IMPACT OF ALGINATE ON THE BIOCOMPATIBILITY OF MAGNETITE NANOPARTICLES UTILIZED FOR DRUG DELIVERY

Alina M. HOLBAN[1], Mihaela VLAD[2], Valentina GRUMEZESCU[3], Anton FICAI[4], Coralia BLEOTU[5], Ioana Madalina ALDEA (PITICA)[6], Alexandru Mihai GRUMEZESCU[7]*, Ecaterina ANDRONESCU[8]

The aim of this paper was to obtain, characterize and investigate the in vitro biocompatibility of newly engineered magnetite nanoparticles coated with antimicrobial drugs and alginate. Our growth, morphology and physiological assessments strongly suggest that the obtained nanosystems present improved biocompatibility, mainly due to the use of the alginate coating. Because of these characteristics and drug delivery versatility, alginate coated nanoparticles could be efficiently utilized as improved antimicrobial agents and may reduce the cytotoxicity related with the use of high amounts of antibiotics.

Keywords: improved biocompatibility, magnetite nanoparticles, alginate, antimicrobial therapy, drug delivery

[1] PhD, Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, and Microbiology Immunology Department, Faculty of Biology, University of Bucharest, Romania

[2] PhD student, Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania

[3] PhD student, Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania

[4] PhD, Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania

[5] PhD, S. Nicolau Institute of Virology; Bucharest, Romania

[6] PhD student, S. Nicolau Institute of Virology; Bucharest, Romania

[7] PhD, Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: grumezescu@yahoo.com

[8] Prof., Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania

1. Introduction

Nanotechnology has revealed a great impact on the biomedical field, bringing significant challenge for drug delivery and targeted release of bioactive drugs. Among all types of nanomaterials intended for biomedical purpose, magnetite nanoparticles represent the most investigated type of material for drug design and improved delivery. Their properties such as superparamagnetism, high surface energy, biocompatibility, and very low toxicity to human body recommend these nanostructures as suitable candidates for multiple biomedical applications [1]. Studies demonstrated that magnetite nanoparticles have an enhanced capacity to stabilize several therapeutic agents (such as volatile oils, natural antimicrobials), to enhance the effect of the active drug (i.e potentate the activity of anti-tumor drugs and antibiotics) and to ensure a targeted and controlled delivery, prolonging the release of the therapeutic drug [2]. However, these nanoparticles have an increased tendency to aggregate and form clusters and are also highly susceptible to leaching under acidic conditions, which may interfere with their biological efficiency [3]. Therefore, magnetite nanoparticles functionalized or coated with different organic or inorganic compounds are preferred in order to overcome these disadvantages. Polymeric substances are preferentially utilized for coating nanoparticles for medical use, since they can stabilize magnetite nanoparticles and can be easily used for further functionalization. Moreover polymers enhance the hydrophilicity and improve the biocompatibility; the core/shell structures composed of nanoparticles/polymers have a high adsorption capacity and chemical and thermal stability [4]. Magnetic nanoparticles coated with a polymeric shell have the potential to serve as drug carriers that can selectively target cancer cells or difficult to reach microbial infections and provide controlled release of chemotherapeutics [5]. In recent years, functionalized magnetic nanoparticles have been extensively utilized in the development of novel antimicrobial agents. Magnetite nanoparticles have been functionalized with various natural and synthetic antibiotics and were proved to stabilize and enhance their antimicrobial effect [6, 7]. This approach is intended to bring significant contribution for reducing the therapeutic dose of antimicrobial drugs and to fight against infections caused by resistant pathogens. Usually, infections caused by resistant or tolerant (biofilm embedded) microorganisms require high amounts of antibiotics for treatment and can be extremely toxic for the human body (i.e. neurotoxicity, nephrotoxicity, hepatotoxicity, etc) [8]. Moreover, severe infections affecting difficult to reach sites are sometimes impossible to treat by classical antibiotic treatment. In this context, nanosized drug delivery shuttles loaded with antimicrobial drugs could be efficiently utilized to target particular infections, improve drug effect and reduce toxicity effects by reducing the therapeutic dose of the active drug. Recent studies revealed that

magnetite nanoparticles functionalized with different classes of antibiotics, such as Penicillins, Cephalosporins, Aminoglycosides, Quinolones and Tetracyclines significantly improve the antimicrobial effect of these drugs and reduce the *in vitro* and *in vivo* minimum inhibitory concentrations necessary for the eradication for both planktonic and biofilm embedded bacterial cells [9, 10, 11]. However, cytotoxicity and biodistribution of these nanostructured shuttles with antimicrobial effect should be carefully addressed prior to their utilization in clinical trials.

The aim of this study was to synthesize, characterize and assess the cytotoxicity of magnetite nanoparticles functionalized with different antibiotics and coated with the polymer alginate, in order to increase the biocompatibility of the functionalized nanosystem.

2. Materials and methods

2.1. Materials

Ferrous sulfate 7-hydrate ($FeSO_4 \bullet 7H_2O$), ferric chloride ($FeCl_3$), ammonia (NH_3, 25%), antibiotics (amoxicillin (AMO), ampicillin (AMP), erythromycin (ERI), tetracycline (TET) and penicillin (PEN)), calcium chloride ($CaCl_2$), sodium alginate (AlG) were purchased from Sigma-Aldrich. All chemicals were of analytical purity and used with no further purification.

2.2. Preparation of modified magnetite nanoparticles

Fe_3O_4 was prepared according to our previously published papers [12, 13, 14, 15]. Prepared magnetite nanoparticles (90 mg), sodium alginate (900 mg) and 10 mg of antibiotics (AMO, AMP, ERI, TET, PEN) were mixed in a grinding mortar with 3 mL of methanol that contains 10% $CaCl_2$ until complete evaporation of the solvent. The mixture was washed 3 times (using a 100 kgf Nd-Fe-B external magnetic field) in order to remove the excess of $CaCl_2$. For *in vitro* tests, as a control, we prepared and utilized also samples composed from same amount of magnetite and antibiotics (Fe_3O_4/ATBs).

2.3. Characterization methods

2.3.1. XRD

X-ray diffraction analysis was performed on a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu Kα radiation from a Cu X-ray tube (run at 15 mA and 30 kV) was used. The samples were scanned in the Bragg-Brentano geometry with 2θ angle range of 20-80 degree [16].

2.3.2. SEM

SEM analysis was performed on a FEI electron microscope, using secondary electron beams with energies of 30 keV, on samples covered with a thin gold layer [17].

2.3.4. IRM

IR mappings were recorded on a Nicolet iN10 MX FT-IR microscope with MCT liquid nitrogen cooled detector in the measurement range 4000–700 cm^{-1}. Spectral collection was made in reflection mode at 4 cm^{-1} resolution. For each spectrum, 32 scans were co-added and converted to absorbance using OmincPicta software (Thermo Scientific). Approximately 250 spectra were analyzed for each sample. One absorption peak known as being characteristics for the prepared material was selected as spectral marker.

2.4. In vitro cell viability

2.4.1. Apoptosis detection

Apoptosis detection was performed using Annexin V-FITC Apoptosis Detection Kit I (BD Bioscience Pharmingen, USA) according to the manufacturer's protocol. 3 x 10^5 cells were seeded in 3.5 cm diameter tissue culture dishes and treated with 100 µg/mL magnetite nanoparticles for 24 hour. The total cells were resuspended in 100 µL of binding buffer and stained with 10 µL Annexin V-FITC and 10 µL propidium iodide for 10 minutes in dark. At least 10,000 events from each sample were acquired using a Beckman Coulter flow cytometer.

2.4.2. Cell cycle analysis

Cultured cells were harvested after the treatment with 1 mg/mL of nanoparticles for 24 h, washed in a cold phosphate saline buffer (PBS) solution (pH 7.5), and then fixed overnight in 70% ethanol, at –20^{o}C. The samples were washed with PBS, treated with RNase A (1 mg/mL) and labelled with propidium iodide (100 µg/mL), at 37^{0}C for 1 hour. The cellular DNA content was quantified on a Beckman Coulter EPICS XL flow cytometer and analyzed using FlowJo 8.8.6 software (Ashland, Oregon, USA).

2.4.3. Cloning assay

Hep cultured cells were seeded in 6-well plates at a concentration of 1x10^3/ well, in RPMI supplemented with 10% FBS (foetal bovine serum) and 1mg/ml nanoparticles. The culture medium was replaced with complete medium (RPMI supplemented with 10% FBS (Sigma) and 400 IU/ml penicillin, 200 µg/mL streptomycin) after 24 hours of treatment and then every 2 days. The growth of colonies was monitored for 7 days. Colonies were counted after fixation with methanol and staining with cresyl violet.

3. Results

The XRD measurements were performed to determine the mineral phase present in the samples. Fig. 1 shows the X-ray diffraction patterns of Fe_3O_4, Fe_3O_4@AlG and Fe_3O_4@Alg/AMP. By comparison with standard diffraction

pattern (ICDD card No. 19-0629) the analyzed samples are crystalline magnetite. The magnetite peaks corresponding to (220), (311), (400), (422), (511) and (440) crystallographic planes were identified. In the case of bulk magnetite the peaks are sharp and intense indicating a high degree of crystallinity. When the magnetite is coated with Alg and Alg/ATBs the peaks present a moderate intensity that indicates a lower cristallinity [18, 19].

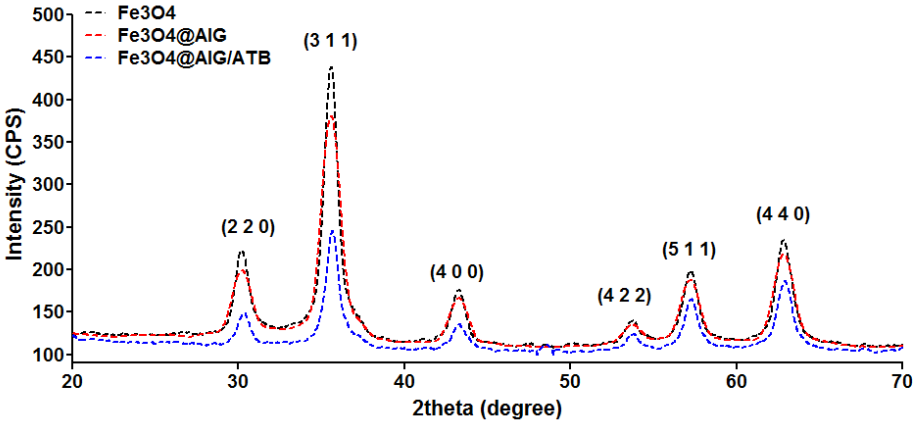

Fig. 1 XRD patterns of Fe_3O_4, Fe_3O_4@AlG and Fe_3O_4@AlG/ATB, where ATB is ampicillin

SEM images of bulk magnetite and alginate coated Fe_3O_4/ATBs are shown in Fig. 2..

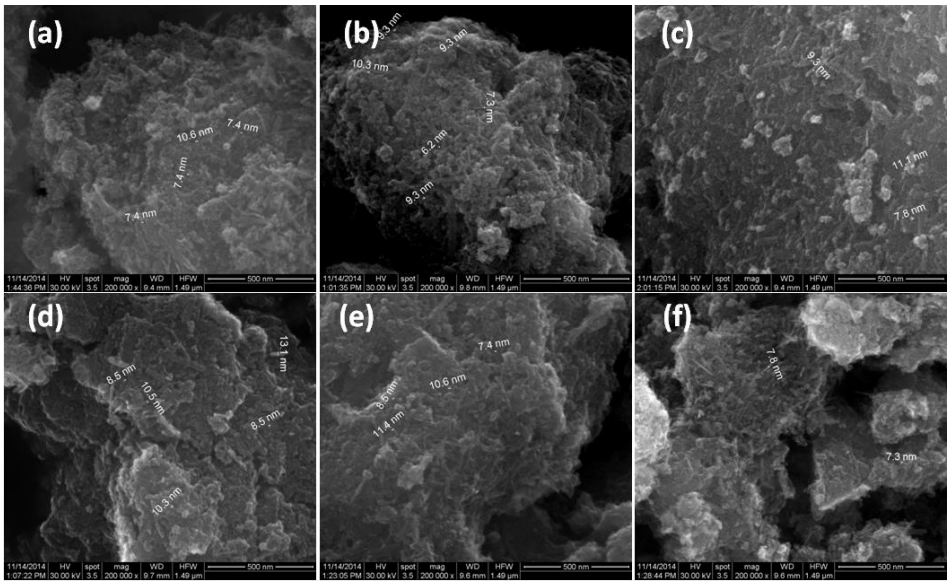

Fig. 2 SEM images of prepared samples: (a) Fe_3O_4; (b) Fe_3O_4@AlG/AMO; c) Fe_3O_4@AlG/AMP; (d) Fe_3O_4@AlG/ERI; (e) Fe_3O_4@AlG/TET; (f) Fe_3O_4@AlG/PEN.

As it can be seen in the case of uncoated Fe_3O_4, the particles have a nearly acicular shape with average particle dimension of 25 ± 5 nm long and 8 ± 2 nm wide. In the case of coated Fe_3O_4/ATBs, the dimensions are quite similar the polymer covering the particles.

Second derivative infrared mapping is used as a quick, easy, inexpensive, non-destructive and reproducible tool in order to estimate the structural integrity of material surface [20, 21]. Fig. 3 presents FTIR images based on the absorbance at 1026 cm^{-1} which represent the C-O-C (cyclic ether) band characteristic to sodium alginate from the samples. The pseudo-colour represents the real absorbance distribution in the FTIR map with an order of blue < green < yellow < red, where the blue area represents an approximate zero absorbance (due to the cavity from the samples), while red area represents a maximum absorbance [22]. By analysing the colours intensity of IR maps for each sample it can be concluded that the preparation of the coated nanostructures was done successfully, without the degradation of sodium alginate. Due to the small amount of antibiotics used in the experiment (under the detection limit of the spectrometer), the peaks assigned to antibiotics could not be identified.

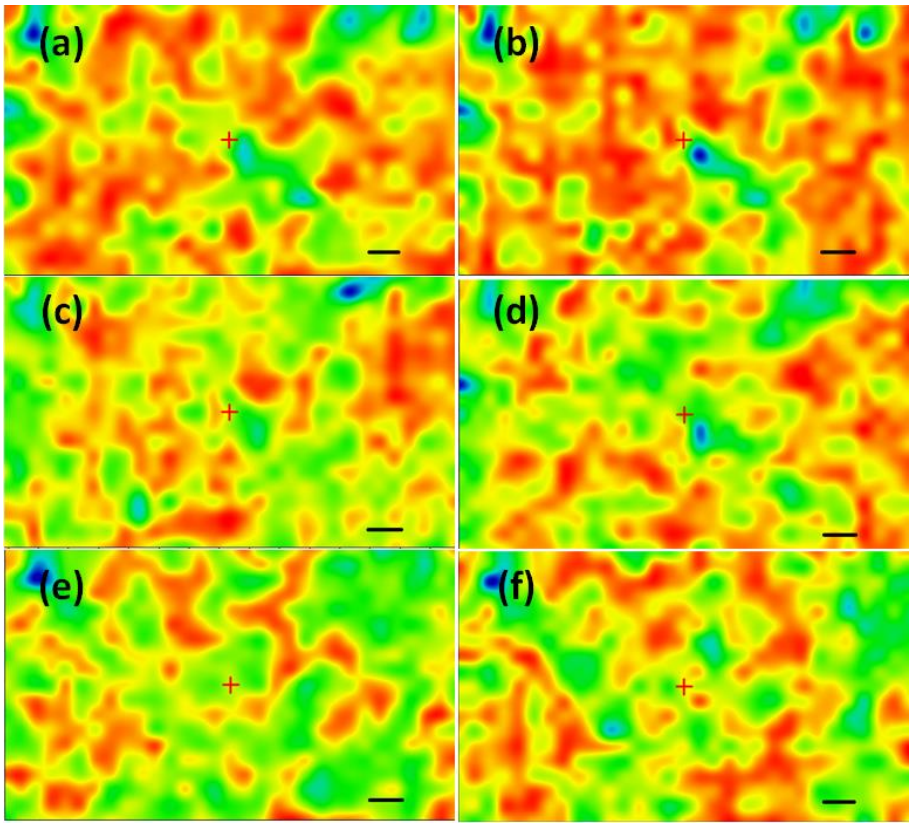

Fig. 3 Second derivate IR mapping of prepared samples (a)Fe_3O_4@AlG; (b) Fe_3O_4@AlG/AMO; (c) Fe_3O_4@AlG/AMP; (d) Fe_3O_4@AlG/ERI; (e) Fe_3O_4@AlG/TET; (f) Fe_3O_4@AlG/PEN

In order to evaluate the cytotoxic effects of the prepared nanoparticles, Annexin V-FITC Apoptosis Detection Kit was used. We have observed that the treatment with 100 µg/ml nanoparticles has no significant toxic effects on the cells after 24 hours of incubation. For the Fe_3O_4/AMO, Fe_3O_4@AlG/AMO and Fe_3O_4/AMP, Fe_3O_4@AlG/AMP the differences between the viability of cells treated with uncoated magnetite nanoparticles and cells treated with alginate coated magnetite nanoparticles were undetectable (lower than 1%).

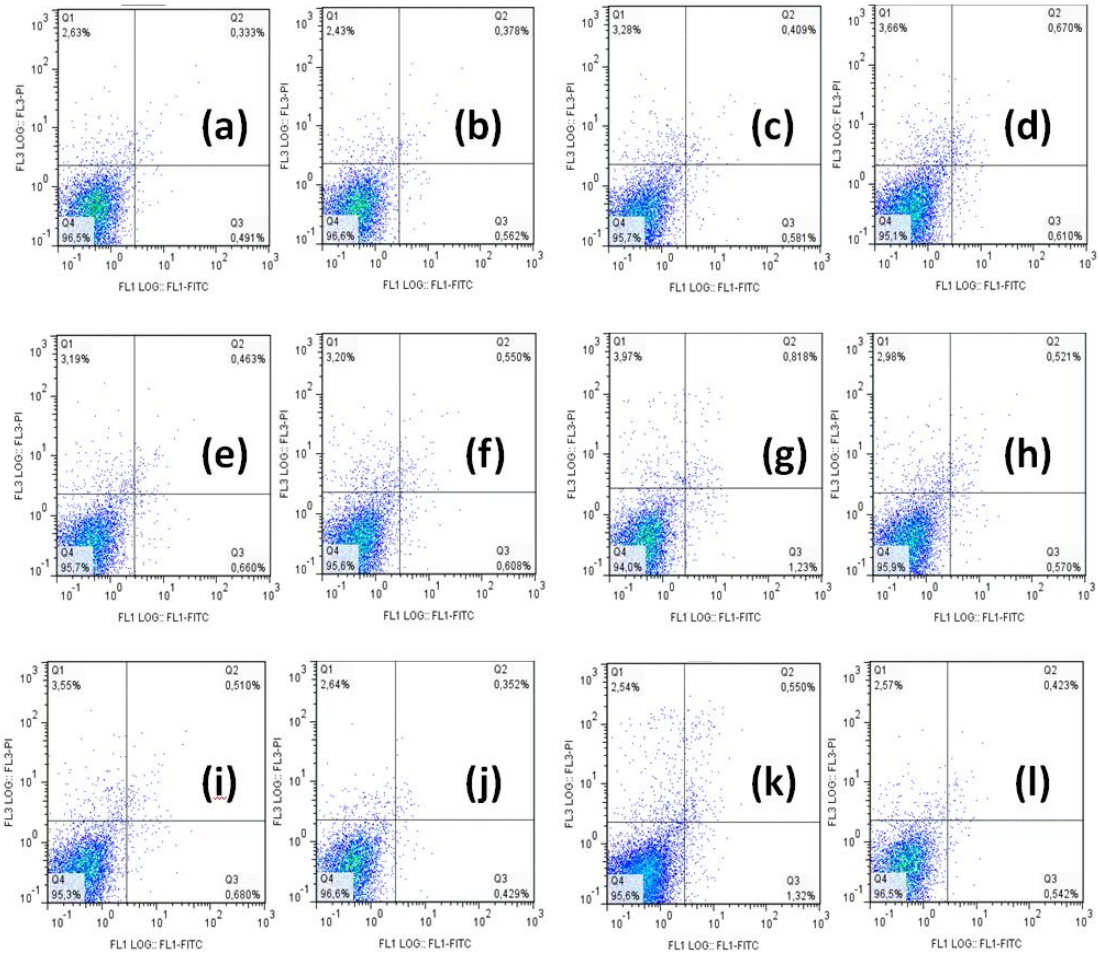

Fig. 4 Evaluation of nanoparticles toxicity using Annexin V-FITC Apoptosis Detection Kit for revealing the number of viable, apoptotic and necrotic Hep cells following the treatment with the obtained nanoparticles. Control. Hep cells grown in control conditions without any modification: (a) Hep cells grown in control conditions without any modification; (b) Hep cells grown in the presence of the same amount of sterile distilled water, utilized for obtaining the dispersion of concentrated nanoparticles); (c) Fe_3O_4/AMO; (d) Fe_3O_4@AlG/AMO; (e) Fe_3O_4/AMP; (f) Fe_3O_4@AlG/AMP; (g) Fe_3O_4/ERI; (h) Fe_3O_4@AlG/ERI; (i) Fe_3O_4/TET; (j) Fe_3O_4@AlG/TET; (k) Fe_3O_4/PEN; (l) Fe_3O_4@AlG/PEN

However, Fe_3O_4/ERI, Fe_3O_4@AlG/ERI, Fe_3O_4/TET, Fe_3O_4@AlG/TET and also Fe_3O_4/PEN, Fe_3O_4@AlG/PEN the results demonstrated differences of more than 1% between the viability of cultured cells treated with uncoated magnetite nanoparticles and alginate coated magnetite nanoparticles. Therefore, in the case of samples Fe_3O_4/ERI the cellular viability was of 94%, while in the case of cells treated with and Fe_3O_4@AlG/ERI the viability was 95,9%; for Hep cells treated with Fe_3O_4/TET the viability was of 95,3% and for cells treated with Fe_3O_4@AlG/TET the viability was of 96,6%; while in the case of Fe_3O_4/PEN the viability of treated cells was of 95,6% and the cellular viability following the treatment with Fe_3O_4@AlG/PEN was of 96,5% (Fig. 4).

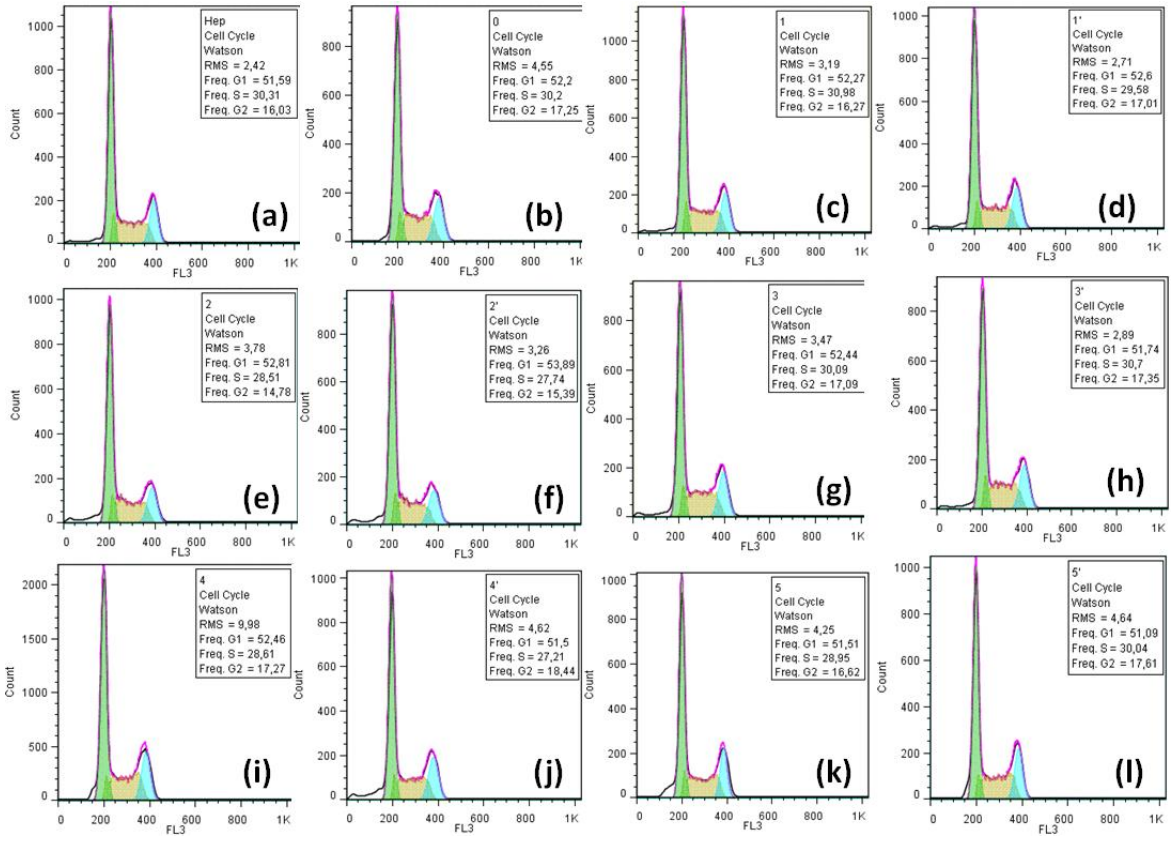

Fig. 5 The effects of nanoparticles on the Hep cell cycle. DNA histogram analysis was performed using Watson model deconvolution and FlowJo software. Hep. Hep cells grown in control conditions without any modification: (a) Hep cells grown in control conditions without any modification; (b) Hep cells grown in the presence of the same amount of sterile distilled water, utilized for obtaining the dispersion of concentrated nanoparticles; (c) Fe_3O_4/AMO; (d) Fe_3O_4@AlG/AMO; (e) Fe_3O_4/AMP; (f) Fe_3O_4@AlG/AMP; (g) Fe_3O_4/ERI; (h) Fe_3O_4@AlG/ERI; (i) Fe_3O_4/TET; (j) Fe_3O_4@AlG/TET; (k) Fe_3O_4/PEN; (l) Fe_3O_4@AlG/PEN;

Flow cytometry assay also revealed that nanoparticles do not cause drastic changes in the cell cycle phases. The progression of cellular cell cycle looks normal for all tested samples, the Sub-G0 peaks specific for apoptotic cells being reduced, and slightly observable only in the case of Fe_3O_4/AMP, Fe_3O_4@AlG/AMP, Fe_3O_4/TET, Fe_3O_4@AlG/TET and Fe_3O_4/PEN (Fig. 5). Also, the results revealed that the Fe_3O_4/TET stimulate the growth of the Hep cultured cells, the treatment of the cells with Fe_3O_4@AlG/TET inducing the highest rate of cells in G2 phase (growing cells) (18.44% as compared with 16.02% observed in the case of control Hep cells) (Table 1).

Table 1

The effects of coated or uncoated nanoparticles on the cell cycle of Hep cultured cells. (Percentage of cells in G1, S (synthesis) and G2 (growing cells) phases are revealed).

	a	b	c	d	e	f	g	h	i	j	k	l
G1	51.59	52.2	52.27	52.6	52.81	53.89	52.44	51.74	52.46	51.5	51.51	51.09
S	30.31	30.2	30.98	29.58	28.51	27.74	30.09	30.7	28.61	27.21	28.95	30.04
G2	16.02	17.25	16.27	17.01	14.78	15.39	17.09	17.05	17.27	18.44	16.62	17.61

Finally, we investigated the cytotoxicity of the obtained nanoparticles by using the cloning assay. No significant differences were observed between the numbers of viable colonies developed in the presence of nanoparticles comparing with the number of colonies developed without them. On the other hand, no significant differences were observed between the numbers of viable colonies developed in the presence of uncoated magnetite nanoparticles, or alginate coated nanoparticles (Fig. 6).

All results strongly suggest that these nanoparticles do not produce toxic effects *in vitro*, excluding cumulative effects which should be further investigated.

Even though we could not find a specific mechanism of the observed differences, the results suggest that alginate may increase the biocompatibility of the functionalized magnetite nanoparticles strictly depending on the therapeutic agent loaded on the nanoparticles.

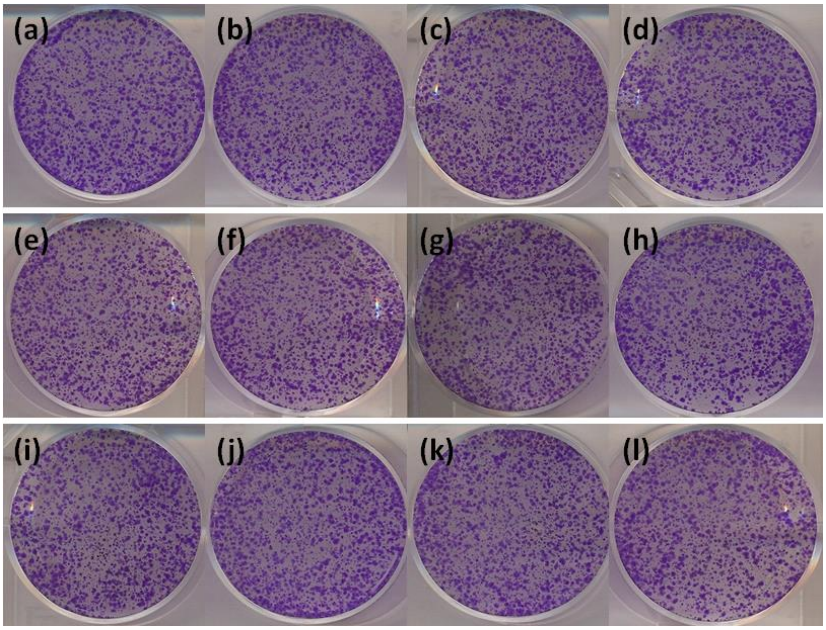

Fig. 6 The effects of concentrated (1 mg/ml) nanoparticles on the growth of Hep cell clones: (a) Hep cells grown in control conditions without any modification; (b) Hep cells grown in the presence of the same amount of sterile distilled water, utilized for obtaining the dispersion of concentrated nanoparticles); (c) Fe_3O_4/AMO; (d) Fe_3O_4@AlG/AMO; (e) Fe_3O_4/AMP; (f) Fe_3O_4@AlG/AMP; (g) Fe_3O_4/ERI; (h) Fe_3O_4@AlG/ERI; (i) Fe_3O_4/TET; (j) Fe_3O_4@AlG/TET; (k) Fe_3O_4/PEN; (l) Fe_3O_4@AlG/PEN

However, the final dimension of the coated nanosystem may also represent a key factor for the biocompatibility of the nanomaterials [23].

4. Conclusions

In this study, we report on the successful preparation and characterization of alginate coated magnetite nanoparticles containing different antibiotics intended to be utilized in targeting severe and difficult to reach infections. Even though all the obtained formulations proved to be biocompatible, the cytotoxicity tests performed *in vitro* demonstrate that the alginate coating may efficiently improve the biocompatibility of the nanoparticles. Our data reveal that alginate coatings may be utilized for reducing cytotoxicity of nanosized drug delivery shuttles and further studies are required in order to prove if these coatings interfere with the release of the active compounds.

Acknowledgments

A.M. Holban, V. Grumezescu and A.M. Grumezescu acknowledge the support of the Sectorial Operational Programme Human Resources Development

2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132397 (ExcelDOC). M. Vlad acknowledges the support of the Sectorial Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/134398 (Knowledge). I.M. Aldea (Pitica) was financed from the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390.

R E F E R E N C E S

[1] *I. Liakos, A.M. Grumezescu, A.M. Holban*, "Magnetite nanostructures as novel strategies for anti-infectious thepray", Molecules, **19**, 2014, 12710-12726.

[2] *V. Grumezescu, E. Andronescu, A.M. Holban, L. Mogoantă, G.D. Mogoşanu, A.M. Grumezescua, A. Stănculescu, G. Socol, F. Iordache, H. Maniu, M.C. Chifiriuc*, "MAPLE fabrication of thin films based on kanamycin functionalized magnetite nanoparticles with anti-pathogenic properties", Applied Surface Science, **336**, 2015, 188–195.

[3] *M.S.A. Darwish, N.H.A. Nguyen, A. Ševců, I. Stibor*, "Functionalized magnetic nanoparticles and their effect on Escherichia coli and Staphylococcus aureus", Journal of Nanomaterials, **2015**, 2015, 416012.

[4] *H. Xuemei, Y. Hao*, "Fabrication of polystyrene/detonation nanographite composite microspheres with the core/shell structure via pickering emulsion polymerization", Journal of Nanomaterials, **2013**, 2013, 751497.

[5] *C.C. Gonzalez, C.A. M. Pérez, A.M. Martínez*, "Development of antibody-coated magnetite nanoparticles for biomarker immobilization", Journal of Nanomaterials, **2014**, 2014, 978284.

[6] *C. Limban, A.V. Missir, A. M. Grumezescu, A.E. Oprea, V. Grumezescu, B.Ş. Vasile, G. Socol, R. Truşcă, M.T. Caproiu, M.C. Chifiriuc, B. Gălăţeanu, M. Costache, L. Moruşciag, G. Pîrcălăbioru, D.C. Nuţă*, "Bioevaluation of novel anti-biofilm coatings based on PVP/Fe_3O_4 nanostructures and 2-((4-ethylphenoxy)methyl)-N-(arylcarbamothioyl)benzamides", Molecules, **19**, 2014, 12011-12030.

[7] *A.G. Anghel, A.M. Grumezescu, M. Chirea, V. Grumezescu, G. Socol, F. Iordache, A.E. Oprea, I. Anghel, A.M. Holban*, "MAPLE fabricated Fe3O4@Cinnamomum verun antimicrobial surface for improved gastrostomy tubes", Molecules, **19**, 2014, 8981-8994.

[8] *M.F. Grill, R.K. Maganti*, "Neurotoxic effects associated with antibiotic use: management considerations", Br. J. Clin. Pharmacol., **72**, 2011, 381–393.

[9] *A.M. Grumezescu, M.C. Gestal, A.M. Holban, V. Grumezescu, B.Ş. Vasile, L. Mogoantă, F. Iordache, C. Bleotu, G.D. Mogoşanu*, "Biocompatible Fe3O4 increases the efficacy of amoxicillin delivery against Gram-positive and Gram-negative bacteria", Molecules, **19**, 2014, 5013-5027.

[10] *A. Bolocan, D.E. Mihaiescu, E. Andronescu, G. Voicu, A.M. Grumezescu, A. Ficai, B.S.Vasile, C. Bleotu, M.C. Chifiriuc, C.S. Pop*, „Biocompatible hydrodispersible magnetite nanoparticles used as antibiotic drug carriers". Rom J Morphol Embryol, **56**(2), 2015, 365-70.

[11] *M.M. Mihai, A.M. Holban, C. Giurcaneanu, L.G. Popa, R.M. Oanea, V. Lazar, M.C. Chifiriuc, M. Popa, M.I. Popa*, Microbial biofilms: impact on the pathogenesis of periodontitis, cystic fibrosis, chronic wounds and medical device-related infections. Curr Top Med Chem, **15**(16), 2015, 1552-76.

[12] *A.M. Prodan, C.S. Ciobanu, C.L. Popa, S.L. Iconaru, D. Predoi*, "Toxicity evaluation following intratracheal instillation of iron oxide in a silica matrix in rats", BioMed Research International, 2014, doi:10.1155/2014/134260.

[13] *A.M. Prodan, S.L. Iconaru, C.S. Ciobanu, M.C. Chifiriuc, M. Stoicea, D. Predoi*, "Iron oxide magnetic nanoparticles: Characterization and toxicity evaluation by in vitro and in vivo assays," Journal of Nanomaterials, 2013, doi:10.1155/2013/587021.

[14] *M.C. Chifiriuc, A.M. Grumezescu, E. Andronescu, A. Ficai, A.I. Cotar, V. Grumezescu, E. Bezirtzoglou, V. Lazar, R. Radulescu*, "Water dispersible magnetite nanoparticles influence the efficacy of antibiotics agains planktonic and biofilm embedded *Enterococcus faecalis* cells", Anaerobe, **22**, 2013, 14-19.

[15] *A.M. Grumezescu, A.I. Cotar, E. Andronescu, A. Ficai, C.D. Ghitulica, V. Grumezescu, B.S. Vasile, M.C. Chifiriuc*, "*In vitro* activity of the new water dispersible Fe_3O_4@usnic acid nanostructure against planktonic and sessile bacterial cells", Journal of Nanoparticle Research **15**, 2013, 1766.

[16] *A.I. Cotar, A.M. Grumezescu, K.-S. Huang, C.M. Chifiriuc, R. Radulescu*, "Magnetite nanoparticles influence the efficacy of antibiotics against biofilm embedded *Staphylococcus aureus* cells", Biointerface Research in Applied Chemistry, **3**, 2013, 559-565.

[17] *A.I. Cotar, A.M. Grumezescu, E. Andronescu, G. Voicu, A. Ficai, K.-L. Ou, K.-S. Huang, M.C. Chifiriuc*, "Nanotechnological solution for improving the antibiotic efficiency against biofilms developed by Gram-negative bacterial strains", Letters in Applied NanoBioScience **2**, 2013, 97-104.

[18] *O. Ivashchenko, M. Lewandowski, B. Peplińska, M. Jarek, G. Nowaczyk, M. Wiesner, K. Załęski, T. Babutina, A. Warowicka, S. Jurga*, "Synthesis and characterization of magnetite/silver/antibiotic nanocomposites for targeted antimicrobial therapy", Materials Science and Engineering: C, **55**, 2015, 343-359.

[19] *N. Habibi*, "Preparation of biocompatible magnetite-carboxymethyl cellulose nanocomposite: Characterization of nanocomposite by FTIR, XRD, FESEM and TEM", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, **131**, 2014, 55-58.

[20] *V. Grumezescu, G. Socol, A. M. Grumezescu, A. M. Holban, A. Ficai, R. Truşcă, C. Bleotu, P. C. Balaure, R. Cristescu, M. C. Chifiriuc*, "Functionalized antibiofilm thin coatings based on PLA–PVA microspheres loaded with usnic acid natural compounds fabricated by MAPLE", Applied Surface Science, 302, **30**, 2014, 262-267.

[21] *V. Grumezescu, E. Andronescu, A. M. Holban, L. Mogoantă, G. D. Mogoşanu, A. M. Grumezescu, A. Stănculescu, G. Socol, F. Iordache, H. Maniu, M. C. Chifiriuc*, "MAPLE fabrication of thin films based on kanamycin functionalized magnetite nanoparticles with anti-pathogenic properties", Applied Surface Science, **336**, 2015, 188-195.

[22] *Q. Wang, X.-T. Jiang, Y.-Z. Xin, J.-X. Cui, P.-D. Zhang*, "Characterization of in vitro mineralization of porous poly (L-lactic acid)/bioactive glass composites by Attenuated Total Reflectance-Fourier Transform Infrared Mapping", Chinese Journal of Analytical Chemistry, **42**, 2014, 221-226.

[23] *X. Li, L. Wang, Y. Fan, Q. Feng, F.-Z.Cui*, "Biocompatibility and toxicity of nanoparticles and nanotubes", Journal of Nanomaterials, **2012**, 548389.