Decellularization of codfish skin by supercritical carbon dioxide for biomedical application

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Abstract

Marine by-products represent a sustainable and underexploited source of biomaterials, namely through the development of extracellular matrix (ECM)-based scaffolds in tissue engineering. Decellularization is a critical step in preparing such scaffolds, aiming to remove cellular components while preserving the biochemical and structural integrity of the native ECM. In this study, codfish (*Gadus morhua*) skin was processed using a supercritical carbon dioxide (scCO) system with ethanol as a co-solvent to explore an eco-friendly, solvent-free alternative to conventional decellularization techniques. The influence of various treatment protocols (time exposure and different depressurization/pressurization cycles) over cell removal and ECM preservation were evaluated through qualitative ((immuno)histochemistry and scanning electron microscopy) and quantitative biochemical assays. Additional analyses, including FTIR-ATR, thermogravimetric analysis, and mechanical testing, provided insight into the structural and functional properties of the resulting matrices. HaCaT keratinocytes were cultured on the matrices up to 5 days to assess biocompatibility. Among the protocols tested, the 3S30 treatment (1 hour + 4 cycles of 30 minutes of pressurization) emerged as the most effective, enabling efficient decellularization while best preserving ECM integrity, with the resulting matrix supporting HaCat adhesion and proliferation. Results suggest this scCO treatment as a promising strategy for marine-derived scaffold fabrication in regenerative medicine.

Keywords: Codfish Skin, Decellularization, Supercritical Fluids

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