Extended in-vitro protocols for TEHV : implanted by minimally invasive procedures

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Extended *in-vitro* protocols for TEHV

-Implanted by Minimally Invasive Procedures-

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Introduction

Tissue engineering of heart valves, based on rapid degrading polymer scaffolds, and minimally invasive valve replacement procedures represent promising technologies for patients with valvular heart disease. The successful merging of these novel technologies was demonstrated in a large animal model previously [1]. However, local irregularities and thickening of the TEHV were observed after explantation (Fig. 4), of which the first may be partly due to separation of the leaflets shortly before implantation (Fig.1&2).

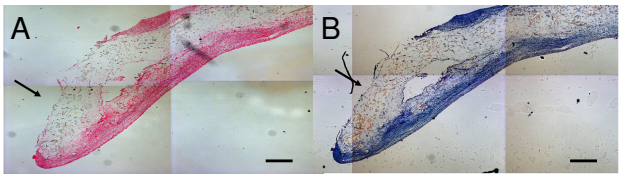

Figure 1: Typical results of H&E (A) and Masson trichrome (B) staining of control leaflets of the previous culture protocol. Separation areas in the leaflets are indicated by arrows. Scale bars represent 500 µm.

We hypothesized that overgrowth of the separation areas before implantation could reduce the *in-vivo* development of irregularities on the leaflets tips. Therefore, we investigated the influence of an advanced *in-vitro* methodology on valve performance.

Figure 2: Separation of the leaflets, crimping of the TEHV, and the TEHV inserted into the implantation device.

Methods

Trileaflet heart valves (n=4, $Ø_{ID}$28mm), based on rapidly degrading polymer scaffolds and self-expandable stents, were engineered from ovine vascular derived autologous cells. Valves were grown *in-vitro* for 13 days, as described previously [1]. To overgrow the separation areas, TEHV were cultured for an additional 6 days after separation of the leaflets utilizing combined strain-flow bioreactor systems (an adaptive version of the diastolic pulse duplicator systems [2]). After crimping (Fig.1) two valves were delivered minimally invasively (mini-thoracotomy, trans-apical approach) in pulmonary position in sheep. Valves were explanted after 4 and 7 weeks.

Results

The advanced culture methodology indeed demonstrated the expected overgrowing of the separation areas (Fig.3).

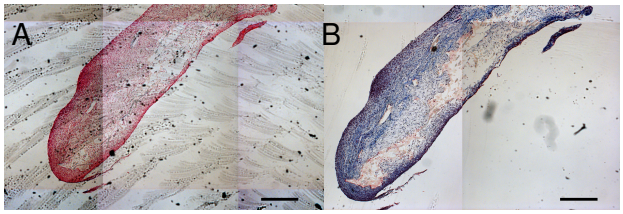

Figure 3: Typical results of H&E (A) and Masson trichrome (B) staining of control leaflets of the advanced culture protocol. Separation areas in the leaflets are overgrown by tissue. Scale bars represent 500 µm.

Moreover, improved *in-vivo* performance (initial pressure gradients were 10 mmHg instead of 20 mmHg) was evident directly after implantation. However, the explant outcome was similar to the previous explants (Fig.4) and excessive remodeling was observed.

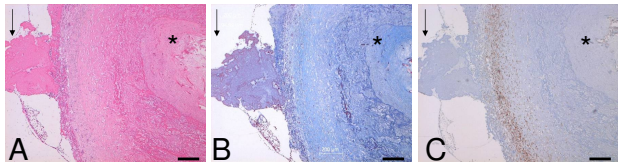

Figure 4: Typical results of H&E (A), Masson trichrome (MT; B), and α-SMA (C) staining of explanted TEHV. H&E staining demonstrates cellular tissue formation (A). MT reveals abundant amounts of collagen (blue, B). α-SMA produced by active fibroblasts is shown in brown (C). Arrows indicate irregularities, stars indicate the implanted tissue. Explants do not reveal large differences independent of the *in-vitro* methodology. Scale bars represent 200 µm.

Discussion

The improved *in-vitro* methodology, to reduce the *in-vivo* thickening response, covered the leaflet tips with a sufficient tissue layer. Moreover, the initial *in-vivo* valve performance was promising. Nevertheless, there was no improvement regarding the excessive remodeling previously found after explantation.

Although representing only a preliminary *in-vivo* study, we conclude that the local irregularities after implantation are not directly related to the separation of the leaflets and further research is necessary. Moreover, extended studies have to be initiated to clarify the *in-vivo* remodeling response.

References

[1] Dijkman PE et al. AHA scientific sessions 2007
[2] Mol A et al., Ann Biomed Eng 2005; 33:1778-88