SCALING UP OF A PROCEDURE TO PRODUCE 'LIVING' PERICARDIUM MATERIAL FOR PERSONALIZED AORTIC VALVE RECONSTRUCTION

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Introduction

The number of heart valve procedures is expected to triple by 2050 [1], increasing the impact on public health [2]. Mechanical prostheses are still subjected to thromboembolic complications, while biological valves encounter structural deterioration, with recurrence of failure in the mid-long term [2]. Here we introduce the possibility of processing pericardium into a valve. In the present contribution, we describe the results of a small-scale recellularization procedure aiming at obtaining an in-vitro engineered pericardial tissue amenable to adult and pediatric valve surgery and the future experimental program to scale-up recellularization procedure exploiting a custom-designed perfusion bioreactor.

Methods

The porcine pericardium was decellularized with a complete aldehyde and xenoantigen residue-free procedure previously described [3, 4]. Human adipose-derived stem cells (hADSC) were purchased (Lonza, Switzerland). Decellularized pericardial matrices were recellularized under perfusion flow using $0.65x10^6$ cells per bioreactor and a flow-rates of 3 ml/min for the first 72 hours and 0.03 ml/min for additional 18 days. Samples were then harvested and prepared for immunofluorescence staining using α-SMA, Vimentin, and proliferating cell nuclear antigen (PCNA) to evaluate cell phenotype and proliferation. The proteomic assessment was performed after 14 and 21 days of culture. A custom-made bioreactor was designed and prototyped to scale up the procedure using an SLA 3D printer (Form 3B+, Formlabs, USA) with a highly biocompatible resin (Biomed Clear, Formlabs). The system is designed to meet the specifications of a complete standalone bioreactor suitable for clinical employment in a GMP-compliant setting.

Results

Histology sectioning and immunofluorescence analysis of the recellularized samples indicated increased human valvular-like protein production at late stages of culture. These results were confirmed by untargeted proteomic analysis of recellularized tissues that showed a generalized increase in cellular protein content of the tissue construct over time after the beginning of the culture. The expression of α-SMA, one of the pro-pathologic markers for VIC was mainly assessed in cells confined in the surface of the recellularized pericardium but not in cells present in the decellularized matrix. According to PCNA staining, the proliferation rate of the cells penetrating the scaffold was lower than in cells covering the scaffold surface. Conversely, elastin and pro-Collagen I expression was higher in the cells in the inner part of the pericardium.

Discussion

Our results show that culture conditions described here induce a valve-like phenotype in hADSCs colonizing the decellularized scaffold. We are now in the progress of redesigning the perfusion bioreactor to scale up the recellularization procedure and adapt it to a GMP-compliant setting using autologous stem cells to deliver a fully recellularized pericardial tissue amenable for surgical reconstruction of a patient-tailored aortic valve.

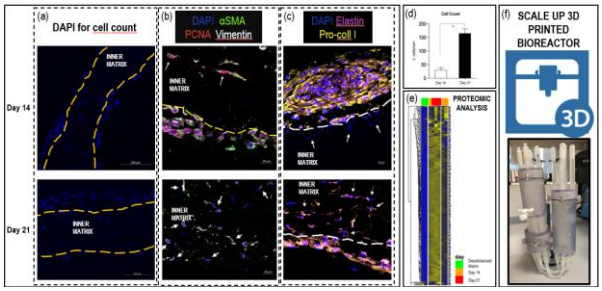

Figure 1: Cell number in the inner part of the scaffold (a,d) denoted a significant increase until day 21, along with protein production (e). Immunofluorescence staining (b) shows reduced α-SMA expression, while vimentin, elastin, and pro-collagen I (c) confirm the pro-physiological valve-like protein production when proliferation (PCNA) (b), slowed down. The scaled-up bioreactor (f) will allow us to recellularize patches suitable to produce aortic valves.

References

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