ANTIBACTERIAL EFFECT OF HYDROXYAPATITE AND SILVER

Alexandra AVRAM[1], Aurora MOCANU[1], Ossi HOROVITZ[1], Gheorghe TOMOAIA[2,3], Maria TOMOAIA-COTISEL[1,3]

REVIEW

Abstract. *Synthetic hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) is widely used in regards to orthopedic applications due to its similarity to the mineral component in bone. However, while HAP aids with osseointegration it does nothing when it comes to orthopedic infections. Moreover, the prevalence of antibiotic resistance makes treatment even more difficult. In view of this, adding silver to hydroxyapatite has been a focus of many studies due to the combined bioactivity of HAP and excellent antibacterial properties of Ag. The present work brings a brief introduction to more recent studies regarding the HAP-silver combination and its effect on different pathogenic strains. The effect of silver on benign cells is also discussed based on in vitro cultures and in vivo studies.*

Keywords: hydroxyapatite, silver ions, silver nanoparticles, antibacterial activity

https://doi.org/10.56082/annalsarsciphyschem.2022.2.7

1. Introduction

The ability of pathogens to resist treatment has become the most prevalent problem, with 700,000 people dying each year due to drug-resistant pathogens [1]. Moreover, orthopedic infections are one of the most common complications after surgery, especially when metallic implants are involved, due to the tendency of bacteria to form a biofilm on their surface. Tissue contamination and inflammation might lead to implant failure, especially since antibiotic resistance has to be taken into account, in terms of treatment. Biocompatible coatings on such metallic implants are quite common as they tend to help with the osseo-integration of said implants. Thus, combining these coatings with an antimicrobial agent would be an important and useful step. In particular, one in vivo study on New Zeeland rabbits [2], dealing with hydroxyapatite, HAP, and silver nanoparticles, AgNPs, deposited on a Ti6Al4V titanium alloy, revealed that the addition of silver nanoparticles influenced the implant stabilization in a positive

[1] *Babes-Bolyai University of Cluj-Napoca, Faculty of Chemistry and Chemical Engineering, Research Centre of Physical Chemistry, 11 Arany Janos Str., RO-400028, Cluj-Napoca, Romania*
[2] *Iuliu Hatieganu University of Medicine and Pharmacy, Department of Orthopedics and Traumatology, 47 General Traian Mosoiu Str., RO-400132, Cluj-Napoca, Romania*
[3] *Academy of Romanian Scientists, 3 Ilfov Str., RO-050044, Bucharest, Romania*
[*] *Corresponding author: Maria Tomoaia-Cotisel, mcotisel@gmail.com*

manner. Here, silver nanoparticles helped to reduce the period of inflammation and accelerated healing, when compared to the implants without AgNPs.

This is of no surprise, considering the fact that hydroxyapatite is highly biocompatible and bioactive, being extensively researched for biomedical applications. However, the properties and applications of HAP may differ due to different parameters such as particle size, morphology or surface characteristics. In terms of drug delivery, nano hydroxyapatite is preferred due to its larger surface area and thus higher loading capacity. The intracellular transportation capability of nanoparticles has to also be taken into account, as smaller nanoparticles tend to be more successful.

On the other hand, silver is a well-known antibacterial agent with little to no toxicity to human cells at low concentrations. While the full mechanism is not yet fully understood, it does depend on the form in which silver is administered. Literature proposes three mechanisms of action: i) disruption of DNA replication through influencing adenosine triphosphate, ATP, production; ii) prevention of proton transportation through accumulation within the membrane; iii) through the generation of oxygen reactive species (ROS) [3]. In addition, silver has been reported to have a prolonged effect. It was found that bacteria tend to absorb silver as they die, silver which can then leach out killing viable bacteria. So, it can be said that dead pathogens kill viable ones which is why this effect was titled as the "zombies effect" by Wakshlak et al. [4].

Hydroxyapatite may also help with ROS generation as calcium is an important controller in mitochondrial function, specifically within the synthesis of adenosine triphosphate [5]. There is a fragile balance between the positive and negative effects of calcium ions. An overload of mitochondrial Ca^{2+} ions can cause an amplified generation of ROS with cytochrome c release and enhanced permeability, in the end, leading to apoptosis [6]. Of course this is only helpful for pathogens that do contain a mitochondria.

In this context, the following will focus on antibacterial studies dealing with hydroxyapatite and silver, namely hydroxyapatite substituted with silver ions and hydroxyapatite to which silver nanoparticles were added. Studies with different pathogens will be analyzed to see if the way in which these two components interact and play a role on the final antibacterial effect. To eliminate all possible variables, studies that contained any other elements (i.e. secondary substitution ion alongside Ag^{+} or antimicrobial drugs) have not been considered.

2. Silver ions versus silver nanoparticles

A feature of hydroxyapatite that has been in focus recently is its ability for ionic substitution, with cations, anions or both at once [7-26]. Of course, to preserve structural stability, especially in terms of further processing, said ions

have to be similar in size and charge to the ones they substitute. Another thing worth mentioning is that while substitution can be performed in small amounts, in some cases, a total substitution is possible (i.e. calcium substituted with strontium). Regarding Ag^+ ions, they substitute Ca^{2+} with a preference for Ca(I) sites in the HAP lattice [$Ca_{10-x/2}Ag_x(PO_4)_6(OH)_2$] which increases unit cell parameters due to the larger ionic radius of Ag^+ (1.28 Å) when compared to Ca^{2+} (1.12 Å) [27]. A Rietveld study performed on silver-substituted hydroxyapatite is in favor of a substitution mechanism at both calcium sites, namely Ca(I) and Ca(II), with occupancies of about 3-5% and not the expected 10% [28]. While the theoretical limit of Ca^{2+} substitution with Ag^+ ions is 20%, in practice this might not be attainable as silver substitution tends to decrease the stability of the hydroxyapatite structure, as well as increase its solubility, depending on the added amount.

Here, structural stability is crucial in regards to further processing for medical implants. For example, as per ISO 13779-3: 2018 [29, 30] hydroxyapatite for implants has to have the Ca/P atomic ratio in the range 1.67–1.76 and be stable for 15 hours while undergoing a thermal treatment at 1000 ^oC. As for hydroxyapatite deposition on metallic substrates, in order to avoid metal oxidation with air treatments, the temperature is generally maintained up to 550 ^oC for the annealing; however with controlled atmospheres the temperature does reach up to 1000 ^oC [31]. Regardless of the chosen method, if intended for medical use, the final coated implant has to be tested in accordance to ISO standards [32, 33].

The substitution of HAP with silver ions is understandable considering they have been proven to have a slightly stronger activity than silver nanoparticles on four specific strains, namely *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermis* with all bacteria showing cell alterations after a 2 μg/mL exposure for 5 hours [34]. In the case of substituted hydroxyapatite, the consensus is that that Ag^+ ions would leach from within the HAP structure and would then interact with microorganisms. For example, Zhao et al. determined that the release of silver ions from Ag-HAP was 0.2 ppm after 1h, 0.32 ppm after 3 h and 0.34 ppm after 6h [35]. Ag^+ ions can deactivate the bacterial cell membrane by exchanging the H in the thiolic groups in proteins [36, 37]. This leads to the impossibility of the bacterial cell to perform its most basic functions. Once they enter the microorganism cell, Ag^+ ions also tend to bind to cytoplasm components or nucleic acids [38].

Many studies take another route and adsorb silver nanoparticles onto hydroxyapatite. Their mechanism of action is more or less the same, as AgNPs do tend to produce silver ions when in contact with aqueous environments. For example, one study [39] reported that 0.05 mg/L (50 ppb) silver ions were continuously released from AgNPs. When it comes to silver nanoparticles the consensus is that the production of reactive oxygen species (ROS) and their

interaction with the cellular membrane of bacteria which leads to an increased permeability is the main mechanism of action. Still, exposure to high concentrations of silver nanoparticles can induce bioaccumulation, toxicity and histological alterations [40, 41]. However, while silver nanoparticles might lead to accumulation and damage to mammalian cells, with smaller ones having a deeper effect, forming composites with biomaterials would dampen bioaccumulation while still allowing a continuous release of antibacterial Ag^+ ions [42]. In view of this, the following will present studies regarding silver coupled with hydroxyapatite and their effect on a variety of pathogens.

3. In vitro antibacterial studies

In vitro studies on pathogens are usually performed for any new potential antimicrobial agent in order to determine its lowest concentration for bacterium inhibition (MIC=minimum inhibitory concentration). However, to obtain accurate reproducible results with as little bias as possible, several factors have to be taken into account. Firstly, the choice of study method, either solid or liquid, plays an important factor as each have their pros and cons. The standard methods employed by scientific literature are agar diffusion and broth dilution.

The type of bacteria on which the antibacterial testing is performed has to also be considered as gram positive and gram negative pathogens have different structures. Typically, gram negative bacteria are harder to destroy due to their outer membrane. Fungal infections require attention as well, as fungi can be difficult to avoid. Though surgical site fungal infections are less than prevalent they might occur in certain hospital conditions.

Another crucial aspect is the fact that results tend to greatly differ with the composition of the used media. If said medium is very rich in components, silver ions tend to either form oxides, hydroxides or salts with them, thereby limiting their activity against cultured pathogens [43].

3.1. Agar diffusion method

This method has the advantage to allow the testing of multiple concentrations or even different antibacterial agents at the same time. Nevertheless, access to nutrients for bacteria can be quite limited depending on the gel content [44]. After incubation, the result is interpreted based on the measured diameter of the inhibition zone. The bigger the diameter the more susceptible that particular strain is to the tested agent. However, while agar diffusion provides a result, it does not also provide the MIC. Also, while this method is quite straightforward, certain factors have to be considered such as the form of the tested agent (pellet, loose powder, impregnated

disks) or its chemical nature (polar vs non-polar) as these can influence diffusion. Moreno et al. [45] reports that compounds with less polarity tend to have a slower diffusion when compared to their more polar counterparts, which limits the use of this method for more natural agents.

In view of this, Table 1 provides some examples of more recent studies on hydroxyapatite and silver combinations that employ agar diffusion as the method of choice for antibacterial studies. These studies were chosen as they address many different types of pathogens, gram positive and gram negative bacteria or fungi.

Table 1. Antibacterial effects of hydroxyapatite and silver performed by the disk diffusion

Amount of silver	**Addition method**	**Microorganism**	**Zone of inhibition (mm)**	**Ref.**
0.54 wt%	Silver substitution	*S. aureus* *S. epidermis* *E. coli* *P. aeruginosa*	*S. aureus:* 12 *S. epidermis:* 13 *E. coli:* 11 mm *P. aeruginosa:* 12	[46]
(0.1 M $AgNO_3$ for 0.1 M $CaNO_3$)	Silver substitution	*E. coli* *P. aeruginosa* *S. aureus* *B. subtilis* *C. albicans* *C. neoformans*	*E. coli:* 14.0±0.55 *P. aeruginosa:* 12.8±0.40 *S. aureus:* 15.5±0.85 *B. subtilis:*13.2±0.98 *C. albicans:*12.8±0.72 *C. neoformans:* 12.1±0.68	[47]
$Ca_{10-x/2}$ $Ag_x(PO_4)_6(OH)_2$ Where x=1.0	Silver substitution	*S. aureus* *B. cereus* *B. subtilis* *E. coli* *P. aerugnosa*	**For 10 mg/mL** *S. aureus:* 13.31±0.21 *B. cereus:* 11.68±0.00 *B. subtilis:* 8.44±0.26 *E coli:* no detection *P. aeruginosa:* no detection **For 30 mg/mL** *S. aureus:* 13.58±0.40 *B. cereus:* 12.65±0.24 *B. subtilis:* 10.35±0.08 *E coli:* no detection *P. aeruginosa:* no detection	[48]
Ag/[Ag + Ca] = 0.2 [Ca + 2Ag]/P = 1.67	hydroxyapatite doped silver nanoparticles (HAp – AgNPs)	*K. pneumonie* *S. aureus* *B. cereus*	**Black Sumatra bone derived HAp/AgNPs:** *K. pneumonie:*28 *S. aureus:*26 *B. cereus:*24 **Fighting cock bones derived HAp/AgNPs:** *K. pneumonie:*22 *S. aureus:*25 *B. cereus:*23	[49]

AgNPs 1, 3, 5 %	AgNPs decorated HAP	*S. aureus*	Visible zone of inhibition (exact zone not mentioned)	[50]
0.5, 1, 2.5 and 5 mM AgNPs	HAP-AgNPs powders	*E. coli* *K. oxytoca* *P. aeruginosa* *S. aureus* *S. mutans* *B. subtilis*	The higher the concentration the higher the inhibition zone (exact zone not mentioned)	[51]
$Ca_{10-x/2}Ag_x(PO_4)_6(OH)_2$ Where x=0.3; 0.4; 0.5	Silver substitution	*E. coli*	x=0.3: 2.00±0.025 x=0.4: 4.00±0.012 x=0.5: 5.00±0.016	[52]
1.07, 3.13, and 9.72 wt% Ag	silver nanoparticle-decorated hydroxyapatite (HA@Ag)	*E. coli* *P. aeruginosa* *S. aureus*	*E. coli:* 19.0 *P. aeruginosa:* 21.0 *S. aureus:* 21.0	[53]

Crystallinity of hydroxyapatite is also an important parameter to consider as it can affect its solubility and antimicrobial activity. For example, one study [54] confirmed that a 3%Ag-HAP with a degree of crystallinity of 0.064 and less silver led to a larger zone of inhibition compared to the 5%Ag-HAP with a higher degree of crystallinity (0.110) and more silver.

Contrary to agar diffusion, agar dilution is able to provide the MIC of tested agents. Tested bacteria is seeded onto agar plates supplemented with different concentrations of said antimicrobial agents. After incubation, the colony forming units (CFU) are counted. One particular benefit of this method is the ability to work with multiple strains at the same time as it is possible to stain only the resistant ones [55]. However, this method does not seem to be extremely popular for testing hydroxyapatite-silver materials, at least to the authors' knowledge.

3.2. Broth dilution method

Broth dilutions, on the other hand, provide good access to nutrients, especially considering the incubation under stirring which leads to a renewal of said nutrients [44]. The presence of bacteria in this case is provided by the appearance of turbidity. Moreover, broth dilutions are preferred in order to determine the minimal inhibitory concentration (MIC) of the tested antibacterial agent. These types of dilutions can be carried out either in glass test tubes (macrodilution) or in plastic plates with 96 wells (microdilutions). Microdilutions are preferred for the testing of multiple strains and antimicrobial agent concentrations as they require less effort and materials.

Table 2 provides the results of some more recent studies dealing with the influence of HAP-silver combinations on different types of pathogens tested by the broth dilution method.

Table 2. Antibacterial effects of hydroxyapatite and silver performed by the broth microdilution method

Amount of silver	**Addition method**	**Microorganism**	**MIC***	**MBC****	**Ref.**
(0.1 M $AgNO_3$ for 0.1 M $CaNO_3$)	Silver substitution	*E. coli* *P. aeruginosa* *S. aureus* *B. subtilis* *C. albicans* *C. neoformans*	*E. coli:* 0.321 μg/mL *P. aeruginosa:* 1.250 μg/mL *S. aureus:* 0.156 μg/mL *B. subtilis:* 0.625 μg/mL *C. albicans:* 1.250 μg/mL *C. neoformans:* 2.50 μg/mL	-	[47]
Ag/[Ag + Ca] = 0.2 [Ca + 2Ag]/P = 1.67	hydroxyapatite doped silver nanoparticles (HAp – AgNPs)	*K. pneumonie* *S. aureus* *B. cereus*	**Black Sumatra bone derived HAp/AgNPs:** *S. aureus:* 45 ± 1.2 μg/ml *K. pneumonie:* 49.5 ± 3.1 μg/ml *S. pyrogenes:* 63 ± 2.5 μg/ml *E. coli:* 72 ± 0.5 μg/ml *B. cereus:* 46 ± 1.25 μg/ml *E. aerogenes:* 89 ± 5 μg/ml **Fighting cock bones derived HAp/AgNPs:** *S. aureus:* 49 ± 2.5 μg/ml *K. pneumonie:* 53 ± 1.8 μg/ml *S. pyrogenes:* 68 ± 0.35 μg/ml *E. coli:* 79 ± 2.5 μg/ml *B. cereus:* 68 ± 1.25 μg/ml *E. aerogenes:* 104 ± 0.128μg/ml	-	[49]
0.5, 1, 2.5 and 5 mM AgNPs	HAP-AgNPs powders	*E. coli* *S. mutans* *E. aureus* *K. oxytoca* *P. aeruginosa* *B. subtilis*	*E. coli* / >0.5 mM *S. mutans* / > 0.5 mM *E. aureus* / > 0.5 mM *K. oxytoca* / > 0 5 nM *P. aeruginosa* / > 0.5 mM *B. subtilis* / > 0.5 mM	-	[51]
1.07, 3.13, and 9.72 wt% Ag	silver nanoparticle-decorated hydroxyapatite (HA@Ag)	*E. coli* *P. aeruginosa* *S. aureus*	*E. coli:* 7.8 μg/ml *P. aeruginosa:* 15.6 μg/ml *S. aureus:* 3.9 μg/ml	*E. coli:* 15.6 μg/ml *P. aeruginosa:* 62.5 μg/ml *S. aureus:* 7.8 μg/ml	[53]

$Ag_x Ca_{10-x/2}(PO_4)_6(OH)_2$ where x=1, 3, 5	Silver substitution	*E. coli* *S. aureus*	**Muller Hinton broth** *E coli:* x=1: >1,500 μg/ml x=3: 220 μg/ml x=5: 160 μg/ml *S.aureus:* x=1: >1,500 μg/ml x=3: 450 μg/ml x=5: 300 μg/ml **M9 minimal media** *E coli:* x=1: 10 μg/ml x=3: 3 μg/ml x=5: 2 μg/ml *S.aureus:* x=1: 17 μg/ml x=3: 4 μg/ml x=5: 3 μg/ml	**Muller Hinton broth** *E coli:* x=1: >1,500 μg/ml x=3: 240 μg/ml x=5: 180 μg/ml *S.aureus:* x=1: >1,500 μg/ml x=3: 475 μg/ml x=5: 350 μg/ml **M9 minimal media** *E coli:* x=1: 15 μg/ml x=3: 5 μg/ml x=5: 3 μg/ml *S.aureus:* x=1: 23 μg/ml x=3: 6 μg/ml x=5: 4 μg/ml	[54]
Ag/[Ag+ Ca] at 1.0; 1.6; 2.4%	Hydroxyapatite doped with AgNPs	*E. coli* *S. aureus* *K. pneumonie* *S. pyogenes*	**Ag1.0/HA:** *E. coli:* 5 μg/mL *S. aureus:* 20 μg/mL *K. pneumonie:*20 μg/mL *S. pyogenes:* 40 μg/mL **Ag1.6/HA:** *E. coli:* 10 μg/mL *S. aureus:* 10 μg/mL *K. pneumonie:*20μg/mL *S. pyogenes:* 20 μg/mL **Ag2.4/HA:** *E. coli:* 10 μg/mL *S. aureus:* 10 μg/mL *K. pneumonie:*20μg/mL *S. pyogenes:* 20 μg/mL	-	[56]
2%		*E. faecalis*	75 μg/ml after 24 hours of incubation	-	[57]

*MIC is the minimum inhibition concentration

**MBC is the minimum bactericidal concentration (the concentration required to kill 99.9% bacteria after incubation)

While parameters such as the amount of silver, the type of incorporation or characteristics of used hydroxyapatite might influence MIC values, there is also a correlation between MIC and the used medium. For example, one study [54] reported silver-substituted hydroxyapatite MIC concentrations for both *E. coli* and *S. aureus* using two mediums, namely Muller Hinton broth and M9 minimal media. As can be observed from Table 2 (ref [54]), MIC values are firmly lower in minimal media when compared to Muller Hinton broth. This is the case for both tested strains, gram positive *S. aureus* and gram negative *E. coli.*

This can be explained by the nutrient composition of each media and the interaction of Ag^+ ions with each individual component. M9 minimal broth only contains salts and nitrogen, being supplemented with vitamins, amino acids and glucose as needed. On the other hand, Muller Hinton broth is much more complex, having all nutrients necessary for bacterial growth. An experiment on how different metal ions interact with different culture media [58] revealed that, silver ions are quite active in both simple and more complex media, producing precipitates (when in M9 media, Ag^+ ions precipitate to AgCl). It could be deduced that the more nutritious the medium, the more compounds there are for silver to interact with, thus reducing its antibacterial properties. While it could be said that testing could be done in less nutritious environments, this is not entirely attainable in reality as certain bacterial strains do need more nutrients to survive.

4. Silver toxicity and cell compatibility

Silver has been successfully applied in clinical practice in different forms and it is generally considered safe in certain doses. This becomes even more accurate when it is coupled with hydroxyapatite, the quintessential biomaterial. In one instance, it is reported that when immersed in PBS (1-42 days), HAP/AgNPs (1, 3, 5 % AgNPs) nanocomposites release a silver ion concentration below the cytotoxicity level (10 mg/L) toward human cells [50]. However, when creating a new type of material for antibacterial purposes the focus should not be only on its effect on pathogens. Cytocompatibility studies are advisable when working with new materials.

Fortunately, there are some studies dealing with the response of different types of cells in regards to the hydroxyapatite-silver combination. For example, Rajendran et al. evaluated the cytocompatibility of an Ag-HAP (10 wt% Ag) ceramic against the NIH3T3 mouse fibroblast line, revealing no cytotoxicity in the 5 - 200 μg/ml concentration range [59]. One other study researching the effect of 2 - 5 % silver-HAP on L929 cells, osteoblast and VERO cells determined that a decrease in cell viability is observed with an increase in the amount of silver within the system while a very good cell progression is recorded for 2%Ag-HAP [60].

Even so, in vitro studies permit a choice in terms of cultures, cells are isolated, with free access to nutrients and there is a lack of an immune system. However, the toxicity or lack thereof of silver-HAP combinations has also been studied in vivo. One group [61] implanted both simple HAP and 3%Ag-HAP coated pure titanium disks that were inoculated with Methicillin resistant *S. aureus* (MRSA, UOEH6 isolated from the blood of a septic patient) into rats. Although the experiment duration was quite short (7 days) which does impose a limitation, it was showed that the 3%Ag-HAP could reduce, even if not prevent biofilm formation. On the other hand, another study [62] performed in vivo testing for 12 weeks and reported that 3%Ag-HAP offered high osteoconductivity and low toxicity, while 50% Ag-HAP coating inhibited bone formation. Here, the silver serum concentrations (at 2 weeks) were 1.1 ppb for 3%Ag-HAP and 5.3 ppb for 50%Ag-HAP which would not lead to any harmful side effects.

A comparison between HAP coatings with more or less silver was performed in vivo, on Sprague–Dawley rats by Eto [63], revealing that the implants coated with 50%Ag-HAP required less force in pull-out tests than those with 3%Ag-HAP. A clinical study by the same group [64] assessed the total hip arthroplasty of Ag-HAP coated implants on 20 patients with a total silver of 2.9 mg/implant. The study determined that the blood silver level was within a normal range, unlikely to cause any damaging effects. It is worth mentioning here that although the silver amount might appear high, generalized argyria (a rare skin condition) is only developed with a minimal dose of 4-5 g of silver [65].

5. Metal resistance, the new multi-drug resistance?

All the studies presented show that when coupled with silver, hydroxyapatite exhibits either an inhibitory or bactericidal effect on different types of strains, depending on silver concentrations. This effect is indeed promising especially considering the major problem of bacterial resistance to antibiotics. However, while not an extensively researched area, it is worth mentioning that pathogens can also develop resistance to silver nanoparticles after repeated exposure. Panacek studied this effect on different types of Gram negative bacteria, namely: *Escherichia coli* 013, *Pseudomonas aeruginos*a CCM 3955 and *E. coli* CCM 3954, observing a gradual increase in MIC values during 20 consequent cultures [66].

Over time, bacteria are capable to evolve metal-resistance mechanisms to protect itself from unpleasant effects such as disruption of membrane permeability, DNA damage or protein inactivation [67]. As concisely presented by Imran in a study dealing with metal and microplastic contaminated environments [68], there are a few of these mechanisms for metals, namely:

- ***Intracellular sequestration*** **–** this processed is controlled by the cysteine-rich protein called metallothionein. Several studies have examined this for silver, for example in the case of *Amanita strobiliformis* [69] or *Hebeloma mesophaeum* [70].
- ***Extracellular sequestration*** **–** the immobilization of metals through the extracellular secretion of polymeric substances that contain negative functional groups (EPS), thus possessing a perfect affinity for metal ions which are positively charged. One study on *Escherichia coli* confirmed that silver ions are reduced to nanoparticles and entrapped by EPS secretions [71]. In this case, cell growth was actually enhanced in the presence of silver ions (up to 0.19 mg/L). Cytochrome c (a key participant in ATP mitochondrial synthesis) found in EPS from *Shewanella oneidensis, Aeromonas hydrophila, and Pseudomonas putida* is thought to be highly involved in Ag^{+} reduction to nanoparticles [72].
- ***Bioprecipitation and biotransformation*** **–** this mechanism transforms metals into insoluble complexes that are stable. One particular study [73] observed three silver-tolerant bacterial strains, namely BAgAK-6, BAgBK-1.1, and BAgBK-3, isolated from silver-craft waste in Indonesia. BAgBK-3 especially can transform silver ions and precipitate AgNPs through NADH-dependent nitrate reductase.
- ***The alteration of morphology and the production of pigment*** **–** over time and frequent exposure, pathogens suffer alterations in morphology. For example *E. coli* 013 and *P. aeruginosa* CCM 3955 have been shown to avoid nanoparticles (20 nm) by agglomeration through the overexpression of a flagellin matrix [66]. On the other hand, *Pseudomonas aeruginosa* develops a resistance to silver nanoparticles through a phenazine pigment generation [74].
- ***Efflux mechanism*** **–** excessive metal ions are released through an efflux pump. This mechanism can be dealt with through the use of an efflux-pump blocking agent such as Verapamil used successfully on silver-resistant pathogens such as *Vibrio alginolyticus, Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis* [75].

It is quite alarming that pathogens are very adaptive and versatile when it comes to finding ways to protect themselves against attacks from antibacterial agents. Undoubtely, there is an imperative need to rethink and redesign antibacterial systems, in order to offer the best effect possible and avoid such pathogenic resistance.

6. Future trends

Evidently, silver has a good antimicrobial effect and its combination with hydroxyapatite does not impair it. This is quite promising for orthopedic applications in terms of combining osseointegration properties with strong antibacterial properties. Different drugs have also been studied in relation to hydroxyapatite and silver for quite some time – nitroxoline [76], lidocaine [77], vancomycin [78, 79], and dexamethasone [80], with increased activity. This is understandable as silver ions do tend to enhance the activity of drugs, such as antibiotics [81] and they are generally used to prevent a *surgical site infection, SSI, which* might occur after surgery in a particular part of the body.

Also, considering the prevalence of silver-based materials in more and more clinical applications and its overuse, more studies are needed to address the emergence of silver-resistant bacteria. This is of particular importance especially when it comes to hospital burn units (i.e. silver sulfadiazine, nanocrystalline silver dressings), dental care or with catheters and endotracheal tubes [82, 83].

In the case of orthopedic implants and surgical site infections, composites where each component would exhibit a form of antibacterial effect would be more practical. For example, taking hydroxyapatite and its predilection for ionic substitution, an addition of Mg^{2+} [84-88], Zn^{2+} [89-92], or Sr^{2+} [93], ions proven to exhibit certain antimicrobial properties, would be quite attainable. Forsterite (FS, Mg_2SiO_4), a biocompatible and bioactive magnesium silicate that can form hydroxyapatite in biological environments [94-99], has also been proven to hold antibacterial properties [97, 100-102]. It was also studied in combination with silver [103] so it would be a good addition to hydroxyapatite silver composites as potential coatings on metallic implants.

In addition, a further functionalization of these ceramic coating with more natural substances that would not hold any side effects, such as carotenoids [104-122], would also be beneficial. Some carotenoids have been proven to exhibit antibacterial properties [123-128]. For example, a methicillin resistant strain of *S. aureus* was inhibited alongside a multi-drug resistant strain of *E. coli* with bacterial carotenoids [129]. This is of particular importance considering the constant increase in drug-resistant strains and the emergence of ones resistant to silver.

An addition of some secondary nanoparticles such as gold nanoparticles (GNPs, AuNPs) [130-145] could also be of interest due to their outstanding surface functionalization chemistry that allows them to be functionalized with almost all electron-donating molecules [146]. Moreover, different formulations of GNPs have been proven as quite efficacious against some pathogenic strains such as *E. coli, S. aureus, B. subtilis, K. pneumoniae, C. albicans* or *P. aeruginosa*

[147-149]. Due to their photothermic properties they could also help exterminate bacteria through photothermic treatments [150].

Of course, such improvements to hydroxyapatite and silver combinations could only help enhance their efficacy. Nevertheless, considering the difference in the cell structure of pathogens, with gram positive bacteria having a thick layer of peptidoglycan and gram negative ones having a thin peptidoglycan layer but also an outer membrane, more studies on model membranes would be advisable. A potential way forward here would be the interaction of either hydroxyapatite-silver combinations or even more complex systems with Langmuir-Blodgett layers [151-158], monolayers [158-167], bilayers and blood red cells [168-171].

7. Conclusions

The hydroxyapatite-silver combination does tend to produce excellent results in terms of antibacterial effect and silver ions can get sustainably released over time, whether it be from the lattice of substituted hydroxyapatite or from AgNPs added to HAP. All in vitro antibacterial studies have promising results although the chosen method of analysis does impose certain limitations. These limitations may come in the form of poor access to nutrients and limited diffusion in solid agar and broth composition for broth dilutions.

The characteristics of the used hydroxyapatite itself are also of consequence as crystallinity was proven to affect solubility and thus antibacterial properties, with lower crystallinity HAP giving better results even at lower silver concentrations. While the antibacterial effect is mainly attributed to silver, hydroxyapatite is also a contributor, mainly through the calcium in its structure. It has been proven that an overload of mitochondrial Ca^{2+} ions can cause an amplified generation of ROS with cytochrome c release and enhanced permeability which leads to apoptosis for pathogens that possess a mitochondria.

However, cytotoxicity studies are also important alongside antimicrobial ones. In vitro studies on viable cells show a good cell progression and no toxicity. Still, a decrease in cell viability is recorded with an increase in the amount of silver. No toxicity is also documented in vivo with a 3%Ag-HAP offering high osteo-conductivity and low toxicity, while a 50% Ag-HAP coating inhibited bone formation. Also, this higher amount of silver led to a lesser force being needed in push-out tests.

In terms of clinical studies, a total hip arthroplasty of Ag-HAP coated implants (total silver of 2.9 mg/implant) on 20 patients, determined that silver blood levels were within the normal range which is reassuring. Of course, more studies are needed to better understand the interactions of silver ions and nanoparticles with different types of bacteria in terms of membrane and

organelles, and to better determine what combinations with drugs or natural substances would minimize or potentially eliminate different types of bacterial resistance.

Acknowledgment
This work was supported by the grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI-UEFISCDI, project number 186 PCE 2021.

R E F E R E N C E S

[1] A. Vasala, V.P. Hyntonen, O.H. Laitinen, Tools for rapid diagnostics of antimicrobial resistance, Frontiers in Cellular and Infection Microbiology, **10**, 308 (2020). https://doi.org/10.3389/fcimb.2020.00308

[2] J. Sulej-Chojnacka, W. Wozniak, D. Andrejewski, The effect of hydroxyapatite with silver nanoparticles on osseointegration of titanium implants, Engineering of Biomaterials **154**, 9-15 (2020). https://doi.org/10.34821/eng.biomat.154.2020.9-15

[3] J. Kolmas, E. Groszyk, D. Kwiatkowska-Róhycka, Substituted hydroxyapatites with antibacterial properties, BioMed Research International, **2014**, 178123, (2014). http://dx.doi.org/10.1155/2014/178123

[4] R.B-K. Wakshlak, R. Pedahzur, D. Avnir, Antibacterial activity of silver-killed bacteria: the "zombies" effect, Scientific Reports, 5, 9555 (2015). https://doi.org/10.1038/srep09555

[5] B.D. Fink, F. Bai, L. Yu, W.I. Sivitz, Regulation of ATP production: dependence on calcium concentration and respiratory state, American Journal of Physiology – Cell Physiology, **313**, C146-C153 (2017). https://doi.org/10.1152/ajpcell.00086.2017

[6] P.S. Brookes, Y. Yoon, J.L. Robotham, M.W. Anders, S.-S. Sheu, Calcium, ATP, and ROS: a mitochondrial love-hate triangle, American Journal of Physiology – Cell Physiology, **287**, C817-C833 (2004). https://doi.org/10.1152/ajpcell.00139.2004

[7] Gh. Tomoaia, M. Tomoaia-Cotisel, L.-B. Pop, A. Pop, O. Horovitz, A. Mocanu, N. Jumate, L.-D. Bobos, Synthesis and characterization of some composites based on nanostructured phosphates, collagen and chitosan, Revue Roumaine de Chimie, **56**(10-11), 1039-1046 (2011).

[8] C. Prejmerean, M. Tomoaia-Cotisel, E. Vasile, G. Furtos, L. Pop, M. Moldovan, C. Sarosi, I. Petean, Characterisation of surface organisation and morphology of some new experimental dental resin-based composites, International Journal of Nano and Biomaterials, **3**(4), 344-359 (2011). https://doi.org/10.1504/IJNBM.2011.045886

[9] Gh. Tomoaia, O. Soritau, M. Tomoaia-Cotisel, L.-B. Pop, A. Pop, A. Mocanu, O. Horovitz, L.-D. Bobos, Scaffolds made of nanostructured phosphates, collagen and chitosan for cell culture, Powder Technology, **238**, 99-107 (2013). https://doi.org/10.1016/j.powtec.2012.05.023

[10] G. Furtos, M. Tomoaia-Cotisel, C. Garbo, M. Senila, N. Jumate, I. Vida-Simiti, C. Prejmerean, New composite bone cement based on hydroxyapatite and nanosilver, Particulate Science and Technology, **31**(4), 392-398 (2013). https://doi.org/10.1080/02726351.2013.767293

[11] E. Forizs, F. Goga, A. Avram, A. Mocanu, I. Petean, O. Horovitz, M. Tomoaia-Cotisel, Thermal analysis of pure and multisubstituted hydroxyapatite pastes, Studia UBB Chemia, **62**(4), 173-180 (2017). https://doi.org/10.24193/subbchem.2017.4.14

[12] Gh. Tomoaia, A. Mocanu, I. Vida-Simiti, N. Jumate, L.-D. Bobos, O. Soritau, M. Tomoaia-Cotisel, Silicon effect on the composition and structure of the composition and structure of nanocalcium phosphates in vitro biocompatibility to human osteoblasts, Materials Science & Engineering C: Materials for Biological Applications, **37**, 37-47 (2014). https://doi.org/10.1016/j.msec.2013.12.027

[13] A. Mocanu, G. Furtos, S. Rapuntean, O. Horovitz, C. Flore, C. Garbo, A. Danisteanu, Gh. Rapuntean, C. Prejmerean, M. Tomoaia-Cotisel, Synthesis characterization and antimicrobial effects of composites based on multi-substituted hydroxyapatite and silver nanoparticles, Applied Surface Science, **298**, 225-235 (2014). https://doi.org/10.1016/j.apsusc.2014.01.166

[14] P.T. Frangopol, A. Mocanu, V. Almasan, C. Garbo, R. Balint, G. Borodi, I. Bratu, O. Horovitz, M. Tomoaia-Cotisel, Synthesis and structural characterization of strontium substituted hydroxyapatites, Revue Roumaine de Chimie, **61**(4-5), 337-344 (2016).

[15] A. Mocanu, R. Balint, C. Garbo, L. Timis, I. Petean, O. Horovitz, M. Tomoaia-Cotisel, Low crystallinity nanohydroxyapatite prepared at room temperature, Studia UBB Chemia, **62**(2), 95-103 (2017). https://doi.org/10.24193/subbchem.2017.2.07

[16] F. Goga, E. Forizs, A. Rotaru, A. Lucian, I. Petean, A. Mocanu, M. Tomoaia-Cotisel, Synthesis and thermal treatment of hydroxyapatite doped with magnesium, zinc and silicon, Revista de Chimie, **68**(6), 1193-1200 (2017). https://doi.org/10.37358/RC.17.6.5640

[17] C. Garbo, M. Sindilaru, A. Carlea, Gh. Tomoaia, V. Almasan, I. Petean, A. Mocanu, O. Horovitz, M. Tomoaia-Cotisel, Synthesis and structural characterization of novel porous zinc substituted nanohydroxyapatite powders, Particulate Science and Technology, **35**(1), 29-37 (2017). https://doi.org/10.1080/02726351.2015.1121180

[18] A. Avram, M. Gorea, R. Balint, L. Timis, S. Jitaru, A. Mocanu, M. Tomoaia-Cotisel, Portland cement enriched with hydroxyapatite for endodontic applications, Studia UBB Chemia, **62**(4), 81-92 (2017). https://doi.org/10.24193/subbchem.2017.4.07

[19] F. Goga, E. Forizs, G. Borodi, Gh. Tomoaia, A. Avram, R. Balint, A. Mocanu, O. Horovitz, M. Tomoaia-Cotisel, Behavior of doped hydroxyapatites during the heat treatment, Revista de Chimie, **68**(12), 2907-2913 (2017). https://doi.org/10.37358/RC.17.12.6004

[20] S. Rapuntean, P.T. Frangopol, I. Hodisan, Gh. Tomoaia, D. Oltean-Dan, A. Mocanu, C. Prejmerean, O. Soritau, L.Z. Racz, M. Tomoaia-Cotisel, In vitro response of human osteoblasts cultured on strontium substituted hydroxyapatites, Revista de Chimie, **69**(12), 3537-3544 (2018). https://doi.org/10.37358/RC.18.12.6787

[21] D. Oltean-Dan, G.B. Dogaru, M. Tomoaia-Cotisel, D. Apostu, A. Mester, H.R.C. Benea, M. G. Paiusan, E. M. Jianu, A. Mocanu, R. Balint, C.O. Popa, C. Berce, G.I. Bodizs, A.M. Toader, Gh. Tomoaia, Enhancement of bone consolidation using high-frequency pulsed electromagnetic short-waves and titanium implants coated with biomimetic composite embedded into PLA matrix: in vivo evaluation, International Journal of Nanomedicine, **14**, 5799-5816 (2019). https://doi.org/10.2147/IJN.S205880

[22] C. Garbo, J. Locs, M. D'Este, G. Demazeau, A. Mocanu, C. Roman, O. Horovitz, M. Tomoaia-Cotisel, Advanced Mg, Zn, Sr, Si multi-substituted hydroxyapatites for bone

regeneration, International Journal of Nanomedicine, **15**, 1037-1058 (2020). https://doi.org/10.2147/IJN.S226630

[23] R. Balint, I. Petean, P.T. Frangopol, A. Mocanu, G. Arghir, S. Riga, Gh. Tomoaia, O. Horovitz, M. Tomoaia-Cotisel, Biomimetic nanocomposite structures designed for coating of orthopedic implants: AFM investigation, Studia UBB Chemia, **66**(3), 141-160 (2021). https://doi.org/10.24193/subbchem.2021.3.08

[24] D. Oltean-Dan, G.-B. Dogaru, E.-M. Jianu, S. Riga, M. Tomoaia-Cotisel, A. Mocanu, L. Barbu-Tudoran, Gh. Tomoaia, Biomimetic composite coatings for activation of titanium implant surfaces: methodological approach and in vivo enhanced osseointegration, Micromachines, **12**(11), 1325 (2021). https://doi.org/10.3390/mi12111352

[25] A. Mocanu, P.T. Frangopol, R. Balint, O. Cadar, I.M. Vancea, R. Mantiu, O. Horovitz, M. Tomoaia-Cotisel, Higuchi model applied to ions release from hydroxyapatites, Studia UBB Chemia, **66**(3), 195-207 (2021). https://doi.org/10.24193/subbchem.2021.3.12

[26] A. Mocanu, O. Cadar, P.T. Frangopol, I. Petean, Gh. Tomoaia, G.-A. Paltinean, C.P. Racz, O. Horovitz, M. Tomoaia-Cotisel, Ion release from hydroxyapatite and substituted hydroxyapatites in different immersion liquids: in vitro experiments and theoretical modelling study, Royal Society Open Science, **8**(1), 201785 (2021). https://doi.org/10.1098/rsos.201785

[27] C.J. Wilcock, G.P. Stafford, C.A. Miller, Y. Ryabenkova, M. Fatima, P. Gentile, G. Mobus, P.V. Hatton, Preparation and antibacterial properties of silver-doped nanoscale hydroxyapatite pastes for bone repair and augmentation. Journal of Biomedical Nanotechnology, **13**, 1168–1176 (2017). https://doi.org/10.1166/jbn.2017.2387

[28] A. Jacobs, M. Gaulier, A. Duval, G. Renaudin, Silver doping mechanism in bioceramics—from Ag^{+}: Doped HAp to Ag^{o}/BCP nanocomposite, Crystals, **9**(7), 326 (2019). https://doi.org/10.3390/cryst9070326

[29] ISO 13779-3, Implants for surgery-hydroxyapatite-Part 3: Chemical analysis and characterization of crystallinity and phase purity, ISO: Geneva, Switzerland, 2018.

[30] T. Bazin, A. Magnaudeix, R. Mayet, P. Carles, I. Julien, A. Demourgues, M. Gaudon, E. Champion, Sintering and biocompatibility of copper-doped hydroxyapatite bioceramics, Ceramics International, **47**(10A), 13644-13654 (2021). https://doi.org/10.1016/j.ceramint.2021.01.225

[31] R. Drevet, H. Benhayoune, Electrodeposition of calcium phosphate coatings on metallic substrates for bone implant applications: a review, Coatings, **12**(4), 539 (2022). https://doi.org/10.3390/coatings12040539

[32] ISO 13779-2, Implants for surgery-Hydroxyapatite-Part 2: Thermally sprayed coatings of hydroxyapatite, ISO: Geneva, Switzerland, 2018.

[33] ISO 13779-4; Implants for surgery-hydroxyapatite-Part 4: Determination of coating adhesion strength. ISO: Geneva, Switzerland, 2018.

[34] W.-R. Li, T.-L. Sun, S.-L. Zhou, Y.-K. Ma, Q.-S. Shi, X.-B. Xie, X.-M. Huang, A comparative analysis of antibacterial activity, dynamics, and effects of silver ions and silver nanoparticles against four bacterial strains, International Biodeterioration & Biodegradation, **123**, 304-310 (2017). http://dx.doi.org/10.1016/j.ibiod.2017.07.015

[35] Y. Zhao, C.Y. Wee, H. Zhang, Z. Yang, W.E.J. Wang, E.S. Thian, Silver-substituted hydroxyapatite inhibits Pseudomonas aeruginosa outer membrane protein F: A potential

antibacterial mechanism, Biomaterials Advances, **134**, 112713 (2022). http://dx.doi.org/10.1016/j.msec.2022.112713

[36] S.Y. Liau, D.C. Read, W.J. Pugh, J.R. Furr, A.D. Russell, Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions, Letters in Applied Microbiology, **25**(4), 279–283 (1997). https://doi.org/10.1046/j.1472-765x.1997.00219.x

[37] K. Mijnendockx, N. Leys, J. Mahillon, S. Silver, R. van Houdt, Antimicrobial silver: uses, toxicity and potential for resistance, Biometals, **26**(4), 609–621 (2013). https://doi.org/10.1007/s10534-013-9645-z

[38] A. Kedziora, M. Speruda, E. Krzyzewska, J. Rybka, A. Lukowiak, G. Bugla-Ploskonska, Similarities and differences between silver ions and silver in nanoforms as antibacterial agents, International Journal of Molecular Sciences, **19**(2), 444 (2018). https://doi.org/10.3390/ijms19020444

[39] J.R. Swathy, M.U. Sankar, A. Choudhary, S. Aigal, Anshup, T. Pradeep, Antimicrobial silver: An unprecedented anion effect, Scientific Reports, **4**, 7161 (2014). https://doi.org/10.1038/srep07161

[40] M.A. Kakakhel, F. Wu, W. Saijad, Q. Zhang, I. Khan, K. Ullah, W. Wang, Long-term exposure to high-concentration silver nanoparticles induced toxicity, fatality, bioaccumulation, and histological alteration in fish (Cyprinus carpio), Environmental Sciences Europe, **33**, 14 (2021). https://doi.org/10.1186/s12302-021-00453-7

[41] Z. Ferdous, A. Nemmar, Health impact of silver nanoparticles: a review of the biodistribution and toxicity following various routes of exposure, International Journal of Molecular Sciences, **21**(7), 2375 (2020). https://doi.org/10.3390/ijms21072375

[42] J. Zhang, F. Wang, S.S.K. Yalamarty, N. Flipczak, Y. Kin, X. Li, Nano silver-induced toxicity and associated mechanisms. International Journal of Nanomedicine, **17**, 1851-1864 (2022). https://doi.org/10.2147/IJN.S355131

[43] Y. Ando, H. Miyamoto, I. Noda, F. Miyaji, T. Shimazaki, Y. Yonekura, M. Miyazaki, M. Mawatari, T. Hotokebuchi, Effect of bacterial media on the evaluation of the antibacterial activity of a biomaterial containing inorganic antibacterial reagents or antibiotics, Biocontrol Science **15**(1), 15-9 (2010). https://doi.org/10.4265/bio.15.15

[44] M. Bonnet, J.C. Lagier, D. Raoult, S. Khelaifa, Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology, New Microbes and New Infections, **34**, 100622 (2020). https://doi.org/10.1016/j.nmni.2019.100622

[45] S. Moreno, T. Scheyer, C.S. Romano, A.A. Vojnov, Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition, Free Radical Research, **40**(2), 223–231 (2006). https://doi.org/10.1080/10715760500473834

[46] K. Pajor, A. Michalicha, A. Belcarz, L. Pajchel, A. Zgadzaj, F. Wojas, J. Kolmas, Antibacterial and cytotoxicity evaluation of new hydroxyapatite-based granules containing silver or gallium ions with potential use as bone substitutes, International Journal of Molecular Sciences, **23**(13), 7102 (2022). https://doi.org/10.3390/ijms23137102

[47] S. Elbasuney, G.S. El-Sayyad, S.M. Radwan, M.A. Correa-Duarte, Antimicrobial, and antibiofilm activities of silver doped hydroxyapatite: a novel bioceramic material for dental filling. Journal of Inorganic and Organometallic Polymers and Materials, **32**, 4559-4575 (2022). https://doi.org/10.1007/s10904-022-02459-x

[48] P. Phatai, N. Prachumrak, S. Kamonwannasit, A. Kamcharoen, W. Roschat, S. Phewphong, C.M. Futalan, P. Khemthong, T. Butburee, S. Youngjan, J.C. Millare, O. Prasitnok, Zinc-silver doped mesoporous hydroxyapatite synthesized via ultrasonic in combination with sol-gel method for increased antibacterial activity, Sustainability, **14**(18), 11756, (2022). https://doi.org/10.3390/su141811756

[49] P. Vijayaraghavan, M.A. Rathi, K.S. Almaary, F.S. Alkhattaf, Y.B. Albadawi, S.W. Chang, B. Ravindran, Preparation and antibacterial application of hydroxyapatite doped Silver nanoparticles derived from chicken bone. Journal of King Saud University – Science, **34**(2), 101749 (2022). https://doi.org/10.1016/j.jksus.2021.101749

[50] S.-H. Bee, Y. Bustami, A. Ul-Hamid, K. Lim, Z.A.A. Hamid, Synthesis of silver nanoparticle-decorated hydroxyapatite nanocomposite with combined bioactivity and antibacterial properties, Journal of Materials Science: Materials in Medicine, **32**, 106 (2021). https://doi.org/10.1007/s10856-021-06590-y

[51] P.N. Silva-Holguin, S.Y. Reyes-Lopez, Synthesis of hydroxyapatite-ag composite as antimicrobial agent. Dose-Response: An International Journal, **18**(3), 1-14 (2020). https://doi.org/10.1177/1559325820951342

[52] H. Wahid, A. Singh, Study of antimicrobial activity of silver-doped hydroxyapatite, Asian Journal of Pharmaceutical and Clinical Research, **12**(8), 243-245 (2019). http://dx.doi.org/10.22159/ajpcr.2019.v12i8.34603

[53] Z. Ni, X. Gu, Y. He, Z. Wang, X, Zhou, Y. Zhao, L. Sun, Synthesis of silver nanoparticle-decorated hydroxyapatite (HA@Ag) poriferous nanocomposites and the study of their antibacterial activities, RCS Advances, **8**, 41722-41730 (2018). https://doi.org/10.1039/c8ra08148d

[54] S. Jadalannagari, K. Deshmukh, S.R. Ramanan, M. Kowhik, Antimicrobial activity of hemocompatible silver doped hydroxyapatite nanoparticles synthesized by modified sol–gel technique, Applied Nanoscience, **4**, 133-141 (2014). https://doi.org/10.1007/s13204-013-0197-x

[55] A. Schumacher, T. Vranke, A. Malhotra, J.J.C. Arts, P. Habibovic, In vitro antimicrobial susceptibility testing methods: agar dilution to 3D tissue-engineered models, European Journal of Clinical Microbiology & Infectious Diseases, **37**, 187–208 (2018). https://doi.org/10.1007/s10096-017-3089-2

[56] I. Fatimah, H. Hidayat, G. Purwiandono, K. Khoirunisa, H.A. Zahra, R. Audita, S. Sagadevan, Green synthesis of antibacterial nanocomposite of silver nanoparticle-doped hydroxyapatite utilizing curcuma longa leaf extract and land snail (achatina fulica) shell waste, Journal of Functional Biomaterials, **13**(2), 84 (2022). https://doi.org/10.3390/jfb13020084

[57] V. Gonzales-Torres, E. Hernandez-Guevara, N.A. Castillo-Martinez, M. Rosales-Aguilar, G.C. Diaz-Trujillo, Antibacterial activity analysis of hydroxyapatite based materials with fluorine and silver. Revista Mexicana de la Ingineria Biomedica, **42**(2), 49-57 (2021). https://dx.doi.org/10.17488/RMIB.42.2.4

[58] J. Rewak-Soroczynska, A. Dorotkiewicz-Jach, Z. Drulis-Kawa, R.J. Wiglusz, Culture media composition influences the antibacterial effect of silver, cupric, and zinc ions against *Pseudomonas aeruginosa*, Biomolecules, **12**, 963 (2022). https://doi.org/10.3390/biom12070963

[59] A. Rajendran, R.C. Barik, D. Natarajan, M.S. Kiran, D.K. Pattanayak, Synthesis, phase stability of hydroxyapatite–silver composite with antimicrobial activity and cytocompatability, Ceramics International, **40**, 10831-10838 (2014). http://dx.doi.org/10.1016/j.ceramint.2014.03.075

[60] U. Erdem, B.M. Bozer, M.B. Turkoz, A.U. metin, G. Yildirim, M. Turk, S. Nezir, Spectral analysis and biological activity assessment of silver doped hydroxyapatite, Journal of Asian Ceramic Society, **9**(4), 1524-1545 (2021). https://doi.org/10.1080/21870764.2021.1989749

[61] M. Ueno, H. Miyamoto, M. Tsukamoto, S. Eto, I. Noda, T. Shobuike, T. Kobatake, M. Sonohata, M. Mawatari, Silver-Containing Hydroxyapatite Coating Reduces Biofilm Formation by Methicillin-Resistant Staphylococcus aureus In Vitro and In Vivo, BioMed Research International, **2016**, 8070597 (2016). http://dx.doi.org/10.1155/2016/8070597

[62] Y. Yonekura, H. Miyamoto, T. Shimazaki, Y. Ando, I. Noda, M. Mawatari, T. Hotokebuchi, Osteoconductivity of thermal-sprayed silver-containing hydroxyapatite coating in the rat tibia. The Journal of Bone and Joint Surgery—British, **93**(5), 644–649 (2011). https://doi.org/10.1302/0301-620x.93b5.25518

[63] S. Eto, H. Moyamoto, T. Shobuike, I. Noda, T. Akiyama, M. Tsukamoto, M. Ueno, S. Someya, S. Kawano, M. Sonohata, M. Mawatari, Silver oxide-containing hydroxyapatite coating supports osteoblast function and enhances implant anchorage strength in rat femur, Journal of Orthopaedic Research, **33**(9), 1391–1397 (2015) https://doi.org/10.1002/jor.22903

[64] S. Eto, S. Kawano, S. Someya, H. Miyamoto, M. Sonohata, M. Mawatari, First clinical experience with thermal-sprayed silver oxide-containing hydroxyapatite coating implant, Journal of Arthroplasty, **31**(7), 1498–1503 (2016). https://doi.org/10.1016/j.arth.2015.12.034

[65] J.K. McKenna, C.M. Hull, Argyria associated with colloidal silver supplementation, International Journal of Dermatology, **42**, 549-549 (2003). https://doi.org/10.1046/j.1365-4362.2003.01899.x

[66] A. Panacek, L. Kvitek, M. Smekalova, R. Vecerova, M. Kolar, M. Roderova, F. Dycka, M. Sebela, R. Prucek, O. Tomanec, R. Zboril, Bacterial resistance to silver nanoparticles and how to overcome it, Nature Nanotechnology, **13**, 65-71 (2018). https://doi.org/10.1038/s41565-017-0013-y

[67] N. Nino-Martinez, M.F. Salas Orozco, G.-A. Martinez-Castanon, F. Torres Mendez, F. Ruiz, Molecular mechanisms of bacterial resistance to metal and metal oxide nanoparticles, International Journal of Molecular Sciences, **20**(11), 2808 (2019). https://doi.org/10.3390/ijms20112808

[68] M. Imran, K.R. Das, M.M. Naik, Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat, Chemosphere, **215**, 846-857 (2019). https://doi.org/10.1016/j.chemosphere.2018.10.114

[69] M. Osobova, V. Urban, P.L. Jedelsky, J. Borovicka, M. Gryndler, T. Ruml, P. Kotrba, Three metallothionein isoforms and sequestration of intracellular silver in the hyper accumulator Amanita strobiliformis, New Phytologist, **190**, 916-926 (2011). https://doi.org/10.1111/j.1469-8137.2010.03634.x

[70] J. Sacky, T. Leonhardt, J. Borovivka, M. Gryndler, A. Briksi, P. Kotrba, Intracellular sequestration of zinc, cadmium and silver in Hebeloma mesophaeum and characterization of its metallothionein genes, Fungal Genetics and Biology, **67**, 3-14 (2014). http://dx.doi.org/10.1016/j.fgb.2014.03.003

[71] F. Kang, P.J. Alvarez, D. Zhu, Microbial extracellular polymeric substances reduce ag+ to silver nanoparticles and antagonize bactericidal activity, Environmental Science & Technology, **48**(1), 316-322 (2014). https://doi.org/10.1021/es403796x

[72] S.-W. Li, X. Zhang, G.-P. Sheng, Silver nanoparticles formation by extracellular polymeric substances (EPS) from electroactive bacteria, Environmental Science and Pollution Research, **23**, 8627-8633 (2016). https://doi.org/10.1007/s11356-016-6105-7

[73] A.P. Sari, F. Nurhidayati, harjoso, E.S. Soertarto, Silver nanoparticles biotransforming bacteria isolated from silver-craft waste, Transactions on Science and Technology, **4**(2), 92-97 (2017).

[74] D.H. Ellis, E.I. Maurer-Gardner, C.E. Sulentic, S.M. Hussain, Silver nanoparticle antibacterial efficacy and resistance development in key bacterial species. Biomedical Physics & Engineering Express, **5**, 015013 (2018). https://doi.org/10.1088/2057-1976/aad5a7

[75] J. Jose, A. Anas, B. Jose, A.B. Puthirath, S. Athiyanathil, C. Jasmin, M.R. Anantharaman, S. Nair, C. Subrahmanyam, Bijiu, Extinction of antimicrobial resistant pathogens using silver embedded silica nanoparticles and an efflux pump blocker, Applied Biomaterials, **2**(11), 4681-4686 (2019). https://doi.org/10.1021/acsabm.9b00614

[76] A. Danistean, M. Gorea, A. Avram, S. Rapuntean, Gh. Tomoaia, A. Mocanu, C. Garbo, O. Horovitz, M. Tomoaia-Cotisel, Antimicrobial activity of ceramic disks loaded with silver ions and nitroxoline, Studia UBB Chemia, **61**(3(1)), 275-283 (2016).

[77] A. Dubnika, D. Loca, V. Rudovica, M.B. Parekh, L. Berzina-Cimdina, Functionalized silver doped hydroxyapatite scaffolds for controlled simultaneous silver ion and drug delivery, Ceramics International, **43**, 3698-3705 (2017). http://dx.doi.org/10.1016/j.ceramint.2016.11.214

[78] A. Hashimoto, H. Miyamoto, T. Kobatake, T. Nakashima, T. Shobuike, M. Ueno, T. Murakami, I. Noda, M. Sonohata, M. Matawari, The combination of silver-containing hydroxyapatite coating and vancomycin has a synergistic antibacterial effect on methicillin-resistant Staphylococcus aureus biofilm formation, Bone & Joint Research, **9**(5), 211-218 (2020). https://doi.org/10.1302%2F2046-3758.95.BJR-2019-0326.R1

[79] A. Hashimoto, H. Moyamoto, S. Kii, T. Kobatake, T. Shobuike, I. Noda, M. Sonohata, M. Mawatari, Time-dependent efficacy of combination of silver-containing hydroxyapatite coating and vancomycin on methicillin-resistant Staphylococcus aureus biofilm formation in vitro, BMC Research Notes, **14**, 81 (2021). https://doi.org/10.1186/s13104-021-05499-7

[80] A. Dubnika, D. Loca, L. Berzina-Cimdina, Cross-linked dexamethasone sodium phosphate delivery systems based on silver doped hydroxyapatite scaffolds, Key Engineering Materials, **721**, 192-196 (2017). https://doi.org/10.4028/www.scientific.net/KEM.721.192

[81] J.R. Morones-Ramirez, J.A. Winkler, C.S. Spina, J.J. Collins, Silver enhances antibiotic activity against gram-negative bacteria, Science Translational Medicine, **5**, 190 (2013). https://doi.org/10.1126/scitranslmed.3006276.190ra81

[82] I. Khansa, A.R. Schoenbrunner, C.T. Kraft, J.E. Janis, Silver in Wound care—friend or foe?: A comprehensive review, Plastic and Reconstructive Surgery - Global Open, **7**(8), e2390 (2019). https://doi.org/10.1097/GOX.0000000000002390

[83] C. Couto-Fernandez, A.R. Sokolonski, M. Santos-Fonseca, D. Stanisic, D. Barral Araujo, V. Azevedo, R. Dias-Portela, L. Tasic, Applications of silver nanoparticles in dentistry: advances and technological innovation, International Journal of Molecular Sciences, **22**, 2485 (2021). https://doi.org/10.3390/ijms22052485

[84] Y. Xie, L. Yang, Calcium and magnesium ions are membraneactive against stationary-phase *Staphylococcus aureus* with high specificity, Scientific Reports, **6**, 20628 (2016). https://doi.org/10.1038/srep20628

[85] S. Subramani, Perdreau-Dahl, J.P. Morth, The magnesium transporter A is activated by cardiolipin and is highly sensitive to free magnesium in vitro, eLife **5**, e11407 (2016). https://doi.org/10.7554/eLife.11407

[86] A. Som, L. Yang, G.C.L. Wong, G.N. Tew, Divalent metal ion triggered activity of a synthetic antimicrobial in cardiolipin membranes, Journal of the American Chemical Society, **131**(42), 15102–15103 (2009). https://doi.org/10.1021/ja9067063

[87] S. Sennato, F. Bordi, C. Cametti, C. Coluzza, A. Desideri, S. Rufini, Evidence of domain formation in cardiolipin-glycerophospholipid mixed monolayers, A thermodynamic and AFM study, The Journal of Physical Chemistry B, **109**(33), 15950–15957 (2005). https://doi.org/10.1021/jp051893q

[88] Q. Li, S. Mahendra, D.Y. Lyon, L. Brunet, M.V. Liga, D. Li, P.J.J. Alvarez, Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications, Water Research, **42**(18), 4591–4602 (2008). https://doi.org/10.1016/j.watres.2008.08.015

[89] D. Boyd, H. Li, D.A. Tanner, M.R. Towler, J.G. Wall, The antibacterial effects of zinc ion migration from zinc-based glass polyalkenoate cements, Journal of Materials Science: Materials in Medicine, **17**, 489-494 (2006). https://doi.org/10.1007/s10856-006-8930-6

[90] G. Jin, H. Cao, Y. Qiao, F. Meng, H. Zhu, X. Liu, Osteogenic activity and antibacterial effect of zinc ion implanted titanium, Colloids and Surfaces B: Biointerfaces, **117**, 158-165 (2014). http://dx.doi.org/10.1016/j.colsurfb.2014.02.025

[91] R.-J. Chung, M.-F. Hsieh, C.-W. Huang, L.-H. Perng, H.-W. Wen, T.-S. Chin, Antimicrobial effects and human gingival biocompatibility of hydroxyapatite sol–gel coatings, Journal of Biomedical Materials Research B: Applied Biomaterials, **76**(1), 169–178 (2006). https://doi.org/10.1002/jbm.b.30365

[92] A. Anwar, S. Akbar, A. Sadiqa, M. Kazmi, Novel continuous flow synthesis, characterization and antibacterial studies of nanoscale zinc substituted hydroxyapatite bioceramics, Inorganica Chimica Acta, **453**, 16–22 (2016). https://doi.org/10.1016/j.ica.2016.07.041

[93] A.D. Anastasiou, M. Nerantzaki, E. Gounari, M.S. Duggal, P.V. Giannoudis, A. Jha, D. Bikiaris, Antibacterial properties and regenerative potential of Sr^{2+} and Ce^{3+} doped fluorapatites; a potential solution for periimplantitis. Scientific Reports, **9**, 14469-14480 (2019). https://doi.org/10.1038/s41598-019-50916-44

[94] M.A. Naghiu, M. Gorea, E. Mutch, F. Kristaly, M. Tomoaia-Cotisel, Forsterite nanopowder: structural characterization and biocompatibility evaluation, Journal of Materials Science & Technology, **29**(7), 628–632 (2013). https://doi.org/10.1016/j.jmst.2013.04.007

[95] G. Furtos, M.A. Naghiu, H. Declerq, M. Gorea, C. Prejmerean, O. Pana, M. Tomoaia-Cotisel, Nano forsterite biocomposites for medical applications: mechanical properties and bioactivity, Journal of Biomedical Materials Research Part B: Applied Biomaterials, **104B**(7), 1290–1301 (2016). https://doi.org/10.1002/jbm.b.33396

[96] M. Gorea, M.-A. Naghiu, A. Avram, I. Petean, A. Mocanu, M. Tomoaia-Cotisel, Novel porous forsterite ceramics biocompatibility and bioactivity evaluation, Revista de Chimie, **71**(2), 343–351 (2020). https://doi.org/10.37358/RC.20.2.7935

[97] R. Choudhary, P. Manohar, J. Vecstaudza, M.J. Yanez-Gascon, H. Perez Sanchez, R. Nachimuthu, J. Locs, S. Swamiappan, Preparation of nanocrystalline forsterite by combustion of different fuels and their comparative in-vitro bioactivity, dissolution behavior and antibacterial studies, Materials Science and Engineering C, **77**, 811–822 (2017). https://doi.org/10.1016/j.msec.2017.03.308

[98] S.K. Venkatraman, R. Choudhary, N. Vijayakumar, G. Krishnamurithy, H.R.B. Raghavendran, M.R. Murali, T. Kamarul, A. Suresh, J. Abraham, S. Swamiappan, Investigation on bioactivity, mechanical stability, bactericidal activity and in-vitro biocompatibility of magnesium silicates for bone tissue engineering applications, Journal of Materials Research, **37**, 608–621 (2022). https://doi.org/10.1557/s43578-021-00450-9

[99] A. Avram, A. Mocanu, Gh. Tomoaia, M. Tomoaia-Cotisel, Review on the Biocompatibility and Bioactivity of Forsterite: In Vitro and In Vivo Studies, Academy of Romanian Scientists Annals Series on Biological Sciences, **11**(1), 167-190 (2022).

[100] R. Choudhary, S.K. Venkatraman, I. Bulygina, A. Chatterjee, J. Abraham, F. Senatov, S. Kaloshkin, A. Ilyasov, M. Abakumov, M. Knyazeva, D. Kukui, F. Walther, S. Swamiappan, Impact of forsterite addition on mechanical and biological properties of composites, Asian Journal of Ceramic Societies, **8**(4), 1051-1065 (2020). https://doi.org/10.1080/21870764.2020.1807695

[101] R. Choudhary, A. Chaterjee, S.K. Venkatraman, S. Koppala, J. Abraham, S. Swamiappan, Antibacterial forsterite (Mg_2SiO_4) scaffold: a promising bioceramic for load bearing applications, Bioactive Materials, **3**(3), 218–224 (2018). https://doi.org/10.1016/j.bioactmat.2018.03.003

[102] A. Avram, S. Rapuntean, M. Gorea, Gh. Tomoaia, A. Mocanu, O. Horovitz, Gh. Rapuntean, M. Tomoaia-Cotisel, In vitro antibacterial effect of forsterite nanopowder: synthesis and characterization, Environmental Science and Pollution Research, **29,** 77097-77112 (2022). https://doi.org/10.56082/annalsarscibio.2022.1.167

[103] A. Avram, M. Gorea, S. Rapuntean, A. Mocanu, G.A. Paltinean, Cs. Varhely Jr., I. Petean, O. Horovitz, M. Tomoaia-Cotisel, In-vitro Antibacterial Activity of Novel Nanostructured Composites Based on Forsterite and Silver Nanoparticles, Revista de Chimie, **71**(1), 13-21 (2020).

[104] E. Chifu, M. Tomoaia-Cotisel, Z. Andrei, Mixed monolayers of canthaxanthin with lipids, Studia UBB Chemia, **24**(2), 63-67 (1979).

[105] E. Chifu, M. Tomoaia-Cotisel, Insoluble monolayers of lecithin and carotenoid pigments, Revue Roumaine de Chimie, **24**(7), 979-986 (1979).

[106] E. Chifu, M. Tomoaia, A. Ioanette, Behaviour of canthaxanthin at the benzene/water and air/water interfaces, Gazzetta Chimica Italiana, **105**(11-12), 1225-1232 (1975).

[107] E. Chifu, M. Tomoaia, E. Nicoară, A. Olteanu, Isozeaxanthin films at the oil/water and air/water interfaces, Revue Roumaine de Chimie, **23**(8), 1163-1169 (1978).

[108] E. Chifu, M. Tomoaia-Cotisel, Z. Andrei, E. Bonciu, β-apo-8-carotenoic acid ethyl ester films at fluid interfaces, Gazzetta Chimica Italiana, **109**(6-7), 365-369 (1979).

[109] E. Chifu , M. Tomoaia-Cotisel, A. Ioanette, Mixed insoluble monolayers of cholesterol and β-apo-8-carotenal, Gazzetta Chimica Italiana, **109**(6-7), 397-398 (1979).

[110] E. Chifu, J. Zsako, M. Tomoaia-Cotisel, Xanthophyll films. I. Single-component monolayers at the air/water interface, Journal of Colloid and Interface Science, **95**(2), 346-354 (1983).

[111] M. Tomoaia-Cotisel, E. Chifu, Carotenoid pigment films at fluid interface, Revista de Chimie, **32**(11), 1063-1069 (1981).

[112] M. Tomoaia-Cotisel E. Chifu, Mixed insoluble monolayers with β-apo-8-carotenoic acid ethyl ester, Gazzetta Chimica Italiana, **109**(6-7), 371-375 (1979).

[113] M. Tomoaia-Cotisel, E. Chifu, J. Zsako, Mixed monolayers of egg lecithin and carotenoids, Colloids and Surfaces, **14**, 239-246 (1985).

[114] M. Tomoaia-Cotisel, J. Zsako, E. Chifu, Ejection curves and miscibility of egg lecithin with some carotenoid derivatives, Revue Roumaine de Chimie, **32**(7), 663-670 (1987).

[115] M. Tomoaia-Cotisel, I. Albu, E. Chifu, Adsorption of carotene and albumin at the oil/water interface, Studia UBB Chemia, **24**(2), 68-73 (1979).

[116] M. Tomoaia-Cotisel, J. Zsako, M. Salajan, E. Chifu, Interaction of unimolecular films of some carotenoids with electrolytes at the air/water interface, in Water and Ions in Biological Systems (A. Pullman, V. Vasilescu, and L. Packer Eds.), Union of Societies for Medical Sciences, Bucharest, Romania, pp. 371-381 (1985).

[117] M. Tomoaia-Cotisel, E. Chifu, Xanthophyll films. II. Two-component monolayers of some xanthophylls and egg lecithin at the air/water interface, Journal of Colloid and Interface Science, **95**(2), 355-361 (1983).

[118] L.Z. Racz, G.-A. Paltinean, I. Petean, Gh. Tomoaia, L. C. Pop, G. Arghir, E. Levei, A. Mocanu, Cs.-P. Rácz, M. Tomoaia-Cotisel, Curcumin and Whey Protein Binding And Structural Characteristics of Their Complex Evidenced by Atomic Force Microscopy, Studia UBB Chemia, **67**(3), 61-74 (2022). https://doi.org/10.24193/subbchem.2022.3.05

[119] L.Z. Rácz, Cs.-P. Rácz, O. Horovitz, Gh. Tomoaia, A. Mocanu, I. Kacso, M. Sárközi, M. Dan, S. Porav, G. Borodi, M. Tomoaia-Cotisel, Complexation of Curcumin using Whey Proteins to Enhance Aqueous Solubility, Stability and Antioxidant Property, Studia UBB Chemia, **67**(3), 75-99 (2022). https://doi.org/10.24193/subbchem.2022.3.06

[120] L.Z. Rácz, Cs. P. Rácz, L. C. Pop, Gh. Tomoaia, A. Mocanu, I. Barbu, M. Sárközi, I. Roman, A. Avram, M. Tomoaia-Cotisel, V.-A. Toma, Strategies for improving bioavailability, bioactivity, and physical-chemical behavior of the curcumin, Molecules, **27**, 6854 (2022). https://doi.org/10.3390/molecules27206854

[121] J. Zsako, E. Chifu, M. Tomoaia-Cotisel, Rotating rigid-plate model of carotenoid molecules and the behaviour of their monolayers at the air/water interface, Gazzeta Chimica Italiana, **109**(11-12), 663-668 (1979).

[122] P. Joos, A. Tomoaia-Cotisel, A.J. Sellers, M. Tomoaia-Cotisel, Adsorption kinetics of some carotenoids at the oil/water interface, Colloids and Surfaces B: Biointerfaces, 37(3-4), 83-91 (2004). https://doi.org/10.1016/j.colsurfb.2004.03.019

[123] T.M. Karpinski, A. Adamczak, Fucoxanthin - an antibacterial carotenoid, Antioxidants, **8**(8), 239 (2019). https://doi.org/10.3390/antiox8080239

[124] A.Y. Yoo, M. Alnaeeli, J.K. Park, Production control and characterization of antibacterial carotenoids from the yeast Rhodotorula mucilaginosa AY-01, Process Biochemistry, **51**(4), 463-473 (2016). https://doi.org/10.1016/j.procbio.2016.01.008

[125] T.M. Karpinski, M. Ozarowsky, R. Alam, M. Lochynska, What do we know about antimicrobial activity of Astaxanthin and Fucoxanthin, Marine Drugs, **20**(1), 36 (2022). https://doi.org/10.3390/md20010036

[126] M. Dias Moreira, M. Magalhaes Melo, J. Marques Coimbra, K.C. dos Reis, Solid coffee waste as alternative to produce carotenoids with antioxidant and antimicrobial activities, Waste Management, **82**, 93-99 (2018). https://doi.org/10.1016/j.wasman.2018.10.017

[127] S.Y. Abdulhadi, R.N. Gergees, G.Q. Hasan, Molecular Identifification, antioxidant effifficacy of phenolic compounds, and antimicrobial activity of beta-carotene isolated from fruiting bodies of Suillus sp, Karbala International Journal of Modern Science, **6**(4), 365-374 (2020). : https://doi.org/10.33640/2405-609X.1966

[128] T.M. Kekeli, Z. Erginkaya, E. Turkkan, U. Kaya, Antioxidant and antibacterial effects of carotenoids extracted from rhodotorula glutinis strains, Asian Journal of Chemistry, **25**(1), 42-46 (2013). http://dx.doi.org/10.14233/ajchem.2013.12377

[129] L. Kusmita, Y.A. Tatsa, Y.D. Franyoto, A. Sabdono, A. Trianto, O.K. Radjasa, Antibacterial activity of carotenoid from bacterial symbiont Virgibacillus salarius strain 19.PP.Sc.1.6 against MDR E. coli and MRSA, Egyptian Journal of Aquatic Biology & Fisheries, **25**(3), 147-157 (2021).

[130] R.D. Pasca, A. Mocanu, S.C. Cobzac, I. Petean, O. Horovitz, M. Tomoaia-Cotisel, Biogenic syntheses of gold nanoparticles using plant extracts, Particulate Science and Technology, **32**(2), 131-137 (2014). https://doi.org/10.1080/02726351.2013.839589

[131] L. Barbu-Tudoran, Gh. Tomoaia, O. Horovitz, A. Mocanu, M. Tomoaia-Cotisel, Self-assembly characteristics of gold nanoparticles in the presence of arginine, Journal of Optoelectronics and Advanced Materials, **10**(9), 2293-2297 (2008).

[132] A. Avram, Gh. Tomoaia, A. Mocanu, M. Tomoaia-Cotisel, Gold nanoparticles and chemotherapeutic agents, Academy of Romanian Scientists, Annals Series on Biological Sciences, **5**(2), 23-64 (2020). https://doi.org/10.56082/annalsarsciphyschem.2020.2.23

[133] M. Tomoaia-Cotisel, A. Mocanu, O. Horovitz, E. Indrea, Gh. Tomoaia, I. Bratu, Self-assembly of gold nanoparticles functionalized with amino acids and aleurone globular protein, in Book series (Proceedings of SPIE): Advanced Topics in Optoelectronics, Microelectronics, and Nanotechnologies IV, edited by P. Schiopu, C. Panait, G. Caruntu, A. Manea, (Constanta, Romania, 2009), **7297**, 729708 (2009). https://doi.org/10.1117/12.823616

[134] O. Horovitz, Gh. Tomoaia, A. Mocanu, T. Yupsanis, M. Tomoaia-Cotisel, Protein binding to gold colloids, Gold Bulletin, **40**(3), 213-218 (2007). https://doi.org/10.1007/BF03215583

[135] O. Horovitz, A. Mocanu, Gh. Tomoaia, L. Olenic, Gh. Mihailescu, O. Borostean, A. Popoviciu, C. Craciun, T. Yupsanis, M. Tomoaia-Cotisel, Synthesis, characterization and properties of gold nanoparticles in colloidal aqueous solutions in the absence and in the presence of globular proteins. Auto-assembled gold nanostructures in thin films, in Convergence of Micro-Nano-Biotechnologies, edited by M. Zaharescu, E. Burzo, L. Dumitru, I. Kleps and D. Dascalu (Romanian Academy Press, Bucharest, Romania, 2006), Vol. 9, pp. 132-146.

[136] Gh. Tomoaia, P.T. Frangopol, O. Horovitz, L.D. Bobos, A. Mocanu, M. Tomoaia-Cotisel, The effect of arginine on gold nanoparticles in colloidal solutions and in thin films, Journal of Nanoscience and Nanotechnology, **11**(9), 7762-7770 (2011). https://doi.org/10.1166/jnn.2011.4734

[137] O. Horovitz, Gh. Tomoaia, A. Mocanu, T. Yupsanis, M. Tomoaia-Cotisel, Protein binding to gold auto assembled films, Gold Bulletin, **40**(4), 295-304 (2007). https://doi.org/10.1007/BF03215603

[138] M. Tomoaia-Cotisel, Gh. Tomoaia, E. Indrea, L.-D. Bobos, O. Horovitz, A. Mocanu, Interfacial nanomaterials based on gold nanoparticles, protein and chitosan, Journal of Optoelectronics and Advanced Materials, Symposia, **2**(1), 125-130 (2010).

[139] A. Mocanu, I. Cernica, Gh. Tomoaia, L.D. Bobos, O. Horovitz, M. Tomoaia-Cotisel, Self-assembly characteristics of gold nanoparticles in the presence of cysteine, Colloids and Surfaces A: Physicochemical and Engineering Aspects, **338**(1-3), 93-101 (2009). https://doi.org/10.1016/j.colsurfa.2008.12.041

[140] I. Petean, Gh. Tomoaia, O. Horovitz, A. Mocanu, M. Tomoaia-Cotisel, Cysteine mediated assembly of gold nanoparticles, Journal of Optoelectronics and Advanced Materials, **10**(9), 2289-2292 (2008).

[141] E. Indrea, S. Dreve, I. Bratu, Gh. Mihailescu, L. Olenic, Gh. Tomoaia, A. Mocanu, O. Horovitz, M. Tomoaia-Cotisel, Multifunctional materials based on chitosan, globular protein and gold nanoparticles, Technical Proceedings of the 2007 NSTI-Nanotech, Santa Clara, California, USA, Vol. 4, Chapter 3, pp. 461-464 (2007).

[142] Gh. Tomoaia, O. Horovitz, A. Mocanu, A. Nita, A. Avram, C.P. Racz, O. Soritau, M. Cenariu, M. Tomoaia-Cotisel, Effects of doxorubicin mediated by gold nanoparticles and resveratrol in two human cervical tumour cell line, Colloids and Surfaces B: Biointerfaces, **135**, 726-734 (2015). https://doi.org/10.1016/j.colsurfb.2015.08.036

[143] O Horovitz, A. Mocanu, Gh. Tomoaia, M. Crisan, L. D. Bobos, C. Racz, M. Tomoaia-Cotisel, Amino acids binding to gold nanoparticles, Studia UBB Chemia, **52**(3), 53-71 (2007).

[144] O. Horovitz, A. Mocanu, Gh. Tomoaia, L. D. Bobos, D. Dubert, I. Daian, T. Yupsanis, M. Tomoaia-Cotisel, Lysine mediated assembly of gold nanoparticles, Studia UBB Chemia, **52**(1), 97-108 (2007).

[145] A. Mocanu, R.D. Pasca, Gh. Tomoaia, A. Avranas, O. Horovitz, M. Tomoaia-Cotisel, Selective effect of procaine tetracaine and dibucaine on gold nanoparticles, Journal of Nanoscience and Nanotechnology, **12**(12), 8935-8939 (2012). https://doi.org/10.1166/jnn.2012.6707

[146] N.R.S. Sibuyi, K.L. Moabelo, A.O. Fadaka, S. Meyer, M.O. Onani, A.M. Madiehe, M. Meyer, Multifunctional gold nanoparticlesor improved diagnostic and therapeutic applications: a review, Nanoscale Research Letters, **16**, 174 (2021). https://doi.org/10.1186/s11671-021-03632-w

[147] X. Li, S.M. Robinson, A. Gupta, K. Saha, Z. Jang, D.F. Moyano, A. Sahar, M.A. Riley, V.M. Rotello, Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria, ACS Nano, **8**(10), 10682-10686 (2014). https://doi.org/10.1021/nn5042625

[148] S. Sharmaila, N. Zafar, S. Riaz, R. Sharif, J. Nazir, S. Naseem, Gold nanoparticles: an efficient antimicrobial agent against enteric bacterial human pathogen, Nanomaterials, **6**(4), 71 (2016). https://doi.org/10.3390/nano6040071

[149] E. Piktel, L. Suprewicz, J. Depciuch, S. Chmielewska, K. Sklodowski, T. Daniluk, G. Krol, P. Kolat-Brodecka, P. Bijak, A. Pajor-Swierzy, K. Fiedoruk, M. Parlinska-Wojtan, R. Bucki, Varied-shaped gold nanoparticles with nanogram killing efficiency as potential antimicrobial surface coatings for the medical devices, Scientific Reports, **11**, 12546 (2021). https://doi.org/10.1038/s41598-021-91847-3

[150] C. Su, K. Huang, H.-H. Li, Y.-G. Lu, S.-L. Zheng, Antibacterial properties of functionalized gold nanoparticles and their application in oral biology, Journal of Nanomaterials, **2020**, 5616379 (2020). https://doi.org/10.1155/2020/5616379

[151] M. Tomoaia-Cotisel, V. D. Pop, Gh. Tomoaia, A. Mocanu, Cs. Racz, C.R. Ispas, O. Pascu, O.C. Borostean, Atomic force microscopy studies of Langmuir-Blodgett films. 4. The influence of aluminum substrate on dipalmitoyl phosphatidylcholine nanolayers, Studia UBB Chemia, **50**(1), 23-37 (2005).

[152] M. Tomoaia-Cotisel, L.C. Stewart, M. Kates, J. Zsako, E. Chifu, A. Mocanu, P.T. Frangopol, L.J. Noe, P.J. Quinn, Acid dissociation constants of diphytanyl glycerol phosphorylglycerol-methylphosphate, and diphytanyl glycerol phosphoryl glycerophosphate and its deoxy analog, Chemistry and Physics of Lipids, **100**, 41-54 (1999).

[153] M. Tomoaia-Cotisel, Gh. Tomoaia, V.-D. Pop, A. Mocanu, O. Cozar, N. Apetroaei, Gh. Popa, Atomic force microscopy studies of Langmuir-Blodgett films. 3. Phase behaviour of dipalmitoyl phosphatidyl choline monolayers, Revue Roumaine de Chimie, **50**(6), 471-478 (2005).

[154] L.J. Noe, M. Tomoaia-Cotisel, M. Casstevens, P. N. Prasad, Characterization of Langmuir-Blodgett films of 3,4-didecyloxy-2,5-di(4-nitrophenylazomethine) thiophene in a stearic acid matrix, Thin Solid Films, **208**, 274-279 (1992).

[155] M. Tomoaia-Cotisel, Gh. Tomoaia, V.-D. Pop, A. Mocanu, O. Cozar, N. Apetroaei, Gh. Popa, Atomic force microscopy studies of Langmuir-Blodgett films. The effect of some drugs on dipalmitoyl phosphatidylcholine, Studia UBB Physica, **49**(3), 141-152 (2004).

[156] M. Tomoaia-Cotisel, A. Tomoaia-Cotisel, T. Yupsanis, Gh. Tomoaia, I. Balea, A. Mocanu, Cs. Racz, Coating layers of major storage protein from aleurone cells of barley studied by atomic force microscopy, Revue Roumaine de Chimie, **51**(12), 1181-1185 (2006).

[157] I. Cojocaru, A. Tomoaia-Cotisel, A. Mocanu, T. Yupsanis, M. Tomoaia-Cotisel, The effects of globular protein from aleurone cells of barley on stearic acid monolayers, Revista de Chimie, **68**(7), 1470-1475 (2017).

[158] A. Mocanu, P.J. Quinn, C. Nicula, S. Riga, Gh. Tomoaia, C.-A. Bardas, M. Tomoaia-Cotisel, Interaction of local anesthetic procaine with phospholipid model membrane, Revue Roumaine de Chimie, **66**(10-11), 855-869 (2021). https://doi.org/10.33224/rrch.2021.66.10-11.09

[159] M. Tomoaia-Cotisel, J. Zsako, E. Chifu, Dipalmitoyl lecithin and egg lecithin monolayers at an air/water interface, Annali di Chimica (Rome), **71**(3-4), 189-200 (1981).

[160] M. Tomoaia-Cotisel, J. Zsako, A. Mocanu, M. Lupea, E. Chifu, Insoluble mixed monolayers. III. The ionization characteristics of some fatty acids at the air/water interface, Journal of Colloid and Interface Science, **117**(2), 464-476 (1987).

[161] M. Tomoaia-Cotisel, A. Sen, P. J. Quinn, Surface active properties of 1,2-distearoylgalactosylglycerols, Journal of Colloid and Interface Science, **94**, 390-398 (1983).

[162] J. Zsako, M. Tomoaia-Cotisel, A. Mocanu, E. Chifu, Insoluble mixed monolayers. II. Protolytic equilibria and the influence of the pH on the collapse pressure, Journal of Colloid and Interface Science, **110**(2), 317-334 (1986).

[163] M. Tomoaia-Cotisel, J. Zsako, E. Chifu, P. J. Quinn, Influence of electrolytes on the monolayers properties of saturated galactolipids at the air/water interface, Chemistry and Physics of Lipids, **34**(1), 55-64 (1983).

[164] P.T. Frangopol, D. A. Cadenhead, M. Tomoaia-Cotisel, A. Mocanu, Procaine effects on surface topography of spread dipalmitoylphosphatidylcholine monolayers, Studia UBB Chemia, **54**(1), 23-35 (2009).

[165] P.T. Frangopol, D. A. Cadenhead, Gh. Tomoaia, A. Mocanu, M. Tomoaia-Cotisel, The effect of procaine on lipid domains investigated by contact mode atomic force microscopy, Revue Roumaine de Chimie, **60**(2-3), 265-273 (2015).

[166] M. Tomoaia-Cotisel, R.-D. Pasca, O. Horovitz, A. Mocanu, Surface potentials of cholesterol and dimyristoyl phosphatidylcholine monolayers at the air/water interface, Revue Roumaine de Chimie, **56**(10-11), 1047-1053 (2011).

[167] Gh. Tomoaia, A. Tomoaia-Cotisel, M. Tomoaia-Cotisel, A. Mocanu, Kinetic study of adsorption of some biocompounds at the oil/water interface, Central European Journal of Chemistry, **3**(2), 347-360 (2005).

[168] J. Zsako, M. Tomoaia-Cotisel, E. Chifu, Insoluble mixed monolayers. I. Phase equilibria at the collapse of binary monolayers at gas/liquid interfaces, Journal of Colloid and Interface Science, **102**(1), 186-205 (1984).

[169] J. Zsako, M. Tomoaia-Cotisel, E. Chifu, Insoluble mixed monolayers. V. Molecular associations in binary films: The regular association approach, Journal of Colloid and Interface Science, **146**(2), 353-362 (1991).

[170] M. Tomoaia-Cotisel, I.W. Levin, Thermodynamic study of the effects of ursodeoxycholic acid and ursodeoxycholate on aqueous dipalmitoyl phosphatidyl choline bilayer dispersions, The Journal of Physical Chemistry B, **101**(42), 8477-8485 (1997).

[171] U.V. Zdrenghea, Gh. Tomoaia, D. -V. Pop-Toader, A. Mocanu, O. Horovitz, M. Tomoaia-Cotisel, Procaine effect on human erythrocyte membrane explored by atomic force microscopy, Combinatorial Chemistry & High Throughput Screening, **14**(4), 237-247 (2011).