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BIOLOGICAL STUDIES ON DEXTRIN COATED IRON OXIDE NANOPARTICLES

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Abstract. In this study, dextrin coated iron oxide nanoparticles were obtained using an adapted chemical co-precipitation method. The size and morphology of the dextrin coated iron oxide nanoparticles (DIO-NPs) were analyzed by transmission electron microscopy (TEM). The scanning electron microscopy (SEM) analysis depicted information on the morphology of DIO-NPs. The elemental analysis was conducted by Energy-dispersive X-ray spectroscopy (EDAX). The iron oxide particles coated with dextrin have a spherical shape at nanometric scale with a narrow size distribution. The cytotoxicity assay was performed by quantification of HeLa cells

viability, while the antimicrobial activities of the DIO-NPs were determined against ATCC reference and clinical microbial strains, *i.e.* Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538) and yeast (*Candida albicans* 393). The obtained nanoparticles did not have cytotoxic effect on HeLa cells after a 24 h exposure to DIO-NPs and the morphology of the cells was not affected. A low toxic effect on HeLa cells was noticed after 48 h. The minimal inhibitory concentration of DIO-NPs was 1 mg/mL for both tested microbial strains.

Key words: iron oxide, biocompatible nanoparticles, dextrin, antimicrobial activity.

1. INTRODUCTION

Nowadays, various technological and medical challenges have been resolved due to nanotechnology and to the development of materials science. The use of nanotechnology in biomedical applications has shown promising results [1–3]. The area of engineered nanoparticles is a branch of materials science which exhibits great potential in solving pressing problems at a global scale by developing new materials with controllable and reproducible properties. In the past decade, magnetic multifunctional nanoparticles (MNPs) have been investigated for important biomedical applications such as targeted drug delivery, magnetic resonance [1–5], cell labelling, [2], hyperthermia, detoxification of blood toxins [3] etc. The use of MNPs as contrast agents in magnetic resonance is one of the well-established promising areas of pharmaceutical development of the last years [4]. Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), the most common forms of iron oxides, have been the subject of numerous studies concerning their use in biomedical applications [5–9]. Although their biological properties have been proven along the years in many studies [10–13], there are still concerns regarding the administration of these types of nanoparticles to the human body. In recent studies iron oxide nanoparticles have been also promoted as antimicrobial agents [13–15].

Therefore the development of MNPs with spherical shape, special surface and biological properties are still a priority among the scientific communities [15–16]. In order to overcome or minimize any potential risk of toxicity, iron oxide nanoparticles are frequently functionalized using biocompatible polymers [16–17]. Because they present chemical stability and do not show any toxicity to the human body, polymers such as dextran, dextrin, cellulose, chitosan and sucrose have been intensively studied as coatings for iron oxide nanoparticles [18].

The aim of this study was to synthesize dextrin coated iron oxide nanoparticles (DIO-NPs) using an adapted chemical co-precipitation method. The obtained nanoparticles were investigated by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The bio-evaluation of DIO-NPs consisted in the investigation of their antimicrobial properties and their cytotoxicity on HeLa cells.

2. MATERIALS AND METHODS

2.1. MATERIALS

Ferrous chloride tetrahydratate ($FeCl_2 \cdot 4H_2O$), ferric chloride hexahydratate ($FeCl_3 \cdot 6H_2O$), natrium hydratate (NaOH) and dextrin $(C_6H_{10}O_5)n$ where purchased from Merck and used without further purification. Deionized water was also used in the synthesis of nanoparticles and in the rinsing of clusters.

2.2. SYNTHESIS AND CHARACTERIZATION OF DIO-NPs

The synthesis of DIO-NPs was performed according to [19–21]. SEM studies were realized with a HITACHI S2600N-type microscope equipped with an energy dispersive X-ray attachment (EDAX/2001 device).

Cell viability assay

The quantification of cell viability was performed on HeLa cells with propidium iodide (PI) and fluorescein diacetate (FdA). HeLa cells, (5×10^4) were seeded in each well of a plate with 24 wells and were treated after 72 h with a suspension of DIO-NPs (200 μL) 10 times diluted. The effects on cell viability were evaluated after 24 h, 48 h, and 72 h. The fluorescence was quantified using an Observer D1 Carl Zeiss microscope [23–25].

In vitro antibacterial and antifungal activity

The antimicrobial activities of the DIO-NPs were determined against ATCC reference and clinical Gram-positive (*Staphylococcus aureus* ATCC 6538) and yeast (*Candida albicans* 393) microbial strains. 0.5 McFarland density microbial suspensions of 1.5×10^8 CFU/mL obtained from 15–18 h bacterial cultures developed on solid media were used in the experiments. The antimicrobial activity was tested using a Mueller-Hinton Agar (MHA) medium for the Gram-positive and a Yeast Peptone Glucose (YPG) medium for the *Candida albicans* yeast.

The qualitative screening of the DIO-NPs antimicrobial activity was performed by an adapted disk diffusion method [25–26]. The quantitative assay of the antimicrobial activity against planktonic microbial strains was performed by the liquid medium microdilution method, in 96 multi-well plates. The minimal inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) have been thus established. The MIC and MBEC values were considered the lowest concentrations of the tested compound that inhibited the visible growth or biofilm development by the microbial strains [26–28].

3. RESULTS AND DISCUSSIONS

The size of the magnetic DIO-NPs were analyzed by TEM. The TEM photomicrographs (Fig. 1A) showed that DIO-NPs have a spherical structure with a narrow size distribution.

The HRTEM image (Fig. 1B) proved an arrangement of atomic planes of amorphous dextrin coated iron oxide nanoparticles. Moreover, the electron diffraction patterns (Fig. 1C) confirmed the existence of atomic planes characteristic to magnetite. The SEM and EDAX analyses were conducted on dextrin coated iron oxide powders. SEM analysis of dextrin coated iron oxide powders depicted information on the morphology of these particles (Fig. 2 A–B).

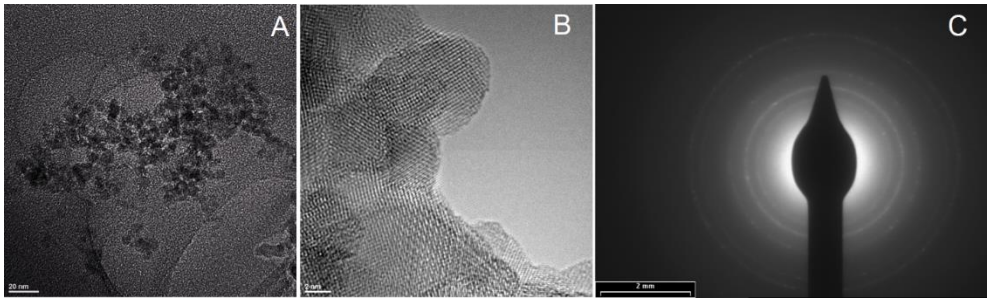

Fig. 1 – TEM (A), HRTEM (B) and electron diffraction patterns (C) photomicrographs of DIO-NPs.

Dextrin coated iron oxide composite showed particles with spherical shape at nanometric scale, in good agreement with SEM studies (Fig. 2B). Figure 3 describes the EDAX analysis of dextrin coated iron oxide powders which consists of carbon (C), oxygen (O) and iron (Fe) peaks.

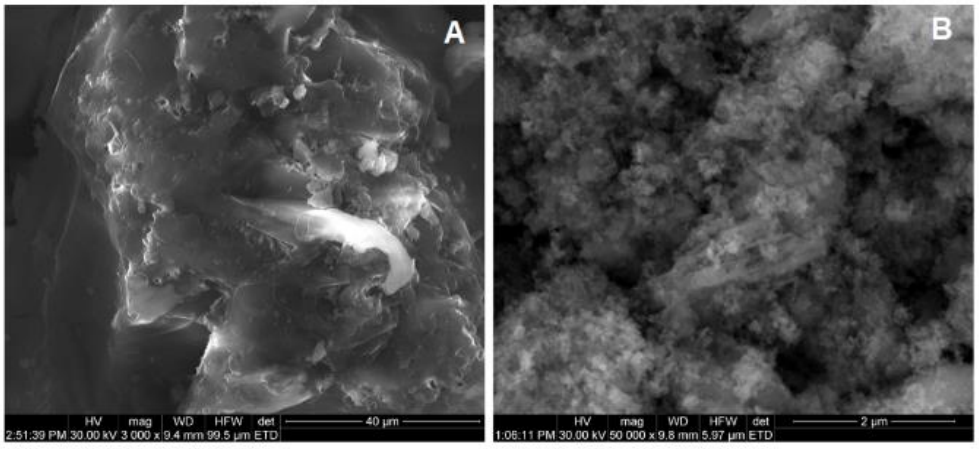

Fig. 2 – SEM micrographs of dextrin coated iron oxide powders.

Despite the considerable number of papers reporting the synthesis, characterization and coating of iron oxide nanoparticles with optimal properties for different medical applications, the development of biocompatibility assays ensuring their safe clinical use is still at the beginning [28–32]. Therefore one of the purposes of this study was to investigate the cytotoxicity of the obtained DIO-NPs on HeLa cells (Fig. 4). The cellular viability of HeLa cells in the presence of 200 μL suspension of DIO-NPs diluted 10 times was determined after an exposure of 24 h, 48 h and 72 h.

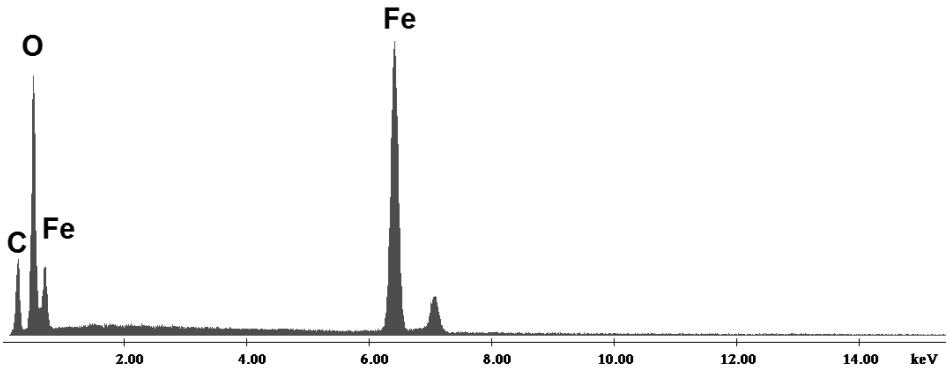

Fig. 3 – EDAX analysis of dextrin coated iron oxide powders.

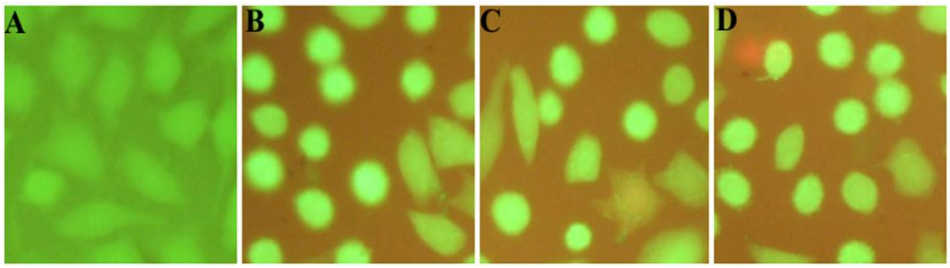

Fig. 4 – Fluorescence microscopy image of HeLa control cells (A).
HeLa cells after 24 h exposure to a suspension of DIO-NPs (200 μL) diluted 10 times (B).
HeLa cells after 48 h exposure to a suspension of DIO-NPs (200 μL) diluted 10 times (C).
HeLa cells after 72 h exposure to a suspension of DIO-NPs (200 μL) diluted 10 times (D).

The fluorescence microscopy images revealed that the obtained nanoparticles were not cytotoxic on HeLa cells after 24 h exposure to DIO-NPs (200 μL) suspension 10 times diluted (Fig. 4B). The HeLa cells exhibited normal morphological features and no cell degradation after exposure to the DIO-NPs

suspension. A slight toxic effect against the cells was noticed after 48 h when, as it could be observed in Fig. 4C, the morphology of the cells started to be affected. The DIO-NPs suspension proved to be toxic and led to cellular death only after an exposure of 72 h. As it can be noticed in the fluorescence microscopy image Fig. 4D, after 72 h, dead red cells stained with PI have been observed, thus proving the toxic effect of the DIO-NPs suspension on HeLa cells.

Although iron is not considered a conventional antimicrobial agent, a lot of studies have been demonstrated the antimicrobial effect of iron compounds (FeO, Fe_2O_3). The antibacterial activity of iron oxide nanoparticles is related to their ability to cross the bacterial wall, to interfere with the cellular membrane and to induce the production of reactive oxygen species. Apart from the antibacterial properties, iron oxide nanoparticles also exhibit superparamagnetic properties that could facilitate their introduction and orientation into the body by a magnetic field [32]. The aim of this study was to demonstrate that the DIO-NPs are not cytotoxic on HeLa cells as well as antimicrobial activity of DIO-NPs against *Staphylococcus aureus* ATCC 6538 and *Candida albicans* 393 yeast.

In order to investigate the biological properties of DIO-NPs, their antimicrobial activity was also investigated using strains belonging to the most common bacterial Gram-positive (*S. aureus* ATCC 6538) and yeast (*C. albicans* 393) pathogens. The results of the qualitative antimicrobial activity evaluation of DIO-NPs are shown in Fig. 5.

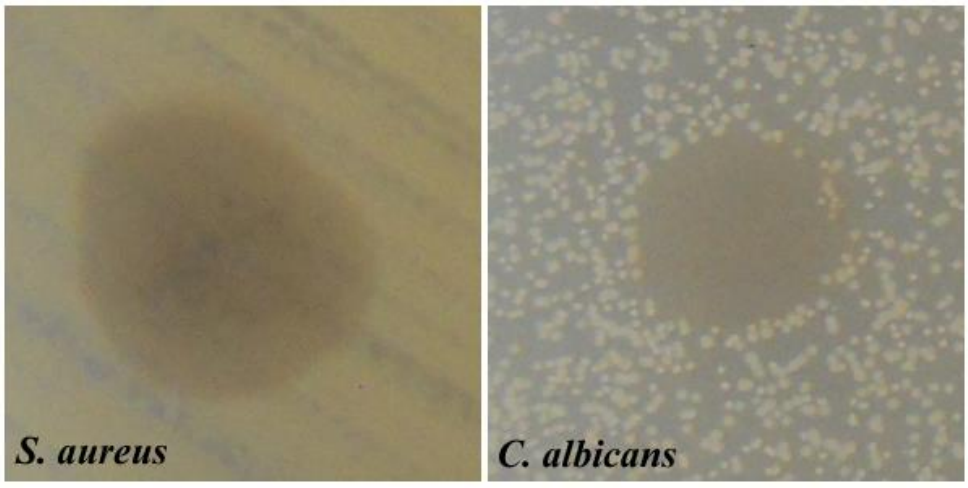

Fig. 5 – Qualitative assay of the inhibitory activity of DIO-NPs on Gram-positive *Staphylococcus aureus* ATCC 6538 and yeast *Candida albicans* 393.

The antimicrobial activity of DIO-NPs was demonstrated by the considerable inhibition zones obtained against *Staphylococcus aureus* ATCC 6538 (~1 cm) and *Candida albicans* 393 yeast (0.8 cm). After the qualitative screening, the microbial strains which proved to be susceptible to the tested DIO-NPs have been investigated in the quantitative assay for establishing the MIC value. The MIC values obtained in the case of the susceptibility of microbial planktonic cells, as well as the MBEC against those developed in biofilms towards DIO-NPs are presented in Table 1. The MIC value for the planktonic bacterial cells growth was equal to 1 mg/mL both in the case of *S. aureus* 6538 and *C. albicans* 393. Meanwhile, the value of the initial biofilm active concentration was higher for *S. aureus* 6538 than for *C. albicans*.

Table 1

MIC and MBEC of DIO-NPs on the microbial strains susceptible to the compounds in the qualitative screening assay

DIO-NPs	*Staphylococcus aureus* ATCC 6538	*Candida albicans* 393
MIC [mg/mL]	1	1
MBEC [mg/mL]	1	0.125

The biocompatibility correlated with the antimicrobial properties of DIO-NPs showed their potential use in the design of materials and surfaces with biomedical applications.

4. CONCLUSIONS

The dextrin coated iron oxide nanoparticles were prepared by an adapted co-precipitation method. TEM and SEM micrographs showed spherical dextrin coated iron oxide nanoparticles.

The fluorescence microscopy investigations revealed that the HeLa cells viability was not influenced by the presence of DIO-NPs after the first 24 h of exposure, the morphology of the cells being preserved. The results showed a decrease in cell viability and the apparition of dead cells only after 72 h of incubation with a DIO-NPs solution 10 times diluted.

The antimicrobial activity of the DIO-NPs was also presented in this study. The results of the quantitative and qualitative assays regarding the antimicrobial and antibiofilm activity revealed that the DIO-NPs successfully inhibited the growth of both Gram-positive *Staphylococcus aureus* ATCC 6538 bacteria and *Candida albicans* 393 yeast.

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