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Reconstruction of aortic and pulmonary valves by acellular matrix and circulating stem cells

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Introduction. Tissue-engineered heart valves represent a promising strategy for the treatment of diseases such as valvulopathies, valvular insufficiency and congenital cardiophaties. Constructs obtained using bone marrow stem cells and acellular matrix from valve leaflets are recently tested as optimized tissue substitutes. In this study, we tested in vitro mesenchymal stem cells from minipig peripheral blood to engineer homologous acellular aortic valves.

Materials and methods. Acellular valve matrix. For decellularization, porcine aortic (AVs) and pulmonary valves (PVs) were treated with protease inhibitors for 12 hours at 4°C and then incubated in 0.5M NaCl at 4°C. After washing with detergents, the samples were treated with 10% isopropanol solution. Residual nucleic acids were removed by Benzonase at 37°C for 24h.

Circulating stem cells (CSCs). Isolated from minipig peripheral blood using Ficoll density gradient separation, mononucleate cells were seeded in α-MEM, 16,5% FBS, 1% antibiotic solution. The obtained fibroblastoid populations were characterized by cytometry, differentiation tests and doubling population study.

Seeding of CSCs on valve matrices. Populations with a defined immunophenotype (CD44high, CD106+, CD90low, SLA-DRlow, CD45-, CD34-, CXCR4-) were seeded (6.5x105 cell/cm2) on matrices and cultured in DMEM, 10% FBS, 1%, 1% APS for 35 days using static conditions. At different time points (7, 14, 28, 35 days), the samples were fixed for SEM analysis and genic expression by RT-PCR for collagen III, fibrillin, emilin 1, tenascin C, CD31, caldesmon, MMP 2, MMP13.

Results. The analysis by SEM demonstrated that CSCs cells colonized rapidly the surface of valve matrices and produced continuous monolayers. After 28 days from seeding, the cell morphology was typically elongated on AVs and poliedric on PVs suggesting a different differentiative stimulus induced by the matrices. Consistent with morphological study, RT-PCR showed a specific expression profile of CSCs on acellular valves detecting mRNAs for MMP 2, MMP 13 in AV constructs and for emilin, fibrillin, caldesmon in PVs samples. In both constructs, the cells showed a positive expression for collagen III and tenascin C.

Keywords: Acellular valve matrix, circulating stem cells, tissue-engineered heart valve