Journal of Colloid and Interface Science 476 (2016) 79–86

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Regular Article

Processing and size range separation of pristine and magnetic poly(L-lactic acid) based microspheres for biomedical applications

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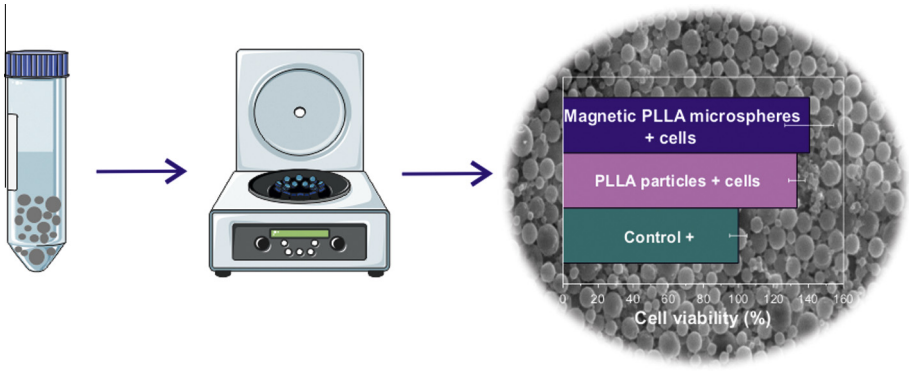
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 March 2016
Revised 5 May 2016
Accepted 11 May 2016
Available online 11 May 2016

Keywords:
Biocompatibility
Poly(L-lactic acid) microspheres
Oil in water emulsion technique
Cell viability
Magnetic nanocomposites

ABSTRACT

Biodegradable poly(L-lactic acid) (PLLA) and PLLA/$CoFe_2O_4$ magnetic microspheres with average sizes ranging between 0.16–3.9 μm and 0.8–2.2 μm, respectively, were obtained by an oil-in-water emulsion method using poly(vinyl alcohol) (PVA) solution as the emulsifier agent. The separation of the microspheres in different size ranges was then performed by centrifugation and the colloidal stability assessed at different pH values. Neat PLLA spheres are more stable in alkaline environments when compared to magnetic microspheres, both types being stable for pHs higher than 4, resulting in a colloidal suspension. On the other hand, in acidic environments the microspheres tend to form aggregates. The neat PLLA microspheres show a degree of crystallinity of 40% whereas the composite ones are nearly amorphous (17%). Finally, the biocompatibility was assessed by cell viability studies with MC3T3-E1 pre-osteoblast cells.

1. Introduction

Smart polymer microspheres have received increasing attention for biomedical applications such as drug delivery systems and support for cell expansion and differentiation [1,2].

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http://dx.doi.org/10.1016/j.jcis.2016.05.012
0021-9797/

In particular, the production of piezoelectric microspheres has been already achieved [2]. Piezoelectric polymers can be used as a bioactive electromechanically responsive materials for improving tissue engineering strategies [3]. Studies reveal that electrical stimulation influences cell proliferation, differentiation and regeneration [2] both under static [4,5] and dynamic conditions [6]. These results show the potential of such materials for the development of a new generation of wireless electrically active scaffolds and structures for biomedical applications [2].

Magnetic nanoparticles and, in particular magnetostrictive ones, offer also strong application potential in the biomedical field, including drug delivery and biomolecular targeting, among others, triggered by external magnetic field [7]. These applications are based on the large surface area of the nanoparticles, good tissue diffusion and reduced dipole-dipole interaction [8]. When combined with the piezoelectric effect, magnetostrictive nanoparticles offer the possibility of developing magnetoelectric composites [9] that convert magnetic stimuli into electrical ones, leading to novel tissue engineering strategies [6,10]. In particular, cobalt ferrite magnetic particles have been explored for biomedical applications due to its large magnetostriction, high Curie temperature and effective anisotropy and moderate saturation magnetization, which can be taken to advantage both for tissue engineering and drug delivery applications [11]. However, they should be coated with organic or inorganic materials in order to ensure their biocompatibility, nontoxicity and colloidal stability [8].

One of the most used polymers for biomedical applications is poly(L-lactic acid) (PLLA), which is a biocompatible and biodegradable aliphatic synthetic polyester. PLLA is also piezoelectric and its electroactive properties have been successfully explored for technological applications, being the β-form the main responsible for the piezoelectric properties [12,13].

PLLA microspheres can be produced by several physicochemical methods, including solvent extraction/evaporation from an emulsion, aggregation by pH adjustment or heat, coacervation (phase separation), interfacial polymerization, ionic gelation, electrospray and spray drying, among other techniques [2,14–16]. Emulsion-solvent evaporation method is perhaps the most commonly used one [17]. The main advantages of the emulsion-solvent extraction/evaporation method (oil in water (O/W) or water in oil (W/O) emulsions) is its simple implementation using a stirrer device, without high temperatures or phase separating agents. Further, it allows the control of the size of the spheres in the nanometer to micrometer range comparatively to other methods such as the electrospray method [18], which does not allow such a control of sphere size. It is to notice that the sphere size is important for biomedical applications such as tissue engineering and drug delivery, in the later the microspheres size influencing the release rate, due to the encapsulation efficiency [19].

In this process, a solution containing the polymer is emulsified in a non-solvent continuous phase, in the presence of a stabilizing agent, using a variety of physical methods such as homogenization and sonication [20]. PLLA microspheres of relatively small size and uniform size distribution can be obtained from an aqueous continuous phase containing poly(vinyl alcohol) (PVA) as stabilizer [20,21].

The potential for application of neat or composite PLLA microspheres has been demonstrated in the treatment of ocular pathologies, namely in regenerative medicine for retinal repair or related intraocular pathologies [14] and drug delivery [7,22–24].

Despite these interesting proofs of concept, a systematic study reporting the effect of the processing parameters on the overall microspheres physico-chemical properties as well as on the encapsulation efficiency and biocompatibility of composite spheres with magnetic nanoparticles is still lacking. In particular, the introduction of highly magnetostrictive nanoparticles, such as $CoFe_2O_4$, has not been reported. Thus, this work, reports on an effective method for the preparation of pristine and magnetic PLLA microspheres based on $CoFe_2O_4$ nanoparticles.

2. Experimental section

2.1. Materials

Poly(L-lactic acid) (PLLA) with an average molecular weight of 217.000–225.000 g mol^{-1}, (Purasorb PL18) and poly(vinyl alcohol) (PVA) with an average molecular weight of 13.000–23.000, 98% hydrolyzed were supplied by Purac and Sigma Aldrich. Chloroform (CF, from *Merck*) was used for the dissolution of the polymer. $CoFe_2O_4$ nanoparticles were purchased from Nanoamor [25] with dimensions between 35 and 55 nm, and used as received.

2.2. Preparation of neat and magnetic PLLA microspheres

PLLA was dissolved in chloroform (CF) to achieve a polymer concentration of 3% (w/v). CF is commonly used to dissolve PLLA due to its ability to solubilize large amounts of polymer [26], and low miscibility with water, leading to a decrease of the amount of PVA adsorbed to the polymer-organic solvent–water interface [21].

The polymer solution was dissolved at room temperature under constant stirring until complete polymer dissolution. Then, the mixture was added into 0.5% (w/v) PVA solution. Polymer and surfactant concentrations were selected based on previous works [27]. The emulsified suspension was mechanically stirred (RS lab) and in the same time the solvent was continuously evaporated overnight at room temperature. The resulting microspheres were washed with distilled water and isolated by centrifugations at either 1000, 2500 and 4000 rcf's, for 5, 10 and 15 min, respectively. This procedure was repeated 5 times. Finally, polymer microspheres were freeze dried (Christ Alpha 2-4 LD Plus from BioBlock Scientific) for 48 h. Magnetic microspheres were also prepared by the aforementioned method. In this case, after complete polymer dissolution, 10% (w/w) of $CoFe_2O_4$ nanoparticles were added to the solution. The next steps were the same as used for neat microspheres preparation.

2.3. Characterization of the PLLA microspheres

The morphology of the microspheres was analyzed using a scanning electron microscope (SEM, Quanta 650, from FEI) with an accelerating voltage of 5 kV. The samples were previously coated with a thin gold layer using a sputter coating (Polaron, model SC502). The average diameter and distribution was calculated over approximately 60 microspheres using SEM images with 50,000× magnification and the *ImageJ* software.

Infrared measurements (FTIR) were performed at room temperature in a Jasco 4100 apparatus in attenuated total reflectance (ATR) mode from 4000 to 400 cm^{-1} using 64 scans with a resolution of 4 cm^{-1}. Differential scanning calorimetry measurements (DSC) were performed in a Mettler Toledo 823e apparatus using a heating rate of 10 °C min^{-1} under nitrogen purge. The degree of crystallinity of the PLLA microspheres was calculated by Eq. (1):

$$\Delta X_c = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \quad (1)$$

where ΔH_m is the melting enthalpy, ΔH_{cc} the enthalpy of cold crystallization and ΔH_m^0 represents the PLLA theoretical value of the melting enthalpy of a fully crystalline sample (93.1 J g^{-1}) [28,29].

Dynamic light scattering (DLS) was used to obtain the average hydrodynamic size and size distribution of the microspheres. A Zetasizer NANO ZS-ZEN3600 (Malvern) was used and measurements were performed at 25 °C using the appropriated sample dilution in ultrapure water to prevent multi scattering