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Ciobanu, Carmen Steluta; Iconaru, Simona Liliana; Gyorgy, Eniko; Radu, Mihaela; Costache, Marieta; Dinischiotu, Anca; Le Coustumer, Philippe; Lafdi, Khalid; and Predoi, Daniela, "Biomedical Properties and Preparation of Iron Oxide-Dextran Nanostructures by MAPLE Technique" (2012). *Chemical and Materials Engineering Faculty Publications*. 11. https://ecommons.udayton.edu/cme_fac_pub/11

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RESEARCH ARTICLE



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Biomedical properties and preparation of iron oxide-dextran nanostructures by MAPLE technique

Carmen Steluta Ciobanu¹, Simona Liliana Iconaru¹, Eniko Gyorgy², Mihaela Radu³, Marieta Costache³, Anca Dinischiotu³, Philippe Le Coustumer⁴, Khalid Lafdi⁵ and Daniela Predoi^{1*}

Abstract

Background: In this work the chemical structure of dextran-iron oxide thin films was reported. The films were obtained by MAPLE technique from composite targets containing 10 wt. % dextran with 1 and 5 wt.% iron oxide nanoparticles (IONPs). The IONPs were synthesized by co-precipitation method. A KrF* excimer laser source ($\lambda = 248 \text{ nm}, \tau_{\text{FWHM}}\cong 25 \text{ ns}, \nu = 10 \text{ Hz}$) was used for the growth of the hybrid, iron oxide NPs-dextran thin films.

Results: Dextran coated iron oxide nanoparticles thin films were indexed into the spinel cubic lattice with a lattice parameter of 8.36 Å. The particle sized calculated was estimated at around 7.7 nm. The XPS shows that the binding energy of the Fe $2p_{3/2}$ of two thin films of dextran coated iron oxide is consistent with Fe³⁺ oxides. The atomic percentage of the C, O and Fe are 66.71, 32.76 and 0.53 for the films deposited from composite targets containing 1 wt.% maghemite and 64.36, 33.92 and 1.72 respectively for the films deposited from composite targets containing 5 wt.% maghemite. In the case of cells cultivated on dextran coated 5% maghemite γ -Fe₂O₃, the number of cells and the level of F-actin were lower compared to the other two types of thin films and control.

Conclusions: The dextran-iron oxide continuous thin films obtained by MAPLE technique from composite targets containing 10 wt.% dextran as well as 1 and 5 wt.% iron oxide nanoparticles synthesized by co-precipitation method presented granular surface morphology. Our data proved a good viability of Hep G2 cells grown on dextran coated maghemite thin films. Also, no changes in cells morphology were noticed under phase contrast microscopy. The data strongly suggest the potential use of iron oxide-dextran nanocomposites as a potential marker for biomedical applications.

Keywords: Iron oxide, Polysaccharides, MAPLE, Thin films, HepG2 cells

Background

Iron oxide nanoparticles and their composites have received increasing attention for their promising biomedical applications [1-7]. The material is highly biocompatible and can be easily conjugated with bioactive molecules. Recently, nanoscale iron oxide nanoparticles have been applied as light scattering labels and luminescent optical markers [1-3] because of their potential applications as contrasting materials for magnetic resonance imaging (MRI) [4-7], in vitro cell separation [8,9], targeted drug delivery [10], hyperthermia [11,12], etc.

Nanophase composite materials exhibit physical and chemical properties which differ considerably from bulk materials. The size effect [13] and the surface chemistry [14] play a major role in the biological applications. To control the surface properties of iron oxide nanoparticles, coating is applied with a biocompatible polymer during or after the synthesis process [15,16]. To overcome any potential risk of toxicity and high-level accumulation in the target tissue or organ, the iron oxide nanoparticles (IONPs) may be subjected to further functionalization using bioactive molecules [17].

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Pulsed Laser Deposition (PLD) is a well know method for laser processing of inorganic materials structures and thin films. This technique is however with few exceptions unsuitable for the immobilization of biomaterials, like polymers, biopolymers and proteins [18,19]. UV laser organic material interactions can lead to irreversible photochemical transformations of the transferred material. For these reasons, the development of other methods was necessary. One of these methods is called Matrix Assisted Pulsed Laser Evaporation (MAPLE). It provides a gentle mechanism to transfer small and large molecular weight species from condensed phase into the vapor phase. In this technique, the organic and/or nanomaterial are diluted in a volatile non-interacting solvent, with concentration of a few percent (in weight), and frozen at liquid nitrogen temperature. The frozen target is irradiated with a pulsed laser beam, whose energy is principally absorbed by the solvent and converted to thermal energy, allowing the solvent to vaporize and to be evacuated by the vacuum system. The solute material collects on a suitable substrate placed in front of target [20-22]. Since the laser energy is absorbed mainly by volatile solvent matrix, the photochemical decomposition of the organic material can be minimized or even eliminated. The evaporation process is defined by thermodynamic parameters of the volatile solvent and not by the organic material. The deposition is conducted at lower energy densities than in the case of conventional PLD, as with additional precaution to avoid thermal decomposition of organic materials.

First investigations have shown that MAPLE technique offers the possibility to deposit complex materials without significant modifications in their chemical structure and their functional properties if laser parameters are adequately selected regarding the wavelength, fluence and pulse duration. The type of solvent substrate temperature and pressure in the reaction chamber are also important.

In this paper, the physico-chemical and biological properties of polymer nanocomposites thin films containing IONPs in a dextran matrix were investigated. To study the structural and morphological state of the films, X-ray analysis and scanning electron microscopy were used. Adsorption of dextran on the surface of IONPs was investigated by X-ray Photoelectron Spectroscopy (XPS), X-ray diffraction (XRD), Scanning Electron Microscope (SEM) coupled with an Energy Dispersive X-ray detector (EDX), Glow Discharge Optical Emission Spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy.

Experimental section Materials

Ferrous chloride tetrahydrate (FeCl₂·4H₂O), ferric chloride hexahidrate (FeCl₃·6H₂O), natrium hydroxide (NaOH), dextran $H(C_6H_{10}O_5)_xOH$, ferric nitrate (FeNO₃), nitric acid (HNO₃) and chlorhidric acid (HCl) where purchased from Merck. Deionized water was used in the synthesis of nanoparticles, and for rinsing of clusters.

Synthesis of iron oxide ferrofluid

IONPs were prepared by co-precipitation [23-26]. Ferrous chloride tetrahydrate (FeCl₂·4H₂O) in 2 M HCl and ferric chloride hexahydratate (FeCl₃·6H₂O) were mixed at 100° C (Fe²⁺/Fe³⁺ = 1/2). The mixture was dropped into 200 ml of NaOH (2 mol·L⁻¹) solution under vigorous stirring for about 30 min. The precipitate of magnetite (black precipitate immediately formed) was converted into γ - Fe₂O₃ particles by repeated treatment with HNO₃ (2 mol·L⁻¹) and FeNO³ (0.3 mol·L⁻¹) solutions [27]. The acidic precipitate was isolated by decantation on a magnet, separated by centrifugation (6000 rpm), then washed in acetone and dispersed in deionized water at pH = 2.5. The final ion concentration was 0.38 mol·L⁻¹. In a final step, the obtained product was mixed at various ratios with different polymer solutions to obtain iron oxide coated with dextran. For biological investigations, the pH was adjusted to 7 using aqueous amonia. The iron content of the suspensions was determined by redox-titration [27].

Immobilisation in form of thin films of dextran and dextran coated maghemite nanoparticles

The UV-MAPLE deposition setup include a vacuum deposition chamber and a UV KrF* excimer laser (Lambda Physics Coherent, COMPexPro 205 model; $\lambda = 248$ nm, $\tau_{FWHM} \approx 25$ ns, $\nu = 10$ Hz) [28]. The laser beam was focused onto the target surface trough a 30 cm FD MgF2 cylindrical lens placed outside the reaction chamber. The incident angle between the laser beam and the target was 45°. Before each deposition the SiO₂ glass substrate was cleaned with ethanol in ultrasonic bath for 10 min and then placed inside the deposition chamber parallel with the target at 4 cm distance. To avoid significant changes in the surface morphology of the target this was rotated during the multipuls laser irradiation with a frequency of 10 Hz.

In our experiments we used solutions consisting of maghemite NPs (0-5 wt. % concentration), dextran (10 wt.% concentration), and distilled water as matrix solvent, prepared by a chemical co-precipitation method.

Before each deposition, 5 ml of the obtained solution were dropped in a cooper holder with 3 cm diameter and 5 mm height and converted into solid (the future MAPLE target) freezing the solution in liquid nitrogen (77 K). The cryogenic process is induced by immersing the target in liquid nitrogen and/or maintained in direct contact with a device connected through cooper pipes at a liquid nitrogen reservoir. In this way, the rapid vaporization of MAPLE target inside the reaction chamber is greatly slowed down.

The irradiation chamber was evacuated down to a residual pressure of 13 Pa. We applied 25×10^3 subsequent laser pulses to deposit each film. The incident laser fluence on the target surface was 0.5 J/cm² for each structure.

Characterization methods

X-ray photoelectron spectroscopy (XPS)

The spectra were measured on a VG ESCA 3 MK II XPS installation using monocromatic Al K α irradiation (1486.7 eV). The vacuum in the analyzer chamber was p ~ 3 × 10-8 torr. The X-rays are emitted by an anti-cathode of Al, U = 12.5 kV, filament emission current I = 20 mA, flood gun: 2 V, electron current I = 0.3 mA, voltage on electron multiplier U = 2.8 kV. The XPS recorded spectrum involved an energy window w = 20 eV with the resolution R = 50 eV, and with 256 recording channels. The XPS recorded spectra were processed using Spectral Data Processor v2.3 (SDP) software.

X-ray diffraction (XRD)

The samples were characterized for phase content by X-ray diffraction (XRD) with a Bruker D8-Advance X-ray diffractometer in the scanning range 10-60° using CuK_{α 1} (1.5416 Å) incident radiation.

Scanning electron microscope (SEM) coupled with an energy dispersive X-ray detector (EDX) and glow discharge optical emission spectroscopy

The morphology of the material was studied using a HITACHI S2600N-type scanning electron microscope (SEM), operating at 25 kV in vacuum on powder samples. The elemental local analysis was performed using an energy dispersive spectroscopy (EDS) detector from EDAX. Operating conditions were an accelerating voltage between 2 up 25 kEv (depending of the ratio signal/noise) with samples tilted at 25° to get the optimal take off angle (30°) allowing a dead time around 20-30% and a collecting time of 90-120 s. The nature of the sample avoids a conductive thin film deposition previously.

The top surface analysis of the samples was studied by the Glow Discharge Optical Emission Spectroscopy (GDOES) using the GD5000 from Horiba/Jobin-Yvon. The technique is dedicated for thin film analysis and helps in determining the chemical gradient composition from the surface to the bulk and -if the ablation rate can be estimated - to precise the thickness of the different layers of the nanocomposite materials [29].

Fourier transform infrared (FTIR) spectroscopy

The functional groups present in the prepared nanoparticles and thin films were identified by FTIR using a Spectrum BX spectrometer. To obtain the nanoparticles spectra 1% of nano-powder was mixed and ground with 99% KBr. Tablets of 10 mm diameter were prepared by pressing the powder mixture at a load of 5 tons for 2 min. The spectrum was taken in the range of 500 to 4000 cm⁻¹ with 4 cm⁻¹ resolution. All the second derivative IR spectra were obtained after 49 -point smoothing of the original IR spectra at room temperature.

Cell culture and treatment

Human liver hepatocellular carcinoma, Hep G2 cells were maintained in minimal essential medium (MEM) containing 3,7 g/L sodium bicarbonate, 4,5 g/L D-glucose, 4,7 g/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 4 mM L-glutamine, 0,1 mM sodium pyruvate, 100 U/ml penicillin, 100 U/ml streptomycin and 10% (v/v) foetal bovine serum. Cells were grown in 5% CO₂ at 37°C, as monolayers in 75 cm² cell culture flasks. Then, HepG2 cells were seeded in six well plates, at a density of 5×10^5 cells/ml and incubated on previously UV sterilized thin films of dextran nanoparticles as well as dextran coated maghemite γ -Fe₂O₃ nanoparticles obtained from composite targets containing 1% (B) and 5% (C) maghemite γ -Fe₂O₃.

Analysis of the actin cytoskeleton

The Hep G2 cells were incubated on the three types of thin films for 24 and 72 h. After the removal of the cell medium, the cells were washed by 70% cold methanol for 15 minutes on shaking. After three washes with phosphate buffer saline, 500 µl/mL of 20 µg/mL phaloidin coupled with FITC (fluorescein -isothiocyanate) in 1.2% bovine serum albumin on each thin film were added. After 1.5 hours of incubation in dark, this reagent was removed and three washes with phosphate buffer saline (five minutes each) were done. Then 500 µL of 10 µg/ml DAPI (4'-6-Diamino-2-phenylindole) were added for 15 minutes in order to stain the nuclei. The cells were analysed using Olympus IX71 microscope with an excitation wavelength of 495 nm and an emission of 513 nm for FITC and 358 nm respectively 461 nm for DAPI.

Results and discussions

Few studies were reported on the formation of dextran coated iron oxide thin films using MAPLE technique.

The XRD peaks of iron oxide nanoparticles (Figure 1A) and dextran coated iron oxide nanoparticles thin films (Figure 1B and 1C) can be indexed into the spinel cubic lattice type (ICSD card no.01-083-0112) with a lattice parameter of 8.36 Å. Diffraction peaks at (111), (220), (311), (400), (422), (511) and (440) are readily recognized from the XRD pattern in the iron oxide powders. The refinement of XRD spectra indicated that no other phases except the maghemite are detectable. The XRD showed a finite broadening of the diffraction lines. The particle sized calculated using the Scherer



[30] formula was estimated at around 7.7 nm. The DRX analyses (Figure 1(B-C)) show that the structure of the thin solid films of dextran coated maghemite nanoparticles obtained from composite targets containing 1 wt.% (B) and 5 wt.% (C) maghemite γ -Fe₂O₃ are monophase maghemite γ -Fe₂O₃ with different particle size and different degree of crystallinity.

X-ray photoelectron spectroscopy (Figure 2) and X-ray diffraction (Figure 1) results are consistent with the expected composition of dextran coated maghemite γ -Fe₂O₃thin films. In the XPS general spectra of dextran thin film Figure 2(A) the binding energy of O (1 s, 532.55 eV) and C (1 s, 286.43 eV) were found. The XPS shows that the binding energy of the Fe $2p_{3/2}$ of two thin films of dextran coated iron oxide (Figure 2(B-C)) is consistent with Fe³⁺ oxides.

In addition existence of doublet spin orbit component corresponding to Fe $2p_{3/2}$ and Fe $2p_{1/2}$ is presented in the spectra of the thin solid films of dextran coated



maghemite nanoparticles obtained from composite targets containing 1 wt.% (Figure 3A) and 5 wt.% (Figure 3B). The Fe $2p_{3/2}$ peak was found to have binding energy between energy 711.2 eV and 711.6 eV and the Fe $2p_{1/2}$ peak from 725.2 to 725.8 eV. The results indicate that iron was completely in the Fe³⁺ state. This result is also supported by the XRD results which indicate that γ -Fe₂O₃ phase contains only Fe³⁺ cations.

The broadened shape of the O 1 s spectra (Figure 4) suggests that the oxygen is present in at least two states.



Figure 4 showed a typical O 1 spectrum fitted with two Gaussian components. The binding energy separation is ~ 2.31 eV in good accord with literature [31]. The main peak is located at 530.58 eV. This component corresponds to O^{2^-} in the iron oxide lattice [32,33]. The second component is represented by a peak located at 532.89 eV and could be attributed to OH⁻ [34-38] and physisorbed H₂O, respectively. On the other hand, the O 1 s photoelectrons in both thin films of dextran coated maghemite are due to dextran (C-O-C [or H]) contributions [39].

The major components of the C 1 s at 286,3 eV (Figure 4) in the spectra of thin films of dextran coated

maghemite γ -Fe₂O₃ are due primarily to the dextran CHOH groups. A smaler peak at ~ 288 eV is assigned to the anomeric carbon of dextran. The components of the Si 2p in all spectra are due to the substrate contributions.

The GDOES Spectra respectively performed on thin films of dextran coated maghemite nanoparticles are obtained from composite targets containing 1 wt.% (A) and 5 wt.% (B) maghemite γ -Fe₂O₃ deposited on a silica glass substrate. The two spectra (Figure 5A and 5B) reveal the presence of a material composed mainly of carbon, iron and oxygen. Top surface (before 10s of



analytical time) corresponds to a contaminated layer (carbon oxygen mainly) frequently observed in as received material [29]. Between 10s and 180 s (Figure 5A) or 140 s (Figure 5B), carbon, iron and oxygen signals increased to reach a maximum before decreasing. This zone corresponds to the nanocomposite of dextran spiked with maghemite γ -Fe₂O₃. After 180 s or 140 s, the signal of silicon increases corresponding to the glass

substrate. Thus the signal observed between 10 s and respectively 180 s for A and 140 s for B, exhibit the same pattern differing only by the intensity measured. In the case of the 1% sample (A) the iron signal is lower than carbon which is opposite with the sample doped by 5% of iron (B). The carbon signal corresponds to the dextran while iron is related to the maghemite. However, oxygen is included in both phases. Thus the



sequences observed on the Figure 5 tends to reveal that maghemite is embedded by the dextran regardless the concentration. Average rate of ablation was around 2s/ min. By measuring the depth of the GD crater using profilometry, the thin film thickness was estimated about 280 nm (sample B) and 360 nm (sample A).

In Figure 6A, B and 6C are showed SEM micrographs (magnification 10000) of surface morphology for thin films of dextran (A) and thin films of dextran coated maghemite nanoparticles obtained from composite targets containing 1 wt.% (B) and 5 wt.% (C) maghemite. SEM images provide the direct information about the size and typical shape of the as prepared samples. The SEM observations for the thin film of maghemite-dextran showed that the samples consist of regular grains with some aggregation. Additionally, the grain size increased when the concentration of the maghemite NPs increased from 1 wt.% (Figure 6(B)) to 5 wt.% (Figure 6(C)). Surface SEM observation of thin films investigated show a granular structure with grain having an average particles dimensions ranging in the 40-150 nm for composite targets containing 1 wt.% maghemite and 60-200 nm for composite targets containing 5 wt.% maghemite. EDAX (Figure 7) images of thin films of dextran and thin films of dextran coated maghemite nanoparticles obtained from composite targets containing 1 wt.% (B) and 5 wt.% (C) maghemite are shown. Elemental maps (Figure 7) of C, O and Fe for the samples prepared from composite targets containing 1 wt.% (B) and 5 wt.% (C) γ -Fe₂O₃ are also shown. X-EDS spectra from the specimen confirm the composition of thin films samples. The carbon (C), oxygen (O) and iron (Fe) contents of the films were measured by X-EDS. The atomic percentage of the C, O and Fe are 66.71, 32.76 and 0.53 for the films deposited from composite targets containing 1 wt.% maghemite and 64.36, 33.92 and 1.72 respectively for the films deposited from composite targets containing 5 wt.% maghemite.

FT-IR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures. IR spectrum of all thin films shows lot of structural information of major constituents. As shown in Figure 8 in the 3600-1600 cm⁻¹ region four bands appear: a broad band centered at 3400 cm⁻¹ and 1700 cm⁻¹ assigned to the OH stretching (vOH) and HOH (δ OH) vibrational bands due to the adsorbed water molecules in the sample [40], the weak signal at 2927 cm⁻¹ [41-43]. The band at 1434 cm⁻¹ may by due to C-OH deformation vibration with contributions of O-C-O symetric stretching vibration of carboxylate group [44,45]. The stronger peaks appear in the range of 1150-900 cm⁻¹ mainly attributed to the stretching vibration of C-O-C [46]. Those characters of peaks intensities and positions at 1150, 1020, 912 and 731 in



Figure 6 SEM micrographs of dextran thin films (A) and thin films of dextran coated maghemite nanoparticles obtained from composite targets containing 1 wt.% (B) and 5 wt.% (C) maghemite.



the IR spectrum display the characteristic absorptions of polysaccharides. The bands observed in the 650-550 cm⁻¹ corresponds to the Fe-O vibrations modes of γ -Fe₂O₃ [47-52]. The FT-IR spectra and the second derivative spectra gives more information than classical IR for polysaccharides and other biomolecules contained in different organism such as red seaweeds, fungi and bacteria [53-57]. Furthermore, in the second derivative spectrum the bands observed are assigned to polysaccharides.

Our previous data proved a good viability of Hep G2 cells grown on dextran powder [58] as well as dextran coated maghemite thin films [59]. Also, no changes in

cells morphology were noticed under phase contrast microscopy [60]. In this study, we were interested in the expression of F-actin in Hep G2 cells adhered to dextran nanoparticles and dextran coated maghemite γ -Fe₂O₃ nanoparticles obtained from composite targets containing 1 wt.% and 5 wt.% maghemite γ -Fe₂O₃ thin films. Actins are highly conserved proteins that are ubiquitously expressed in all eukaryotic cells. F-actin microfilaments are essential for the maintenance of cell shape and permeability of tight junctions [61]. Figure 9 shows that, after 24 hours of cultivation, the expression of Factin in Hep G2 cells adhered on dextran nanoparticles









representative for five independent experiments.

thin films (B) and dextran coated 1% maghemite γ -Fe₂O₃ (C) was similar and less than in control cells.

In the case of cells cultivated on dextran coated 5% maghemite γ -Fe₂O₃ (D1), the number of cells and the level of F-actin were lower compared to the other two types of thin films and control. After 24 hours, the F-actin distribution in the cells from the thin films was

especially around the nuclei. Later on, at 72 hours (Figure 10) after the cells cultivation there was no significant difference between the three experimental alternatives from the point of view of cell numbers and F-actin expression. It might be worthwhile to mention that F-actin is spread until the periphery of the hepatocytes, which is in accordance with other scientific results [60,61].

Conclusions

The dextran-iron oxide continuous thin films obtained by MAPLE technique from composite targets containing 10 wt.% dextran as well as 1 and 5 wt.% iron oxide nanoparticles synthesized by co-precipitation method presented granular surface morphology. This represented an advantage in the adhesion and growth of living HepG2 cells. Our results proved that Hep G2 cells adhered very well to thin films of dextran (coated with 1% and 5% maghemite) and exhibited a normal actin cytoskeleton, which suggest that these cells underwent normal cell cycle progression. As a result, hepatocytes adhered to these thin films could be used as biosenzors for different xenobiotics.

Acknowledgements

This study was financially supported by Grant POSDRU 88/1.5/S/61150/2010 co-financed from European Social Fund by the Sectorial Operational Program for Development of Human Resources 2007-2010.

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Authors' contributions

KL, MC, and AD conceived the experiments. SLI synthesised the iron oxide ferofluid. CSC performed FT-IR and XPS studies. PLC performed structural studies. MR reported all biological experiments. DP wrote the manuscript. All the authors read and approved the manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 8 November 2011 Accepted: 13 March 2012 Published: 13 March 2012

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doi:10.1186/1752-153X-6-17

Cite this article as: Ciobanu *et al.*: **Biomedical properties and preparation of iron oxide-dextran nanostructures by MAPLE technique.** *Chemistry Central Journal* 2012 **6**:17.

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