CHITIN NANOFIBROUS THREE-DIMENSIONAL SCAFFOLD PREPARED BY SUPERCRITICAL ANTISOLVENT PRECIPITATION. J.F. Louvier-Hernández(1), R.A. Mauricio-Sánchez (1), G.A. Camacho-Bragado(2), G. Luna-Bárcenas (1) and R.B. Gupta(3).
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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 since there are reports of Mesenchymal stem cells that have been seeded into a chitin scaffold showing good compatibility [2]. 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, including N,N-Dimethylacetamide with 5 w/v% LiCl and 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP). The latter 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 ($scCO_2$). 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 [3]. 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}. Fig. 1 shows (A) the chitin flakes raw material, and (B) the SAS processed fibrous chitin. 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. SAS processed chitin fibers have an average diameter of 55 microns (Fig. 2A). Each of these fibers is formed of nanofibers (Fig. 2B) which average diameter was found to be 84 nm with standard deviation of 26 nm. The novel three-dimensional nanofibrous network (Fig. 2C) might be a useful scaffold for cell culture. Detailed photographs of a fiber group (Fig. 2D), and a fiber alone (Fig. 2E) were obtained using TEM, and it is possible to observe in these images the open space between fibers evidencing high surface area and porosity. 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.

References

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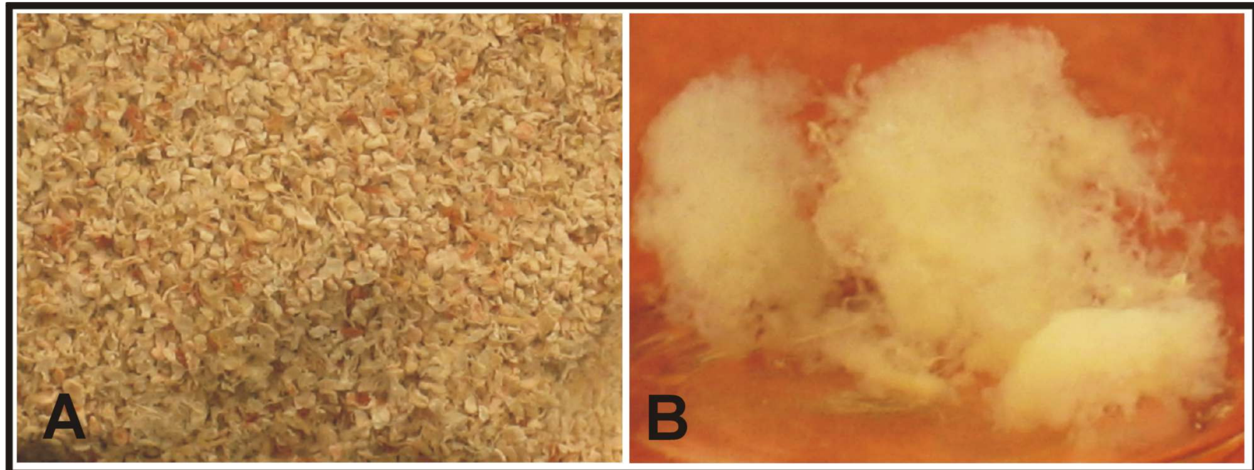

Figure 1. (A) Chitin flakes raw material, and (B) Chitin fibers after SAS processing.

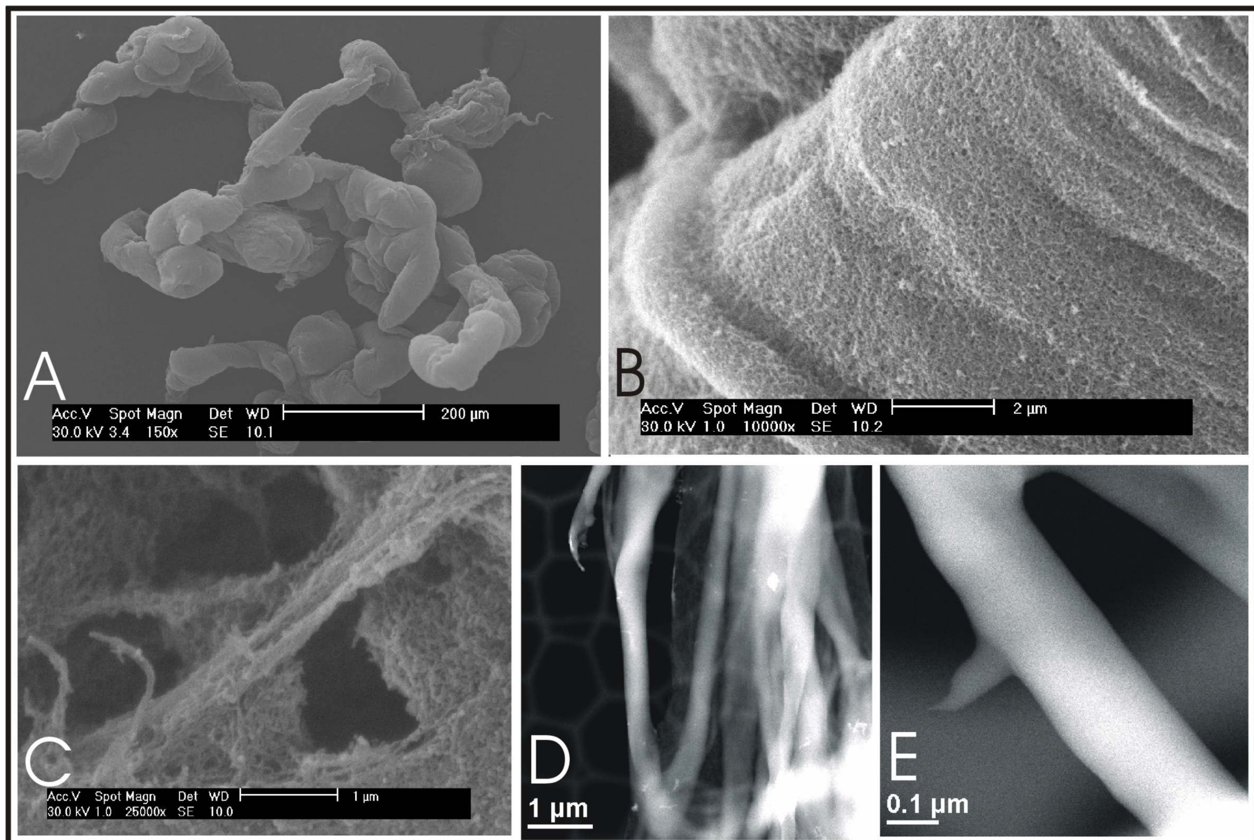

Figure 2. A,B,C are SEM photographs; D,E are TEM photographs. (A) Fiber structure of the biopolymer after SAS process; (B,C) Nanofibrous three-dimensional network. Porosity can be observed in C; (D) Nanofibers arranged in a parallel fashion with open pores between them; and (E) detailed nanofiber.