CHITIN NANOFIBROUS THREE-DIMENSIONAL SCAFFOLD PREPARED BY SUPERCRITICAL ANTISOLVENT PRECIPITATION

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Abstract- Chitin, the second most abundant natural polymer after cellulose, is commonly found in the exoskeletons or cuticles of many invertebrates and in the cell walls of most fungi and some algae. Chitin and its derivatives are biodegradable and biocompatible to humans. Chitin is a viable candidate to be used as a scaffold in Tissue Engineering since there are reports of Mesenchymal stem cells that have been seeded into a chitin scaffold showing good compatibility. In this work we intend to prepare a three-dimensional nanofibrous network of chitin biopolymer by means of supercritical carbon dioxide. The supercritical antisolvent (SAS) processed biomaterial may present high porosity and high surface area to volume ratio. In these SAS experiments, temperature and pressure were set to 40 °C and 103.4 bar, respectively. When the chitin/HFIP solution is injected into supercritical carbon dioxide, a fast precipitation of chitin in fiber form occurs. The resultant is a fibrous, white-yellowish, fluffy and sticky material, with an estimated bulk density of about 0.01 g cm^{-3}. We believe that combining the bioactive properties of chitin with the SAS ability for obtaining porous structures, a novel biomaterial can be successful used as scaffold in tissue engineering.

Introduction

Chitin, the second most abundant natural polymer after cellulose, is commonly found in the exoskeletons or cuticles of many invertebrates and in the cell walls of most fungi and some algae. Chitin and its derivatives are biodegradable and biocompatible to humans [1]. Chitin is a viable candidate to be used as a scaffold in Tissue Engineering. There are reports of Mesenchymal stem cells that have been seeded into a three-dimensional nanofibrous scaffold for cartilage repairment [2], and also the use of chitin as scaffold showing good compatibility [3]. In this work we intend to prepare a three-dimensional nanofibrous network of chitin biopolymer by means of supercritical carbon dioxide. The supercritical antisolvent (SAS) processed biomaterial may present high porosity and high surface area to volume ratio. There are only a few solvents that can solubilize chitin, 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was used in this work since it appears to provide solubilization without alteration of the chitin molecular structure, and it is miscible with supercritical carbon dioxide ($scCO_2$).

Experimental

In SAS, a solution is sprayed through a fine nozzle into supercritical carbon dioxide which acts as an antisolvent. The process is operated at conditions in which the solvent (HFIP) and antisolvent ($scCO_2$) are miscible, and solvent has more affinity for antisolvent than solute (chitin), forming a homogeneous phase. Due to the rapid extraction of the solvent from the solution, super-saturation occurs, causing the solute precipitation. After the solute is precipitated, it is further washed with the supercritical antisolvent to remove any residual solvent, and then the system is depressurized for product collection. HFIP from SynQuest Labs (>99% purity) was used after filtered through a 0.2 micron syringe filter. Chitin from crab shells was purchased from Sigma (Practical grade) with 20 mesh size and a 96% degree of acetylation. Liquid carbon dioxide from BOC gases (Grade 5.5) was used as received. Chitin practical grade was purified prior to use. A 2.0 mg/ml of chitin solution in

HFIP was prepared stirring for 48 h and then filtering through a 0.2 micron PTFE syringe filter. A detailed description of the supercritical antisolvent procedure is provided elsewhere [4]. In these SAS experiments, temperature and pressure were set to 40 °C and 103.4 bar, respectively. When the chitin/HFIP solution is injected into supercritical carbon dioxide, a fast precipitation of chitin in fiber form occurs. The resultant is a fibrous, white-yellowish, fluffy and sticky material, with an estimated bulk density of about 0.01 g cm^{-3}. The biomaterial was analyzed using a Phillips SEM (ESEM XL30), and a JEOL 2010-F Transmission Electron Microscope (TEM) equipped with an EDS unit. Samples for SEM were sputter-coated with gold; samples for TEM were dispersed in acetone. XRD analysis was performed using a 2100-Rigaku diffractometer, equipped with the CuK_{α} radiation; and FTIR measurements on a Perkin Elmer spectrophotometer model Spectrum GX, equipped with a KBr beamsplitter and a DTGS detector. Spectra were obtained using an ATR accessory in the range 400-650 cm^{-1}, resolution was set to 4 cm^{-1} and the spectra shown are an average of 32 scans.

Results

SAS processed chitin fibers have an average diameter of 55 microns (Fig. 1A). Each of these fibers is formed of nanofibers (Fig. 1B) which average diameter was found to be 84 nm with standard deviation of 26 nm. The novel three-dimensional nanofibrous network (Fig. 1C) might be a useful scaffold for cell culture. Detailed photographs of a fiber group (Fig. 1D), and a fiber alone (Fig. 1E) were obtained using TEM, and it is possible to observe in these images the open space between fibers evidencing high surface area and porosity.

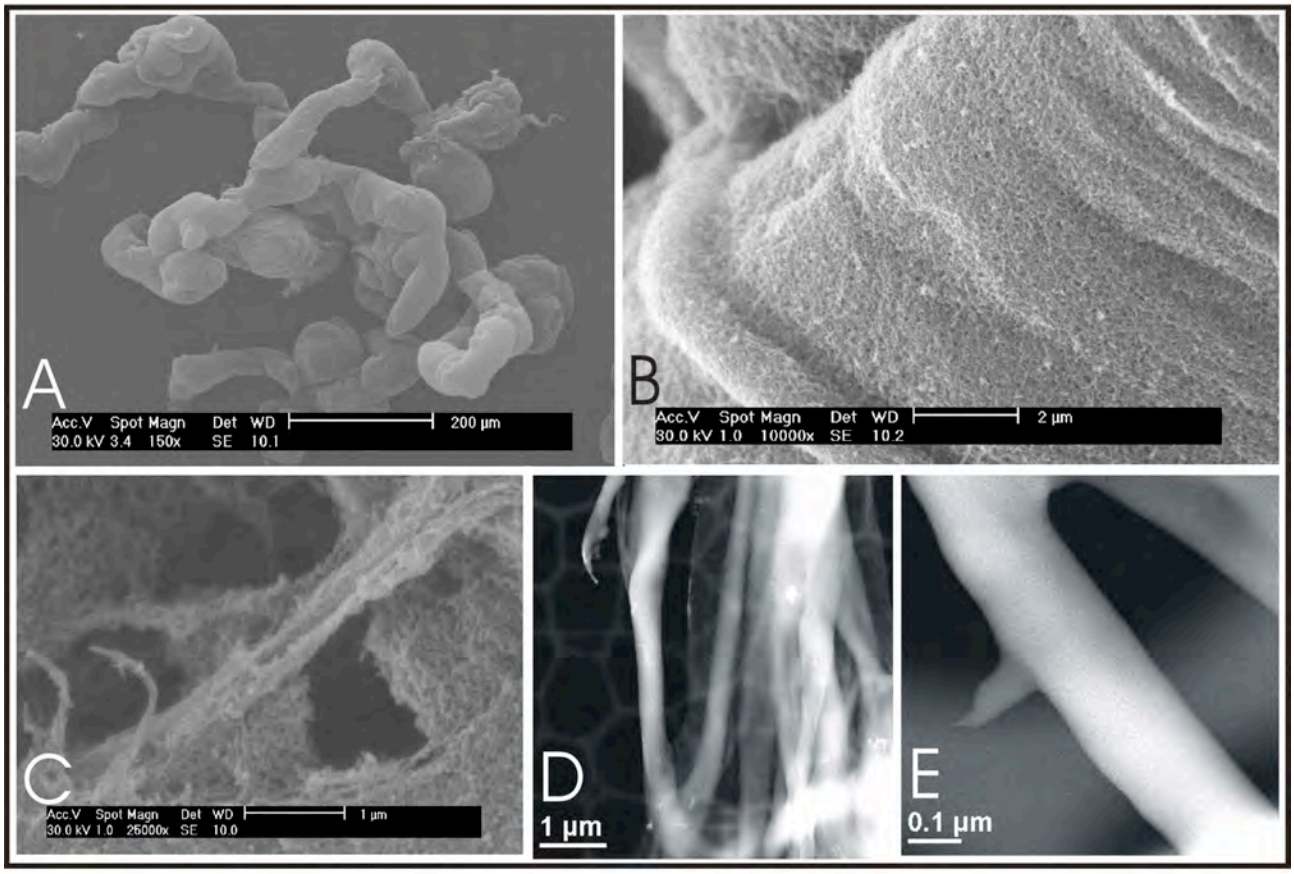

Figure 1. A,B,C are SEM photographs; D,E are TEM photographs. (A) Fiber structure of the biopolymer after SAS process; (B,C) Nanofibrous three-dimensional network. Porosity can be observed in C; (D) Nanofibers arranged in a parallel fashion with open pores between them; and (E) detailed nanofiber.

An important feature of the precipitated nanofibers by supercritical antisolvent process is the change in crystallinity, which can be appreciated in the infrared and x-ray diffraction analysis. The infrared spectra of the purified chitin raw material (solid line) and nanofibrous chitin (dash line) are shown in Figure 2. Purified chitin exists as the α-polymorph, since the infrared spectrum shows the two bands in the Amide I region at around 1660 and 1626 cm^{-1}[5,6]. In contrast, chitin nanofibers do not show these two bands but only one centered at 1639 cm-1; the presence of only one band in the Amide I region is usually related to β-chitin[6], but here is more likely to be related with an amorphous chitin since the x-ray diffraction pattern indicates so.

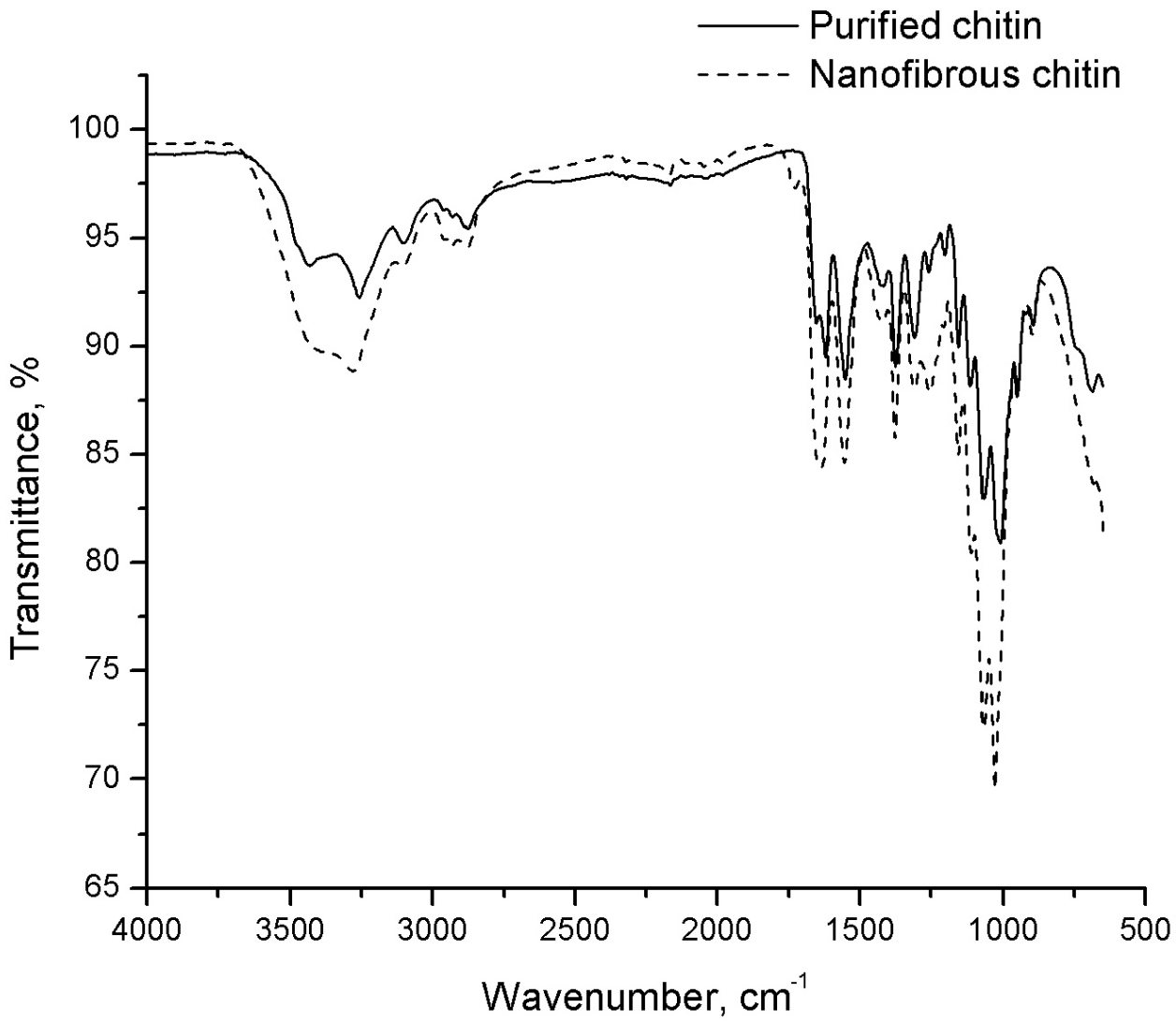

Figure 2 Infrared spectra of purified chitin raw material (solid line) and chitin nanofibers after SAS processing (dash line).

The most common polymorph of chitin is the α-chitin. In Figure 3, the diffraction pattern of purified chitin is shown with well resolved intense peaks at around 9.8 and 19.9 of 2θ degrees, due to the presence of (020) and the mixture of (110) and (040), respectively. Less intense peaks appear at 13.14 and between 20 and 27 of 2θ degrees, owing to the presence of (021), (101), (130), and (013) [7,8]. The diffraction pattern of purified chitin was also compared with the diffraction pattern of a commercial purified chitin (not shown) and they are in good agreement. Supercritical

antisolvent process performs a rapid precipitation of chitin yielding an amorphous fibrous material, as indicated by the low intensity very broad diffraction pattern of Figure 3. The infrared spectra and x-ray diffraction pattern suggest that the a-chitin polymorph is physically transformed into an amorphous chitin with a three-dimensional fibrous structure, which is proposed to be used as scaffold for cell culture in tissue engineering.

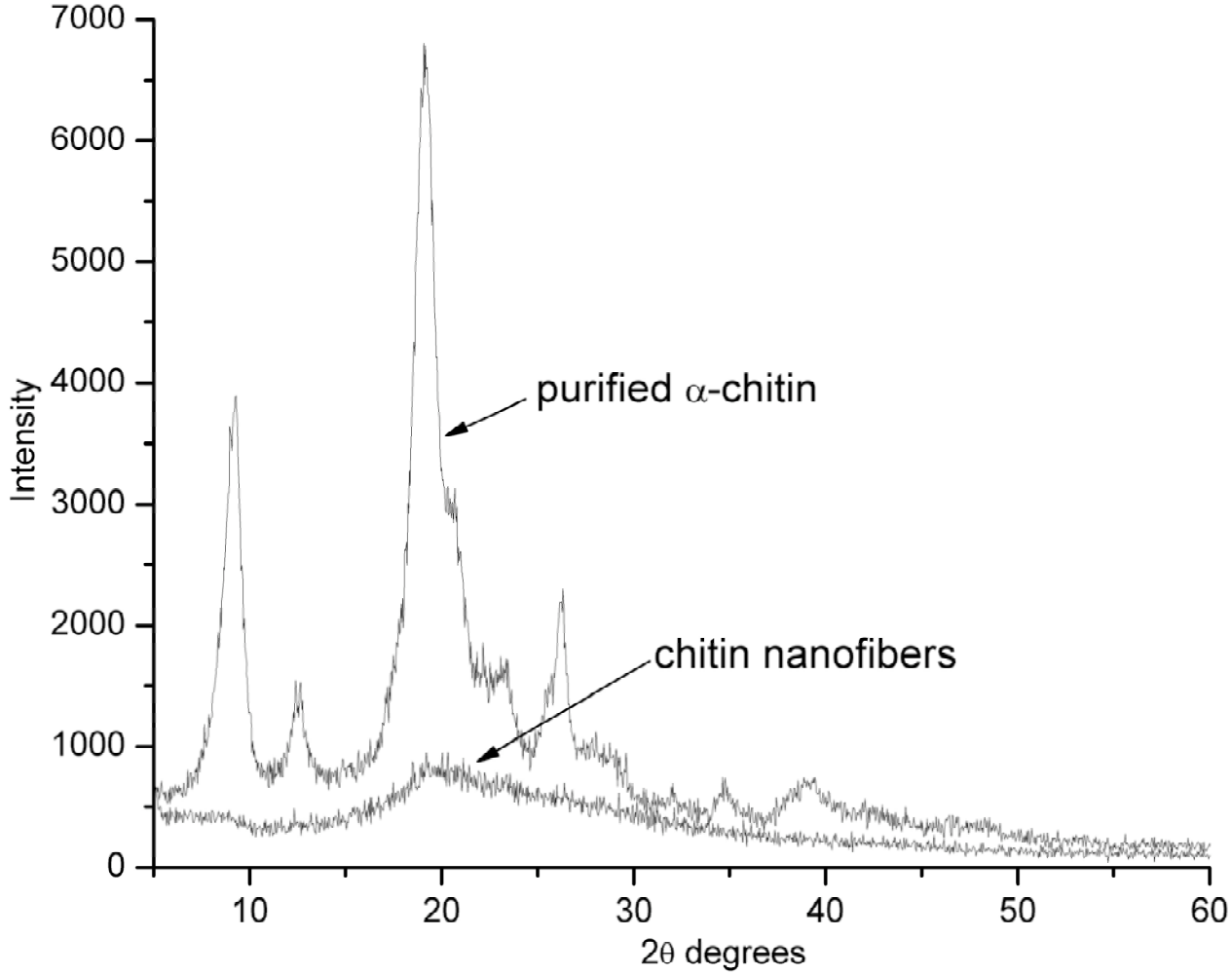

Figure 3 Diffraction patterns of purified α-chitin raw material and chitin nanofibers.

Conclusions

SAS processed chitin fibers are a fibrous, white-yellowish, fluffy and sticky material, with an estimated bulk density of about 0.01 g cm^{-3} and average diameter of 55 microns. The nanofibrous structure have average diameter of 84 nm with standard deviation of 26 nm. An important feature of the precipitated nanofibers by supercritical antisolvent process is the change in crystallinity. The infrared spectra and x-ray diffraction pattern suggest that the α-chitin polymorph is physically transformed into an amorphous chitin with a three-dimensional fibrous structure. It is proposed that, by combining the bioactive properties of chitin with the SAS ability for obtaining porous structures, a novel biomaterial can be successful used as scaffold in tissue engineering.

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References

1. **E. Khor,** *Chitin: Fulfilling a biomaterials promise*, **Elsevier, 2001**.
2. **W-J. Li; R. Tuli; Ch. Okafor; A. Derrfoul; K.G. Danielson; D.J. Hall; R.S. Tuan** *Biomaterials* **2005**, *26*, **599**.
3. **L. Li; J.H.P. Hui; J.Ch.H. Goh; F. Chen; E.H. Lee** *J. Pediatr. Orthop*. **2004**, *24*, **205**.
4. **J.F. Louvier-Hernández, G. Luna-Bárcenas, R. Thakur, R.B. Gupta** *J. Biomed. Nanotech*. **2005**, *1*, **109**.
5. **F.G. Pearson; R.H. Marchessault; C.Y. Liang** *J. Polym. Sci*. **1960**, *43*, **101**.
6. **B. Focher; A. Naggi; G. Torri; A. Cosani; M. Terbojevich** *Carbohydr. Polym.* **1992**, *17*, **97**.
7. **S.S. Kim; S.H. Kim; Y.M. Lee** *J. Polym. Sci. B: Polym. Phys*. **1996,** *34*, **2367**.
8. **C.T. Andrade; K.M.P. Silva; M.I. Tavares; R.A. Simão; C. Achete; C.A. Pérez** *J. Appl. Polym. Sci*. **2002**, *83*, **151**.