Manufacturing of sterile drug-loaded scaffolds in a single-step process

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Introduction: The sterilization treatment of medical devices must ensure a sterility assurance level (SAL-6) against bacterial endospores prior to their usage, according to the current legal framework. This scenario represents a major hurdle in the development and commercialization of new generation biomedical products, including scaffolds (1). Gold-standard sterilization techniques for the processing of biomaterials may not only complex, expensive and hazardous, but also lead to significant morphological and physicochemical modifications in the treated products. Carbon dioxide under supercritical (sc-) conditions incorporating low contents of H_2O_2 is able to inactivate bacterial endospores while preserving the physicochemical properties of the treated biomaterial (2). On the other hand, the sc-foaming technology allows the production of drug-loaded scaffolds in the absence of solvents. By the fine control of the processing parameters, scaffolds with morphological characteristics matching those of the natural bone tissue can be obtained (3). In this work, vancomycin-loaded poly(ε-caprolactone) (PCL) scaffolds were obtained through an integrated sterilization and foaming procedure based on $scCO_2$ technology where mild pressure and temperature conditions along with the addition of H_2O_2 ensured log-6 reduction levels against dry bacterial endospores of biological indicators (*B. stearothermophilus*, *B. pumilus* and *B. atrophaeus*).

Methods: 1200 ppm of hydrogen peroxide were added in liquid form in a high-pressure autoclave (100 mL) containing scaffolds components (PCL, PCL-vancomycin 5 wt.%). The system was heated to 39 ºC and pressurized at 140 bar, maintaining a continuous CO_2 flow for 2.5 h. Finally, the system was depressurized at a constant venting rate of 3 bar/min until atmospheric pressure. The characterization of the scaffolds was performed in physicochemical and morphological terms. Vancomycin release kinetics were evaluated for 14 days in PBS pH 7.4 medium (37 ºC, 100 rpm). On the other hand, the safety and biocompatibility of the scaffolds was *in vitro* and *in ovo* assessed.

Results: The developed method successfully achieved log-6 reduction levels against dry spores of *B. stearothermophilus*, *B. pumilus* and *B. atrophaeus*. These three microorganisms are the biological indicators used in steam and hydrogen peroxide vapor sterilization, radiation sterilization and ethylene oxide or dry heat sterilization, respectively. Sterile PCL scaffolds loaded with vancomycin with a porous architecture in the 100-600 µm range were obtained, which is coherent for their application in bone tissue regeneration. In addition, scaffolds showed good cytocompatibility and a two-stage vancomycin release pattern suitable for the prophylaxis of infections at the grafted area, even those caused by methicillin-resistant *Staphylococcus aureus* (MRSA).

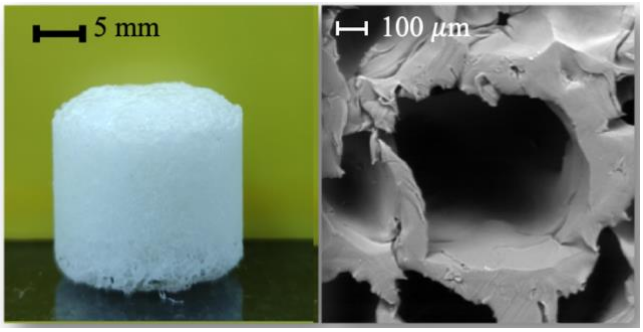

Figure 2. Gross view of sterile PCL scaffolds loaded with vancomycin with a detail of the pore structure processed at 39ºC, 140 bar in presence of 1200 ppm of H_2O_2. Scale bars 5mm (black), 100 µm (white).

Conclusion: A simultaneous sterilization and fabrication method for scaffold development based on the use of $scCO_2$ technology is presented for the first time. Sterile PCL scaffolds loaded with vancomycin were obtained, meeting the morphological requirements to be used as bone grafts substitutes while ensuring a sustained release of the drug for two weeks. In addition, the safety of the manufactured scaffolds was *in ovo* verified.

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References:

1. Ribeiro N. et al. J Biomed Mater Res. 2020; 108B: 399.
2. Santos-Rosales V. et al. Int. J. Pharm. 2021, 605, 120801.
3. Santos-Rosales V. et al. J. Supercrit. Fluids 2020, 166, 105012.