Noda I, Shobuiken T, Kobatake T, Sonohata M, Mawatari M. Silver-containing hydroxyapatite coating reduces biofilm formation by methicillin-resistant *Staphylococcus aureus* in vitro and in vivo. BioMed Research International. 2016;2016:8070597. DOI: 10.1155/2016/8070597
- [90] Eto S, Kawano S, Someya S, Miyamoto H, Sonohata M, Mawatari M. First clinical experience with thermal-sprayed silver oxide-containing hydroxyapatite coating implant. The Journal of Arthroplasty. 2016;31:1498-1503. DOI: 10.1016/j.arth.2015.12.034
- [91] Lim PN, Shi Z, Neoh KG, Ho B, Tay BY, Thian ES. The effects of silver, silicon-containing apatite towards bacteria and cell responses. Biomedical Materials. 2014;9:015010. DOI: 10.1088/1748-6041/9/1/015010
- [92] Xu Z, Lei Y, Yin W, Chen Y, Ke Q, Guo Y, Zhang C. Enhanced antibacterial activity and osteoinductivity of Ag-loaded strontium hydroxyapatite/chitosan porous scaffold for bone tissue engineering. Journal of Materials Chemistry B. 2016;4:7919-7928. DOI: 10.1039/C6TB01282E
- [93] Aksakal B, Demirel M. The effect of Zirconia/Yttria/Silver substitutions on mechanostructure and cell viability of the synthesized bioceramic bone grafts. Ceramics International. 2017;43:7482-7487. DOI: 10.1016/j.ceramint.2017.03.026
- [94] Kolmas J, Piotrowska U, Kuras M, Kurek E. Effect of carbonate substitution on physicochemical and biological properties of silver containing hydroxyapatites. Materials Science and Engineering C. 2017;74:124-130. DOI: 10.1016/j.msec.2017.01.003
- [95] Huo K, Zhang X, Wang H, Zhao L, Liu X, Chu PK. Osteogenic activity and antibacterial effects on titanium surfaces modified with Zn-incorporated nanotube arrays. Biomaterials. 2013;34:3467-3478. DOI: 10.1016/j.biomaterials.2013.01.071
- [96] Cai R, Wang H, Cao M, Hao L, Zhai L, Jiang S, Li X. Synthesis and antimicrobial activity of mesoporous hydroxylapatite/zinc oxide nanofibers. Materials and Design. 2015;87:17-24. DOI: 10.1016/j.matdes.2015.08.004
- [97] Fang J, Zhao J, Sun Y, Ma H, Yu X, Ma Y, Ni Y, Zheng L, Zhou Y. Biocompatibility and antibacterial properties of zinc-ion implantation on titanium. Journal of Hard Tissue Biology. 2014;23:35-44
- [98] Anwar A, Akbar S, Sadiqa A, Kazmi M. Novel continuous flow synthesis, characterization and antibacterial studies of nanoscale zinc substituted hydroxyapatite bioceramics. Inorganica Chimica Acta. 2016;453:16-22. DOI: 10.1016/j.ica.2016.07.041
- [99] Chen X, Tang QL, Zhu YJ, Zhu CL, Feng XP. Synthesis and antibacterial property of zinc loaded hydroxyapatite nanorods. Materials Letters. 2012;89:233-235. DOI: 10.1016/j.matlet.2012.08.115
- [100] Predoi D, Iconaru SL, Deniaud A, Chevallet M, Michaud-Soret I, Buton N, Prodan AM. Textural, structural and biological evaluation of hydroxyapatite doped with zinc at low concentrations. Materials. 2017;10:229. DOI: 10.3390/ma10030229

- [101] Radovanović Ž, Veljović D, Jokić B, Dimitrijević S, Bogdanović G, Kojić V, Petrović R, Janaćković D. Biocompatibility and antimicrobial activity of zinc(II)-doped hydroxyapatite, synthesized by a hydrothermal method. *Journal of Serbian Chemical Society*. 2012;**77**:1787-1798. DOI: 10.2298/JSC121019131R
- [102] Thian ES, Konishi T, Kawanobe Y, Lim PN, Choong C, Aizawa M. Zinc-substituted hydroxyapatite: A biomaterial with enhanced bioactivity and antimicrobial properties. *Journal of Materials Science: Materials in Medicine*. 2013;**24**:437-445. DOI: 10.1007/s10856-012-4817-x
- [103] Tank KP, Chudasama KS, Thaker VS, Joshi MJ. Pure and zinc doped nano-hydroxyapatite: Synthesis, characterization, antimicrobial and hemolytic studies. *Journal of Crystal Growth*. 2014;**401**:474-479. DOI: 10.1016/j.jcrysgro.2014.01.062
- [104] Zhang J. Biocompatibility and anti-bacterial activity of Zn-containing HA/TiO₂ hybrid coatings on Ti substrate. *Journal of Hard Tissue Biology*. 2013;**22**:311-318
- [105] Colombo M, Beltrami R, Rattalino D, Mirando M, Chiesa M, Poggio C. Protective effects of a zinc-hydroxyapatite toothpaste on enamel erosion. SEM study. *Annali di Stomatologia (Roma)*. 2017;**10**:38-45. DOI: 10.11138/ads/2016.7.3.038
- [106] Samani S, Hossainalipour SM, Tamizifar M, Rezaie HR. *In vitro* antibacterial evaluation of sol-gel-derived Zn-, Ag-, and (Zn + Ag)-doped hydroxyapatite coatings against methicillin-resistant *Staphylococcus aureus*. *Journal of Biomedical Materials Research Part A*. 2013;**101A**:222-230. DOI: 10.1002/jbm.a.34322
- [107] Livitska O, Strutynska N, Zatovsky I, Nikolenko I, Slobodyanik N, Prytutskyy Y, Epple M, Prymak O, Byeda A. Copper (II), zinc (II) and copper (II)/zinc (II)-containing carbonate-substituted hydroxyapatite: synthesis, characterization and thermal behavior. *Materialwissenschaft und Werkstofftechnik*. 2016;**47**:85-91. DOI: 10.1002/mawe.201600460
- [108] Kaygili O, Keser S. Sol-gel synthesis and characterization of Sr/Mg, Mg/Zn and Sr/Zn co-doped hydroxyapatites. *Materials Letters*. 2015;**141**:161-164. DOI: 10.1016/j.matlet.2014.11.078
- [109] Friederichs RJ, Chappell HF, Shepherd DV, Best SM. Synthesis, characterization and modelling of zinc and silicate co-substituted hydroxyapatite. *Journal of Royal Society Interface*. 2015;**12**:20150190. DOI: 10.1098/rsif.2015.0190
- [110] Uysal I, Severcan F, Evis Z. Characterization by Fourier transform infrared spectroscopy of hydroxyapatite co-doped with zinc and fluoride. *Ceramics International*. 2013;**39**:7727-7733. DOI: 10.1016/j.ceramint.2013.03.029
- [111] Vincent M, Hartemann P, Engels-Deutsch M. Antimicrobial applications of copper. *International Journal of Hygiene and Environmental Health*. 2016;**219**:585-591. DOI: 10.1016/j.ijheh.2016.06.003

- [112] Li Y, Ho J, Ooi CP. Antibacterial efficacy and cytotoxicity studies of copper (II) and titanium (IV) substituted hydroxyapatite nanoparticles. Materials Science and Engineering C. 2010;30:1137-1144. DOI: 10.1016/j.msec.2010.06.011
- [113] Sahithi K, Swetha M, Prabaharan M, Moorthi A, Saranya N, Ramasamy K, Srinivasan N, Partridge NC, Selvamurugan N. Synthesis and characterization of nanoscale-hydroxyapatite-copper for antimicrobial activity towards bone tissue engineering applications. Journal of Biomedical Nanotechnology. 2010;6:333-339. DOI: 10.1166/jbn.2010.1138
- [114] Shanmugam S, Gopal B. Copper substituted hydroxyapatite and fluorapatite: Synthesis, characterization and antimicrobial properties. Ceramics International. 2014;40:15655-15662. DOI: 10.1016/j.ceramint.2014.07.086
- [115] Stanić V, Dimitrijević S, Antić-Stanković J, Mitić M, Jokić B, Plečaš IB, Raičević S. Synthesis, characterization and antimicrobial activity of copper and zinc-doped hydroxyapatite nanopowders. Applied Surface Science. 2010;256:6083-6089. DOI: 10.1016/j.apsusc.2010.03.124
- [116] Radovanović Ž, Jokić B, Veljović D, Dimitrijević S, Kojić V, Petrović R, Janaćković D. Antimicrobial activity and biocompatibility of Ag⁺- and Cu²⁺-doped biphasic hydroxyapatite/α-tricalcium phosphate obtained from hydrothermally synthesized Ag⁺- and Cu²⁺-doped hydroxyapatite. Applied Surface Science. 2014;307:513-519. DOI: 10.1016/j.apsusc.2014.04.066
- [117] Tran PL, Hammond AA, Mosley T, Cortez J, Gray T, Colmer-Hamood JA, Shashtri M, Spallholz JE, Hamood AN, Reid TW. Organoselenium coating on cellulose inhibits the formation of biofilms by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Applied Environmental Microbiology. 2009;75:3586-3592. DOI: 10.1128/AEM.02683-08
- [118] Tran PA, Webster TJ. Selenium nanoparticles inhibit *Staphylococcus aureus* growth. International Journal of Nanomedicine. 2011;6:1553-1558. DOI: 10.2147/IJN.S21729
- [119] Rodríguez-Valencia C, López-Álvarez M, Cochón-Cores B, Pereiro I, Serra J, González P. Novel selenium-doped hydroxyapatite coatings for biomedical applications. Journal of Biomedical Materials Research Part A. 2013;101:853-861. DOI: 10.1002/jbm.a.34387
- [120] Uskoković V, Iyer MA, Wu VM. One ion to rule them all: the combined antibacterial, osteoinductive and anticancer properties of selenite-incorporated hydroxyapatite. Journal of Materials Chemistry B. 2017;5:1430-1445. DOI: 10.1039/C6TB03387C
- [121] Kolmas J, Groszyk E, Piotrowska U. Nanocrystalline hydroxyapatite enriched in selenite and manganese ions: Physicochemical and antibacterial properties. Nanoscale Research Letters. 2015;10:278. DOI: 10.1186/s11671-015-0989-x
- [122] Kolmas J, Groszyk E, Kwiatkowska-Różycka D. Substituted hydroxyapatites with antibacterial properties. BioMed Research International. 2014;2014:178123. DOI: 10.1155/2014/178123

- [123] Ivashenko OA, Perekos AO, Ulianchych NV, Uvarova IV, Protsenko LS, Budylina OM, Holovkova MY, Yarmola TM. Interaction of Ag-free and Ag-doped hydroxyapatite with ciprofloxacin solutions. *Materialwissenschaft und Werkstofftechnik*. 2011;42: 98-108. DOI: 10.1002/mawe.201100739
- [124] Xiong Z-C, Yang Z-Y, Zhu Y-J, Chen F-F, Zhang Y-G, Yang R-L. Ultralong hydroxyapatite nanowires-based paper co-loaded with silver nanoparticles and antibiotic for long-term antibacterial benefit. *ACS Applied Materials and Interfaces*. 2017;9(27):22212-22222. DOI: 10.1021/acsmami.7b05208
- [125] Predoi D, Popa CL, Chapon P, Groza A, Iconaru SL. Evaluation of the antimicrobial activity of different antibiotics enhanced with silver-doped hydroxyapatite thin-films. *Materials*. 2016;9(9),778:1-18. DOI: 10.3390/ma9090778
- [126] Suvannapruk W, Thammarakcharoen F, Phanpiriya P, Suwanprateeb J. Development of antibiotic impregnated nanosized silver phosphate-doped hydroxyapatite bone-graft. *Journal of Nanomaterials*. 2013, Article ID:542584:1-9. <http://dx.doi.org/10.1155/2013/542584>
- [127] Sampath Kumar TS, Madhumathi K, Rubalya Y, Doble M. Dual mode antibacterial activity of ion substituted calcium phosphate nanocarriers for bone infections. *Frontiers in Bioengineering and Biotechnology*. 2015;3(59):1-10. DOI: 10.3389/fbioe.2015.00059
- [128] Yu W, Sun T, Ding Z, Qi C, Zhao H, Chen F, Shi Z, Zhu Y, Chen D, He J. Copper-doped mesoporous hydroxyapatite microspheres synthesized by a microwave-hydrothermal method using creatine phosphate as an organic phosphorus source: application in drug delivery and enhanced bone regeneration. *Journal of Materials Chemistry B*. 2017;5:1039-1052. DOI: 10.1039/C6TB02747D

