STRUCTURĂ BIOHIBRIDĂ BAZATĂ PE ULEI ESENȚIAL DE *EUGENIA CARYOPHYLLATA* ŞI SiO_2 PENTRU POTENȚAREA ACTIVITĂȚII ANTIBIOTICELOR

EUGENIA CARYOPHYLLATA ESSENTIAL OIL-SiO_2 BIOHYBRID STRUCTURE FOR THE POTENTIATION OF ANTIBIOTICS' ACTIVITY

ALEXANDRU MIHAI GRUMEZESCU[1], ECATERINA ANDRONESCU[1], ANTON FICAI[1], GEORGETA VOICU[1]*, OCTAVIAN COCOŞ[2], MARIANA CARMEN CHIFIRIUC[2]

[1]*Universitatea Politehnica Bucureşti, Str. G. Polizu nr. 1, sector 1, cod 011061, Bucureşti, România*

[2]*Universitatea Bucureşti, Facultatea de Biologie, Departamentul de Microbiologie şi Imunologie, Aleea Portocalelor nr 1-3, sector 6, cod 060101, Bucureşti, România*

The aim of this paper was to obtain a hybrid biostructure, combining the Eugenia caryophyllata essential oil and a silica network and to demonstrate by in vitro studies its potential chemotherapeutic value to improve the efficacy of different classes of antibiotics currently used against Gram-positive and Gram-negative bacterial strains. The obtained hybrid structure significantly improved the activity of topic antibiotics, such as bacitracin and neomycin sulfate, by significantly decreasing their minimal inhibitory concentration. This antibiotics potentiating activity, together with no measurable in vitro cytotoxicity, make this hybrid structure potentially relevant for biomedicine applications, especially in the antimicrobial therapy.

Scopul acestei lucrări a fost obținerea unei biostructuri hibride, combinând uleiul esențial de Eugenia caryophyllata şi o rețea de silice şi demonstrarea prin studii in vitro, a potențialului chemoterapeutic de a imbunătăți eficiența diferitelor clase de antibiotice asupra tulpinilor bacteriene Gram-pozitive şi Gram-negative. Structura hibridă obținută a îmbunătățit semnificativ activitatea antibioticelor topice, cum ar fi bacitracina şi sulfatul de neomicină, prin reducerea semnificativă a concentrației minime inhibitorii. Această activitate de potențare a activității antibioticelor, asociată cu lipsa toxicității in vitro, demonstrează potențialul relevant al structurii hibride obținute pentru aplicații în biomedicină, în special în terapia antimicrobiană.

Keywords: *hybrid material, silica, drug delivery, biocompatibility, antimicrobial therapy*

1. Introduction

The alarming rates of the occurrence and spreading of multiple antibiotic resistant bacteria have triggered the attention of global surveillance authorities and public media and a huge demand for novel effective antimicrobial drugs [1]. The materials science and biotechnology are recently exploited for the design of new strategies for the improvement of the activity of the existing arsenal of drugs [2-10]. Plant secondary metabolites (phytochemicals) have already demonstrated their potential as antibacterials when used alone and as synergists or potentiators of other antibacterial agents [11]. Phytochemicals frequently act through different mechanisms than conventional antibiotics and could, therefore be of use in the treatment of infections produced by resistant bacteria [12,13]. The essential oil extracted from the dried flower buds of clove, *Eugenia caryophyllata* L. Merr. & Perry (Myrtaceae), has as main constituents phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde, with inhibitory activity against several microorganisms and parasites, including pathogenic bacteria, fungi, Herpes simplex and hepatitis C viruses, as well as antioxidant, antiinflammatory, cytotoxic, insect repellent and anaesthetic properties [14,15].

In the present paper we report the fabrication, characterization and the bio-evaluation of a new hybrid biostructure based on *E. caryophyllata* and silica network, to establish its potential chemotherapeutic value by improving the efficacy of different classes of antibiotics currently used against Gram-positive and Gram-negative bacterial strains.

2. Materials and Methods

Sodium metasilicate and sulfuric acid (ACS reagent 95-98%), from Sigma-Aldrich, were used without any further purification.

The *E. caryophyllata* essential oil (EO) microwave assisted extraction was performed in a Neo-Clevenger type apparatus [16] and its chemical composition was settled by GC-MS, the results being presented in previous [17].

EO was dispersed in 100 mL ultrapure water

* Autor corespondent/Corresponding author,
Tel.: +40 21 402.38.85, e-mail: getav2001@yahoo.co.uk

(1 %, w/v) (sol A) and sodium metasilicate (20 mL) was dissolved in 100 mL ultrapure water (sol B). The sol A was dropped in the sol B under vigorous stirring. Thereafter, a solution consisting of 5 % H_2SO_4 was dropped under permanent stirring up to pH = 7, leading to the formation of transparent hydrogels. The product (EO-SiO_2) were filtered and repeatedly washed with ultrapure water and subsequently dried at room temperature.

The amount of the antibiotic adsorbed on the EO-SiO_2 support was 10 %. The EO-SiO_2 and the respective antibiotics (bacitracin, neomycin sulfate, kanamycin sulfate and amoxicillin) to be adsorbed were mixed in the presence of 2 mL of ultrapure water until the latter completely evaporated at 40^{o}C.

SEM analysis was performed on a HITACHI S2600N electron microscope, at 15 or 20keV, in primary electrons fascicle, on gold covered samples.

A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FT-IR spectra of hybrid materials. The samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25^{o}C). FT-IR spectra were collected in the frequency range of 4,000–650 cm^{-1} by co-adding 32 scans and at a resolution of 4 cm^{-1} with strong apodization. The spectra were recorded as absorbance values at each data point in triplicate.

The Brunauer–Emmett–Teller (BET) analysis was performed on a Micrometrics Gemini V2 model 2380, surface area and pore size analyzer. The adsorption isotherms were obtained by measuring the amount of gas adsorbed across a wide range of relative pressures at a constant temperature (N_2, 77K and pressure between 780 and 7.8 mmHg). Conversely desorption isotherms are achieved by measuring gas removed as pressure is reduced.

X-ray diffraction analysis was performed on a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu Kα radiation (λ=1,5406Å at 15 mA and 30 kV) was used. The samples were scanned in the Bragg angle 2θ range of 10-80 degree.

Staphylococcus aureus ATCC 25923 and *Escherichia coli* ATCC 25922 reference bacterial strains were used in this study. Quantitative testing of the antimicrobial activity of hybrid structure with the establishment of minimum inhibitory concentration (MIC) was performed by microdilution method in liquid medium (Mueller Hinton broth), using 96 multiwell plates [18-20]. Two-fold serial microdilutions were achieved in 200 µL medium, the dilution range varying, depending on the tested antibiotic and the bacterial strain, in accordance with CLSI breakpoints [21]. Subsequently, the wells were seeded with 50 µL of each bacterial suspension, adjusted to 0.5 MacFarland density. Positive and negative controls were used. After incubating the plates at 37^{o}C for 24 h, the results were macroscopically assessed for bacterial growth, MIC corresponding to the well with clear content, thus without no visible microbial growth.

3. Results

Inorganic materials have been reported as drug delivery systems (DDS) with improved antimicrobial activity [22-28]. Inorganic DDS proved to be ideal supporting materials, because they are easily obtained from several precursors and retained stability in most chemical and biological environments [29].

FT-IR spectrum of EO-SiO_2 is plotted in Figure 1. The FT-IR spectrum show absorption bands arising at 1045 cm^{-1} from asymmetric vibration of Si–O, at 964 cm^{-1} asymmetric vibration of Si–OH, and at 793 cm^{-1} from symmetric vibration of Si–O [30], as well as the absorption band of alkyl groups at 2936 and 2854 cm^{-1},

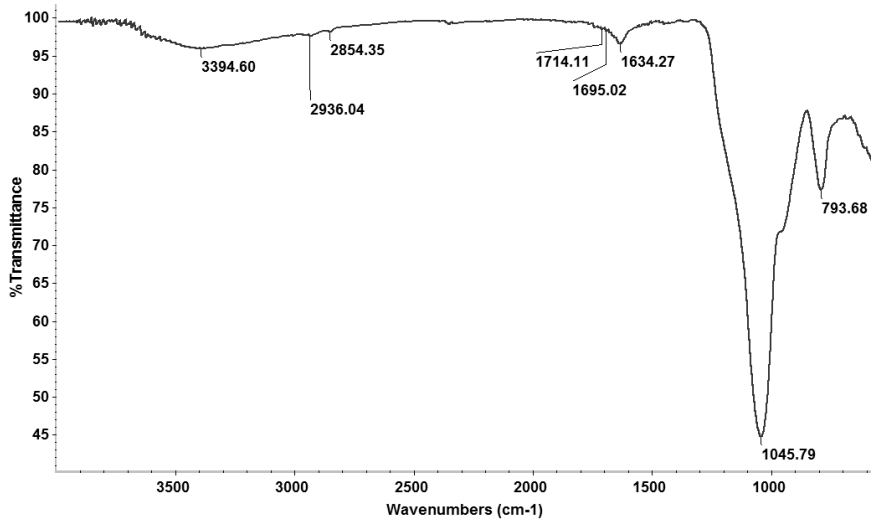

Fig. 1- FT-IR spectrum of EO-SiO_2 / *Spectrul FT-IR pentru EO-SiO_2*

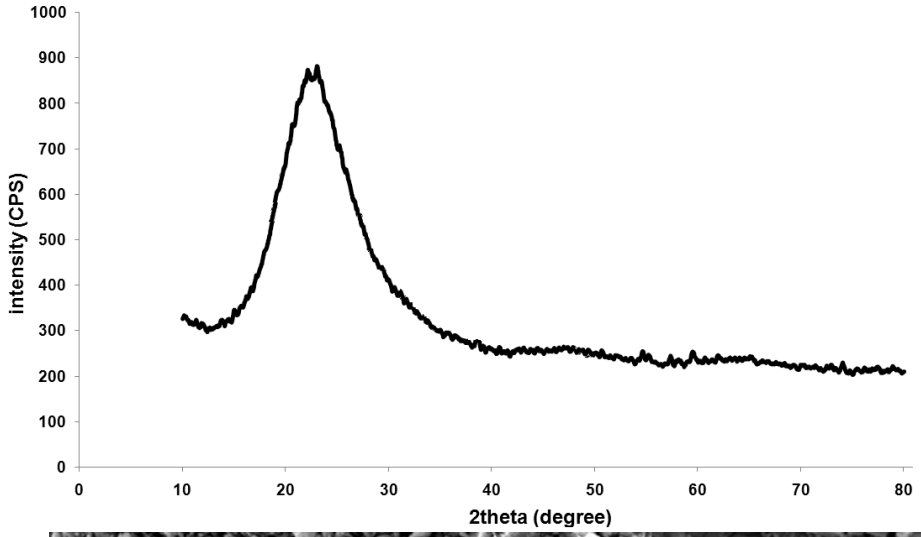

Fig. 2 - XRD difractogram of EO-SiO_2 / *Difractograma XRD pentru EO-SiO_2.*

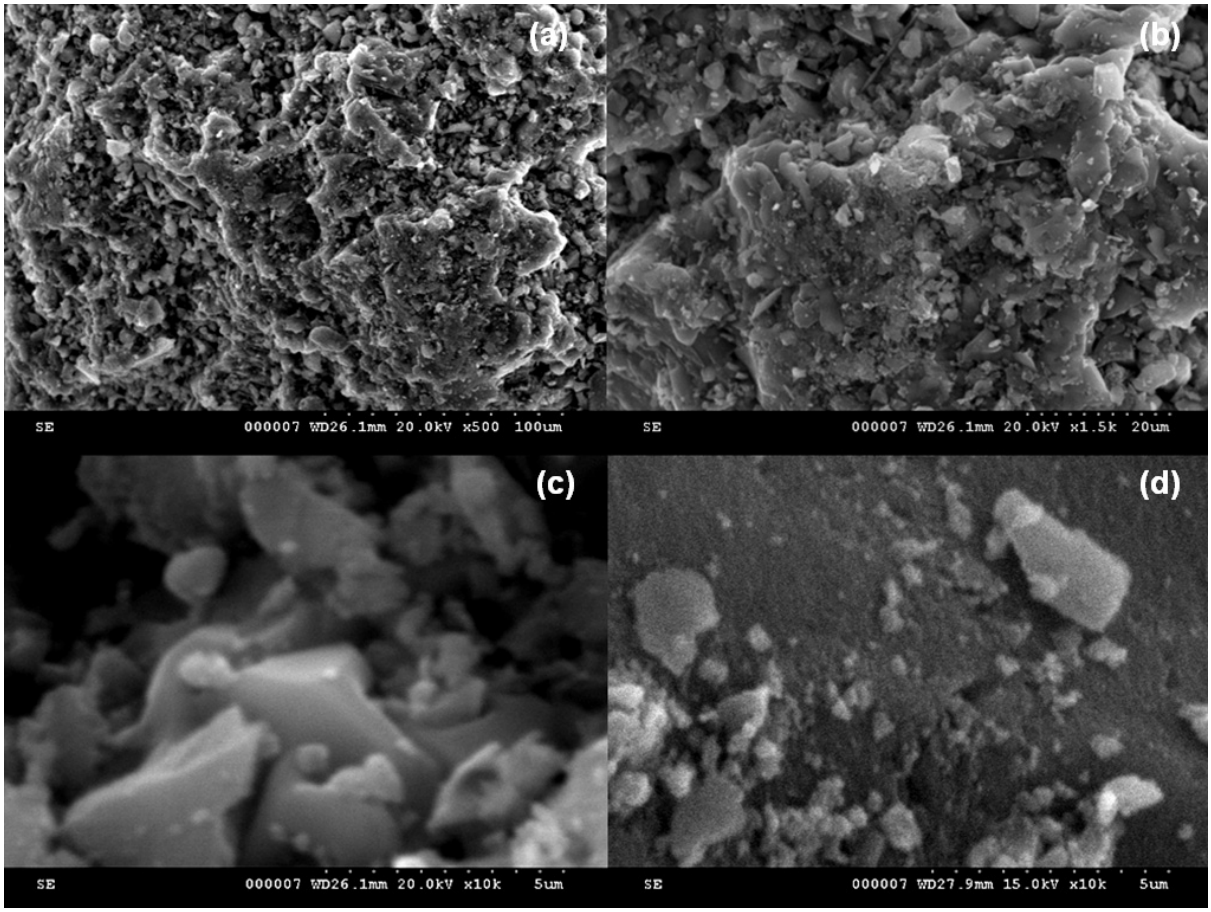

Fig. 3 - SEM micrographs of EO-SiO_2; a. x500; b. x1500; c. x10000 (20kV); d. x10000 (15kV)/*Micrografii SEM pentru EO-SiO_2; a. x500; b. x1500; c. x10000 (20kV); d. x10000 (15kV).*

absorption band of cis olefin groups or carbonyl groups from EO at 1634 cm^{-1}, and a broad absorption bands indicating hydroxyl groups from EO and SiO_2 between 3200-3600 cm^{-1}. The presence of residual silanol (Si–OH) groups is frequently observed in many sol-gel derived materials, reflecting the incomplete polycondensation [31].

The XRD pattern of the biohybrid structure EO-SiO_2 is plotted in Figure 2 and exhibit a broad peak in the range of 15–35° (2θ), which indicates an amorphous structure.

The SEM images exhibit characteristic sharp edges (Fig. 3) – or agglomerates composed by nanometric particles (Fig. 3). The co-existence of the two types of structures can be explained by taking into account that SiO_2 have limited crystallinity, the sharp edged structures being resulted by grinding.

The structure of this sample is further characterized by N_2 adsorption. The isotherm (Figure 4) of this sample is type-IV with a capillary

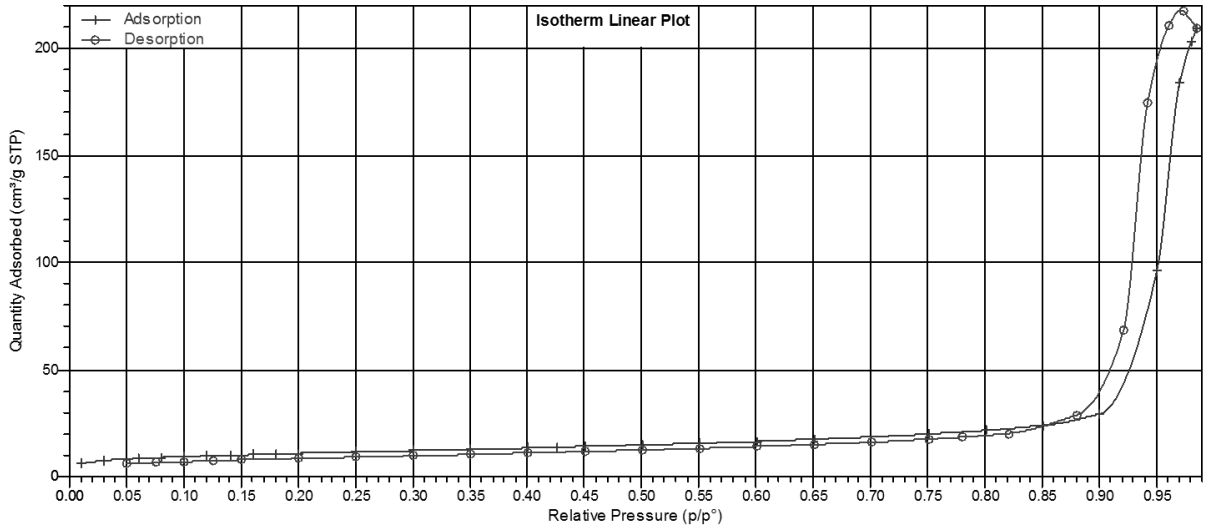

Fig. 4 - BET analysis of EO-SiO_2 / *Analiza BET pentru EO-SiO_2.*

Table 1

Centralized BET data of EO-SiO_2 / *Datele BET centralizate pentru EO-SiO_2*

	EO-SiO_2
Single point surface area at p/p° = 0.300423966/*Aria suprafeței într-un singur punct la p/p° = 0,300423966*	36.92 m²/g
BET Surface Area / *Aria suprafeței BET*	37.87 m²/g
Langmuir Surface Area / *Aria suprafeței Langmuir*	54.85 m²/g
BJH Adsorption cumulative surface area of pores between 1.7 nm and 300nm diameter / *Aria suprafeței cumulative de adsorbție BJH a porilor cu diametru între 1,7 nm şi 300 nm*	36.97 m²/g
BJH Adsorption average pore diameter (4V/A) / *Diametrul mediu al porilor determinat prin adsorbție BJH (4V/A)*	34.65 nm

condensation step at large relative pressures P/P^o of 0,30. The sample exhibit a BET surface area of 37,87 m^2/g and Langmuir surface area of 54,85 m^2/g. The average pore size is about 34,65 nm. Based on the BET analysis estimations about surface area and pore diameters were done, the most important data being centralized in Table 1.

Silica-based hybrid materials have been previously reported to be effective in the antibiotic drugs release, i.e. silica-coated Fe_2O_3 nanoparticles for the covalent immobilization and release of antimicrobial drug sparfloxacin [32]; sodium alginate, chitosan and silica network [33]; composite particles of polymeric magnetic silica [34]; silver and zinc nanoparticle-doped SiO_2 microspheres exhibited effective inhibition against proliferation of *E. coli* and *Streptococcus faecalis* [35 - 37]; SiO_2-Cu core-shell composite proved to be more efficient in killing *E. coli* as compared with *S. aureus* and *Candida albicans*, also inducing morphological changes revealed by TEM [38,39]; ciprofloxacin-encapsulated silica nanoshells synthesized from gold@silica core-shell nanoparticles had an improved MIC towards *E. coli* and *Lactococcus lactis* strains [40]. The TiO_2, SiO_2 and ZnO showed dose dependent antibacterial toxicity under both dark and light conditions indicating that mechanisms additional to reactive oxygen species production were responsible for growth inhibition [41].

In the present study, the EO-SiO_2 biohybrid system showed a significant decrease of the MIC values, as compared with the antibiotic solution, demonstrating its potentiator effect on the antimicrobial activity of bacitracin on *S. aureus* and neomycin sulphate on *E. coli* (Fig. 5 a, b). These results could be significant, taking into account the high cytotoxicity and poor oral bioavailability of these antibiotics, which are used mostly for topical use. The use of such DDS could decrease the required active doses of antibiotics, thus diminishing their adverse effects on the host. The peptidic antibiotics, such bacitracin are acting by disrupting the cell wall and fosfolipids synthesis both in Gram-positive and Gram-negative both bacteria. The EOs are also acting by disrupting the integrity of the bacterial wall, acting thus synergically with the antibiotic. In case of neomycin, an extremely nephrotoxic aminoglycoside antibiotic, the potentiating effect of the biohybrid could be explained by the fact that the Eos is previously inducing lesions in the bacterial cell wall, facilitating the internalization of the neomycin antibiotic and its access to the intracellular target, represented by protein synthesis. For the other two tested antibiotics, i.e. kanamycin sulphate and amoxicillin, no improvement in the antibacterial activity has been observed. The fact that the EO-SiO_2 biohybrid system is differently influencing the activity of antibiotics belonging to the same class, e.g. aminoglycosides, is clearly demonstrating that these antibiotics are differently interacting with the proposed carrier, probably by covalent binding, influencing thus the subsequent antimicrobial activity of the respective drug, which is depending

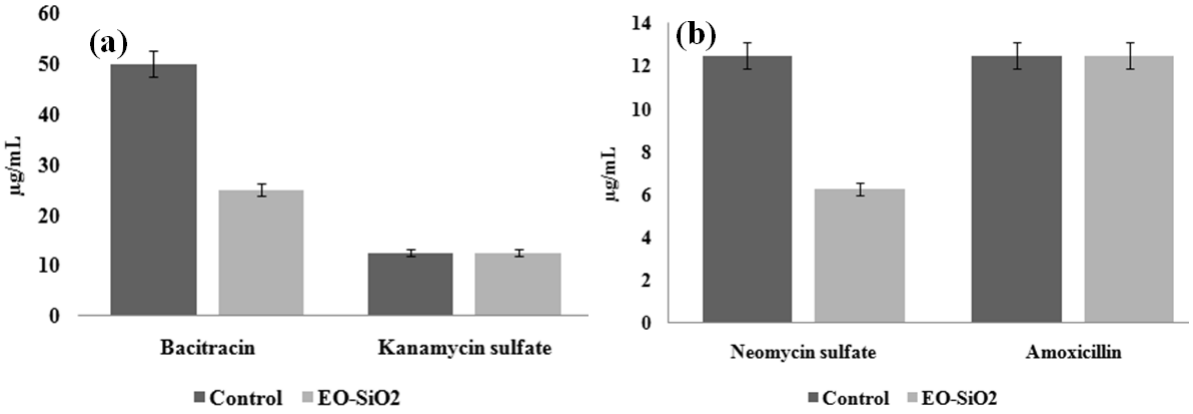

Fig. 5 - Graphic representation of MIC values of antibiotics loaded on the EO-SiO_2 *versus* antibiotic control towards *S. aureus* (a) and *E. coli* (b)/ *Reprezentarea grafică a valorilor CMI ale structurii hibride încărcate cu antibiotice versus soluția de antibiotic față de S. aureus (a) şi E. coli (b).*

on the availability of functional groups, such as amino substituents in case of aminoglycosides and amoxicillin.

The assessment of the EO-SiO_2 biohybrid cytotoxicity and influence on the eukaryotic cellular cycle were performed at 10µL/mL concentration.

After 24 h of treatment, a very low number of dead cells (stained in red), indicating a low cytotoxicity was observed at the fluorescence microscope examination. The monolayer integrity was not affected by the treatment as revealed by the inverted microscope examination (Fig. 6).

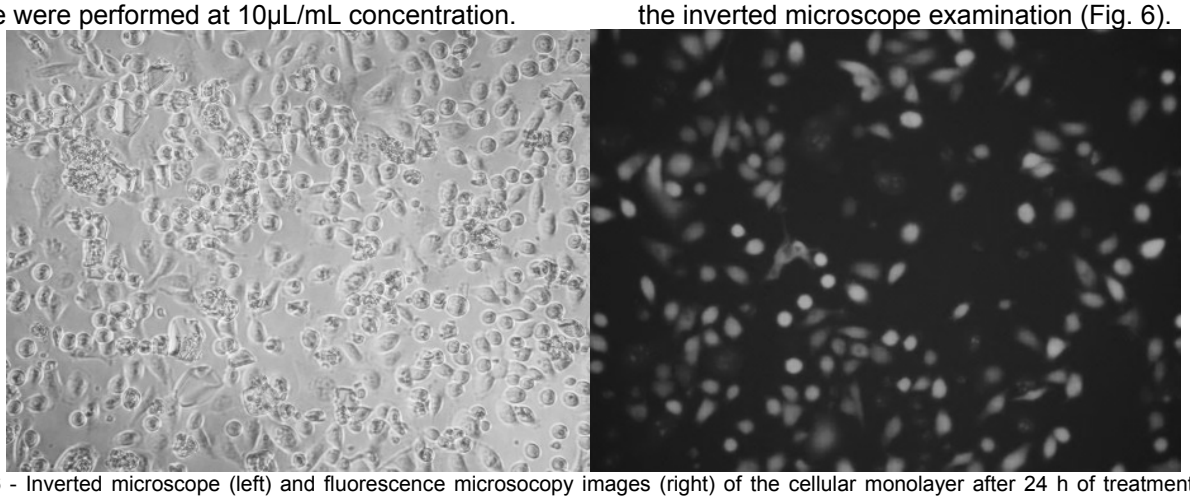

Fig. 6 - Inverted microscope (left) and fluorescence microsocopy images (right) of the cellular monolayer after 24 h of treatment with 10µL/mL EO-SiO_2/ *Imagini de microscopie inversată (stânga) şi de fluorescență (dreapta) ale monostratului celular examinat după 24 h de tratament cu 10µL/mL EO-SiO_2.*

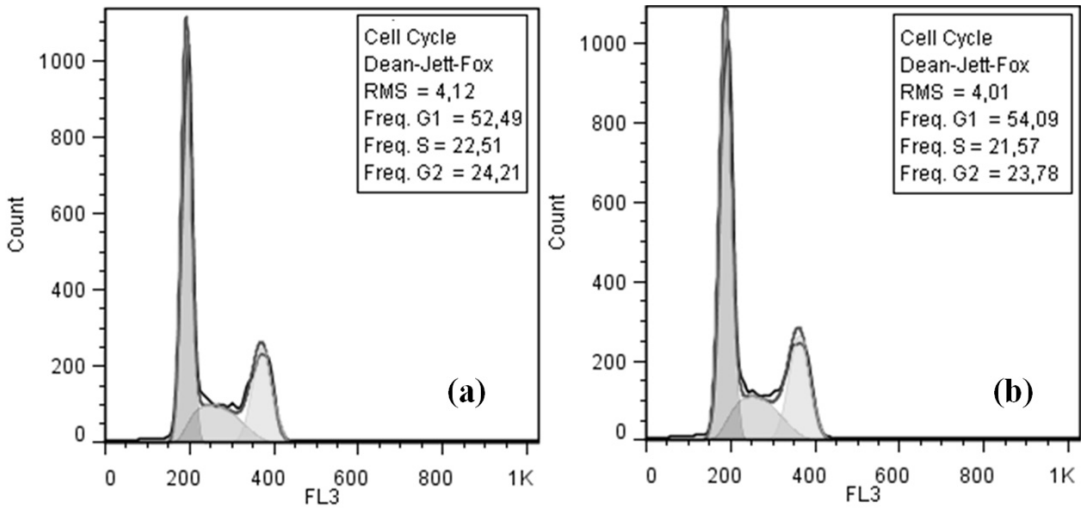

Fig. 7 - Histograms of flow cytometry analysis of the eukaryotic cell cycle after 24 h of (a) control and (b) treatment with 10µL/mL EO-SiO_2/ *Histogramele rezultate în urma analizei prin citometrie în flux a ciclului celular după 24 h pentru control (a) şi tratament cu 10µL/mL EO-SiO_2 (b).*

The cellular cycle was also not affected by the treatment, demonstrating that the obtained hybrid system is highly compatible and could be used for *in vivo* applications (Fig. 7). A variety of flow or cytometric methods to analyze the cell cycle progression have been developed in the past decades. We have used a single time-point measurement revealing the percentage of cells in G 1 vs. S vs. G2/M, the duration of each phase, however, being estimated from the percentage of cells in this phase if the length of the cell cycle (or the doubling time of cells in the culture) is known [42].

The three colors are representing the estimated percentage of cells found in the three celullar phases, i.e. G1, S, G2, after univariate analysis of the cellular DNA content following cell staining with propidium iodide (PI) and deconvolution of the cellular DNA content frequency histograms. No significant difference in the number of apoptotic cells (indicated by the arrow) was observed in treated *versus* untreated cells.

4. Conclusions

The EO-SiO_2 biostructure was synthesized, characterized by FT-IR, XRD, SEM, BET and studied for the potentiation of different antibiotics' activity. The obtained hybrid structure significantly improved the activity of toxic antibiotics, such as bacitracin and neomycin sulfate, by significantly decreasing their MICs. This potentiating activity, together with no measurable *in vitro* cytotoxicity, makes this conjugate potentially relevant for medicine applications.

Acknowledgments

This research was financed by the Human Resources Project no. 135/2010 Contract no. 76/2010).

REFERENCES

1. M. Pieren, and M. Tigges , Adjuvant strategies for potentiation of antibiotics to overcome antimicrobial resistance, Current Opinion Pharmacology, 2012, **12**(5),551.
2. S.K. Prasad, S.L. Kumar, P. Melvin, B. Jayalakshmi, and H. D. Revanasiddappa, Synthesis, spectral characterization, DNA interaction studies, anthelmintic and antimicrobial activity of transition metal complexes with 3-(2 hydroxybenzylideneamino)-2-methylquinazolin-4(*3H*)-one and 1,10-phenanthroline, Biointerface Research in Applied Chemistry, 2011, **1**(4), 127.
3. A. M. Grumezescu, E. Andronescu, A. Ficai, C. Saviuc, D. Mihaiescu, M. C. Chifiriuc, Deae-Cellulose/Fe_3O_4/cephalosporins hybrid materials for targeted drug delivery, Romanian Journal of Materials, 2011, **41**(4), 383.
4. M. Milović, J. Djuriš, L.a Djekić, D. Vasiljević, S. Ibrić, Characterization and evaluation of solid self-microemulsifying drug delivery systems with porous carriers as systems for improved carbamazepine release, International Journal of Pharmaceutics, 2012, **436**(1–2), 58.
5. A.M. Grumezescu, E. Ilinca, C. Chifiriuc, D. Mihăiescu, P. Balaure, V. Trăistaru, and G. Mihăiescu, Influence of magnetic MWCNTs on the antimicrobial activity of cephalosporins, Biointerface Research in Applied Chemistry, 2011, **1**(4), 139.
6. M. Narvekar, H. Y. Xue, and H. L. Wong, A novel hybrid delivery system: Polymer-oil nanostructured carrier for controlled delivery of highly lipophilic drug all-trans-retinoic acid (ATRA), International Journal of Pharmaceutics, 2012, **436**(1–2), 721.
7. S. Dhanasingh, J. Mallesha, and J. Hiriyannaiah, Preparation, characterization and antimicrobial studies of chitosan/silica hybrid polymer, Biointerface Research in Applied Chemistry, 2012, **1**(2), 048..
8. E.K. Efthimiadou, L.A. Tziveleka, P. Bilalis, and G. Kordas, Novel PLA modification of organic microcontainers based on ring opening polymerization: Synthesis, characterization, biocompatibility and drug loading/release properties, International Journal of Pharmaceutics, 2012, **428(**1–2), 134.
9. R. S. Karmali, A. Bartakke, V. P. Borker, and K. S. Rane, Bactericidal action of N doped ZnO in sunlight, Biointerface Research in Applied Chemistry, 2011, **1**(2), 057.
10. B. Vaidya, G.P. Agrawal, and S. P. Vyas, Functionalized carriers for the improved delivery of plasminogen activators, International Journal of Pharmaceutics, 2012, **424**(1-2), 1.
11. C. Saviuc, I. Marinas, A. M. Grumezescu, C. Bleotu, C. Chifiriuc, D. Mihăiescu, and V. Lazăr, Phytochemical composition of the fennel fruits essential oil and its influence on prokariotic cells growth and pathogenic features, Biointerface Research in Applied Chemistry, 2012, **2**(2), 300.
12. A.C. Abreu, A.J. McBain, and M. Simões, Plants as sources of new antimicrobials and resistance-modifying agents, Natural Product Reports, 2012, **9**,1007.
13. A. Wattanasatcha, S. Rengpipat, and S. Wanichwecharungruang, Thymol nanospheres as an effective anti-bacterial agent, International Journal of Pharmaceutics, 2012, **434**(1–2), 360.
14. K. Chaieb, H. Hajlaoui, T. Zmantar, A.B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani, and A. Bakhrouf, The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review, Phytotherapy Research, 2007, **21**(6), 501.
15. I. Marinas, A. M. Grumezescu, C. Saviuc, C. Chifiriuc, D. Mihăiescu, and V. Lazăr, *Rosmarinus officinalis* essential oil as antibiotic potentiator agains *Staphylococcus aureus*, Biointerface Research in Applied Chemistry, 2012, **2**(1), 271.
16. C. Saviuc, A. M. Grumezescu, E. Oprea, V. Rădulescu, L. Dascălu, M. C. Chifiriuc, M. Bucur, O. Banu, V. Lazăr, Antifungal activity of some vegetal extracts on *Candida* biofilms developed on inert substratum, Biointerface Research in Applied Chemistry, 2011, **1**(1), 015.
17. A.M. Grumezescu, M.C. Chifiriuc, C. Saviuc, V. Grumezescu, R. Hristu, D.E. Mihăiescu, G.A. Stanciu, and E. Andronescu, Hybrid nanomaterial for stabilizing the antibiofilm activity of *Eugenia carryophyllata* essential oil., IEEE Transactions on Nanobioscience, 2012, **11**(4), 360.
18. M. C. Chifiriuc, R. Palade, and A. M. Israil, Comparative analysis of disk diffusion and liquid medium microdillution methods fortesting the antibiotic susceptibility patterns of anaerobic bacterial strains isolated from intrabdominal infections, Biointerface Research in Applied Chemistry, 2011, **1**(6), 209.
19. L. Mărutescu, C. Limban, M. C. Chifiriuc, A.V. Missir, I. C. Chiriță, and M. T. Caproiu, Studies on the antimicrobial activity of new compounds containing thiourea function, Biointerface Research in Applied Chemistry, 2011, **1**(6), 236.

20. S.G. Khanage, P.B. Mohite, R.B. Pandhare, and S.A. Raju, Synthesis, characterization and antimicrobial evaluation of 3,5 diphenyl-1H-1,2,4-triazole containing pyrazole function, Biointerface Research in Applied Chemistry, 2012, **2**(3), 313.

21. http://www.rsu.ac.th/medtech/files/CLSI%202011.pdf

22. D.R. Naik, and J.P. Raval, Characteristic and controlled release of antiviral drug: A comparative study on preparative techniques and polymer affected parameter, Biointerface Research in Applied Chemistry, 2012, **2**(5), 409.

23. A.M. Grumezescu, E. Andronescu, A. Ficai, C. Bleotu, M. C. Chifiriuc, Chitin based biomaterial for antimicrobial therapy: fabrication, characterization and in vitro profile based interaction with eukaryotic and prokaryotic cells, Biointerface Research in Applied Chemistry, 2012, **2**(5), 438.

24. E. Andronescu, A. M. Grumezescu, A. Ficai, I. Gheorghe, M. Chifiriuc, D. Eduard Mihăiescu, and V. Lazăr, In vitro efficacy of antibiotic magnetic dextran microspheres complexes against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains, Biointerface Research in Applied Chemistry, 2012, **2**(3), 332.

25. A. M. Grumezescu, D. E. Mihăiescu, and D. Tamaş, Hybrid materials for drug delivery of rifampicin: evaluation of release profile, Biointerface Research in Applied Chemistry, 2011, **1**(6), 229.

26. D. E. Mihăiescu, M. Horja, I. Gheorghe, A. Ficai, A. M. Grumezescu, C. Bleotu, and C. M. Chifiriuc, Water soluble magnetite nanoparticles for antimicrobial drugs delivery, Letters in Applied NanoBioScience, 2012, **1**(2), 45.

27. A. M. Grumezescu, A. M. Holban, E. Andronescu, A. Ficai, C. Bleotu, and M. Carmen Chifiriuc, Water dispersible metal oxide nanobiocomposite as a potentiator of the antimicrobial activity of kanamycin, Letters in Applied NanobioScience, 2012, **1**(4), 77.

28. A. M. Grumezescu, A. M. Holban, E. Andronescu, M. Tomoiaga, A. Ficai, C. Bleotu, and M. C. Chifiriuc, Microbiological applications of a new water dispersible magnetic nanobiocomposite, Letters in Applied NanoBioScience, 2012, **1**(4), 83.

29. C. Wang, J. Yan, X. Cui, and H. Wang, Synthesis of raspberry-like monodisperse magnetic hollow hybrid nanospheres by coating polystyrene template with Fe_3O_4@SiO_2 particles, Journal of Colloid and Interface Science, 2011, 354, 94.

30. A. Beganskienė, V. Sirutkaitis , M. Kurtinaitienė, and R. Juškėnas , A. Kareiva, FTIR, TEM and NMR investigations of Stöber silica nanoparticles, Materials Science (Medžiagotyra), 2004, **10**(4), 287.

31. E.J. Lee, D.S. Shin, H.E. Kim, H.W. Kim, Y.H. Koh, J.H. Jang, Membrane of hybrid chitosan–silica xerogel for guided bone regeneration, Biomaterials, 2009, **30**, 743.

32. N.E. El-Gamel, L. Wortmann, K. Arroub, S. Mathur, SiO_2@Fe_2O_3 core-shell nanoparticles for covalent immobilization and release of sparfloxacin drug, Chemical Communications, 2011, **47**(36), 10076.

33. P. C. Balaure, E. Andronescu, A. M. Grumezescu, A. Ficai, K.S. Huang, C.H. Yang, C. M. Chifiriuc, and Y.S. Lin, Fabrication, characterization and in vitro profile based interaction with eukaryotic and prokaryotic cells of alginate–chitosan–silica biocomposite, International Journal of Pharmaceutics, 2012, http://dx.doi.org/10.1016/j.ijpharm.2012.10.045.

34. A. M. Grumezescu, A. Ficai, D. Ficai, G. Predan, and M. C. Chifiriuc, Polymeric magnetic silica microspheres as a drug loader for antimicrobial delivery substances, Digest Journal of Nanomaterials and Biostructures, 2012, **7**(4), 1891.

35. Z. Ma, H. Ji, D. Tan, G. Dong, Y. Teng, J. Zhou, M. Guan, J. Qiu, and M. Zhang, Large-scale preparation of strawberry-like, AgNP-doped SiO_2 microspheres using the electrospraying method, Nanotechnology, 2011, **22**(30), 305.

36. H. Jia, W. Hou, L. Wei, B. Xu, and X. Liu, The structures and antibacterial properties of nano-SiO_2 supported silver/zinc-silver materials**,** Dental Materials, 2008, **24**(2), 244.

37. G. Gu, J. Xu, Y. Wu, M. Chen, and L. Wu, Synthesis and antibacterial property of hollow SiO_2/Ag nanocomposite spheres, Journal of Colloid Interface Science, 2011, **359**(2), 327.

38. N. Zhang, Y. Gao, H. Zhang, X. Feng, H. Cai, and Y. Liu, Preparation and characterization of core-shell structure of SiO_2@Cu antibacterial agent, Colloinds and Surfaces B: Biointerfaces, 2010, **81**(2), 537.

39. Y.H. Kim, D.K. Lee, H.G. Cha, C.W. Kim, Y.C. Kang, and Y.S. Kang, Preparation and characterization of the antibacterial Cu nanoparticle formed on the surface of SiO_2 nanoparticles, the Journal of Physical Chemistry B, **110**(49), 24923.

40. M.J. Rosemary, I. MacLaren, T. Pradeep, Investigations of the antibacterial properties of ciprofloxacin@SiO_2, Langmuir, 2006, **22**(24), 10125.

41. L.K. Adams, D.Y. Lyon, A. McIntosh, P.J.Alvarez, Comparative toxicity of nano-scale TiO_2, SiO_2 and ZnO water suspensions, Water Science and Technology, 2006, **54**(11-12), 327.

42. P. Pozarowski, and Z. Darzynkiewicz, Analysis of Cell Cycle by Flow Cytometry, Methods in Molecular Biology, vol. 281: Checkpoint Controls and Cancer, Volume 2: Activation and Regulation Protocols Edited by: Axel H. Schönthal ©Humana Press Inc., Totowa, NJ.

****