Efficient Aortic Valve Cell Seeding into Decellularized Pericardial Membrane: Advancements into Manufacture of Engineered Valve Tissue for the Desing of 'off the shelf', Living, Valve Bioimplants

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Statement of Purpose: Aortic valve stenosis is a widely diffused and inhabilitating pathology, finally resulting in surgical replacement with prosthetic devices. Current implants, either mechanical or biological, have a major limitation in the incapability to grow and self-renew. Despite intense efforts at engineering 'off-the shelf' living valve bioimplants, an optimized scaffold/cell combination providing mechanical performance/stability and durability has still to be found. Thanks to its high availability and compatible mechanical properties, animal pericardium, after aldheyde fixation, is commonly used in valve biological implant manufacture. In this work we exploited pericardial membrane decellularized with a fixative-free method (modifyed from Vinci et al.), to engineer a leaflet-like tissue by seeding the cells naturally deputed to valve tissue homeostasis, the valve interstitial cells (VICs). For the first time, this was performed using a direct perfusion bioreactor system, which allows controlled and reproducible seeding through the whole pericardium .

Methods: Following treatment with hypotonic buffer (Tris-HCl), to induce cell lysis, porcine pericardium was incubated with TritonX-100, to remove adipose tissue, then treated with sodium dodecylsulphate (SDS), to wash out cellular debris and, finally, incubated with DNAse I, to remove nucleic acid material. Tissue decontamination was performed by incubation in antibiotic cocktail, commonly used in graft transplant practice (BASE128). A direct perfusion bioreactor (U-CUP, Cellec), designed to perform seeding and long term culture, was employed to seed porcine VICs (6.5E+5 cell/scaffold) into decellularized pericardium patches (6mm diameter). Dynamic cell seeding efficiency (Day 3) and cell proliferation (Day 7) were evaluated by staining with 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), histological analyses (Hematoxylin & Eosin), nuclear fluorescence staining (DAPI) and immunofluorescence staining for **α**SMA.

Results: DAPI and H&E staining showed complete cell removal and relative tissue integrity after decellularization treatment. Perfusion conditions were set to optimize both cell seeding and culture. This led us to performing the seeding phase for 3 days, at a 3ml/min flow rate, followed by perfusion culture at 0.03ml/min up to 14 days. MTT staining revealed homogeneous cell distribution onto both pericardium sides (Fig.1A). DAPI staining showed an efficient cellularization in the inner layer (Fig.1B). Computer-based cell nuclei counting showed a significant increase in cell number from day 3 to day 7 (Fig.1C), not only at the graft surface (t-test, $p<0.0001$), but also inside the whole patch thickness (t-test, $p<0.0001$). Finally, immunofluorescence staining for **α**SMA revealed a decreased polymerization of this cytoskeleton component over time and in cells growing in the inner layers of the pericardium patches (Fig.2).

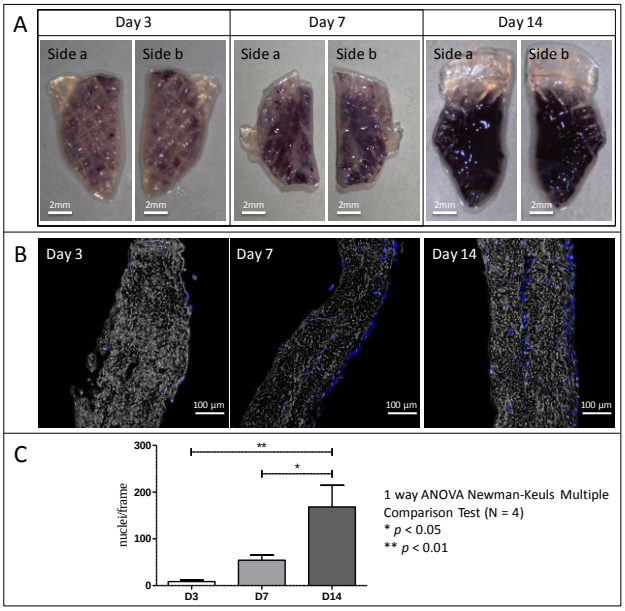

Figure 1. Bioreactor based methods optimizes pericardium grafts cellularization.

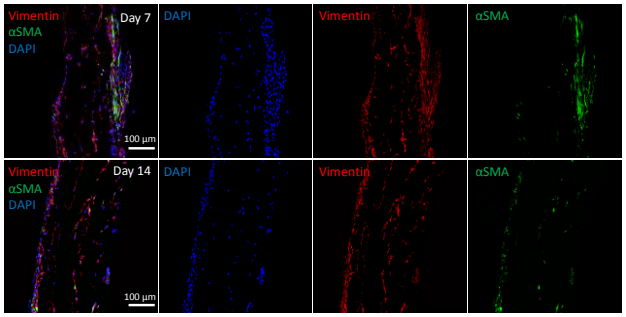

Figure 2. 3D culture into decellularized pericardium supports VICs physiological growth.

Conclusions: Our data show, for the first time, the employment of a direct perfusion bioreactor to seed cell-free pericardium patches with aortic valve-competent cells. While this demonstrates the utility of our approach to favor cell seeding of a the animal-derived pericardium with cells with an unprecedented efficiency, it has also important implications for manufacturing living valve bioimplants with a controllable and reproducible system.

References: Vinci MC, PLoSOne. 2013; 8(5):e64769