PP-229

Continuous conductivity measurement method improves decellularization outcome of bioengineered intrathoracic organs

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Objectives

To improve the decellularization outcome of bioengineered intrathoracic organs, by continuous monitoring and evaluation of the detergent and enzymatic fluids.

Materials and Methods

A modified shortened(23 hours)detergent-enzymatic decellularization protocol using sodium deoxycholate 4% and DNAse was used to decellularize 3 native hearts and 3 native lungs of adult male Lewis rats. Real-time and constant monitoring was completed using the Arduino-based development board, custom-made conductivity sensors, ADC breakout (ADS1115 by Texas Instruments), Kubuntu 14.04 (GNU/Linux OS) and bash-scripts. Decellularized scaffolds were evaluated using histological staining and immunochemical characterization for remaining nuclei, extracellular matrix proteins (ECM) after processing with specialized kits.

Results

The method of continuous conductivity measurement is based on the conductivity difference between the original fluids used in the detergent-enzymatic protocol and the complex solution, which is the result of cells' destruction products, mixed with the fluids during decellularization. Data was acquired from a customized sensor using the voltage divider scheme and was measured by the 16-bit ADC (ADS1115). After raw data analysis, a conductivity graph was obtained, which showed rapid rising of the solution conductivity at the beginning, decreased rate of rise in the middle and a plateau during the last few minutes. Data shows an inverse correlation between conductivity and live cells, which indicates that the conductivity increase is the result of rising intracellular ion concentration after the cells' destruction. Once a plateau in conductivity was reached, perfusion was stopped. Decellularized scaffolds were then evaluated with histological staining and immunochemical characterization, which demonstrated that a decreased perfusion time did not adversely affect the quality of decellularization.

Conclusion

Real-time measurements of conductivity during decellularization could be used to evaluate individual decellularization protocols to minimize the time the organ is exposed to the reagants and thus minimize structural damage to the scaffold. A detailed evaluation of scaffold quality is the goal of further investigation.