Supercritical CO2 Decellularization: A Fast and Residue-free Strategy for ECM Preparation

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Decellularization is essential for producing extracellular matrix (ECM) scaffolds used in regenerative medicine. Conventional detergent-based methods can achieve effective cell removal but often rely on harsh chemicals, generate liquid waste, and require extensive rinsing. This limitation points to the need for a sustainable decellularization approach. Supercritical CO2 (scCO2), the state of CO2 above its critical point (31 °C, 1070 psi), offers a green processing option. In this state, CO2 has both gas-like diffusivity and liquid-like solvating ability: it can penetrate tissues, extract lipids, and then evaporate upon depressurization without leaving chemical residues. This study examined whether these properties could be used, through parameter optimization, to achieve effective and sustainable decellularization. To determine suitable conditions, engineered human dermis tissue was processed under varied parameters. Four variables were evaluated: co-solvent, treatment duration, flow mode, and number of cycles. Decellularization effectiveness was assessed using DAPI fluorescence imaging for nuclear removal, and ECM structural preservation was examined using scanning electron microscopy (SEM). This study identified an optimized scCO2 protocol using a 70% ethanol pre-soak and two 30-minute scCO2 treatment cycles under continuous slow-flow conditions. It achieved effective nuclear removal and preserved native collagen microstructure. The process required about 2.5 hours, compared to conventional methods that often require around 3 days. These results show that optimized scCO2 processing can achieve effective decellularization while preserving ECM structure in a residue-free process, providing a faster and more sustainable approach for ECM preparation.

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