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A simple route to develop transparent doxorubicin-loaded nanodiamonds/cellulose nanocomposite membranes as potential wound dressings

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ABSTRACT

The objective of this study is to develop transparent porous nanodiamonds/cellulose nanocomposite membranes with controlled release of doxorubicin for potential applications as wound dressings, which were fabricated by tape casting method from dispersing carboxylated nanodiamonds and dissolving cellulose homogeneously in 7 wt% NaOH/12 wt% urea aqueous solution. By adjusting the carboxylated nanodiamonds content, various nanocomposite membranes were obtained. The structure and properties of these membranes have been investigated by light transmittance measurements, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), tensile tests, water loss analyses, etc. The drug loading and release was investigated using doxorubicin hydrochloride as a model drug. In vitro cytotoxicity assay of the membranes was also studied. This work presented a proof-of-concept utility of these membranes for loading and release of bioactive compounds to be employed as a candidate for wound dressing.

1. Introduction

Skin plays an important role in homeostasis and the prevention of invasion by microorganisms. Skin generally needs to be covered with a dressing immediately after it is damaged (Jayakumar, Prabaharan, Sudheesh Kumar, Nair, & Tamura, 2011). However, in the absence of adequate repair, damaged skin can lead to prolonged healing time, major disability, and even death (Cardoso et al., 2011). Therefore, it is essential to design proper wound dressings to protect wound from further damage and to promote wound healing (Unnithan et al., 2015).

Natural polymers (cellulose, etc.) have attracted increasing attention due to their non-cytotoxicity (Sell et al., 2010). Cellulose, being the most abundant renewable natural polymer, has been widely explored for the development of biomedical materials such as scaffolds, medical device, drug diagnosis and so on in recent years (Pei et al., 2015). Because of its biocompatibility and porosity, various types of cellulose based wound dressings have been reported (Gustaite et al., 2014; Maxwell, Mazzeo, & Whitesides, 2013). Cellulose membranes are available in a broad range of thicknesses and well-defined pore sizes. They also possess advantages including easy to store and high mechanical property (Credou, Volland, Dano, & Berthelot, 2013). Natural cellulose may not serve as an ideal wound dressing due to the lack of active healing properties by itself. Improvement of cellulose properties is mostly achieved by chemically modifying the polymer structure through introduction of various functional groups for the adsorption of drugs (Fras Zemljič, Persin, & Stenius, 2009; Mikhaylova et al., 2011), and doping with metal ions which have antimicrobial activity (Cady, Behnke, & Strickland, 2011; Jian et al., 2014; Wu et al., 2014). However, several reports pointed out that chemical modification may introduce chemical pollution during complicated modification process and metal release from wound dressings can have cytotoxic effects on the healthy tissue (Peršin et al., 2014).

Incorporation of biocompatible nanoparticles may be an easy and useful way to improve composite material properties (Mukherjee & De, 2014; Unnithan, Gnanasekaran, Sathishkumar, Lee, & Kim, 2014), which is simple, fast, and economical due to without chemical modifications (Credou & Berthelot, 2014). Excellent chemical stability of nanodiamonds makes them the promising nanofillers for composite materials. Moreover,

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the biocompatibility allows these materials for biomedical applications. A wide range of drugs can bind to nanodiamonds due to the abundant surface functional groups (Kirmani et al., 2015; Mochalin, Shenderova, Ho, & Gogotsi, 2011). However, non-purified or aggregated nanodiamonds will affect the toxicity of composites, giving rise to the need of well-dispersed and properly functionalized nanodiamonds (Cai et al., 2016; Kato et al., 2011).

The purpose of this study is to develop transparent porous cellulose-based nanocomposite membranes and evaluate their properties in regard to the applications as wound dressings. Nanodiamonds/cellulose nanocomposite membranes were fabricated by tape casting method through blending a homogeneous dispersion of carboxylated nanodiamonds (CND) and cellulose in NaOH/urea aqueous solution system. The structure and properties of these membranes have been investigated. Drug loading and release process were investigated using doxorubicin hydrochloride (DOX) as a model drug. This work presents a proof-of-concept demonstration of the application of these membranes for loading and release of bioactive compounds (Zhu, Gao, Wang, & Sukhishvili, 2013). In vitro cytotoxicity assay of the membranes was also studied.

2. Experimental

2.1. Materials

Cellulose (cotton linter pulp) with an α-cellulose content of more than 95% was provided by Hubei Chemical Fiber Group Ltd. (Xianfan, 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 ($[\eta] = 3.72 \times 10^{-2} M_\eta^{0.77}$) (Cai, Liu, & Zhang, 2006), to be 8.1×10^4 Da. Detonation synthesized nanodiamond (ND) with size of 50–100 nm was supplied by Huajin Microndiamond Nanotechnology, China. Doxorubicin hydrochloride (DOX·HCl, >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 cells were obtained from Chinese Typical Culture Center (CTCC, Wuhan University) and grown in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin). Cell cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Other chemical reagents of analytical grade were purchased from China, and were used without further purification.

2.2. Preparation of carboxylated nanodiamonds/cellulose nanocomposite membranes (CNM-CND)

The functionalization of the nanodiamonds to its surface oxides was achieved chemically via strong acid treatment (Tu, Perevedentseva, Chung, & Cheng, 2006; Ushizawa et al., 2002). The detonation-synthesized ND (1.0 g) was performed in a heated mixture of sulfuric acid and nitric acid (3:1, 80 mL) for 48 h, then in 0.1 M NaOH aqueous solution at 90 °C for 2 h, and in 0.1 M HCl aqueous solution at 90 °C for 2 h. The resulting diamonds were extensively rinsed with deionized water and separated by sedimentation with a centrifuge at 12,000 rpm and dried for testing, or the products were washed with deionized water until the pH value of the upper layer suspension arrives to neutral. The obtained CND were redispersed into deionized water and ultrasonicated at ultrasonic power of 500 W for 30 min to obtain the homogeneous CND suspension for usage.

To fabricate CNM-CND samples, desired amount of CND powder (0.008–0.120 g) was homogeneously dispersed into 162 mL of deionized water and 14.0 g of NaOH and 24.0 g of urea were added into the above solution. The resultant mixture was treated at ultrasonic power of 500 W for 30 min and pre-cooled to −12.5 °C, and then 8.0 g of cellulose (cotton linter pulp) was added immediately into the mixture with vigorous stirring for 5 min to obtain a cellulose composite solution. After degasification, the mixed solution was cast on a glass plate to obtain a gel like sheet with thickness of about 0.5 mm, which 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 (at least 5 times) (Fu et al., 2015). Finally, the wet membranes were freeze-dried. By adjusting the weight ratio of CND to cellulose, we can obtain CNM-CND with different CND contents: 0.1 wt%, 0.5 wt%, 1.0 wt% and 1.5 wt%, which were coded as CNM-CND01, CNM-CND05, CNM-CND10 and CNM-CND15, respectively, and native cellulose membrane was coded as CM.

2.3. Physicochemical properties of ND and CND

The mean size and the surface charge of the ND and CND nanoparticles in suspension were measured by a Malvern Zetasizer Nano Series Nano-ZS with 1 g/L suspension in water, respectively. The Boehm titration method (Schmidlin et al., 2012) allows us to confirm the presence of carboxylic acid groups on the surface of the ND and CND. The surface area was obtained by a Beckman Coulter SA 3100 Surface Area Analyzer. For this analysis, the samples were outgassed at 225 °C under helium atmosphere for 15 h.

2.4. Structure and morphology of CM and CNM-CND

Optical transmittance (T_r) 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 were presented in our work). The thickness of the membranes was approximately 0.5 mm. Scanning electron microscopy (SEM) micrographs were taken on a Hitachi X-650 scanning electron microscope. The wet membranes were frozen in liquid nitrogen and then vacuum-dried. The free surface (side in direct contact with the coagulant) of the membranes were sputtered with gold, then observed and photographed. Pore diameters were obtained from analyzing SEM images using ImageJ software and average pore diameter was calculated (Raub et al., 2008). The 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 average values were calculated (Zhang, Zhou, Yang, & Chen, 1998).

Fourier-transform infrared spectroscopy (FTIR) 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 the measurements. The test specimens were prepared by the KBr-disk method. The Raman-spectra have been recorded using a DXR Raman microscope (Thermo Fisher Scientific, Madison, WI) equipped with a laser with an excitation wavelength of 532 nm. The samples were analyzed with a 20× objective and an integration time of 0.5 s. The laser power was 1 mW. To improve the signal to noise ratio, 200 accumulations were made. 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 through differential scanning calorimetry (DSC) (Seiko Instruments, DSC6220). Each sample of 10 mg was firstly dried under reduced pressure for 24 h, and then sealed in an aluminum