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Poly(3-hydroxybutyrate)/caffeic acid electrospun fibrous materials coated with polyelectrolyte complex and their antibacterial activity and in vitro antitumor effect against HeLa cells

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ABSTRACT

The purpose of this work was to investigate the possibility for the preparation of new poly(3-hydroxybutyrate) (PHB)/poly(ethylene glycol) (PEG)-based fibrous materials containing natural phenolic compound caffeic acid (CA) of diverse architectures, as well as to study the impact of the fiber composition on the in vitro CA release profile and on the biological properties of the fibrous materials. The application of the one-pot electrospinning enabled the fabrication of nanofibrous materials from PHB and PEG loaded with the CA. Materials with targeted design were obtained by coating with polyelectrolyte complex of alginate (Alg) and *N,N,N*-trimethylchitosan (TMCh). Three different processing paths were used to obtain coated mats: (i) with CA incorporated in the PHB/PEG core; (ii) with CA embedded in the Alg layer; and (iii) with CA included in the TMCh layer. The in vitro release of CA was modulated by controlling the composition and the architecture of the nanofibrous mats. The performed microbiological screening and MTT cell viability studies revealed that in contrast to the bare mats, the CA-containing nanofibrous materials were effective in suppressing the growth of the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* and displayed good cytotoxicity against human cervical HeLa tumor cells. In addition, the proliferation of murine spleen lymphocytes and peritoneal macrophages was increased by the prepared CA-containing nanofibrous materials. The obtained materials are promising for antibacterial wound dressing applications as well as for application in local treatment of cervical tumors.

1. Introduction

Caffeic acid (CA) is a widely distributed natural phenolic compound and is present in honey, fruits and vegetables, etc. It possesses a number of valuable properties such as antioxidant, antithrombogenic, anti-inflammatory, antimicrobial, antiviral, antimutagenic and antitumor activities [1–4]. It has been reported that CA inhibits the proliferation of tumor cells and induces apoptosis [4–8]. Therefore, it is attractive for applications in biomedicine and pharmacy.

Recently, electrospinning has emerged as a very suitable technique for the preparation of drug-loaded nanofibrous materials from synthetic and bio-based polymers [9]. Electrospinning is a simple and cost-effective method to fabricate polymer fibers with diameter ranging from a few nanometers to several micrometers. In electrospinning process, a polymer solution is pumped through a thin nozzle, which simultaneously serves as an electrode, to which a high electric field is applied. The potential difference between the nozzle and the counter electrode (collector) creates a thin jet. During the time of flight the solvent evaporates and ultrafine fibers are deposited on the collector [10,11]. An advantage of electrospinning is that it allows facile and efficacious drug loading, as well as controlling the drug release profile [12–15]. The specific properties of the nanofibrous materials related to their nanoscale size and the possibility of modulating drug release may result in enhancement of the therapeutic effect of the drugs [16,17]. Moreover, electrospun micro- and nanofibrous materials are suitable carriers for enhancing drug bioavailability. Natural polysaccharides (chitosan and its derivatives, alginates) and polyesters (polyhydroxyalkanoates, homo- and copolymers of lactic acid) can be regarded as some of the most promising polymers obtained from renewable sources suitable as drug carriers. Polyhydroxyalkanoates, and in particular, poly(3-hydroxybutyrate) (PHB), are promising for in vitro and in vivo applications [18] because they degrade completely without releasing toxic side products [19]. Furthermore, the electrospinning of PHB is easily feasible

Abbreviations: Alg, sodium alginate; CA, caffeic acid; PEC, polyelectrolyte complex; PHB, poly(3-hydroxybutyrate); TMCh, *N,N,N*-trimethylchitosan iodide.

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and the obtained micro- and nanofibrous materials possess good mechanical properties [20]. The incorporation of alginate (Alg) in nanofibrous materials designed for biomedical purposes is also of interest because this linear anionic polysaccharide is biodegradable, biocompatible and possesses low toxicity. The quaternized derivative of chitosan – *N*,*N*,*N*-trimethylchitosan (TMCh), similarly to chitosan is biodegradable and biocompatible. It displays higher antimicrobial activity compared to chitosan at pH ≥5.5 [21,22], as well as good in vitro antitumor activity [23]. In aqueous solutions TMCh can form polyelectrolyte complexes (PEC) with polyanions [24–28]. It has been demonstrated that the incorporation of quaternized chitosan derivatives in electrospun materials [29–32] or formation of PEC coating from quaternized chitosan derivatives on the surface of fibrous materials [33–35] imparts them antimicrobial activity. Recently, we have reported that nanofibrous materials composed of PEC of TMCh and poly(acrylic acid) or poly(2-acrylamido-2-methylpropanesulfonic acid) prepared by one-step electrospinning display antibacterial activity [36]. Nanofibrous materials based on polylactide coated with a quaternized derivative of chitosan, loaded with the natural polyphenol gossypol manifest good antitumor activity in vitro [37] and in vivo [38]. Up to now, there have been a limited number of reports on the preparation of electrospun mats containing the phenolic compound CA. It has been shown that CA-loaded polycaprolactone nanofibrous materials are effective in suppressing the growth of gastric cancer cells (MKN28) and induce apoptosis in these cells [39]. It has been found that CA-containing polylactide fibrous materials are capable of promoting the adhesion and growth of osteoblast-like cells and manifest protective activity against oxidative stress [40]. Furthermore, it has been reported that these fibrous materials preserve in vitro and in vivo their capacity to protect DNA from oxidative damage [41]. Nanofibrous materials with antioxidant activity and capacity to promote the proliferation of human dermal fibroblasts in which CA is chemically immobilized onto the surface of electrospun poly(L-lactic acid) fibers have been reported [42]. To our knowledge, until now, CA-containing PHB electrospun fibrous materials coated with PEC have not yet been reported. There are no data about antibacterial properties and in vitro antitumor activity on human cervical cancer cell line (HeLa) of fibrous materials containing CA, TMCh and Alg.

The present contribution aims at studying the possibility for the preparation of novel CA-containing fibrous materials from PHB/PEG of various architectures using different processing paths based on electrospinning and dip coating. In order to combine the valuable properties of CA, TMCh and Alg, the obtained electrospun nanofibrous materials were coated with TMCh/Alg PEC. With the purpose of tuning the CA release and the antibacterial or antitumor activity of the nanofibrous materials, CA was incorporated in the PHB/PEG core or in the TMCh/Alg PEC coating. The morphology, the composition, and the thermal characteristics of the fibrous materials were studied. Their antibacterial activity against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* was assessed, as well. The impact of the composition of the polymer matrix on the in vitro release profile of CA as well as on the proliferation of murine spleen lymphocytes and peritoneal macrophages was also studied. An evaluation of the in vitro antitumor activity of the obtained fibrous materials on human cervical HeLa tumor cells was also performed.

2. Experimental

2.1. Materials

PHB (330,000 g.mol^{-1}, Biomer), poly(ethylene glycol) (PEG, 2000 g.mol^{-1}, Fluka), formaldehyde solution (36% in water) (Fluka), CH_3I (Fluka), $NaBH_4$ (Fluka), NaI (Fluka), Alg (Aldrich) and CA (Acros Organics) were of analytical grade of purity and were used as received. Dimethylformamide (DMF) (Merck) and chloroform (Merck) were dried on molecular sieves (4 Å). Prior to use, *N*-methyl-2-pyrrolidone (NMP) (Fluka) was distilled under reduced pressure. Chitosan (Aldrich) with an average viscometric molar mass of 380,000 g.mol^{-1} and a deacetylation degree of 80% was used. TMCh was prepared from chitosan using a known procedure [22]. The quaternization degree of TMCh was determined by ^{1}H NMR and by potentiometric titration with aqueous silver nitrate. The quaternization degree was calculated from the intensity ratio of the signal at 3.39 ppm for -R-N$^+$($\underline{CH_3}$)$_2$I$^-$ (where R = CH_2 or CH_3) to the signals at δ 3.64–4.54 ppm for H-2, *H*-3, H-4, H-5, H-6, H-6′ (6H). This value (56%) is in agreement with the value determined potentiometrically (57%). The degree of methylation of the –OH functions was determined from the intensity ratios of the signal of $\underline{CH_3}$-O at 3.50 and 3.42 ppm for OH at *C*-3 and C-6 positions, respectively, and the H-2, *H*-3, H-4, H-5, H-6, H-6′ (6H) signals at δ 3.64–4.54. The degree of methylation of *H*-3 and H-6 was 97%. 3-(4.5-Dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT), ethidium bromide (EtBr), and acridine orange (AO) were purchased from Sigma-Aldrich, Germany. All culture reagents RPMI-medium (Sigma-Aldrich, Germany), fetal bovine serum (FBS) (Gibso/BRL, Grand Island, NY), glutamine, penicillin and streptomycin (LONZA) were used as received. The disposable consumables were supplied by Orange Scientific, Belgium. *S. aureus* 749 and *E. coli* 3588 were purchased from the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria.

2.2. Preparation of bare and CA-containing PHB/PEG nanofibrous mats

For the preparation of PHB nanofibers, 10 wt% solution of PHB in $CHCl_3$/DMF = 4/1 (v/v) was prepared by heating at 60 °C using a reflux condenser. PHB/PEG nanofibers at weight ratio of PHB:PEG 70:30 were prepared by electrospinning of their mixed solutions in $CHCl_3$/DMF = 4/1 (v/v) at total polymer concentration of 10 wt%. The PHB/PEG nanofibrous materials in which CA was incorporated will be further denoted as CA/PHB/PEG. For their preparation spinning solutions of the PHB/PEG and the CA in a mixed $CHCl_3$/DMF = 4/1 (v/v) solvent system with a total polymer concentration of 10 wt% were used. The amount of the incorporated CA was selected in such a way as to attain CA content of 5, 10 and 20 wt% with respect to the polymer weight. The electrospinning set-up was composed of a custom-made high voltage power supply (10–30 kV), a grounded rotating drum collector, an infusion pump (NE-300 Just InfusionTM Syringe Pump, New Era Pump Systems Inc., USA) for delivering the spinning solution at a constant rate, and a syringe equipped with a metal needle was used. Electrospinning was performed under the following conditions: flow rate of 2.0 mL·h^{-1}, voltage of 25 kV, needle tip-to-collector distance 25 cm, and collector rotating speed of 1200 rpm. The electrospun nanofibrous mats were placed under reduced pressure at 30 °C for 8 h to remove any solvent residues.

The dynamic viscosity of the spinning solutions was measured using a Brookfield DV-II+ Pro programmable viscometer for cone/plate option equipped with a sample thermostated cup and a cone spindle, at 25 ± 0.1 °C. The electrical resistance of the spinning solutions was measured in an electrolytic cell equipped with two rectangular sheet platinum electrodes as previously described [29].

2.3. Preparation of coatings of Alg and Alg/TMCh complex on the surface of PHB/PEG or CA/PHB/PEG nanofibrous mats

Alg-coated PHB/PEG or CA/PHB/PEG mats (further denoted as Alg-*coat*-PHB/PEG or Alg-*coat*-CA/PHB/PEG) were prepared by immersing the PHB/PEG or CA/PHB/PEG mats in 0.1 wt% aqueous solution of Alg for 15 min, and subsequently drying the mats to constant weight. Coatings of TMCh/Alg complex on the surface of the mats were prepared by soaking the Alg-coated mats into 0.1 wt% water/ethanol (3/1, v/v) solution of TMCh at room temperature for 15 min and drying the mats to constant weight.