

C21

Autologous Heart Valve Tissue Engineering: Repopulation Of Homograft-Derived Decellularized Matrices With Human Mesenchymal Stem Cells

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Objectives:

Decellularization techniques and tissue engineering technology with autologous bone-marrow mesenchymal stem cells (BM-MSCs) applied to homograft heart valves might importantly contribute to reduce the risks of calcification, inflammation and immune response.

Method:

Human BM-MSCs were isolated by Ficoll-gradient centrifugation from a young volunteer donor's bone marrow, selected for plastic adherence, expanded and characterized with immunofluorescence and FACS analysis. Pulmonary valve leaflets obtained from human cryopreserved homografts (hPVLs) underwent decellularization with Triton X100-sodium cholate (TRICOL) and nuclear fragments removal. Following coating with FBS and fibronectin, decellularized hPVL were seeded with hBM-MSCs (2×10^6 cells/cm²) and statically cultured for 30 days. Standard histology, Masson trichrome and von Kossa staining, immunohistochemistry for mesenchymal stem cell and valve cell markers, as also transmission electron microscopy were performed on engineered hPVLs.

Results:

Immunohistochemical analysis of seeded hPVLs revealed reconstitution of endothelial lining (von Willebrand factor⁺), and repopulation by fibroblasts, myofibroblasts and smooth muscle cells (vimentin⁺, platelet myosin⁺, smooth muscle alpha-actin⁺, SM22⁺ and smooth muscle myosin⁺) similar to the native leaflet. A small amount of seeded cells did not transdifferentiate and maintained stem cell marker expression (SSEA4⁺, OCT4⁺). No signs of calcification were detected. Ultrastructural analysis consistently confirmed proper cell differentiation.

Conclusions:

These data show that TRICOL decellularization allow the creation of biological scaffolds ready to be cell-colonized under static conditions in vitro while hBM-MSCs are able to transdifferentiate into proper valve cell phenotypes, suggesting the feasibility of a novel, autogeneic tissue-engineered heart valve.