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Electrospun composite cellulose acetate/iron oxide nanoparticles non-woven membranes for magnetic hyperthermia applications

Ricardo J.R. Matos[a], Catarina I.P. Chaparro[a], Jorge C. Silva[b], Manuel Almeida Valente[c], João Paulo Borges[a,*], Paula I.P. Soares[a,*]

[a] CENIMAT/i3N, Departamento de Ciência dos Materiais, Faculdade de Ciências e Tecnologia, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[b] CENIMAT/i3N, Departamento de Física, Faculdade de Ciências e Tecnologia, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[c] Physics Department (i3N), University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

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ABSTRACT

In the present work composite membranes were produced by combining magnetic nanoparticles (NPs) with cellulose acetate (CA) membranes for magnetic hyperthermia applications. The non-woven CA membranes were produced by electrospinning technique, and magnetic NPs were incorporated by adsorption at fibers surface or by addition to the electrospinning solution. Therefore, different designs of composite membranes were obtained. Superparamagnetic NPs synthesized by chemical precipitation were stabilized either with oleic acid (OA) or dimercaptosuccinic acid (DMSA) to obtain stable suspensions at physiological pH. The incorporation of magnetic NP into CA matrix was confirmed by scanning and transmission electron microscopy. The results showed that adsorption of magnetic NPs at fibers' surface originates composite membranes with higher heating ability than those produced by incorporation of magnetic NPs inside the fibers. However, adsorption of magnetic NPs at fibers' surface can cause cytotoxicity depending on the NPs concentration. Tensile tests demonstrated a reinforcement effect caused by the incorporation of magnetic NPs in the non-woven membrane.

1. Introduction

Cancer is a major public health problem worldwide causing nearly 1 in 6 deaths, from which 70% occur in low- or middle-income countries. Chemotherapy has a limited effectiveness, poor distribution and lack of selectivity. Alternatively, magnetic hyperthermia is clinically used as a treatment for various human solid cancers (Assa et al., 2017; Shi, Kantoff, Wooster, & Farokhzad, 2017; Yan et al., 2018). Cancer cells are more sensitive to temperature oscillations than normal cells due to reduced blood flow in the tumor site. Therefore, temperatures around 42–43 °C can cause significant tumor cell death without harming healthy tissues (Kobayashi, 2011; Majewski & Thierry, 2007). The use of magnetic NPs could be an innovative solution to perform magnetic hyperthermia. Magnetic NPs can function at cellular and molecular level of biological interactions and can be manipulated using a magnetic field (Yadollahpour & Hosseini, 2016; Zhong et al., 2015). The application of free magnetic NPs has several limitations due to low solubility, poor cancer targeting, and leakage of NPs from the tumor location. For that reason, a strategy currently being researched involves NPs-loaded electrospun nanofibers for localized magnetic hyperthermia, which is the main purpose of this work (Lee et al., 2015). Magnetite nanoparticles (Fe_3O_4) have attracted broad interest for cancer treatment using magnetic hyperthermia because they are non-toxic and biocompatible (Darwish, Nguyen, Sevcu, Stibor, & Smoukov, 2016). In general, iron oxide nanoparticles (IONPs) become superparamagnetic at room temperature when their size is below about 15 nm, and heat generation is a result of a combination of internal Néel fluctuations of the particle magnetic moment and the external Brownian fluctuations (Wu, Wu, Yu, Jiang, & Kim, 2015). However, aggregation among superparamagnetic IONPs is a common phenomenon (Mahdavi et al., 2013). Hence, for protecting bare IONPs against aggregation, coating agents such as OA and DMSA can be used, without significantly affect their magnetic properties (Soares et al., 2014, 2015).

Electrospinning is a simple, fast and easy to scale-up technique to produce functional materials based on polymeric micro/nanofibers with high surface-to-volume ratio and tunable porosity (Baptista et al., 2011; Baptista, Soares, Ferreira, & Borges, 2013). Electrospinning can be used to produce stimuli-responsive fibers that actively react to changes in the surrounding environment, which are translated into significant changes in their morphological and chemical properties (Almeida, Amaral, & Lobão, 2012). Combining electrospun non-woven membranes with magnetic NPs produces a flexible composite system

* Corresponding authors.
E-mail addresses: jpb@fct.unl.pt (J.P. Borges), pi.soares@fct.unl.pt (P.I.P. Soares).

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with fixed NPs suitable for magnetic hyperthermia applications. In addition, this combination can improve cell adhesion, proliferation, and differentiation when a magnetic field is applied (Rao, Kumar, & Han, 2018). The composite membranes produced in this work are composed by an electrospun matrix of cellulose acetate and functionalized with magnetite NPs. The NPs were conjugated to the polymeric matrix either through adsorption, or by incorporation in the electrospinning solution. Some authors have explored the use of electrospinning to produce controlled drug delivery systems (Aguilar, GhavamiNejad, Park, & Kim, 2017; Qiu et al., 2013; Thakkar & Misra, 2017), while a few studies encapsulated IONPs into polymeric nanofibers for magnetic hyperthermia application (Lin, Lin, & Lin, 2012; Lin, Lin, & Lin, 2013; Zhong et al., 2015). This combination will cause a synergistic effect, thus producing a highly efficient cancer treatment option for easy to access tumors or post-operation treatments, with smaller drug dose and a significant reduction of side effects.

2. Experimental section

All the chemical reagents used in this research work were of analytical grade and used without further purification.

2.1. Synthesis of iron oxide nanoparticles

Iron oxide nanoparticles were produced by a chemical co-precipitation technique using a previously described method (Soares, Machado et al., 2016; Soares et al., 2014, 2015). IONPs without further treatment will be named "Bare-Fe_3O_4 NPs"; the ones stabilized with either DMSA (*Sigma- Aldrich*) 4% or OA (*Fisher Chemical*) 64 mM will be named "DMSA-Fe_3O_4 NPs" and "OA-Fe_3O_4 NPs", respectively.

IONPs stabilization with OA was performed by adding to the NPs suspension an appropriate amount of OA calculated as a percentage of the NPs mass. The mixture reacted for 1 h in an ultrasound bath. In the case of coating with DMSA, the suspension pH was previously adjusted to pH 3 with nitric acid 65% under magnetic stirring. DMSA was added immediately at a 3–4 ratio of [DMSA]/[Fe^{2+}]. DMSA was dissolved in 2 mL of deionized water and the pH adjusted to 5.5 with 0.1 M NaOH solution under vigorous stirring (Fauconnier, Pons, Roger, & Bee, 1997; Massart, 1980). The reaction of DMSA with IONPs was performed in an ultrasound bath during 1 h. In both cases, a part of the suspension was freeze-dried (VaCo2, *Zirbus*) to obtain dry NPs for further characterization.

2.2. Production of CA/Fe_3O_4 composite

To produce CA/Fe_3O_4 composites, two strategies were used: i) Immersion of CA membrane into IONPs solution using different concentrations – Adsorption; ii) electrospinning a solution containing CA and IONPs – Incorporation.

2.2.1. Adsorption of IONPs in CA membranes

A 12 wt% CA solution in 2:1 (w/w) acetone (*Fisher chemical*)/*N,N*-dimethylacetamide (DMAc, *Sigma-Aldrich*) was prepared and used for electrospinning. Electrospinning was performed at 20 °C with humidity around 40%. The polymer solution was placed in a 1 mL syringe, and dispensed by a syringe pump (*KDS100*, *KD Scientific*) at a rate of 0.15 mL h^{-1}. A high voltage supply (*Glassman EL* 30 kV) was connected to the metallic needle (ITEC 21G) with a fixed voltage of 20 kV, and a sheet of aluminum foil was placed 17 cm in front of the tip of the needle to collect the fibers. The electrospun fiber mats were dried at room temperature for 24 h to remove any residual solvent. These parameters were based on the literature (Baptista et al., 2011; Tarus, Fadel, Al-Oufy, & El-Messiry, 2016). However, other combinations of parameters were tested. Table 1 presents the electrospinning parameters tested that resulted in fibers without defects.

To produce CA/Fe_3O_4 composites by adsorption, the CA solution was electrospun during 6 h. CA membranes were cut into 2.5 × 1.5 cm pieces and placed in IONPs solution during 24 h in an orbital shaker. The adsorption method was tested using two concentrations of IONPs, 0.5 and 2.0 mg mL^{-1}, either of DMSA-Fe_3O_4 NPs or OA-Fe_3O_4 NPs. The membranes were washed with ultrapure water to remove the non-adsorbed NPs and were dried at room temperature.

2.2.2. Incorporation of IONPs in CA nanofibers

In this case, a new solvent combination for CA was used: 63:32:5 (w/w/w) acetone/DMAc/ IONPs solution ([OA-Fe_3O_4 NPs] = 6.34 mg mL^{-1}; [DMSA-Fe_3O_4 NPs] = 4.01 mg mL^{-1}). The addition of IONPs to CA solution affected its viscosity, and consequently the optimal conditions for electrospinning also change, as shown in Table 2.

2.3. Characterization

The iron content of the samples was determined using the 1,10-phenantroline colorimetric method (Talelli et al., 2009). To obtain NPs concentration the formula [Fe] = 0.7 × [NPs] was used, obtained from control experiments.

Transmission electron microscopy (TEM) images were obtained using a Hitachi H-8100 II with thermo-ionic emission LaB_6 with a resolution of 2.7 Å. TEM analysis was performed in NPs diluted in pure water placed in a Kevlar 25 mesh grid. The membranes analysis was performed on the thinnest membrane that can be technically extracted.

The crystalline phases of the samples were verified using powder X-ray diffraction (XRD). X'Pert PRO PANAlytical X-ray diffractometer was used to obtain X-ray diffraction patterns of the iron oxide nanoparticles previously freeze-dried. The 2θ values were taken from 15° to 80° using a Cu-Kα radiation (λ = 1.54060 Å) with a step size of 0.033. The Scherrer's equation was used to measure the average crystallite size.

FTIR spectra of the samples were obtained using a Nicolet 6700–Thermo Electron Corporation Attenuated Total Reflectance-Fourier Transform Infrared spectrometer (ATR-FTIR). Measurements were performed in freeze-dried samples in the range of 480–4000 cm^{-1} with a resolution of 2 cm^{-1}.

Dynamic Light Scattering (DLS) measurements were performed using a SZ-100 nanopartica series (Horiba, Lda) with a 532 nm laser and a Peltier temperature control system (25 °C). DLS measurements were carried out for diluted NPs suspensions (five samples for each concentration) using a disposable cell with a scattering angle equal to 90°. Data analysis was performed using cumulants statistics to measure hydrodynamic diameter (D_H) and polydispersity index (PDI) (Soares, Laia et al., 2016).

Scanning electron microscopy (SEM) that allowed fiber morphology analysis was performed using a Carl Zeiss Auriga SEM equipment. The samples were coated with a thin layer of gold.

The magnetic properties of bare-Fe_3O_4 NPs and OA-Fe_3O_4 NPs were performed in previous studies (Soares, Laia et al., 2016) using a 7 T SQUID magnetometer (S700X; Cryogenic Ltd.). The magnetization curves were obtained at 5 K and 320 K, with a variation of the applied field of −5 T up to 5 T. The magnetic properties of DMSA-Fe_3O_4 NPs was performed through Vibrating Sample Magnetometer (VSM) technique using a 10 T VSM magnetometer (Cryogenic-Cryofree). The magnetization curve was obtained at 300 K, with a variation of the applied field of −2 T up to 2 T.

2.4. Mechanical response – tensile tests

The tensile tests were performed on a Rheometric Scientific uniaxial tensile testing machine with 20 N load cell and operable through the Minimat software (Minimat Control Software Version 1.60 February 1994 (c) PL Thermal Science 1984-94 Reometric Scientific Ltd.). A velocity of 1 mm min^{-1} was applied in all experiments, which were performed at room temperature and humidity around 50%. For each