In vitro assay of the antimicrobial activity of Fe_3O_4 and $CoFe_2O_4$ / oleic acid – core/shell on clinical isolates of bacterial and fungal strains

A. M. GRUMEZESCU*, D. E. MIHAIESCU, D. E. MOGOŞANU, M. C. CHIFIRIUC[a], V. LAZĂR[a], I. CĂLUGĂRESCU[a], V. TRĂISTARU

[1]*Organic Chemistry Department, Faculty of Applied Chemistry and Materials Science, "Politehnica" University of Bucharest, 1–7 Polizu Street, 011061 Bucharest, Romania*

[2]*Microbiology Immunology Department, Faculty of Biology, University of Bucharest, Aleea Portocalelor 1-3, Sector 5, 60101-Bucharest, Romania*

The aim of the present study was to evaluate the antimicrobial activity of Fe_3O_4 and $CoFe_2O_4$ / oleic acid – core microwave conditions. The dimensions of Fe_3O_4 and $CoFe_2O_4$ nanoparticles did not exceed the 20 nm range and they were characterized by High Resolution Transmision Electron Microscopy. The nanoparticles were screened for their *in vitro* antimicrobial activity against Gram – positive (*Staphylococcus aureus, Enterococcus faecalis*), Gram–negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) and fungal strains (*Candida albicans*), using both reference and clinical, multidrug resistant strains. The quantitative assay of the antimicrobial activity was performed by both microdilution method in 96-well microplates in order to establish the minimal inhibitory concentration (MIC).

(Received October 27, 2010; accepted November 10, 2010)

Keywords: Antimicrobial activity, Minimum inhibitory concentration, Magnetic nanoparticles, HR-TEM

1. Introduction

Nanoscale materials find use in a variety of different areas such as optoelectronic [1-4], biomedical [5,7], pharmaceutical [6, 8, 9, 10], environmental [11, 12], catalytic and material applications. Particles in the nano-sized range have attracted a lot of attention because of their properties [13]. Their unique higher surface area, surface roughness, altered electron distribution, energetics and biological activity [14] led to an important role in medicine [15] and biomedical engineering [16]. Veerapadian and Kyusiuk [13] presented in their paper nanobiopharmaceutical carrier systems, represented by metal, non-metal, carbon, polymer, lipid, virus and miscellaneous nanostructures.

Dramatic development of nanotechnology in material science and engineering has taken place in the last decade [17]. Nanostructured materials have the capability to be adapted and integrated into biomedical devices, since most biological systems, including viruses, membrane and protein complex, exhibit natural nanostructures. Nanoparticle delivery systems were developed for diagnosis and treatment of dangerous diseases such as cancer [18,19], diabetes [17, 20, 21], and tuberculosis (TB) – reported by Mathuria et al [22]. Therefore, the development of wide spectrum drug delivery systems is of fundamental importance. The efficiency of drug delivery to various parts of the body is directly affected by particle size. Rapid methods for the diagnosis of certain infections are being developed [23].

The mechanisms used to achieve alternative drug delivery typically incorporate one or more of the following nano-materials: biologicals, polymers, silicon-based materials, carbon-based materials, or metals. It is well known that plant extracts are an economic and efficient alternative for the synthesis of nanoparticles. The use of methanolic extract of *Eucalyptus hybrida* leaf in the extracellular biosynthesis of silver nanoparticles was developed by Dubey et al [24]. Ankanna et al. reported in their paper [25] a dried stem bark of *Boswellia ovalifoliolata* extract that was used as the reducing agent to synthesize highly dispersed silver nanoparticles. Tripathi et al [26] also stated a method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of onion (*Allium cepa)* extract. Farooqui [27] synthesized silver nanoparticles using extracts of fresh, sun-dried and hot-air oven dried medicinal *Clerodendrum Inerme* leafs. The high antibacterial activity exhibited by some silver nanoparticles made them suitable for studying their properties' effect on various clinically important microorganisms such as *Escherichia coli, Salmonella typhimurium, Bacillus subtilis and Pseudomonas aeruginosa* [26, 28].

Due to the increasing interest in the antibacterial activity of magnetic nanoparticles [6], [29], this paper presents the synthesis, characterization and the antimicrobial activity against Gram – positive (*Staphylococcus aureus, Enterococcus faecalis*), Gram–negative bacterial (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) and fungal strains

(*Candida albicans*), using both reference and clinical, multidrug resistant strains of Fe_3O_4 and $CoFe_2O_4$ / oleic acid - core/shell nanoparticles.

2. Materials and methods

2.1. Preparation and characterization of Fe_3O_4 and $CoFe_2O_4$ / oleic acid - core/shell

Fe_3O_4 and $CoFe_2O_4$ has been synthesized by Massart adapted method using Fe^{3+}, Fe^{2+} salts and HO^-, under microwave conditions. Core/shell was also synthesized under microwave conditions using oleic acid [30]. High-Resolution Transmission Electron Microscope (HR-TEM) confirmed the formation of magnetic nanoparticles not exceeding 20 nm [30].

2.2. Antimicrobial susceptibility testing

2.2.1. Microbial strains

The antimicrobial activity of the investigated nanoparticles was tested against bacterial and fungal strains recently isolated from clinical specimens as well as reference strains belonging to the following genera and species: Gram positive (*Staphylococcus aureus, Enterococcus faecalis*), Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) and *Candida albicans.* The microbial strains were identified by aid of VITEK I automatic system. VITEK cards for identification and susceptibility testing (GNS-522) were inoculated and incubated according to the manufacturer's recommendations. The results were interpreted by using software version AMS R09.1. In our experiments there were used bacterial suspensions of 1.5×10^8 CFU/ mL or 0.5 McFarland density obtained from 15 - 18 h bacterial cultures developed on solid media. The antimicrobial activity was tested on Mueller- Hinton medium recommended for the bacterial strains and Yeast Peptone Glucose (YPG) medium for *C. albicans*.

2.2.2. Quantitative assay of the antimicrobial activity

It was performed by binary micro dilution method, in 96 multi-well plates, in order to establish the minimal inhibitory concentration (MIC) [29, 31-34]. In this purpose, serial binary dilutions of the tested nanoparticles (ranging between 1000 and 1.9 µg/mL) were performed in a 200 µL volume of nutrient broth and each well was seeded with 50 µL microbial inoculum. The plates were incubated for 24 hrs at 37 °C, and MICs were read as the last concentration of the compound, which inhibited the microbial growth.

3. Results and discussion

The microbial behavior was different in the presence of the two types of tested nanoparticles. The $CoFe_2O_4$ nanoparticles exhibited a general inhibitory effect on the microbial growth rate, but without any dose-effect correlation, the inhibition being noticed for all the 10 two-fold dilutions of the nanoparticles suspension (Fig. 1). This similar inhibitory effect on Gram-positive and Gram-negative strains is probably due to their low dimensions and their hydrophobic shell, favoring the adhesion and free diffusion through the phospholipidic outer and cytoplasmic bacterial membranes. The different degrees of hidrophobicity of Gram negative versus Gram positive bacterial strains' wall is also subject of antibiotic wall diffusion studies [37]. Porin diffusion can be excluded due to the 1500 Da (related to 0.2 nm cross section) permeability limit. These results could account for the large spectrum antimicrobial activity of the tested nanoparticles, against microbial cells grown in suspension (planktonic state) and for their use in the development of new antimicrobial strategies or in the design of new devices with antimicrobial properties, to be used in the medical, industrial or ecological field.

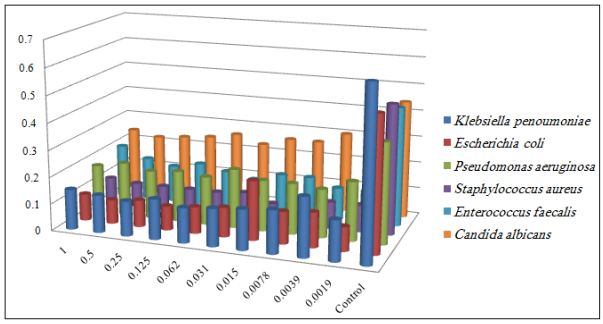

Fig. 1. Influence of $CoFe_2O_4$ / oleic acid – core/shell nanoparticles on the microbial growth rate of different bacterial and fungal strains (the A 600 nm is proportional with the intensity of microbial growth).

Concerning the Fe_3O_4 nanoparticles, they exhibited a dose dependent stimulatory effect on the microbial growth in case of *K. pneumoniae, P. aeruginosa, E. faecalis* and *C. albicans* strains and an inhibitory effect on *S. aureus,* noticed at high concentrations of the tested nanoparticles (the first 5 two-fold dilutions) (Fig. 2).

These results indicate that the Fe_3O_4 nanoparticles interact more specifically with the microbial strains, as compared with the $CoFe_3O_4$ nanoparticles. Further studies will be needed in order to identify the specific mechanism of action and their microbial target. Previous studies have demonstrated the ability of iron oxide/titania (Fe_3O_4/TiO_2) core/shell magnetic nanoparticles to effectively inhibit the cell growth of the different bacterial strains such as *Staphylococcus saprophyticus, Streptococcus pyogenes*, and antibiotic-resistant bacterial strains, such as multiantibiotic-resistant *S. pyogenes* and methicillin-resistant *Staphylococcus aureus* (MRSA), by photokilling, targeted by the nanoparticles under irradiation of a low-power UV lamp within a short period (Chen et al., 2008). Chudasam et al. (2009) demonstrated that the colloidal suspension of narrowly dispersed superparamagnetic

Fe_3O_4/Ag core-shell nanostructures are highly toxic to microorganisms. Antimicrobial activity studies carried out on both Gram negative (*Escherichia coli* and *Proteus vulgaris*) and Gram positive (*Bacillus megaterium* and *Staphylococcus aureus*) bacterial strains have shown a more intensive antimicrobial effect against Gram negative strains, superior to that that observed for silver nanoparticles and even considerably higher than that of commercially available antibiotics. This improved antimicrobial activity was attributed to their stability as a colloid in the medium, which modulates the phosphotyrosine profile of the bacterial proteins and arrests bacterial growth.

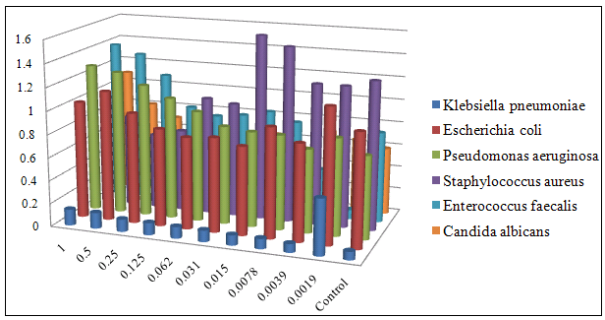

Fig. 2. Influence of Fe_3O_4 / oleic acid – core/shell nanoparticles on the microbial growth rate of different bacterial and fungal strains (the A 600 nm value is proportional with the intensity of microbial growth).

4. Conclusion

The $CoFe_2O_4$ oleic acid - core/shell exhibited large spectrum antimicrobial activity against Gram-positive, Gram-negative bacterial as well as fungal strains and could be used in the design of microbicidal formulations comprising nanoparticles, featuring the great advantage that can be removed from the medium by means of an external magnetic field which provides a mechanism to prevent uncontrolled waste disposal of these potentially hazardous nanostructures.

References

[1] L. C. Chen, C. C. Wang, B. S. Tseng, Journal of Optoelectronics and Biomedical Materials **1**(3), 249 (2009).
[2] R. S. Dubey, D. K. Gautam, Journal of Optoelectronics and Biomedical Materials **1**(1), 8 (2009).
[3] M. Farahmandjou, Journal of Optoelectronics and Biomedical Materials **1**(1), 124, (2009).
[4] R. Sarkar, P. Kumbhakar, A. K. Mitra, Digest Journal of Nanomaterials and Biostructures **5**(2), 491 (2010).
[5] P. Singh, R. M. Tripathi, A. Saxena, Journal of Optoelectronics and Biomedical Materials **2**(2), 91 (2010).
[6] A. S. Buteică, D. E. Mihaiescu, A. M. Grumezescu, B. Ş. Vasile, A. Popescu, D. Călina, O. M. Mihaiescu, Digest Journal of Nanomaterials and Biostructures **5**(3), 651 (2010).
[7] M. A. Farooqui, P. S. Chauhan, P. K. Moorthy, J. Shaik, Digest Journal of Nanomaterials and Biostructures **5**(1), 43, (2010).
[8] K. Natarajan, S. Selvaraj, V. R. Murty, Digest Journal of Nanomaterials and Biostructures **5**(1), 135 (2010).
[9] V. Bayazit, Digest Journal of Nanomaterials and Biostructures **5**(2), 347 (2010).
[10] A. Singh, D. Jain, M. K. Upadhyay, N. Khandelwal, H. N. Verma, Digest Journal of Nanomaterials and Biostructures **5**(2), 483 (2010).
[11] A. Saxena, R. M. Tripathi, R. P. Singh, Digest Journal of Nanomaterials and Biostructures **5**(2), 427 (2010).
[12] M. Behpour, S. M. Ghoreishi, F. S. Razavi, Digest Journal of Nanomaterials and Biostructures **5**(2), 467 (2010).
[13] M. Veerapandian, K. Yun, Digest Journal of Nanomaterials and Biostructures **4**(2), 243 (2009).
[14] R. Nithya, R. Ragunathan, Digest Journal of Nanomaterials and Biostructures **4**(4), 623 (2009).
[15] C. Lazau, L. Mocanu, I. Miron, P. Sfirloaga, G. Tanasiea, C. Tatua, A. Gruiab, I. Grozescu, Digest . Journal of Nanomaterials and Biostructures, **2**(3), 257 (2007).
[16] V. K. Mishra, S. K. Mishra, A. Jha, Digest Journal of Nanomaterials and Biostructures **3**(4), 203 (2008).
[17] P. S. Sona, Digest Journal of Nanomaterials and Biostructures **5**(2), 411 (2010).
[18] B. Kumar, P. R. Yadav, H. C. Goel, M. Moshahid A. Rizvi, Digest Journal of Nanomaterials and Biostructures, **4**(1), 1 (2009).
[19] V. K. R. Mishra, G. Mohammada, S. K. Mishrab, Digest Journal of Nanomaterials and Biostructures **3**(4), 163 (2008).
[20] A. K. Arya, L. Kumar, D. Pokharia, K. Tripathi, Digest Journal of Nanomaterials and Biostructures **3**(4), 221 (2008).
[21] M. Mishra, H. Kumar, R. K. Singha, K. Tripathi, Digest Journal of Nanomaterials and Biostructures **3**(3), 109 (2008).
[22] J. P. Mathuria, Digest Journal of Nanomaterials and Biostructures **4**(2), 309 (2009).
[23] P. Chandra, P. K. Maurya, P. Kumar, P. Tripathi, A. K. Srivastava, Digest Journal of Nanomaterials and Biostructures **4**(4), 645 (2009).
[24] M. Dubey, S. Bhadauria, B. S. Kushwah, Digest Journal of Nanomaterials and Biostructures **4**(3), 537 (2009).
[25] S. Ankanna, T. N. V. K. V. Prasad, E. K. Elumalai, N. Savithramma, Digest Journal of Nanomaterials and Biostructures **5**(2), 369 (2010).
[26] R. M. Tripathi, A. Saxena, N. Gupta, H. Kapoor, R. P. Singh, Digest Journal of Nanomaterials and Biostructures **5**(2), 323 (2010).
[27] V. Arya, Digest Journal of Nanomaterials and Biostructures **5**(1), 9 (2010).
[28] K. Natarajan, S. Selvaraj, V. R. Murty, Digest Journal of Nanomaterials and Biostructures **5**(1), 135 (2010).
[29] M. Singh, S. Singh, S. Prasad, I. S. Gambhir, Digest

Journal of Nanomaterials and Biostructures **3**(3), 115 (2008).
[30] A. S. Buteică, D. E. Mihaiescu, A. M. Grumezescu, B. Ş. Vasile, A. Popescu, O. M. Mihaiescu, R. Cristescu, Digest Journal of Nanomaterials and Biostructures, **5**(4), 927 (2010).
[31] M. C. Balotescu, C. Limban, A. V. Missir, I. C. Chirita, M. G. Nitulescu, Revista de Chimie (Bucuresti) **58**, 1064 (2007).
[32] *** Reference Method For Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition 2007, M27-A3, Vol. 0 No. 0, Replaces M27-A2, Vol. 22 No. 15.
[33] *** Performance Standards For Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement M100-S18, **28**(1), Replaces M100-S17, Vol. 76 No. 1 Informational Supplement 2008.
[34] I. Wiegand, K. Hilpert, R. E. Hancock, Nat Protoc., **3**(2), 163 (2008).
[35] C. Limban, M. Balotescu Chifiriuc, A. V. Missir, I. C. Chiriță, C. Bleotu, Molecules, **13**(3), 567 (2008).
[36] R. Olar, M. Badea, O. Carp, D. Marinescu, V. Lazar, C. Balotescu, A. Dumbrava, J. Therm. Anal. Calor. **92**, 245 (2008).
[37] H. Tsujimoto Et Al. Journal of Infection and Chemotherapy, **74**, 1045 (2003).

*Corresponding author: grumezescu@yahoo.com