11th International Conference
on Advanced Materials

ICAM2009

Rio de Janeiro Brazil
September 20 - 25

Influence of sterilization methods on the properties of porous silk fibroin membranes

R. F. Weska[(1)*], A. C. D. Rodas[(2)], G. M. Nogueira[(1)] and M. M. Beppu[(1)]

(1) School of Chemical Engineering, University of Campinas, UNICAMP, Campinas, SP, Brazil, e-mail: weska@feq.unicamp.br
(2) Biotechnology Center, Energy and Nuclear Research Institute, IPEN, Sao Paulo, SP, Brazil
* Corresponding author.

Abstract – The aim of this study was to analyze the influence of different sterilization methods on the physical, chemical and biological characteristics of porous silk fibroin membranes. The membranes were sterilized by ultraviolet radiation, 70% ethanol, autoclave, ethylene oxide gas and gamma radiation, and were analyzed by SEM, FTIR-ATR, XRD and cytotoxicity tests. The results indicated that the sterilization methods didn't cause degradation of the membranes or formation of cytotoxic substances. Before sterilization, the molecular conformation of the porous SF membranes presented a preponderance of β-sheet structures (silk II), that remained unchanged after sterilization.

Silk fibroin (SF), derived from *Bombyx mori*, is a fibrous protein that has been extensively studied for application in the biomedical field, due to its unique biological properties including good biocompatibility, biodegradability, versatility in processing, and minimal inflammatory reaction. Several methods have been developed to manufacture porous SF fibroin scaffolds for application in tissue engineering. However, the methods presented in the literature demand a dialysis step, which usually takes three days for completion. Our group has developed a new method to obtain a porous three-dimensional SF membrane that dispenses the dialysis step, and since sterilization is a fundamental step in biomaterials processing, that must not affect its functionality, the aim of this study was to analyze the influence of different sterilization methods on the physical, chemical and biological characteristics of lyophilized porous SF membranes.

Raw silk fibers of *Bombyx mori* were degummed three times, to remove sericin, in 0.5% Na_2CO_3 solution at 85 °C, for 30 min. Afterwards, the fibroin fibers were washed with deionized water and dried at room temperature. Dried SF fibers were dissolved in a ternary solvent ($CaCl_2$-ethanol-water), at 85 °C for *ca.* 2 h. Porous membranes were obtained from SF solution, through the compression of a material generated by phase separation [1]. The membranes, prepared with a thickness of *ca.* 4 mm, were treated with 70% ethanol solution for 24 h, rinsed with ultrapure water, frozen in ultrafreezer at –80 °C for 24 h and then lyophilized for 24 h.

To evaluate the effects of sterilization, porous SF membranes were submitted to five different sterilization techniques: ethanol 70%, UV radiation, autoclave, ethylene oxide gas and gamma radiation. The sterilization with ethanol was performed by the immersion of the membranes in 70% ethanol solution for 48 h. The sterilization with UV radiation was performed in a laminar flow chamber with UV lamp. Each side of the membranes was irradiated for 30 min. The sterilization in autoclave was performed at 121 °C for 30 min. The membranes were sterilized with ethylene oxide by the exposure to the gas for 4 h at 55°C. After sterilization, three forced aerations with N_2 were performed to remove residues of the gas. Sterilization with gamma radiation was performed by the irradiation of the membranes with 25 kGy. All samples were analyzed by SEM, FTIR-ATR, XRD and cytotoxicity tests.

The results indicated that the sterilization methods didn't cause degradation of the membranes or formation of cytotoxic substances. By SEM analysis, it was possible to observe that the porosity and surface roughness of the porous SF membranes were not altered by sterilization. In FTIR-ATR analysis of non sterilized and sterilized membranes, the silk II molecular conformation (β-sheet) observed in amide I and II peaks at 1622 cm^{-1} and 1517 cm^{-1}, and silk I (α-helix) observed in amide III peak at 1230 cm^{-1} indicated the coexistence of β-sheet and α-helix, with the predominance of β-sheet crystalline structure. These results were in agreement with XRD results, with diffraction peaks at 9.5 °, 20° and 24.5 ° (silk II), and a peak at 28.5 ° (silk I). All sterilization methods preserved the original characteristics of porous SF membranes.

References

[1] M.M. Beppu, B. Polakiewicz, G.M. Nogueira, title of patent, INPI/Brazil PI: 0601975-7 (2006).