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A novel candidate for wound dressing: Transparent porous maghemite/cellulose nanocomposite membranes with controlled release of doxorubicin from a simple approach

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ABSTRACT

The aim of this study is to develop transparent maghemite/cellulose nanocomposite membranes with high porosity, high adsorption capacity and controlled release of doxorubicin to be used as a candidate for wound dressing. The membranes were fabricated by a tape casting method through blending a homogeneous dispersion of citrate coated maghemite nanoparticles and cellulose in the NaOH/urea aqueous solution system. The prepared membranes were characterized by Light transmittance measurements, Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray Diffractometry (XRD), Differential scanning calorimetry (DSC), Tensile tests and Vibrating sample magnetometer (VSM). Furthermore, porosity, swelling behavior, water loss ratio and Bovine serum albumin adsorption capacity were evaluated. Drug loading and release was investigated using doxorubicin hydrochloride as a model drug. In vitro cytotoxicity and cells morphology assays of cells growth and proliferation were also studied. This study served as a demonstration of the feasibility of maghemite/cellulose nanocomposite membranes for loading and release of bioactive compounds as a candidate for wound dressing.

1. Introduction

Skin is the largest organ of the body and the most important barrier between human body and the external environment. Damaged skin from surgical wounds, burns, chronic skin ulcers and skin cancer may cause the loss of water, electrolytes and lead to wound infection [1]. And without adequate repair, water, electrolyte disturbances and the infection can result in prolonged healing time, major disability, and even death [2,3]. A proper wound dressing is needed to protect the wound from further damage, and to accelerate healing with controlled release of drugs [4].

Among different polymeric materials for the preparation of wound dressings, cellulose has been widely explored for its biocompatibility, biodegradability and high porosity which are attractive for wound healing applications [5,6]. Different forms of cellulose have been developed during the past years such as hydrogels of cellulose nano-whiskers with polyvinyl alcohol [7], hydroxyethyl cellulose nanofibers prepared by electrospinning [8], and membranes from microcrystalline cellulose [9]. However, with no strong chemical or physical interaction, the absorption properties of cellulose materials in the above forms have been found poor and unstable [10], which will lead to fast drug release and influence healing efficiency.

Improvement of absorption properties of cellulose for better healing efficiency [11,12] is mostly achieved by some chemical modification methods [13–16]. However, a large number of data proved that some modification may introduce chemical pollution, and have cytotoxic effects [17]. Incorporation of biocompatible charged nanoparticles may be an easy and useful way to improve drug adsorption property through electrostatic interaction [18], which is simple, fast and economical [19]. In our previous research, nanodiamonds were applied to enhance cellulose adsorption properties [20]. Maghemite nanoparticles attracted great attention due to their biocompatibility, biodegradability and superparamagnetic property [21]. They hold promising applications in various biomedical fields such as drug delivery and tissue engineering [22]. And some reports have showed that maghemite has the ability of promoting wound healing [23]. Therefore, introduction of maghemite nanoparticles is expected to yield benefit of improving cellulose adsorption properties and wound healing properties [24].

The aim of this study was to further investigate the potential of transparent porous maghemite/cellulose nanocomposite membrane

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(MCNM) as a novel candidate for wound dressing. MCNM with high porosity and high drug adsorption capacity was fabricated by a simple tape casting method through blending a homogeneous dispersion of citrate coated maghemite and cellulose in the NaOH/urea aqueous solution system. The structure and properties of these membranes were investigated. As a proof-of-concept of the functionality of MCNM matrix, in vitro cytotoxicity assay of the membranes was also studied using Hela cells as a model.

2. Experimental

2.1. Materials

Cellulose (cotton linter pulp) with α-cellulose content of >95% was provided by Hubei Chemical Fiber Group Ltd. (Xiangfan, China). Its viscosity-average molecular weight (M_η) was determined by using an Ubbelohde viscometer in LiOH/urea aqueous solution at 25 ± 0.05 °C and calculated according to the Mark-Houwink equation ($[\eta] = 3.72\ 10^{-2}\ M_\eta^{0.77}$) [25], to be 8.1×10^4 Da. Maghemite (γ-Fe_2O_3) nanoparticles were purchased from Aladdin (Shanghai, China). The purity is 99.5% and the size of particles is ~20 nm. Bovine serum albumin (BSA) was obtained from Yancheng Saibao Biotechnology Co., Ltd., China. Doxorubicin hydrochloride (DOX, >98%) was purchased from Arking Pharma Scientific Inc., Canada. 3-[4,5-Dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. HeLa and HepG2 cells were purchased from Chinese Typical Culture Center (CTCC, Wuhan University) and cultured in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin) at 37 °C in a humidified atmosphere containing 5% CO_2. Other chemical regents of analytical grade were purchased from Sinopharm Chemical Reagent and used as received.

2.2. Preparation of citrate-modified maghemite nanoparticles (MMNP)

The functionalization of maghemite nanoparticles (MNP) was performed as follows: 5 g MNP was dispersed in 150 mL hydrochloric acid (pH 3.0) and then added to 150 mL of 5 mg/mL sodium citrate under stirring. The mixture was sonicated for 30 min and then further stirred for 2 h. The solid was separated from supernatant by centrifugation, and washed several times with deionized water [26].

2.3. Preparation of MCNM

To fabricate MCNM samples, a desired amount of MMNP (0.42–2.67 g) was homogeneously dispersed into 162 mL of deionized water, and then 14.0 g of NaOH and 24.0 g of urea were added into above solution. The resultant mixture was sonicated at ultrasonic power of 500 W for 30 min and pre-cooled to −12.5 °C, then 8.0 g of cellulose (cotton linter pulp) was added immediately with vigorous stirring for 5 min to obtain a uniform cellulose composite solution. After degasification, the mixed solution was cast on a glass plate to give a gel like sheet with a thickness of about 0.5 mm and the sheet was immediately immersed into 5 wt% H_2SO_4 aqueous solution for 5 min at room temperature to coagulate and regenerate. The resultant membranes were washed with running water and deionized water until the washings were neutral [27]. Finally, the wet membranes were freeze-dried. By adjusting the weight ratio of MMNP to cellulose, MCNM can be obtained with different MMNP contents: 5 wt%, 15 wt% and 25 wt%, which were coded as MCNM05, MCNM15 and MCNM25, respectively, and native cellulose membrane was coded as CM.

2.4. Characterization

2.4.1. Zeta potential of MNP and MMNP

Zeta potential of MNP and MMNP (with 1 g/L suspension in water) as a function of pH was measured by a Malvern Zetasizer Nano Series Nano-ZS. The pH of the buffers used was 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. Each buffer had 25 mL of 0.2 M KCl solution and 75 mL of ultrapure water. The pH was controlled by adding HCl and NaOH solutions [28].

2.4.2. Structure and morphology of CM and MCNM

Scanning electron micrographs (SEM) were taken on a Hitachi TM3030 scanning electron microscope. The wet membranes were frozen in liquid nitrogen and then vacuum-dried. The free surface (the side in direct contact with the coagulant) of the membranes was sputtered with gold, and then observed and photographed. Pore diameters were obtained from analyzing SEM images by using ImageJ software [29]. The volume (V), backbone density (ρ_g), mean pore volume (V_p) and porosity (P_r) of wet membranes were measured and calculated by a pycnometer method at 20 °C. Each membrane was measured for three times and the average was calculated [30]. Optical transmittance (Tr) of the wet membranes was measured with a SpectraMax M2 (Molecular Devices, USA) in the wavelength ranging from 200 to 800 nm (measurements at the wavelength of 800 nm are represented in this work), the thickness of the membranes was approximately 0.5 mm.

Fourier-transform infrared spectroscopy (FT-IR) was recorded with a Fourier transform infrared spectrometer (1600, Perkin-Elmer Co., USA). The membranes were cut into pieces and then vacuum-dried at 60 °C for 24 h before test. The test specimens were prepared by the KBr-disk method. X-ray diffraction studies were performed on an X-ray diffractometer (Bruker D8 Advance, Germany) at ambient temperature with scanning rate of 2°/min in 2θ range of 10–80°, using a CuKα radiation (λ = 1.5406 Å). The thermal analysis was carried out with differential scanning calorimetry (Seiko Instruments, DSC 6220). Each sample of 10 mg was first dried under reduced pressure for 24 h, and then sealed in an aluminum pan for the measurement. The heating rate of 10 °C/min from 50 to 500 °C was used under nitrogen flow.

2.4.3. Magnetic properties of MNP, MMNP and MCNM

The magnetic properties of MNP, MMNP and MCNM were evaluated on a vibrating sample magnetometer (VSM, Westerville, OH, USA) by changing the magnetic field from −20,000 to 20,000 Oe at 25 °C.

2.4.4. Mechanical properties of CM and MCNM

The mechanical properties of the dry membranes were measured with a CMT8202 universal testing machine according to ASTM D882-09 [31]. Rectangular specimens were cut to 60 mm × 5 mm and extended at a constant crosshead speed of 5 mm/min with a 40 mm gauge length. Each specimen was repeated 10 times to determine the mean value; error bars on all plots represent one standard deviation of the mean. The thickness of each specimen was obtained from the average of ten measurements taken along the gauge length with a digital micrometer.

2.4.5. Swelling behavior of CM and MCNM

The swelling studies of CM and MCNM were carried out according to P. T. Sudheesh Kumar's method [32]. Dry membranes were cut into small pieces (2 cm × 2 cm) and immersed in phosphate buffered saline (PBS) (0.1 M, pH = 7.4, 37 °C). After being soaked for 1, 3 and 7 days, the swollen samples were taken out and gently blotted with filter paper to remove surface liquid, and weighed (W_w) immediately. The swollen sample was then dried and weighed (W_d). The swelling ratio was calculated using the following equation:

$$\text{Swelling ratio} = \frac{W_w - W_d}{W_d} \times 100\% \quad (1)$$

2.4.6. Weight loss analyses of CM and MCNM

The weight loss analyses were measured using the shake method [33]. Three wet membranes (with the dimension 2 cm × 2 cm) were quickly shaken twice and then weighed. The wet samples were weighed