

# INVESTIGATION OF ANTIBACTERIAL PROPERTIES OF CERAMIC SUBSTRATES COATED WITH CALCIUM PHOSPHATE AND POLYMERIC NANOPARTICLES LOADED WITH ANTIBIOTICS

ANNA MARSZAŁEK\* , IWONA PUDEŁKO-PRAŻUCH ,  
MAŁGORZATA KROK-BORKOWICZ\* 

AGH UNIVERSITY OF KRAKOW,  
FACULTY OF MATERIALS SCIENCE AND CERAMICS,  
DEPARTMENT OF BIOMATERIALS AND COMPOSITES,  
AL. A. MICKIEWICZA 30, 30-059 KRAKOW, POLAND  
\*E-MAIL: AMARSZALEK@AGH.EDU.PL, KROK@AGH.EDU.PL

## Abstract

This study investigates a biomimetic method of deposition of bioactive calcium phosphate (CaP) layers on zirconium oxide substrates (ZrO<sub>2</sub>). The substrates contained polymer nanoparticles of poly(L-lactide-co-glycolide) (PLGA) obtained using the double emulsion method with solvent evaporation. Three antibiotics were encapsulated within the nanoparticles: bacitracin, gentamicin sulphate, and hydrophobic gentamicin, prepared with the use of the ion pairing method. Nanoparticles were immobilized on the substrates using the drop casting or the co-deposition method. The microstructure of the layers and the distribution of the nanoparticles were assessed by scanning electron microscopy. The nanoparticles size and their zeta potential were measured using the dynamic light scattering method. The release of drugs over time was examined and the antibacterial properties were evaluated in contact with *Staphylococcus aureus* bacteria using the spectrophotometric method and the Kirby-Bauer test. The results show that the layer deposition method is effective and allows to obtain homogenous bioactive coatings. Nanoparticles were agglomerated on the surface or homogeneously distributed in the CaP coating, depending on the process used to immobilize them. The drug release profile and antibacterial properties can also be modified by changing the process – the drop casting method allows to obtain a coating with a stronger antimicrobial effect and faster drug release.

Nanoparticles obtained by the double emulsion method with solvent evaporation have the required size to be immobilized between the CaP crystallites. Additionally, the encapsulation of drugs decreased the zeta potential of the nanoparticles, which was caused by the interaction of the drug and the polymer. Nanoparticles loaded with bacitracin showed weak antibacterial properties, as the growth inhibition zone in the Kirby-Bauer test was barely visible. Two other types of nanoparticles exhibited good antibacterial properties, exceptionally strong for those loaded with hydrophobic gentamicin.

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**Keywords:** ceramic substrates, polymer nanoparticles, poly(L-lactide-co-glycolide) (PLGA), hydrophobic gentamicin, biomimetic coating, calcium phosphate

## Introduction

With increasing life expectancy, diseases of the musculoskeletal system are becoming more frequent. They can be related to joint wear or be the result of complications after invasive orthopedic procedures [1]. In general, bone tissue has a natural ability to regenerate, but not all mechanical injuries or defects caused by diseases such as osteomyelitis, bone cancer, or osteoporosis can be spontaneously healed. In such cases, surgical intervention is required to fill the defect or replace the missing bone. Replacement can be made by bone grafting or by using a synthetic biomaterial, usually ceramic [1,2]. One of the diseases that is increasing in frequency is osteomyelitis, which often develops in the femur or spine. Untreated, it causes irreversible changes in the body, the need for amputation, and even death [3-5]. Osteomyelitis can be caused by many species of bacteria and fungi. *S. aureus* is responsible for the development of the disease in 80-90% of cases [6,7].

Zirconium dioxide is often used as a bone substitute to fill in gaps resulting from diseases or injuries [8,9]. ZrO<sub>2</sub> is an inert biomaterial, but it is possible to provide it with bioactive properties by coating it with another material, such as hydroxyapatite ceramics (HAp), which can bind to bone without creating fibrous tissue after implantation. The ZrO<sub>2</sub> substrate modified in this way allows cells to attach and proliferate on its surface [10]. One of the methods for obtaining such a coating is the biomimetic method, which mimics the first phase of integration of the material with bone tissue *in vivo*. It consists of precipitating calcium phosphate on the surface of the material immersed in simulated body fluid (SBF) and can be used to cover properly prepared metal, ceramic, polymer, or composite surfaces. The coatings obtained in this way have mechanical properties similar to those of natural bone and have high biocompatibility [11,12]. Changes in SBF concentration also allow to control roughness, which is important for the adhesion and proliferation of osteoblasts. The advantages of the method are the high homogeneity of the layers obtained and the possibility to coat devices characterized by various shapes and sizes, but it is necessary to control the process conditions: the temperature and pH of the solution [11-13].

In many applications, it is beneficial for implants to have antibacterial properties, as it reduces the risk of infection that could occur during the implantation process and prevents the adhesion of bacteria to the surface. Implants can also act as drug carriers, for example, by depositing nanoparticles loaded with antibiotics on their surface. Such a modification is valuable in the case of materials used to fill bone defects caused by surgical removal of infected tissue in osteomyelitis treatment [5,6].

The use of drug delivery systems (DDS) is more beneficial than containing the antibiotic itself in the coating. The main difficulty in the treatment of bone infections is the complexity of its tissue structure. The delivery of drugs to the bones, oral or intravenous, is often ineffective. Quite commonly, they are excreted from the body before they have a therapeutic effect at the target site, while the frequent administration of large doses of drugs can cause severe side effects [6]. Conventional antibiotic therapy guarantees that bacteria are eliminated from the whole body, but it has many disadvantages. Both the poor absorption of drugs from the digestive system and their rapid excretion from the body make it difficult to maintain a stable therapeutic level [14].

Meanwhile, DDS allows for a slower release, preventing a sudden increase in drug concentration to potentially toxic. Furthermore, for the same reason, drug levels are more stable over time. The drug can be released only in a specific place, thus avoiding systemic side effects. The behavior of the drug carrier can be modified to release substance only under certain conditions, e.g. local pH or temperature. In addition, prolonged release of the drug makes the absorption of the active substance easier, providing a better therapeutic effect. Targeted antibiotic therapy ensures better penetration of bone tissue by the drug, but attention must be paid to maintaining the appropriate concentration of the antibiotic, because microorganisms can acquire antibacterial resistance if it is not high enough [14,15].

Drug delivery systems are mostly based on nanoparticles (NPs). Particles with nanometric size easily penetrate infected bone tissue. The appropriate functionalization of their surface allows the therapeutic effect to be improved, for example, by providing an additional layer of molecules of another antibiotic or other compounds [6]. Polymer nanoparticles are stable colloidal structures in the form of nanocapsules or nanospheres differing in structure, size, and mechanism the drug is released. The manufacturing methods for both nanocapsules and nanospheres are well established [16,17].

This study investigates a biomimetic method of deposition of bioactive calcium phosphate (CaP) layers on zirconium oxide substrates ( $ZrO_2$ ), containing polymer nanoparticles of poly(L-lactide-co-glycolide) (PLGA) obtained using the double emulsion method with solvent evaporation with three antibiotics encapsulated: bacitracin, gentamicin in the form of gentamicin sulphate and hydrophobic gentamicin, prepared with the use of the ion pairing method. Hydrophobic gentamicin was used in this study due to its higher encapsulation efficiency compared to basic gentamicin [18]. Similar research has already been conducted on the subject of CaP coatings with antibacterial NPs [1,19]. However, we compared different drugs and conducted thorough microbiological tests, which has not been done before.

## Materials and Methods

### Manufacturing nanoparticles

Drug-loaded NPs were prepared using the double emulsion method with solvent evaporation (FIG. 1). In the first step of NPs fabrication, 6 mg of each drug was added to 3 ml of a 2% solution of poly(L-lactide-co-glycolide) (PLGA, Polish Academy of Sciences, Zabrze, Poland) in dichloromethane (DCM, Chemland, Stargard, Poland) and mixed with ultrasounds for 3 min with an amplitude of 40% (Vibra Cell VCX130, Sonics, Newtown, CT, USA).

The obtained solution was added to the 2% aqueous solution of poly(vinyl alcohol) (PVA, Sigma Aldrich, Steinheim, Germany) and mixed with ultrasounds for 3 min with an amplitude of 40% and then on a magnetic stirrer with a speed of 1000 rpm. After 24 h, the solutions were centrifuged for 20 min at a speed of 15 000 rpm (MPW-351R, MPW Med. instruments, Warszawa, Poland). 2 ml of supernatants were taken to investigate the encapsulation efficiency (EE) and drug loading (DL). The remaining volume was removed.

Then 20 ml of ultra-high quality water was added and the particles were centrifuged again. This process was repeated 3 times. After removing the water from the last centrifugation, 3 ml of ultra-high quality water was added and the obtained sample was placed at a temperature of  $-80^{\circ}\text{C}$  for 24 h. The last step in the preparation of particles was the freeze-drying process, which was carried out for 24 h (Christ Alpha 1–2 LDplus, Martin Christ, Osterode am Harz, Germany). To prepare empty NPs, mixing antibiotics with PLGA in the first step was omitted, and PLGA was sonicated alone to keep the same conditions, the rest of the process was conducted without any changes. The drugs used were bacitracin (Sigma Aldrich, Steinheim, Germany), gentamicin sulphate (Sigma Aldrich, Steinheim, Germany), and hydrophobic gentamicin (GEN AOT), obtained as described in [18]. Briefly, gentamicin sulphate was modified into a hydrophobic complex, by hydrophobic ion pairing of gentamicin with the anionic surfactant dioctyl sulfosuccinate sodium salt (AOT, Sigma-Aldrich, Germany).

### Nanoparticles characterization

The size and zeta potential of NPs were measured using the dynamic light scattering method (DLS, Zetasizer nano-ZS, Malvern, UK). Three measurements were made for each type of particles. The encapsulation efficiency and drug loading were investigated using supernatants collected after the first centrifugation of NPs. To quantify the amount of drug in them, an o-phthalaldehyde (OPA, Sigma-Aldrich, Germany) assay was conducted. This test is based on the reaction of OPA with the drug present in the supernatant and the fluorescence measurement. The OPA reagent was prepared by mixing o-phthalaldehyde with methanol, 2-mercaptoethanol, and borate buffer. Then 50  $\mu\text{l}$  of supernatant (three samples for each) was transferred to a black 96-well plate and 50  $\mu\text{l}$  of OPA reagent was added to each. Based on the results of fluorescence measurement, EE and DL were calculated according to formulas (1) and (2), respectively:

$$EE = \frac{\text{mass of used drug} - \text{mass of drug in supernatant}}{\text{mass of used drug}} \cdot 100 [\%] \quad (1)$$

$$DL = \frac{\text{mass of used drug} - \text{mass of drug in supernatant}}{\text{mass of obtained NPs}} \cdot 100 [\%] \quad (2)$$

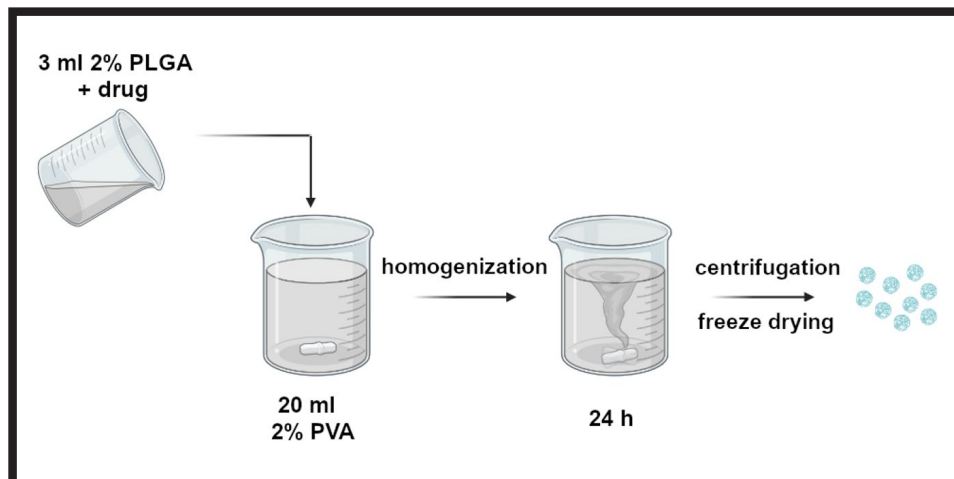
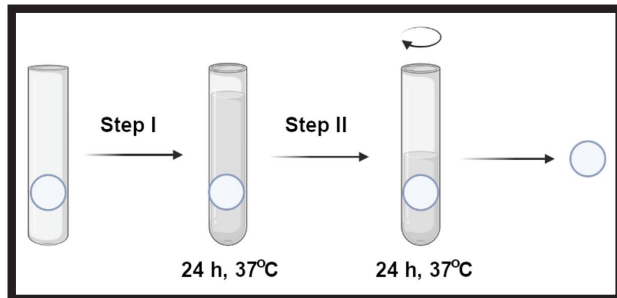


FIG. 1. Schematic process of NPs manufacturing.

### Coating deposition

Round zirconium oxide substrates 0.8 cm in diameter, each obtained by pressing and sintering 1.3 g of zirconium oxide powder, were used. After sintering, they were polished and treated with phosphoric acid to create functional Zr-OH groups, which effectively participate in the process of apatite formation in the body's environment [20].

Bioactive coatings on the surface of the substrates were obtained using the biomimetic method based on the precipitation of CaP crystals from the simulated body fluid (SBF) solution. To coat the substrates with a CaP layer, a two-step precipitation method with the use of solutions similar to concentrated SBF was applied. The first step solution was prepared as detailed by Costa *et al.* [12], while the second one as described in [11] (FIG. 2).



**FIG. 2. Schematic representation of the deposition process of calcium phosphate.**

### Immobilization of nanoparticles

NPs were incorporated into the CaP layers using two methods:

- Co-deposition method

In this method, the dispersion of NPs (10 mg/ml) was added to the solution used during step II of the deposition process.

- Drop casting method

After the second step of the layer deposition process was completed, 100  $\mu$ l of NPs dispersed in water at a concentration of 2 mg/ml was applied to the substrate surface and left to dry at room temperature.

### Layers morphology

Pictures of layers without NPs after steps I and II of different depositions and layers with NPs immobilized by the co-deposition and the drop casting method were taken using a scanning electron microscope (SEM, GeminiSEM 500, Zeiss, Germany).

### Drug release profile

To determine drug release profiles, substrates with NPs were closed in dialysis bags (ZelluTrans, Roth, Germany, cut-off 12-14 kDa) and placed in vials filled with 20 ml of PBS. The experiment was carried out at 37°C for 30 days. In predetermined periods of time, 2 ml of PBS was collected and replaced with the same amount of fresh PBS solution. The amount of drug in the collected solution was then quantified using the OPA assay.

### Antimicrobial effect

To verify the antibacterial properties of the obtained coatings, the tests with *S. aureus* (ATCC MSSA 25923) were performed. Bacteria cultures were passaged twice before analysis by sieving onto slants with nutrient agar and incubated for 24 h at 37°C  $\pm$  1°C. Scaffolds containing empty or loaded NPs samples were incubated in 2 ml of PBS for 24 h at 37°C to obtain the extracts.

### Kirby-Bauer test

A well was cut in the agar in which 100  $\mu$ l of a 500 mg/ml suspension of particles was placed. After 24 h, photos were taken and growth inhibition zones were measured.

### Spectrophotometric method

Extracts were prepared by placing coated substrates with empty NPs and NPs loaded with bacitracin, gentamicin, or hydrophobic gentamicin in 2 ml of PBS. The bacteria were suspended in a sterile solution of nutrient broth and buffered saline in a 1:3 ratio to obtain a bacterial count of  $\sim 10^6$  cell forming units (CFU)/ml. The inoculum was then added to the test tubes containing the extracts of the test samples in a 1:1 ratio. A control sample containing a bacterial solution without the addition of any extract was also prepared. Tubes containing inoculated test materials and control materials were incubated at 36°C  $\pm$  0.1°C for 24 h. The microbial viability was assessed using the spectrophotometric method (UV Vis Shimadzu, Japan) by measuring the optical density of the prepared solutions at a wavelength of 600 nm. Test samples were taken from the incubated solutions at defined time intervals.

Statistical analysis of the results was performed using a one-way analysis of variance (one-way ANOVA).

## Results and Discussions

### Characterization of nanoparticles

TABLE 1 shows the results of the measurements of the zeta potential and nanoparticles size using the DLS method. The size range of the particles and the average size are included. For each type of NPs with drugs, the average size is smaller than that of empty NPs, with the smallest being those with gentamicin. The size of NPs containing gentamicin and those with AOT-modified gentamicin is in the same range, but their average size is significantly different – for NPs with AOT-modified gentamicin it is larger by about 40 nm. The difference between all NPs is statistically significant, which means that they do not belong to the same population. This phenomenon was also observed in [19] for gentamicin and bacitracin loaded NPs and in [21], where encapsulation of gentamicin in PLGA NPs caused a decrease in particle size.

**TABLE 1. Size range (n = 3) and average size of the NPs, zeta potential (n = 3), encapsulation efficiency (EE), and drug loading (DL) of respective NPs.**

	Size range [nm]	Average size [nm] $\pm$ SD	Zeta potential [mV] $\pm$ SD	EE [%] $\pm$ SD	DL [%] $\pm$ SD
Empty NPs	142-396	314 $\pm$ 7	-10.3 $\pm$ 0.7	-	-
GEN NPs	122-459	241 $\pm$ 4	-12.3 $\pm$ 0.4	38.7 $\pm$ 9.9	3.5 $\pm$ 0.9
BAC NPs	122-615	271 $\pm$ 2	-15.1 $\pm$ 0.4	56.9 $\pm$ 2.4	5.2 $\pm$ 0.2
GEN AOT NPs	122-459	278 $\pm$ 2	-11.1 $\pm$ 0.5	99.9 $\pm$ 0.1	9.2 $\pm$ 0.2



The zeta potential decreased for all types of NPs with drugs, which is caused by the interaction of the drug and polymer. The lowest zeta potential was measured for nanoparticles with bacitracin. Moreover, the zeta potential of NPs loaded with hydrophobic gentamicin was higher than that for non-modified gentamicin with a statistically significant difference. However, many studies showed the opposite trend [1,18,19,21,22], suggesting that other external factors influence the properties of NPs, which requires further research.

The results of the calculations of EE and DL are also presented in TABLE 1. For NPs with bacitracin, a higher efficiency of encapsulation and drug loading was achieved than for particles with gentamicin, while the best encapsulation was achieved in the case of NPs with hydrophobic gentamicin, which proves the effectiveness of this modification, also described in other studies, such as [1,18] or [23], where nearly 100% EE was reported.

### Coatings morphology

FIG. 3 shows photos of the obtained CaP layers without the addition of NPs, after the first and after the second step of deposition, respectively. As seen in FIGs 3 A and B, the layer after the first step is inhomogeneous and areas not covered with CaP crystals are visible, as the purpose of the first step is to deposit CaP nuclei.

Because of that, a two-step process is necessary, as FIGs 3 C and D show a homogeneous coating with well-formed CaP crystals. The finished coatings look very similar to those reported in papers [12,19], where the authors used the same precipitation method.

FIG. 4 shows the microstructure of the coatings with NPs, after co-deposition and after application of the drop casting method. Few particles are visible on the surface of the layer produced by the co-deposition process, while after the drop casting method many particles forming clusters can be seen. During co-deposition, the nanoparticle suspension was added to the solution, so the particles can also occur under the CaP crystals. If a drop of the suspension is placed on the surface, the particles do not penetrate the CaP layer and more of them are visible. However, there are fewer particles visible after the use of the co-deposition method; they seem to be more homogeneously distributed between the CaP crystals as compared to NPs immobilized with the drop casting method, which are highly agglomerated. Similar results can be seen in [19], where exactly the same method of NPs immobilization was used.

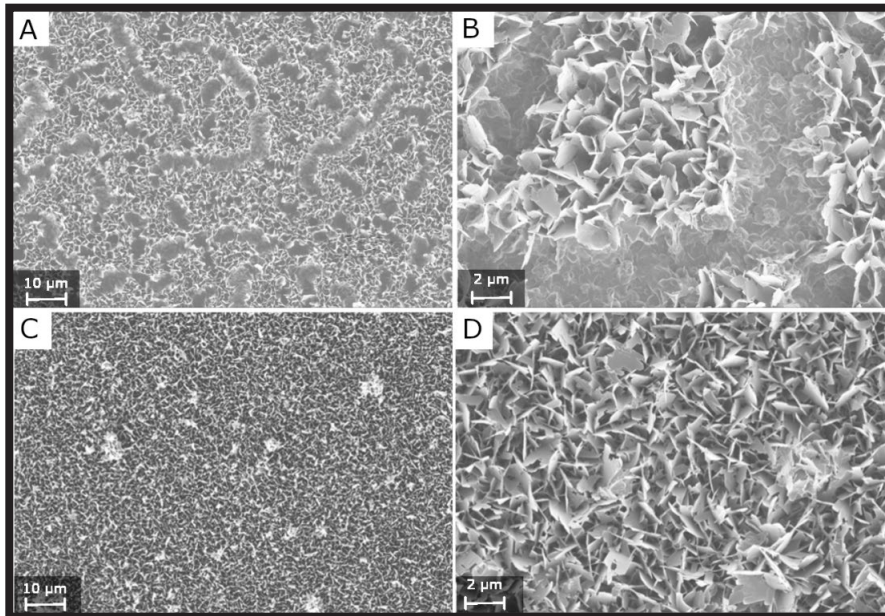


FIG. 3. SEM images of CaP coating morphology after the first (A, B) and second step (C, D) of the deposition process.

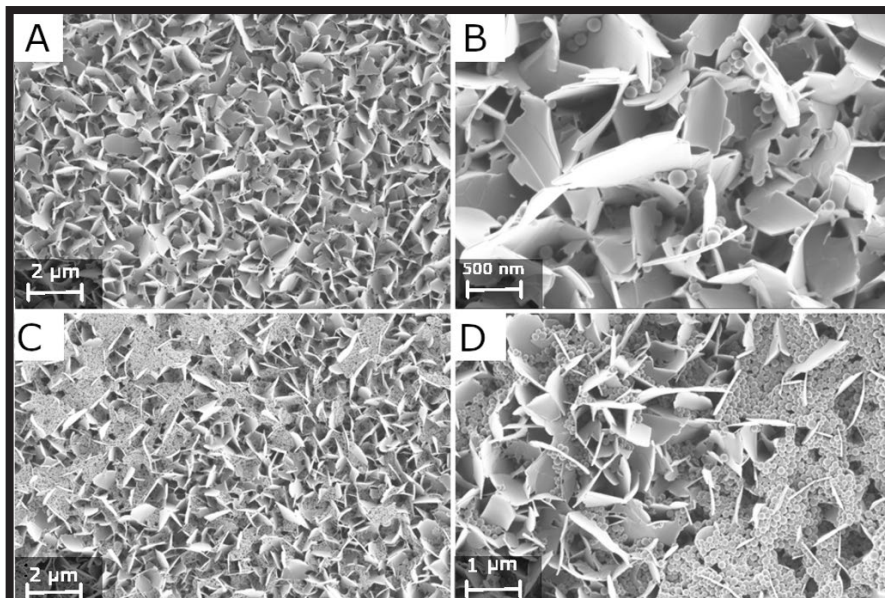


FIG. 4. SEM images of CaP coating with NPs immobilized with co-deposition (A, B) and drop casting method (C, D).

### Drug release profile

FIG. 5 shows the release of drugs from the manufactured  $ZrO_2$  samples surface-modified with CaP with drug-loaded NPs. For each sample, a much higher drug concentration was achieved for the particles deposited using the drop casting method, due to the greater number of particles on the surface of the layer, as shown by the SEM studies (FIG. 4). At the end of the study, it was found that the lowest concentration was achieved with gentamicin and the highest with bacitracin.

For both NPs loaded with GEN and GEN AOT in the beginning, the drug was released in a similar manner. However, the final concentration of hydrophobic gentamicin was higher than that of gentamicin sulphate, which may be due to better encapsulation efficiency of this drug: almost 100% of the GEN AOT was encapsulated in NPs (TABLE 1). A similar drug release profile was obtained in the paper [1] for NPs loaded with GEN and GEN AOT, the latter being released faster and reaching a higher concentration at the end of the study. Also in [22], where PLGA NPs loaded with gentamicin were studied, after a burst release, the drug was slowly released until the end.

### Antimicrobial properties

FIG. 6 shows the growth inhibition zones of *S. aureus* bacteria in contact with suspended NPs according to the Kirby-Bauer test. As expected, the presence of empty NPs did not affect bacterial growth. Bacitracin NPs had a weak effect, so they were not included in further antibacterial studies. Although more drug was released from the nanoparticles containing bacitracin (FIG. 5), the larger inhibition zone of bacterial growth was obtained for both gentamicin and AOT-modified gentamicin, suggesting that those two antibiotics are more effective against this particular strain of bacteria. Moreover, studies report that bacitracin generally has a weaker antibacterial effect on *S. aureus* than gentamicin [24].

Both particles with gentamicin and AOT-modified gentamicin showed good antibacterial activity, with the growth inhibition zone for hydrophobic gentamicin being larger, indicating a stronger antibacterial effect. The same trend was observed in other studies [18,25], proving that the ion pairing of gentamicin with AOT does not affect the potency of the antibiotic in a negative way.

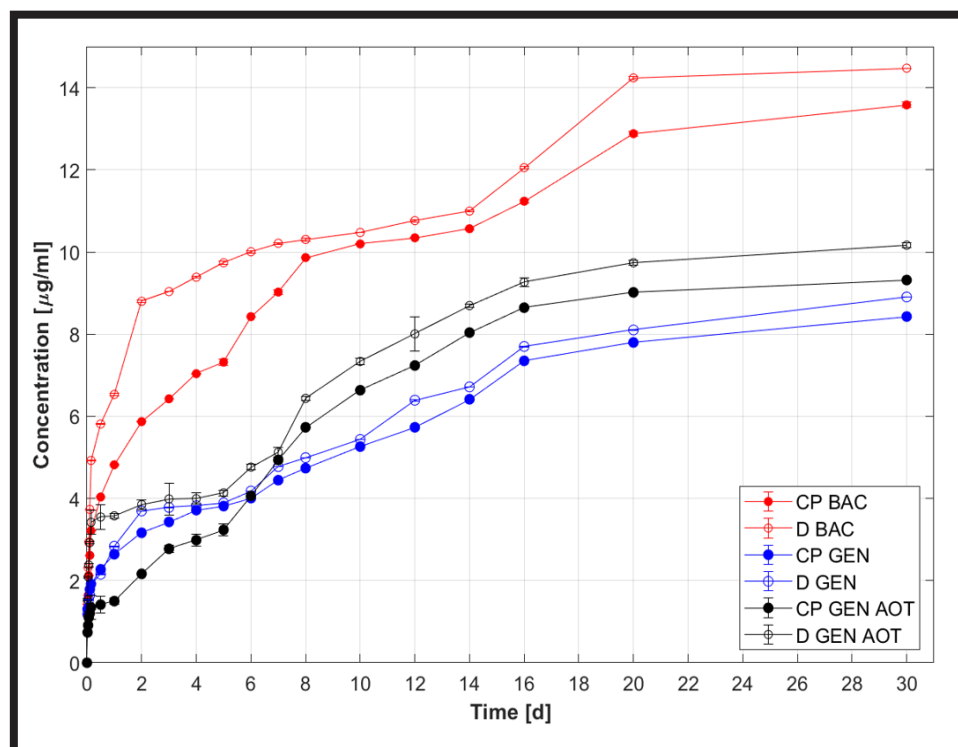


FIG. 5. Drug release profile for all types of substrates prepared using the co-deposition (CP) or drop casting (D) method with particles loaded with bacitracin, gentamicin, and hydrophobic gentamicin ( $n = 3$ ).

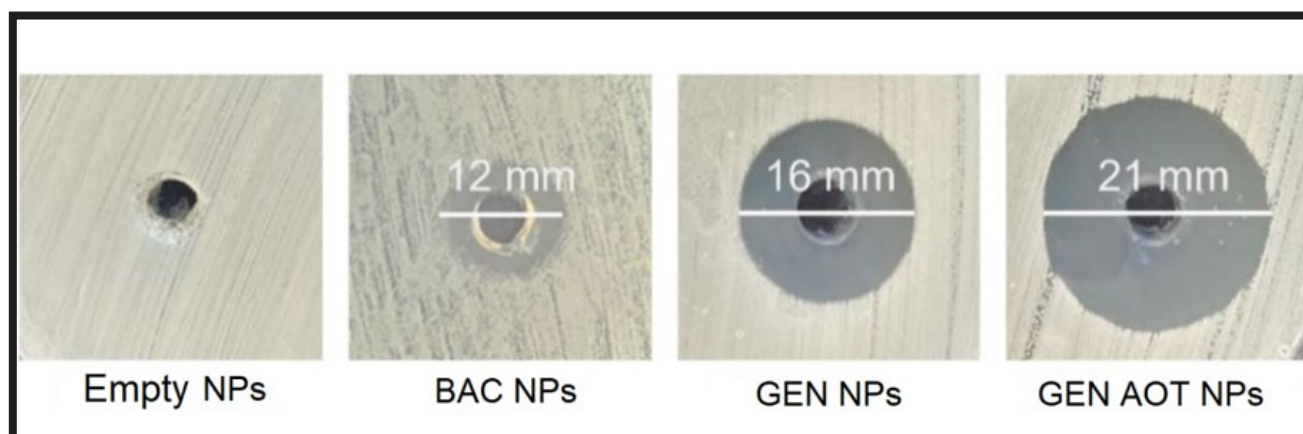


FIG. 6. Growth inhibition zones of *S. aureus* bacteria in contact with different types of NPs ( $n = 3$ ).

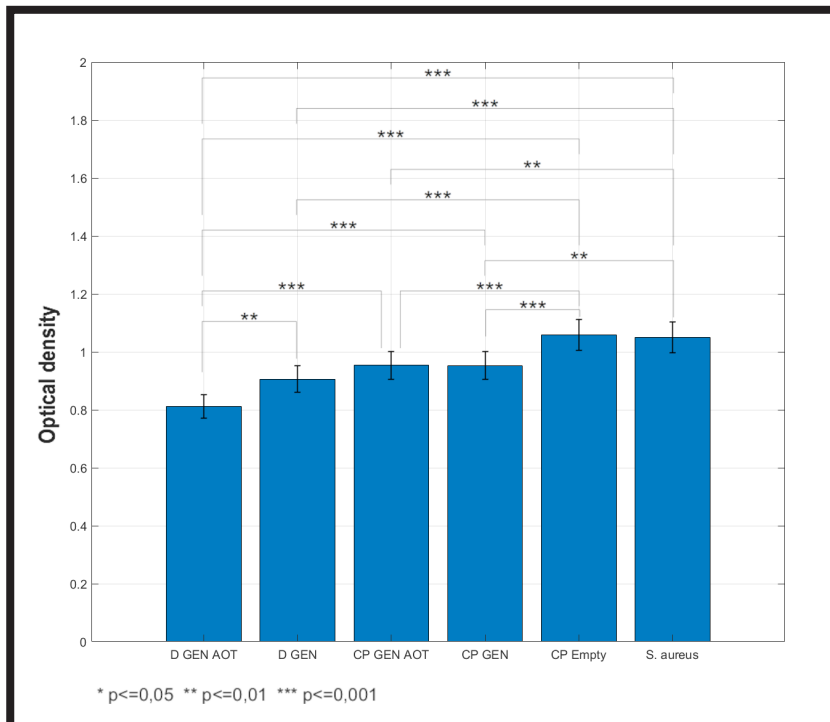


FIG. 7. Optical density for *S. aureus* and *S. aureus* in contact with extracts of samples coated with CaP layer that contains different types of antibiotic-loaded NPs (n = 3).

### Spectrophotometric method

FIG. 7 presents the results of the optical density measurement of extracts at different time points, which represents the growth of bacteria. The highest optical density, and thus the weakest antibacterial properties, showed extracts from substrates with empty NPs, as expected. This confirms the result of the Kirby-Bauer test. In the case of NPs with non-modified gentamicin and AOT-modified gentamicin, inhibition of bacterial growth can be observed.

Regardless of the drug used, extracts from samples coated using the co-deposition method have weaker antibacterial properties due to slower drug release (FIG. 5). It is caused by a more homogenous distribution of NPs in the coating – in the case of the drop casting method, the majority are located on the surface, where release is easier (compare FIGs 4 A, B and C, D).

### Conclusions

The aim of this study was to obtain a CaP coating on ceramic substrates and immobilize PLGA nanoparticles with antibacterial drugs encapsulated: bacitracin, gentamicin, and AOT-modified hydrophobic gentamicin. Their size and zeta potential were characterized, as well as the drug loading and encapsulation efficiency. The release of drugs from the coatings on the ZrO<sub>2</sub> substrates and antibacterial properties were also investigated.

The biomimetic method of depositing bioactive layers on ZrO<sub>2</sub> substrates and incorporating the prepared NPs by two methods: co-deposition and drop casting proved to be effective. SEM pictures show homogenous coating and the presence of the NPs. Particles produced using the double emulsion method have the required nanometric size. Loading them with any of the three tested drugs caused a reduction in both the size and zeta potential due to the interaction between PLGA and antibiotics, with GEN NPs being the smallest and GEN AOT the largest.

The conversion of gentamicin sulphate into hydrophobic gentamicin by ion pairing was found to be an effective way to fabricate particles with significantly improved encapsulation efficiency and drug loading values. NPs with hydrophobic gentamicin coupled with AOT are characterized by a highly improved encapsulation efficiency of  $99.9 \pm 0.1\%$ .

Although both methods of immobilizing NPs were effective, the release of the drug was faster when the drop casting method was used. This is because NPs agglomerate on the surface of the substrate in greater quantity, while in the co-deposition method they are also present below the surface of the deposited layers. Because of this, the method of deposition allows to control the rate and profile of drug release. Antibacterial tests of the obtained materials showed that both particles containing gentamicin sulphate and gentamicin modified with AOT exhibit antibacterial properties, inhibiting the growth of *S. aureus* bacteria. Bacitracin showed weak antimicrobial activity in the Kirby-Bauer test, and thus was not tested with the spectrophotometric method.

In summary, the response of bacteria in contact with either produced NPs or extracts from the substrates depends on the type of drug – GEN AOT being the most efficient. The method used to immobilize nanoparticles directly translates into antibacterial activity of the substrate. By changing manufacturing conditions of NPs and coatings, the antibacterial activity of samples can be adjusted to the desired application.

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### ORCID iD

A. Marszałek: <https://orcid.org/0009-0005-2511-2070>  
 I. Pudelko-Prażuch: <https://orcid.org/0000-0002-1024-9374>  
 M. Krok-Borkowicz: <https://orcid.org/0000-0002-9415-0282>



## References

- [1] Pudełko I., Moskwik A., Kwiecień K., Kriegseis S., Krok-Borkowicz M., Schickle K., Ochońska D., Dobrzyński P., Brzychczy-Włoch M., Gonzales-Julian J., Pamuła E.: Porous zirconia scaffolds functionalized with calcium phosphate layers and PLGA nanoparticles loaded with hydrophobic gentamicin. *International Journal of Molecular Sciences* 24(9) (2023) 8400.
- [2] Glauser R., Schupbach P.: Early bone formation around immediately placed two-piece tissue-level zirconia implants with a modified surface: an experimental study in the miniature pig mandible. *International Journal of Implant Dentistry* 8(1) (2022) 37.
- [3] Nair R., Schweizer M.L., Singh N.: Septic arthritis and prosthetic joint infections in older adults. *Infectious Disease Clinics of North America* 31(4) (2017) 715-729.
- [4] Alder K.D., Lee I., Munger A.M., Kwon H.-K., Morris M.T., Cahill S.V., Back J., Yu K.E., Lee F.Y.: Intracellular *Staphylococcus aureus* in bone and joint infections: a mechanism of disease recurrence, inflammation, and bone and cartilage destruction. *Bone* 141 (2020) 115568.
- [5] Pontes A.P., Welting T.J.M., Rip J., Creemers L.B.: Polymeric nanoparticles for drug delivery in osteoarthritis. *Pharmaceutics* 14(12) (2022) 2639.
- [6] Aguilera-Correa J.J., Gisbert-Garzarán M., Mediero A., Fernández-Aceñero M.J., de-Pablo-Velasco D., Lozano D., Esteban J., Vallet-Regí M.: Antibiotic delivery from bone-targeted mesoporous silica nanoparticles for the treatment of osteomyelitis caused by methicillin-resistant *Staphylococcus aureus*. *Acta Biomaterialia* 154 (2022) 608-625.
- [7] Jiang T., Yu X., Carbone E.J., Nelson C., Kan H.M., Lo K. W.-H.: Poly aspartic acid peptide-linked PLGA based nanoscale particles: potential for bone-targeting drug delivery applications. *International Journal of Pharmaceutics* 475 (2014) 547-557.
- [8] Thu M.K., Kang Y.S., Kwak J.M., Jo Y.-H., Han J.-S., Yeo I.-S.L.: Comparison between bone-implant interfaces of microtopographically modified zirconia and titanium implant. *Scientific Reports* 13(1) (2023) 11142.
- [9] Zhao Y., Li P., Dong P., Zeng Y., Chen J.: Investigation on 3D printing ZrO<sub>2</sub> implant abutment and its fatigue performance simulation. *Ceramics International* 47(1) (2021) 1053-1062.
- [10] Idaszek J., Jaroszewicz J., Chojińska E., Górecka Z., Hyc A., Osiecka-Iwan A., Wieluńska-Kuś B., Świążkowski W., Moskałowski S.: Toward osteomimetic formation of calcium phosphate coatings with carbonated hydroxyapatite. *Biomaterials Advances* 149 (2023) 213403.
- [11] Tas A.C., Bhaduri S.B.: Rapid coating of Ti6Al4V at room temperature with a calcium phosphate solution similar to 10× simulated body fluid. *Journal of Materials Research* 19(9) (2004) 2742-2749.
- [12] Costa D.O., Allo B.A., Klassen R., Hutter J.L., Dixon S.J., Rizkalla A.S.: Control of surface topography in biomimetic calcium phosphate coatings. *Langmuir* 28(8) (2012) 3871-3880.
- [13] Dziadek M., Zagrajczuk B., Menaszek E., Cholewa-Kowalska K.: A new insight into in vitro behaviour of poly(ε-caprolactone)/bioactive glass composites in biologically related fluids. *Journal of Materials Science* 53(6) (2018) 3939-3958.
- [14] Nandi S.K., Bandyopadhyay S., Das P., Samanta I., Mukherjee P., Roy S., Kundu B.: Understanding osteomyelitis and its treatment through local drug delivery system. *Biotechnology Advances* 34(8) (2016) 1305-1317.
- [15] Wang Y., Yuan Q., Feng W., Pu W., Ding J., Zhang H., Li X., Yang B., Dai Q., Cheng L., Wang J., Sun F., Zhang D.: Targeted delivery of antibiotics to the infected pulmonary tissues using ROS-responsive nanoparticles. *Journal of Nanobiotechnology* 17(1) (2019) 103.
- [16] Sur S., Rathore A., Dave V., Reddy K.R., Chouhan R.S., Sadhu V.: Recent developments in functionalized polymer nanoparticles for efficient drug delivery system. *Nano-Structures & Nano-Objects* 20 (2019) 100397.
- [17] Deirram N., Zhang C., Kermaniyan S.S., Johnston A.P.R., Such G.K.: pH-Responsive polymer nanoparticles for drug delivery. *Macromolecular Rapid Communications* 40(10) (2019) 1800917.
- [18] Kwiecień K., Pudełko I., Knap K., Reczyńska-Kolman K., Krok-Borkowicz M., Ochońska D., Brzychczy-Włoch M., Pamuła E.: Insight in superiority of the hydrophobized gentamicin in terms of antibiotics delivery to bone tissue. *International Journal of Molecular Sciences* 23(20) (2022) 12077.
- [19] Pudełko I., Desante G., Pamuła E., Schickle K., Krok-Borkowicz M.: The encapsulation of antibacterial drugs in polymer nanoparticles and their use in drug delivery systems on ZrO<sub>2</sub> scaffold with bioactive coating. *Engineering of Biomaterials* 161 (2021) 21-27.
- [20] Uchida M., Kim H.-M., Kokubo T., Nawa M., Asano T., Tanaka K., Nakamura T.: Apatite-forming ability of a zirconia/alumina nanocomposite induced by chemical treatment. *Journal of Biomedical Materials Research* 60(2) (2002) 277-282.
- [21] Ciocilteu M.-V., Nicolaescu O. E., Mocanu A. G., Nicolicescu C., Rau G., Neamtu J., Amzoiu E., Amzoiu E., Oancea C., Turcu-Stolica A.: Process optimization using quality by design (QBD) approach of a gentamicin loaded PLGA biocomposite. *Journal of Science and Arts* 21(4) (2021) 1069-1080.
- [22] Posadowska U., Brzychczy-Włoch M., Pamuła E.: Gentamicin loaded PLGA nanoparticles as local drug delivery system for the osteomyelitis treatment. *Acta of Bioengineering and Biomechanics* 17(3) (2015).
- [23] Imbuluzqueta E., Gamazo C., Lana H., Campanero M.-Á., Salas D., Gil A.G., Elizondo E., Ventosa N., Veciana J., Blanco-Prieto M.J.: Hydrophobic Gentamicin-Loaded Nanoparticles are Effective against *Brucella melitensis* Infection in Mice. *Antimicrobial Agents and Chemotherapy* 57(7) (2013) 3326-3333.
- [24] Dadpour S., Hosseini Doust R.: Synergistic Effects of Gold Nanoparticles Mixed with Gentamicin, Erythromycin, Clindamycin, Bacitracin, and Polymyxin B against *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococcus faecium* and *Enterococcus faecalis*. *Iranian Journal of Medicinal Microbiology* 16(4) (2022) 324-335.
- [25] Ter Boo G.A., Grijpma D.W., Richards R.G., Moriarty T.F., Eglin D.: Preparation of gentamicin dioctyl sulfosuccinate loaded poly(trimethylene carbonate) matrices intended for the treatment of orthopaedic infections. *Clinical Hemorheology and Microcirculation* 60(1) (2015) 89-98.