

Optimization Of Human Heart Decellularization Method For Cardiac Regenerative Medicine

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Extracellular matrix (ECM) is an intricate mesh of collagenous and non-collagenous proteins, whose presence and amount vary according to type of tissue. ECM drew the attention of regenerative medicine scientists as natural scaffold suitable for stem cell delivery into damaged tissues. Although a multitude of protocols and combinations of chemical agents and physical methods have been tested and proved effective in the decellularization of human heart, none of the ones tried in our setting fulfilled the goal of obtaining a structurally preserved cardiac decellularized ECM (d-ECM). While testing already described procedures, we made several adjustments that led to the development of a novel, simpler and robust protocol to decellularize adult human heart. Specifically, we decellularized cardiac samples of the free wall of both ventricles of adult human hearts scaled down to fit into embedding cassettes used to avoid stirring stress and preserve structure. To shorten the procedure, a combination of SDS, Triton X-100 and antibiotics was used in simple and fast two-step protocol. After decellularization, d-ECM was fixed and processed for histological study or snap-frozen for molecular biology analysis or cytocompatibility test *in vitro*. Histochemistry and immunohistochemistry confirmed the absence of nuclei and the preservation of architecture and composition of d-ECM. Further, while DNA content in d-ECM was well below accepted standards, sGAG, elastin and growth factors were retained and d-ECM scaffolds supported cardiac primitive cell engraftment and survival *in vitro*. Hence, according to our evidence, our protocol is simple, fast, effective and is worth improving for clinical translation.