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Title: Effects of freezing/thawing on the mechanical properties of decellularized lungs

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Body: Lung bioengineering based on decellularized organ scaffolds is a potential alternative for transplantation. Freezing/thawing, which is a usual procedure in organ decellularization and storage, could modify the mechanical properties of the lung scaffold and reduce the performance of the bioengineered lung when subjected to the physiological breathing cycles. The aim of this study was to determine the effects of repeated freezing/thawing on the mechanical properties of decellularized lungs in the physiological pressure-volume regime associated with normal ventilation. To this end, the lungs of 15 mice (C57BL/6, female, 17-22 g) were excised and decellularized using a conventional protocol not involving organ freezing and based on sodium dodecyl sulfate detergent. Subsequently, the mechanical properties of the acellular lungs were measured before and after subjecting them to 3 consecutive cycles of freezing/thawing: room temperature, -20°C, -80°C, -20°C and room temperature for each cycle. The resistance (RL) and elastance (EL) of the decellularized lungs were computed by linear regression fitting of the recorded signals (tracheal pressure, flow and volume) during mechanical ventilation (tidal volume = 10 ml/kg, frequency = 60 breath/min, positive end-expiratory pressure = 2 cmH2O). RL was not significantly modified by freezing-thawing: from 0.88±0.37 to 0.90±0.38 cmH2O• s• mL-1 (mean±SE). EL slightly increased from 64.4±11.1 to 73.0±16.3 cmH2O• mL-1 after the three freeze-thaw cycles (p=0.0013). In conclusion, the process of organ freezing/thawing that is commonly used for both organ decellularization and storage induces only minor changes in the ventilation mechanical properties of the organ scaffold.