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Research Paper

Prominent reinforcing effect of chitin nanocrystals on electrospun polydioxanone nanocomposite fiber mats

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ABSTRACT

The ultra-strong nanocomposite fiber mats based on biodegradable polydioxanone (PDO) and chitin nanocrystals (ChiNCs) were successfully prepared by means of electrospinning. The ChiNCs are uniformly dispersed in the PDO matrix and mostly oriented along fiber long axis, resulting in a significant improvement in mechanical property. Moreover, the introduction of ChiNCs led to the increase of the glass-transition temperature (T_g) and thermal decomposition temperature (T_d) of PDO elucidated by thermal analyses. In addition, the loading of ChiNCs caused very different *In vitro* degradation behavior compared to neat PDO fiber mat. Furthermore, *in vitro* cell culture results indicated that the addition of ChiNCs improved the cellular adhesion and proliferation.

1. Introduction

Chitin is the second most abundant biopolymer next to cellulose and possesses many favorable properties such as non-toxicity, high crystallinity, biocompatibility and biodegradability. Acid-treatment of chitin can dissolve away regions of low lateral order, resulting in elongated rod-like crystalline nanoparticles, termed "nanocrystals" (otherwise called nanowhiskers, or nanofibrils) (Marchessault et al., 1959). Chitin nanocrystal (ChiNC) has shown one of the most promising nanofillers to biomedical materials due to its good biocompatibility and biodegradability, as well as nanodimension, high surface area, low density, and high modulus. However, ChiNCs were thought only well disperse in aqueous solution, and poorly disperse in organic solvents. As a result, the ChiNC based nanocomposites were not extensively explored due to the processing difficulties. There are two routes often used to obtain a good dispersion of ChiNCs within polymer matrix. One requires the use of either an aqueous suspension of polymer, *i.e.* latex, or an aqueous solution of polymer (Uddin et al., 2012a, 2012b; Junkasem et al., 2010;

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Lu et al., 2004; Li et al., 2011; Hariraksapitak and Supaphol, 2010; Watthanaphanit et al., 2008; Huang et al., 2013; Wongpanit et al., 2007; Huang et al., 2011; Paillet and Dufresne, 2001; Morin and Dufresne, 2002; Nair and Dufresne, 2003). Many polymers were used as matrices, such as polyvinyl alcohol (PVA) (Uddin et al., 2012a, 2012b; Junkasem et al., 2010), soy protein isolate (Lu et al., 2004), chitosan (Li et al., 2011), gelatin (Hariraksapitak and Supaphol, 2010), alginate (Watthanaphanit et al., 2008), cellulose (Huang et al., 2013), silk fibroin (Wongpanit et al., 2007) and waterborne polyurethane (Huang et al., 2011) *via* aqueous blending, and poly(ε-caprolactone) (PCL) and poly(styrene-co-butyl acrylate) *via* latex blending (Paillet and Dufresne, 2001; Morin and Dufresne, 2002; Nair and Dufresne, 2003). The other requires modification of the ChiNC surface with functional groups or chains to improve its miscibility with organic solvents or matrix polymers (Feng et al., 2009; Fan et al., 2008). In previous study, we found that ChiNCs could well disperse in 2,2,2-trifluoroethanol (TFE) solvent, and based on that, the ultra-strong ChiNC/PCL nanocomposites were facilely prepared by organic solvent blending (Ji et al., 2012, 2014). In polysaccharide nanocrystal reinforced composites, the matrix structure and the resulting competition between matrix/filler and filler/filler interactions dominate the reinforcing effect of nanofillers. Dufresne (Dufresne, 2008) deduced that higher the affinity between the filler and host matrix was, lower the mechanical performances were, which was contrary to the classical composite science. Considering this point, PCL, a rather hydrophobic polyester and less affinity to ChiNC, was proved to be highly reinforced by ChiNC fillers (Morin and Dufresne, 2002). But more investigations should be taken to prove this viewpoint.

Polydioxanone (PDO), one of FDA-approval suture materials, is a synthetic aliphatic poly(ether ester). The presence of ether bond endows PDO with greater flexibility and hydrophilicity compared to PCL. The excellent properties of good biodegradability, biocompatibility, and bioabsorbability allow PDO to find extensive application in biomedical materials such as suture, tissue fixation, drug delivery systems, and vascular prostheses in tissue engineering (Ray et al., 1981; Makela et al., 2002; Bhattarai et al., 2004; Bahadur et al., 2007). Here, the less hydrophobic PDO was selected as matrix to further test the reinforcing effect of ChiNC filler using TFE as cosolvent *via* electrospinning. In addition, the *in vitro* cellular response to the reinforced nanocomposites was studied to determine the cellular biocompatibility for their future potential application in peripheral nerve repair.

2. Experimental

2.1. Preparation of ChiNC

ChiNC suspension was prepared according to Dufresne's method (Morin and Dufresne, 2002) with minor modification. Briefly, chitin flakes (from shrimp shell, Sigma-Aldrich Co., USA) were hydrolyzed in 3 mol L^{-1} HCl under stirring and refluxing for 6 h. The ratio of the 3 mol L^{-1} HCl solution to chitin was 30 $cm^3\ g^{-1}$. The residue was collected after centrifugation (9000 rpm) and repeated refluxing in 3 mol L^{-1} HCl twice. Then, the residue was washed with deionized water for three times by centrifugating (9000 rpm) and decanting the supernatant. The obtained suspension was further dialyzed in deionized water at room temperature for 3 days, followed by ultrasonic treatment (Whaledent Biosonic Ultrasonicator, USA) for 20 min and subsequent filtration to remove residual aggregates. Finally, the clear suspension was lyophilized to obtain light brown powders. The yield was about 55%.

2.2. Electrospinning of ChiNC/PDO nanocomposite fiber mats

A desired amount of ChiNC powders were redispersed in deionized water by sonicating for 30 min, followed by solvent-exchanging from water to acetone through centrifugation, and then the pasty ChiNCs were well dispersed in TFE (TCI America Inc., USA) by sonicating for 30 min, finally, a colloidal suspension was obtained; PDO (Ethicon, Inc.) was dissolved in TFE under shaking overnight. The two parts were mixed together and further sonicated for 30 min. The mass ratios of ChiNC filler to PDO were controlled at 0:100 (0 wt%), 5:95 (5 wt%), 10:90 (10 wt%), 15:85 (15 wt%) and 20:80 (20 wt%), and the total concentration of ChiNC and PDO was kept 9 wt%. The mixture was loaded into a 5 cm^3 plastic syringe fitted with 18-gauge blunt-tipped needle, and dispensed at 1 $cm^3\ h^{-1}$ using a syringe pump. The applied potential was fixed at 20 kV, the distance between the needle tip and the grounded mandrel (cylindrical, diameter=2.5 cm) was 15 cm, and mandrel rotation speed was 400 rpm.

2.3. Measurement and characterization

The morphology and dispersibility of ChiNCs were evaluated *via* transmission electron microscopy (TEM). A drop of diluted ChiNC suspension in TFE was cast onto a carbon coated copper grid, slowly evaporated at room temperature, and then observed on a JEM-1230 TEM (JEOL, Japan).

The morphologies of electrospun fiber mats were observed on a JSM-5610LV scanning electron microscope (SEM) (JEOL, Japan). The fiber diameters and distributions were determined by the ImageJ 1.42 software. Means and standard deviations were determined from 80 measurements of fiber diameter. The distribution of ChiNCs in a single fiber was observed under SU8010 field emission scanning electron microscope (FESEM) (Hitachi, Japan). The fiber mat was first embedded into epoxy resin, and then cryo-fractured in liquid nitrogen to obtain a cross section of individual fiber.

Uniaxial tensile tests were performed on a MTS Bionix 200 mechanical testing system instrument (MTS Systems Corp., USA) with a crosshead speed of 10 mm min^{-1}. Samples were cut into“dog-bone” shapes by a die punch (narrowest width of 2.67 mm and gage length of 7.49 mm). The results of tensile modulus, peak stress and break at strain were the average of 5 specimens.

Differential scanning calorimetry (DSC) measurements were performed on a DSC Q20 (TA Instruments, USA). Samples were heated from −40 to 150 °C at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere. Each test was repeated three times and obtained data were averaged.

Thermogravimetric analysis (TGA) was carried on a Q5000IR thermal analyzer (TA Instrument, USA) to investigate the thermal stability of the nanocomposites. The scanning range was 50–550 °C with a heating rate of 10 °C min^{-1} under a nitrogen atmosphere.