Journal of Alloys and Compounds 495 (2010) 592–595

Contents lists available at ScienceDirect

Journal of Alloys and Compounds

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

Chitosan/MWCNT composites prepared by thermal induced phase separation

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ARTICLE INFO

Article history:
Received 5 July 2008
Received in revised form 21 July 2009
Accepted 25 October 2009
Available online 29 October 2009

Keywords:
Composite materials
TIPS technique

ABSTRACT

The development of porous materials for the sustained three-dimensional (3D) growth of cells is of particular interest in regenerative medicine and tissue engineering because they can be potentially tailored to mimic the natural extracellular matrix (ECM) in terms of the structure, chemical composition, and mechanical properties. These materials, called scaffolds, require high interconnected porosity in order to allow the vascularization process. In this work the preparation of Chitosan, a natural biocompatible polymer was used as scaffold and prepared by thermal induced phase separation (TIPS). In order to acquire appropriate characteristics, the influence of several parameters as polymer concentration, quenching temperature and the incorporation of different concentration of multi-walled carbon nanotubes (MWCNT) were investigated. It was found that the mechanical properties of the scaffolds can be tuned by the addition of small amount of MWCNT.

1. Introduction

In recent years, biodegradable polymer based scaffolds have been studied intensively for tissue engineering applications; these materials must be biocompatible, biodegradable and bioactive [1,2]. Furthermore, the biomaterials require suitable mechanical properties for the desired application, interconnect and controllable porosity to direct the cellular growth to support the vascularization of the tissue in growth [3]. Chitosan (CH) is a natural polymer that has generated an enormous interest due to its diverse advantages like its low cost, easy access (by deacetylation of Chitin, for being the second most abundant natural polymer), biocompatibility, antimicrobial activity and its hydrophilic nature facilitates cell adhesion, proliferation, and differentiation [4]. It is soluble in weak acids ($pH < 6.3$) and can be processed in films and porous scaffolds. However, the poor flexibility in the regulation of mechanical properties and the limits of degradation restrict its use. Many studies have reported the fabrication of Chitosan-based hybrid system and composites in order to achieve mechanical properties [5,6]. On the other hand, since carbon nanotubes were discovered by Iijima [7], it has attracted interest due to exceptional physical properties: elastic modulus (>1 TPa), small size, high strength and excellent electronic and thermal properties [8]. In order to improve the mechanical properties, the incorporation of carbon nanotubes into a matrix of polymers has been investigated, however the improvements by CNTs incorporation remain modest with regard to what should be expected from a nanometer-scale reinforcement [9,10]. Biosensors are another field where CNTs–CH have found application due to carbon nanotubes, the molecular scale wires with high electrical conductivity, high chemical stability [11]. The active site being the end of the tubes make CNTs hold excellent properties, so CNT modified solid electrode has attracted much attention [11–13], also Chitosan has been used to decorate CNTs [14]. Conductive three-dimensional CH–MWCNT scaffolds prepared by freeze drying process which could be used for a broad range of biomaterial applications, including those that immobilize biomolecules for biosensing and biofuel cell approaches, were reported by Lau et al. [15]. It was found that the MWCNTs embedded with the Chitosan polymer scaffold provided a conductive integrated electrical network that permitted electron transfer between pore walls and electrode surface. The use of CH–MWCNT composites for applications in tissue engineering were presented by Abarrategi et al. [16] where scaffolds composed of a major fraction of multi-wall carbon nanotubes (MWCNTs, up to 89 wt%) and a minor one of Chitosan, the materials were prepared by a freeze drying technique. TIPS process allows the manipulation of pore size and pore structure by varying the preparation conditions, this technique has been shown to be an excellent way to make microporous membranes as well bulk macroporous materials for diverse applications [17,18]. It is only recently, however, that technique has begun to explore the development of porous 3-D nanocomposite scaffolds for biomedical applications [19,20]. Only a very few manuscripts report the use of TIPS technique to prepare polymer/MWCNT composites. In a recent work TIPS proved to be a successful manu-

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doi:10.1016/j.jallcom.2009.10.205

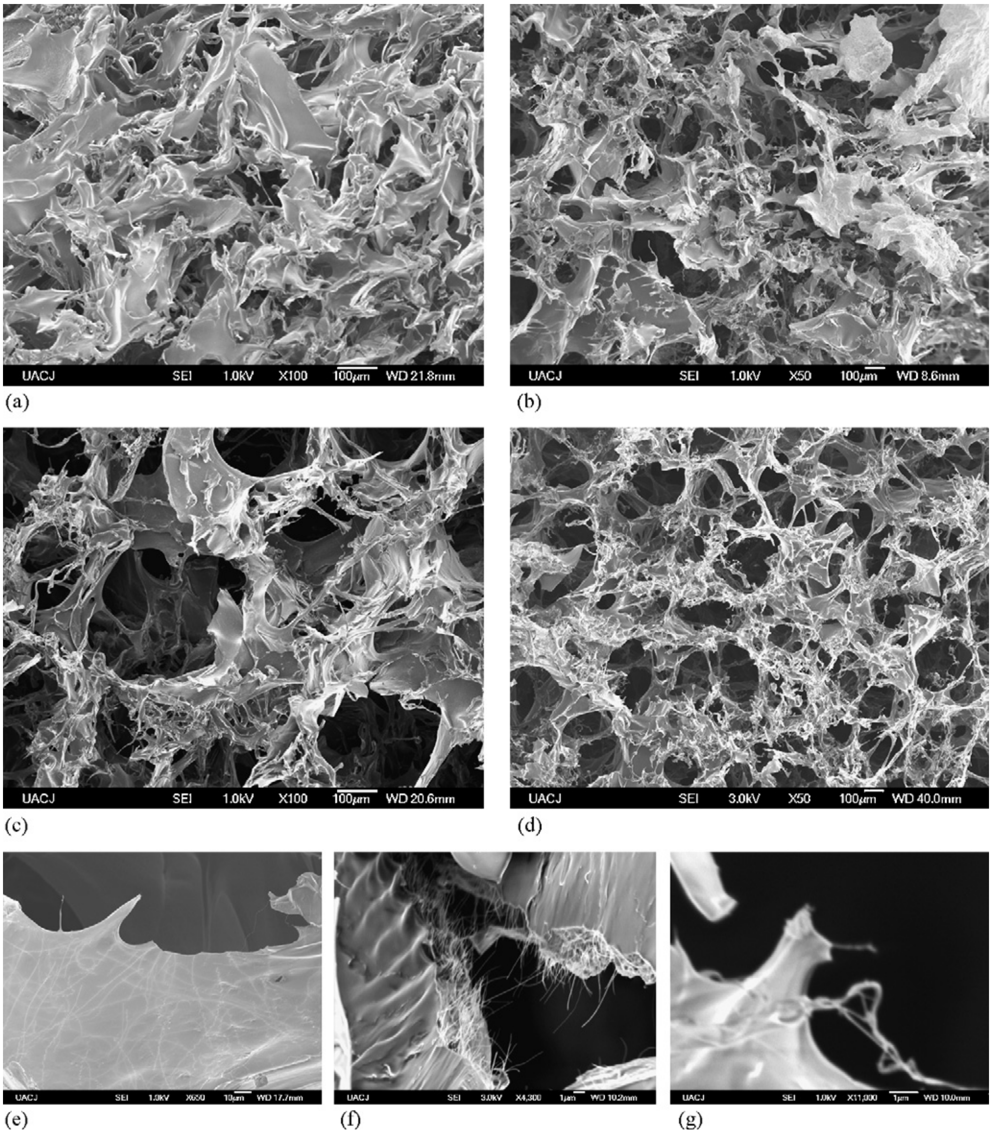

Fig. 1. SEM pictures of Chitosan with MWCNT (a) 0.5 wt%. (b) Magnification of picture (a); (c) 1% wt% and (d) 2 wt% of MWNT.

facturing route to three-dimensional, highly porous polyurethane containing CNT where the mechanical properties were improved with the addition until 5% of CNT, also the composite scaffolds were not only found to be non-toxic but also induce phenotypic changes that may enhance wound healing and bone formation in vivo [21]. In this context, the preparation of 3D porous Chitosan with a small minor fraction of MWCNT by TIPS technique is described.

2. Experimental procedure

2.1. Preparation of the composites

The MWCNTs were fabricated in our laboratory by spray pyrolysis method according to a previous work [22]. Chitosan of high molecular weight was purchased from Carbomer, Inc. (United States) with a degree of deacetylation of 85%. The CNT were treated in a 4 M HNO_3 solution using reflux for 2 h at 100 °C. Then they were stirred at room temperature at 300 rpm. To eliminate HNO_3 and impurities the mixture was washed with distilled water to obtain a pH of 7. The product was dried at 100 °C for 2 h. It was placed with various weight ratios of MWCNTs in 62 mL of 1 M acetic acid solution. The MWCNT was dispersed by sonification for 5 min at room temperature. Afterwards 2 g of Chitosan was added, the mixture was stirred at 1700 rpm to obtain a homogeneous mixture. The mixture was frozen at −40 °C and later the solvent was extracted by a freeze-drying system for 48 h. The composites were neutralized in a aqueous solution of NaOH–CH_3CH_2OH (NaOH was dissolved in 80% ethanol aqueous solution with 0.5 wt% NaOH concentration) at −20 °C for 12 h were immersed in ethanol for 12 h, washed with distilled water until neutrality and dried in a freeze dryer.

2.2. Characterization of the composites

Simultaneous TGA/DSC system (SDT-Q600 TA instruments) was used to study the thermal stability of the composites and Chitosan. For TGA and DSC analysis, the samples were cut in small pieces and heated at constant rate of 10 °C/min under nitrogen atmosphere from room temperature to 310 °C and 750 °C with N_2 flow rate of 20 mL/min. The DSC TA instruments in a nitrogen atmosphere, at heating rate of 10 °C/min were also used. The samples were initially heated to 120 °C to eliminate the thermal history of the sample and then cooled to −75 °C and finally reheated to 120 °C. The infrared spectra were acquired using a infrared spectrometer PerkinElmer model Spectrum GX, using Attenuated total reflectance (ATR) in a SMITHS system, Durasample IRII model, that has a diamond window of 2 mm of diameter. All the samples were scanned within the range 550–4000 cm^{-1}. Morphological characterization was made in a field emission scanning electron microscope Joel JSM7000F. RSA III (Rheometrics Analyzes System) TA was used in a frequency range of 1×10^{-6} to 80 Hz. For each sample the effort–deformation tests were made at 40 °C and a speed of deformation of 0.001 mm/s. Scaffold porosity was determined using a liquid displacement method. Ethanol was used as the displacement liquid because it penetrated easily into the pores and did not induce shrinkage or swelling as a non-solvent of the polymers. A scaffold sample with a volume of 1 mm^3 (V_c) and weight (w_1) was immersed in ethanol with density ρ_e and freeze it at −6 °C for 40 min and weighted (w_2). The volume of the scaffold (V_s), the volume of pores (V_p)

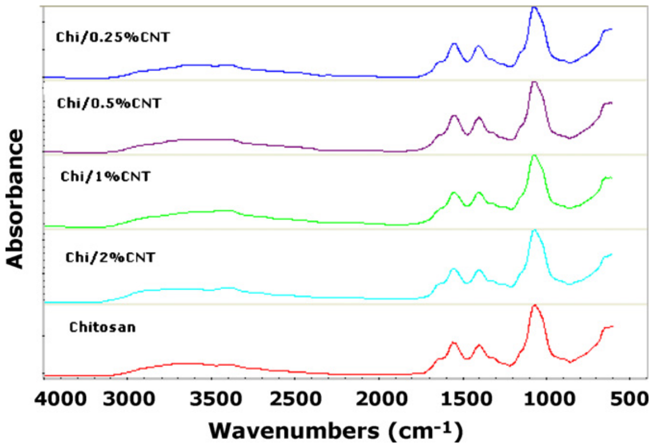

Fig. 2. FTIR spectra of the Chitosan with different content of MWCNT.

that is the same of volume of ethanol (V_e). The porosity of the scaffold was expressed as $P = (1 - V_s/V_c)$; where $V_c = V_s + (w_2 - w_1)/\rho_e$.

3. Results and discussion

Fig. 1a–d shows FE-SEM pictures of Chitosan and composites containing 0.5, 1 and 2 wt% of MWCNT, respectively; a highly porous morphology can be appreciated until 92%; the pore size is between 100 and 300 μm. For scaffold utility, the pore size, porosity and interconnectivity become important parameters for analysis where macroporos between 50 and 300 μm are most useful for cell and tissue ingrowth, and smaller pores contribute to solute diffusion. The porosity and size pore of the CH–MWCNT composites are in the appropriate range for application in tissue engineering. It can be appreciated that CH–2% MWCNTs present pores with better circle form than the scaffolds of pure Chitosan that present pores more elongated. In Fig. 1e–g SEM pictures of the composites at higher magnification are present. CNTs were embedded at the polymer matrix and appeared uniformly distributed throughout the scaffold. The CNT frequently emerged from the pore surface, forming a rough, hairy texture that can be appreciated in Fig. 1f. The alignment of the CNTs to certain directions into the matrix and in the surface perpendicular to it could be attributed to the phase separation during the freeze-drying processes. Even when the CNTs are emerging from the surface, they are coated by Chitosan showing the compatibility between them.

The IR spectra of the Chitosan/MWCNTs at different contents of CNTs are shown in Fig. 2. The typical peaks of the Chitosan that a broad band at 3300 cm^{-1} which is the stretching vibration of N–H and O–H. The peaks at ~1560 and 1410 cm^{-1} can be assigned to the bending vibration of N–H and C–H. The strong peak at 1080 cm^{-1} is due to the stretching vibration of C–O bond. However the main active modes for carbon nanotubes in infrared spectroscopy the A2u and E1u modes that appear around 850 and 1590 cm^{-1} in all CNTs symmetry independently of the diameters were not observed, beyond the small quantities of MWCNT, it could be overlap with the signal due to the N–H vibration due to the fact the CNT are embed and coated by the Chitosan.

In Fig. 3 the TGA results show that the incorporation of MWCNTs affect the thermal stability of the Chitosan. The degradation of the polymer is displaced with the incorporation of MWCNTs but the number of stages in which the thermal degradation occurs is not affected.

The mechanical properties of the composite are affected by the content of MWCNTs as it can be seen in Fig. 4, compressive strength vs. strain where a considerable increment of the strength is observed with the addition of CNT. In general, the compressive stress strain exhibits a linear elastic deformation where the stress sharply increases at small strain, this is attributed to the accommodation of the material into the scaffoldĭs pores. The results exhibit a significant increase in the strength range of the CH/MWCNT composites compared with the pure Chitosan, the compressive modulus was pronouncedly improved with the content of MWCNT as it can be appreciated in Fig. 5. The Young's modulus was increased nearly 100% and 300% with the addition of 0.5 and 1.0 wt% with respect to pure Chitosan. The obtained results are promising and it could be possible with further improve-

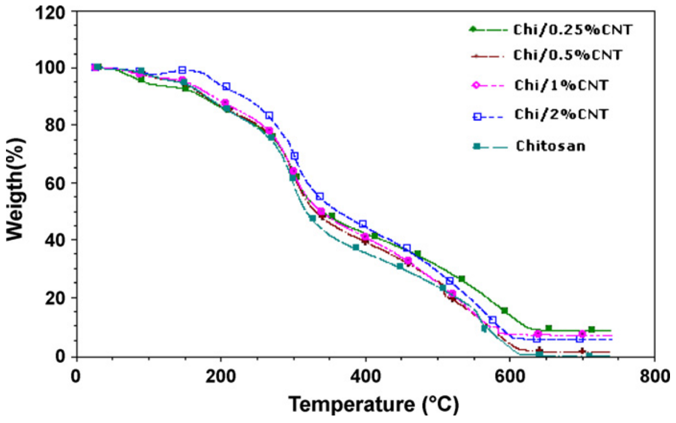

Fig. 3. TGA of the Chitosan with the different content of MWCNT.

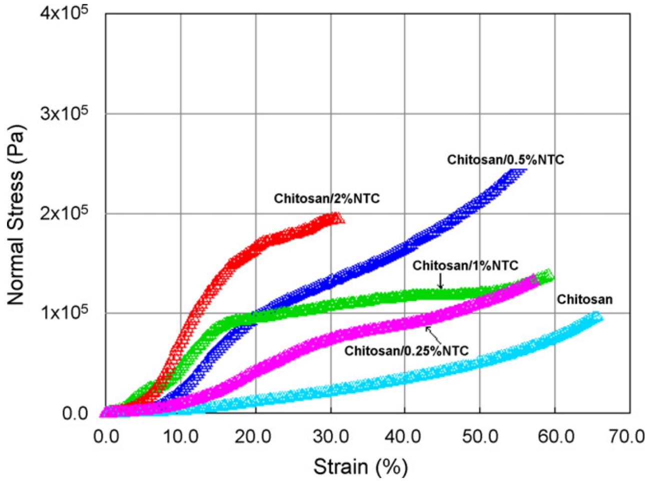

Fig. 4. Compressive stress vs. strain plot of the CH/MWCNT composites.

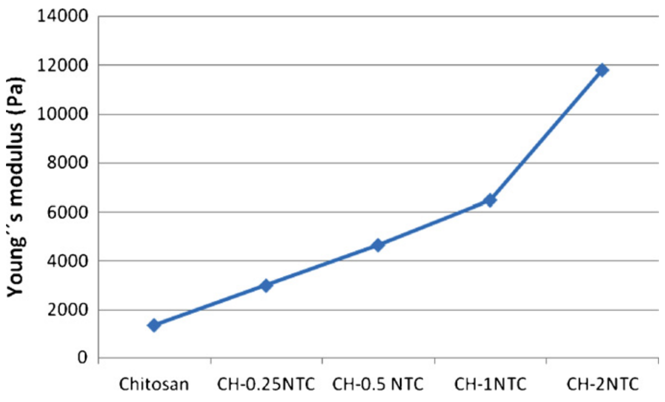

Fig. 5. Variation of the Young's modulus of the Chitosan and Chitosan–MWCNT composites.

ments (as orientation of CNT) to increase the mechanical properties towards the level required for load-bearing implants.

4. Conclusions

In this work, it was demonstrated that is possible to prepare Chitosan/MWCNT by thermal induced phase separation technique. It was found that the mechanical properties of the CH–MWCNT composites were pronouncedly improved compared to the scaffold composed of the pure Chitosan. These composites could be used as scaffold for biomedical applications.

Acknowledgment

The authors want to thank CONACyT México for their financial support.

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