57. QUALITATIVE EVALUATION OF DETERGENT-ENZYMATIC DECELLULARIZED SMALL-CALIBER BLOOD VESSELS

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Introduction. Despite the obvious advantages of decellularized (DC) arteries as optimal vascular graft material, there are still some technical problems regarding the proper evaluation of the quality of obtained scaffolds. Scanning electron microscopy (SEM) and a series of histochemical stains as hematoxylin and eosin (H&E), Elastica van Gieson, Masson's trichrome, and Movat pentachrome can be used for scaffold characterization. H&E staining is indicated for qualitative assessment of remaining cellular components through the matrix, while SEM allows evaluation of the topology of the DC surface on the nano-scale and estimation of the preservation of the basal lamina integrity, thought to be important for cell adhesion and biocompatibility of scaffolds. Obviously, the balance between keeping the vessel architecture and removal of cellular components is crucial.

Aim of study. To evaluate the efficiency of a combined decellularization approach in cell removal from vascular tissue by H&E stain, and to appreciate its impact on the surface structure by SEM analysis.

Methods and materials. Cryopreserved porcine carotid arteries were treated with detergents for 48h under rotation (24h exposure to 0.5% SDS and 0.5% SDC, followed by 24h exposure to 1% TritonX-100) followed by an enzymatic digestion of DNA (48h exposure to 300 U/ml DNase I). The efficacy of DC and structural integrity preservation were evaluated by H&E stain and SEM. The sample preparation for SEM included the specimens' fixation, dehydration, critical point drying and metal coating.

Results. H&E stain revealed no persisting cells in the study group. SEM analysis demonstrated the luminal surface of carotid arteries was free of structural cellular artefacts after the treatment. In addition, the basal lamina of arteries appeared intact, fragmentation with fibers exposition being detected only within a few areas.

Conclusion. Complex characterization of decellularized scaffolds is mandatory, including qualitative and quantitative analysis of remaining cellular elements, structural evaluation of the matrix, and its biomechanical assessment. Histochemical stains, as H&E, allows to determine the presence of intact nuclei, indicative for whole cells, while SEM permits an overview of the morphology of the luminal surface. Additional assessments, such as immunohistochemical staining for basal lamina proteins (laminin, collagen IV, or fibronectin) can be considered beneficial to ensure that a basal lamina is truly present and can be tested for biocompatibility.