Carbohydrate Polymers 91 (2013) 419–427

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Carbon nanotube-incorporated multilayered cellulose acetate nanofibers for tissue engineering applications

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ARTICLE INFO

Article history:
Received 24 March 2012
Received in revised form 11 August 2012
Accepted 19 August 2012
Available online 25 August 2012

Keywords:
Self-assembly
Carbon nanotube
Electrospinning
Nanofiber
Cell culture

ABSTRACT

We report the fabrication of a novel carbon nanotube-containing nanofibrous polysaccharide scaffolding material via the combination of electrospinning and layer-by-layer (LbL) self-assembly techniques for tissue engineering applications. In this approach, electrospun cellulose acetate (CA) nanofibers were assembled with positively charged chitosan (CS) and negatively charged multiwalled carbon nanotubes (MWCNTs) or sodium alginate (ALG) via a LbL technique. We show that the 3-dimensional fibrous structures of the CA nanofibers do not appreciably change after the multilayered assembly process except that the surface of the fibers became much rougher than that before assembly. The incorporation of MWCNTs in the multilayered CA fibrous scaffolds tends to endow the fibers with improved mechanical property and promote fibroblast attachment, spreading, and proliferation when compared with CS/ALG multilayer-assembled fibrous scaffolds. The approach to engineering the nanofiber surfaces via LbL assembly likely provides many opportunities for new scaffolding materials design in various tissue engineering applications.

1. Introduction

Tremendous progress has been witnessed in the development of various tissue engineering scaffolding materials since the pioneering work reported by Langer and Vacanti in 1990s (Langer & Vacanti, 1993, 1999). In the field of tissue engineering, design of suitable bioactive scaffold materials that can mimic the extracellular matrices (ECM) is an essential prerequisite (Harrington et al., 2006). Much effort has been devoted to fabricate such scaffold materials for improved cellular attachment, proliferation, and differentiation (Abraham, Riggs, Nelson, Lee, & Rao, 2010; Rowley, Madlambayan, & Mooney, 1999; Sottile, 2004; Wei & Ma, 2008).

Electrospinning is a simple and versatile technique that can be used to fabricate fibers with a diameter ranging from tens of nanometers to a few microns (Reneker & Chun, 1996). The fabricated nanofibrous mats with ultra-fine fiber diameter, high surface area to volume ratio, three-dimensional (3D) porous structure can well mimic the natural ECM (Bhattarai, Li, Edmondson, & Zhang, 2006; Huang et al., 2010; Li, Laurencin, Caterson, Tuan, & Ko, 2002; Li et al., 2005; Mo, Xu, Kotaki, & Ramakrishna, 2004; Yoshimoto, Shin, Terai, & Vacanti, 2003), providing a useful option for tissue engineering applications. For practical biomedical applications, the formed nanofibrous mats have to be functionalized to improve the surface physicochemical properties, mechanical durability, biocompatibility, and cellular response.

As one of the most important surface modification techniques, layer-by-layer (LbL) self-assembly has been widely utilized to deposit multilayers onto planar substrates (Decher, 1997; Mamedov & Kotov, 2000; Olek et al., 2004; Schlenoff & Decher, 2003) or nanofiber surfaces (Almodóvar & Kipper, 2011; Deng et al., 2010, 2011; Mamedov & Kotov, 2000; Xiao et al., 2009). For improved cellular functionalities of planar substrates, Yang et al. reported the formation of hydrogen-bonded multilayers comprised of polyacrylamide and a weak polyelectrolyte, such as poly(acrylic acid) or poly(methacrylic acid) that have a high resistance to the adhesion (cytophobicity) of mammalian fibroblasts (Yang, Mendelsohn, & Rubner, 2003). Likewise, poly(glutamic acid) and poly(L-lysine) multilayers have been successfully self-assembled on planar substrates (Halthur & Elofsson, 2004; Lavalle et al., 2002) and display very good biocompatibility for implant coatings (Picart et al., 2005). In another study, Caruso and coworkers reported the

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http://dx.doi.org/10.1016/j.carbpol.2012.08.069

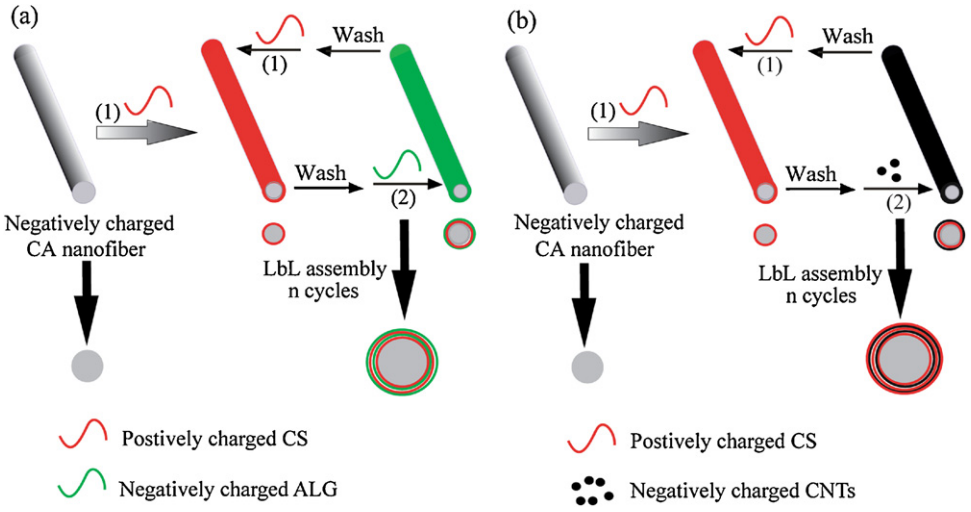

Fig. 1. Schematic illustration of the preparation of multilayered CA/(CS/MWCNTs)$_n$ and CA/(CS/ALG)$_n$ scaffolds.

construction of peptide-functionalized, low-biofouling click multilayers for promoting cell adhesion and growth (Kinnane, Wark, Such, Johnston, & Caruso, 2009). For the fiber surface functionalization, Deng et al. reported the construction of chitosan (CS) and alginate (ALG) multilayers onto cellulose acetate (CA) nanofibers for cell culture. These studies clearly suggest that the LbL self-assembly technique can be used to modify either planar substrates or fiber surfaces to improve the cellular functionalities.

In our previous work, we have shown that incorporating multiwalled carbon nanotubes (MWCNTs) within the electrospun polymer nanofibers is able to promote protein adsorption onto the fibrous substrates, significantly regulating the cellular spreading and proliferation (Liao et al., 2011; Liu et al., 2010). The improved cellular response is likely due to the presence of MWCNTs in the nanofibers that allows favorable protein adsorption from culture media onto the porous membrane structures (Meng et al., 2009). In our reported work, the MWCNTs were incorporated within the polymer nanofibers by simply electrospinning the polymer solution mixed with the MWCNTs (Liao et al., 2011; Liu et al., 2010). This approach does not allow for precise engineering of the nanofiber surface and the surface chemistry of the fibers cannot be easily regulated. Motivated by the versatile LbL self-assembly approach for fiber surface modification and the unique properties of MWCNTs in regulating cellular growth, we attempted to assemble electrospun CA nanofibers with multilayers of CS and MWCNTs to form a novel nanofiber-based scaffolding material for tissue engineering applications since CS is known to be a biocompatible naturally occurring polysaccharide material (Chicatun et al., 2011).

In this present study, we fabricated the MWCNT-containing nanofibrous polysaccharide scaffolds by combining electrospinning and LbL self-assembly techniques. Electrospun CA nanofibrous mats were first formed and used as templates for subsequent deposition of CS/MWCNTs multilayers via electrostatic self-assembly. For comparison, CS/ALG multilayers were also deposited onto the CA nanofibers (Fig. 1) since ALG is a biocompatible naturally occurring polymer and has been widely used in tissue engineering applications (Zhou & Xu, 2011). The formed multilayered CA nanofibers were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and mechanical durability tests. The protein adsorption behavior of the scaffolds was also investigated. Then, the cell attachment and proliferation viabilities onto the nanofibrous scaffolds were evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of cell viability and SEM observation of the cell morphology. Finally, the hemocompatibility of the multilayered CA nanofibrous scaffolds was assessed by hemolysis assay. To our knowledge, this is the first attempt to assemble MWCNTs onto the electrospun nanofiber-based scaffolding materials via an LbL assembly technique for potential tissue engineering applications.

2. Experimental

2.1. Materials

CA (bound acetic acid of 54.5–56.0 wt%, intrinsic viscosity of 300.0–500.0 mPa s), acetone, and CS (medium molecular weight, degree of deacetylation >90%, intrinsic viscosity 50.0–800.0 mPa s) were from Sinopharm Chemical Reagent co., Ltd. ALG with low intrinsic viscosity was from J&K chemical. MWCNTs (diameter 30–70 nm, length 100–400 nm) were obtained according to literature (Petersen, Huang, & Weber, 2008) and were functionalized with carboxyl residues according to a procedure described in literature (Lu & Imae, 2007). In brief, 20 mg of MWCNT was refluxed in 20 mL HNO_3/H_2SO_4 (v/v, 3:1) for 24 h, followed by filtration and drying to render the surface of MWCNTs with carboxylic acid residues. Mouse fibroblasts (L929 cells) were obtained from the Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Hangzhou Jinuo Biomedical Technology (Hangzhou, China). Human blood stabilized with heparin was kindly provided by Shanghai First People's Hospital (Shanghai, China). All other chemicals with reagent grade were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The water used in all the experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18 MΩ cm.

2.2. Electrospinning of CA nanofibers

CA (12.5 wt%) solution was prepared by dissolving 1.8 g CA powder into 15 mL acetone/N,N-dimethylformamide mixture solvent (2/1, v/v) with vigorous magnetic stirring for 12 h at 25 °C, and the solution was sonicated using a water bath ultrasonic cleaner (50 w, 15 × 14 × 10 cm (length × width × height), SK1200H, Shanghai KUDOS Inc., China) for 30 min before use. Freshly prepared CA solution was loaded into a syringe with a needle having an inner