

P165. Ultrastructural Analysis Of 3-dimensional Extracellular Matrix Structure Of Different Decellularization Protocols Of Heart Valve Tissues

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OBJECTIVES: Decellularized xenogeneic matrix represents an interesting scaffold material for tissue engineering. Routine HE and Movat pentachrome staining are usually employed to determine remaining cells and structural changes of extracellular matrix (ECM). Objective of this study was to apply two-photon laser scanning microscopy to visualize potential structural ECM disruption on the submicron level.

METHODS: Porcine pulmonary leaflets were harvested and sterilized with antibiotics prior to decellularization with four different protocols using sodium deoxycholate (Group A), sodium dodecylsulfate (Group B), trypsin/EDTA (Group C), and trypsin-detergent-nuclease (Group D) respectively. Decellularized tissues were studied without fixation, embedding and staining using two-photon laser scanning microscopy, or processed with traditional HE and Movat pentachrome staining. Leaflets without any decellularization served as control.

RESULTS: HE-staining revealed no remaining cells in all four groups. Movat pentachrome staining showed no significant differences in the histoarchitecture of ECM in either groups. Application of two-photon laser microscopy (LSM) enabled clear visualization of collagen, elastic fibers on the submicron level. LSM detected significant ECM alterations in Groups B, C and D. with an almost complete preservation in Group A

CONCLUSIONS: Two-photon laser scanning microscopy enabled detection of ultrastructural ECM alterations in decellularization of heart valve tissues. Sodium deoxycholate proved the only protocol to enable a complete cell removal with almost complete preservation of ECM structures.