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Self-assembled magnetic liposomes from electrospun fibers

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ABSTRACT

A new and facile route for preparing magnetic liposomes from electrospun fibers comprising the hydrophilic polymer polyvinylpyrrolidone K90, phosphatidyl choline and Fe_3O_4 nanoparticles is reported. The composite fibers were found to contain Fe_3O_4 particles dispersed relatively uniformly. Magnetic liposomes formed spontaneously *via* molecular self-assembly when the composite fibers were added to water. The liposomes were found to be generally well dispersed, and stable to degradation. Their size can be controlled by varying the Fe_3O_4 content of the fibers. The magnetic properties of the nanoparticles are maintained through the electrospinning and self-assembly processes, indicating that these materials maybe have very considerable potential in the development of magnetic drug delivery systems and other therapeutic or theranostic applications.

1. Introduction

Over the last two decades, magnetic iron oxide nanoparticles have been increasingly explored for biomedical applications, for instance in targeted drug delivery [1–3], gene delivery [4], magnetic resonance imaging [5–8], magnetic hyperthermia for cancer treatment [9–12], enzyme immobilization [13–15], and the magnetic separations of biological entities [16–18]. A wide variety of substances can be used as coatings to generate more stable particles: polymers (for example dextran [19,20]), proteins [21] and amphiphilic molecules such as fatty acids [22] or phospholipids [23] have all been exploited to this end.

Liposomes are artificially prepared vesicles consisting of lipid bilayers, and comprise one of the safest and most versatile transfer vectors developed to date. They are suitable for use in the medical, pharmaceutical, food, textile, and cosmetic fields owing to their biodegradability, low toxicity and amphiphilic nature [24–29]. Liposomes are highly compatible with biological membranes, being similar in both composition and structure, and their utility as drug delivery systems is well established [30–33].

Much work has been done to prepare magnetic liposomes via a range of routes [34–41]. Liposomes with superparamagnetic iron oxide nanoparticles embedded in their membranes have been prepared by the supercritical carbon dioxide ($scCO_2$) method [35]. Huang et al. [36] have reported the preparation of "dextran-magnetic layered double hydroxide-fluorouracil" liposomes by the reverse evaporation method. The dried-rehydrated vesicle technique (followed by extrusion) was used for the construction of hydrophilic iron oxide encapsulating magnetoliposomes by Skouras et al. [37]. In addition, 5-fluorouracil-loaded magnetoliposomes can be obtained following the thin film hydration method [38], and the layer by layer approach [39] has also found utility.

However, the routes employed in previous studies are generally time-consuming and complex: for instance, the procedure reported by De Cuyper [40,41] requires surfactants such as lauric acid to protect the particles from aggregation. This is a common problem with self-assembly processes, in which it is frequently challenging to manipulate the molecular building blocks in the desired manner. One route which can be used to direct self-assembly is to first encapsulate the building blocks into polymer-based carriers using electrohydrodynamic atomization (EHDA; electrospinning or electrospraying).

Electrospinning is a simple and effective one-step method for the fabrication of ultrathin nanofibers ranging from a few nanometers to several microns in diameter [42,43]. The polymer matrix appears to confine the species being assembled into

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localized regions, helping to control their transport and contact via secondary interactions such as hydrophobic and hydrogen bonding interactions. This results in tightly controlled by molecular self-assembly [44–47]. A variety of polymer-based composite microparticles have been prepared using EHDA [45–47] and subsequently used to drive self-assembly.

In this work, we have extended these studies to create a facile, novel, and reliable route to the production of magnetic liposomes *via* electrospun fibers. The electrospinning technology is generally easy to implement, and thus our work offers enhanced convenience over the currently used technologies. The properties of the magnetic liposomes prepared have been studied, and the liposomes found to be stable in physiological conditions. The results reported herein have the potential to lead to future applications in various fields, including drug delivery or the treatment of tumors.

2. Materials and methods

2.1. Materials

Polyvinylpyrrolidone (PVP) K90 (M_w = 360,000) was purchased from the Shanghai Yun-Hong Pharmaceutical Aids and Technology Co. Ltd., Shanghai, China. Phosphatidyl choline (PC, extracted from soy bean), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), and sodium hydroxide were all purchased from the Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Trichloromethane (TCM) was purchased from Sigma–Aldrich Inc., St. Louis, MO, USA. Water was doubly distilled before use. All other chemicals were analytical grade reagents commercially available, and used without further purification.

2.2. Preparation and characterization of magnetite nanoparticles

Superparamagnetic Fe_3O_4 nanoparticles were prepared according to the method of Kang et al. [48], *via* the chemical coprecipitation of $FeCl_3$ and $FeCl_2$ salts. This method is a simple route to polydisperse Fe_3O_4 nanoparticles with high yields. The resultant polydisperse magnetic nanoparticles have been applied in many fields including Langmuir films, cosmetics, surface coatings, medicine, and optoelectronic applications [49–54].

Firstly, 6.76 g $FeCl_3{\cdot}6H_2O$ and 3.08 g $FeCl_2$ were dissolved in 200 mL deionized water with mechanical stirring under a nitrogen atmosphere. After degassing under nitrogen for 30 min, a 5 M NaOH solution was added dropwise until the pH value of the mixed solution was 10. Following mechanical stirring for 15 min at 40 °C, the mixture was heated at 80 °C for 30 min. The resultant magnetite nanoparticles were washed three times with doubly distilled water and isolated using an external magnetic field. Finally, the water was removed through a freeze drying process and retained for future use.

The magnetic properties of the nanoparticles were measured on a vibrating sample magnetometer (VSM; Model-155, Princeton Applied Research, Oak Ridge, TN, USA) at 300 K.

2.3. Preparation of electrospun fibers

Fibers were prepared from polymer suspensions containing different amounts of Fe_3O_4 nanoparticles. First, PC and PVP were dissolved in TCM at room temperature, with the concentration of PC fixed at 5% w/v and PVP at 6% w/v. The resultant solution was stirred for 2 h at 37 °C to obtain a clear and transparent solution. Fe_3O_4 nanoparticles were then added and the mixture mechanically stirred for 24 h at 37 °C to form homogeneous suspensions for electrospinning. The Fe_3O_4 concentrations were set at 0.2%, 0.4%, 0.5% and 0.6% w/v. The resultant composite fibers were denoted F1 (0.2% Fe_3O_4 w/v), F2 (0.4%), F3 (0.5%), and F4 (0.6%) respectively.

The electrospinning apparatus comprises three major components: a high-voltage power supply (SDW-ZGF60/2, Shanghai Sute Electrical Co., Ltd., Shanghai, China), an earthed collector, and syringe pump (KDS100, Cole-Parmer, Vernon Hills, IL, USA) [55]. During spinning, the flow rate was fixed at 0.5 mL/h, and the voltage at 16.0 kV. Fibers were collected at a constant distance of 25 cm from the needle tip of the spinneret. The internal diameter of the needle was 0.5 mm. Experiments were carried out under ambient conditions (21 ± 2 °C; relative humidity 57 ± 4%). Except for the Fe_3O_4 content of the spinning suspensions, all processing parameters were identical for the preparation of all four composite materials.

2.4. Preparation of self-assembled magnetic liposomes

For liposome preparation, 0.01 g of the Fe_3O_4–PC–PVP fibers was added to 10 mL of water and allowed to stand for 12 h. The solution was then centrifuged, and the lower solute recovered through a freeze drying process and retained for future use.

2.5. Characterization

2.5.1. Microscopy

The morphologies of the fibers and self-assembled magnetic liposomes were assessed using a JSM-5600LV scanning electron microscope (SEM: JEOL, Tokyo, Japan). Samples were rendered conductive by sputter-coating with Pt. The average fiber diameter was determined from SEM images by measuring the diameters of the fibers at more than 50 different points, using the Image J software (National Institutes of Health, Bethesda, MD, USA).

The internal structures of the Fe_3O_4-containing fibers and liposomes were investigated by transmission electron microscopy (TEM; H-800 instrument, Hitachi, Tokyo, Japan). Both electrosun fibers and liposome samples were prepared on a carbon-coated 200 mesh copper grids for TEM analysis.

To prepare self-assembled samples for scanning probe microscopy (SPM), a drop of water from a micro-injector was placed on fibers collected on aluminum foil and the wet fibers allowed to dry at room temperature in air. SPM images were taken on a DI-NSIV scanning force microscope (Digital Instruments, New York, NY, USA).

2.5.2. Structural analysis

Fourier-transform infra-red spectroscopy (FT-IR) was carried out on a Nicolet-Nexus 670 FTIR spectrometer (Nicolet Instrument Corporation, Madison, WI, USA) over the range 500–4000 cm^{-1}. The elemental compositions of the materials were analysed by energy-dispersive X-ray spectroscopy (EDS; Oxford Link IE 300 X probe, Oxford Instruments, Abingdon, UK).

2.5.3. Size analysis

The average hydrodynamic diameter and size distribution of the self-assembled magnetic liposomes were determined using a BI-200SM static and dynamic light scattering (DLS) instrument (Brookhaven Instruments Corporation, Austin, TX, USA). Measurements were performed at 25 °C with a scattering angle of 90°. The breadth of the particle size distribution was expressed in terms of the polydispersity index (PDI).

2.5.4. Zeta potential determination

Zeta potential measurements were obtained with the aid of a Malvern Zetasizer Nano-series Zen 3600 instrument (Malvern Instruments, Malvern, UK). All measurements were performed at least three times after dilution in water.