**Optimization of Decellularization Processes for Renal Structures**

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**Introduction**

Five hundred thousand people in the United States suffer from end stage renal disease (ESRD) annually. Allogeneic transplantation is problematic due to a scarcity of donors and also a high risk of organ rejection. Dialysis treatments are effective but inconvenient and expensive. Tissue-engineered kidneys, which use decellularized scaffolds, reseeded with autologous cells presents a promising solution for obtaining non-immunogenic, transplantable organs from a virtually inexhaustible supply. Proposed decellularization methods require long timesor high flow rates that may damage the native architecture of the extracellular matrix (ECM) and lead to thrombosis upon implantation. Our aim is to optimize the process of decellularization to preserve the integrity of the extracellular matrix for subsequent recellularization and re-implantation.

**Materials and Methods**

Porcine kidneys were harvested at a local abattoir and after initial trimming, tubes were connected to the renal artery. The kidneys were then decellularized by continual perfusion in a bioreactor with a series of “tonic cycles” (alternating from a hypertonic solution to a hypotonic solution and then to sodium dodecyl sulfate (SDS)) or with SDS-only. Flow rate was monitored and increased at a pre-specificed rate, typically 2 RPM every 15 minutes, by a computer. Using SEM imaging, assays, histology and Instron data, samples from native, SDS and tonic cycle tissues were compared to evaluate the viability of the scaffold and determine the optimal decellularization method.

**Results**

Continuous, stepped, antegradeflow of the different “tonic cycles” and SDS-only solutions resulted in a much faster (4-12 hr) decellularization process without damaging the ECM. Tonic cycles proved even milder on the ECM by demonstrating mechanical and DNA quantification similar to SDS, but stood out by exhibiting a significant increase in essential ECM compounds such as GAG's and collagen. Tonic cycles limit the kidneys to a total of only about 4 hour’s worth of 0.05% SDS exposure, thereby limiting the denaturing effects of SDS on ECM proteins.

**Discussion and Conclusions**

Tonic cycle was determined the best at maintaining ECM integrity and therefore the optimal decellularization method. It was also shown that a continuously stepped flow rate significantly accelerated the process without observable damage to the micro structure.

With the optimization afforded by tonic cycles and stepped flow, focus is now being turned to recellularization techniques such as sterilization and cell culturing.