Minimally Invasive Implantation of Tissue Engineered Living Autologous Self-Expandable Heart Valves in an Animal Model

Benedikt Weber, Dörthe Schmidt, René Stenger, Bernhard Odermatt, Gregor Zund, Simon P. Hoerstrup.
Clinic for Cardiovascular Surgery, Department of Surgical Research, University Hospital, Zurich, Switzerland.

Statement of Purpose:
Minimally invasive procedures represent an attractive concept for the treatment of valvular heart disease and have recently been introduced into clinical practice for implantation in the pulmonary as well as the aortic position. However, currently used valve substitutes for minimally invasive implantation are bioprosthetic, suggesting their clinical application primarily in elderly people and for re-interventions. Tissue engineered heart valves, representing living autologous valvular replacements, have been successfully used in several in vitro as well as in vivo experiments [1-3]. A heart valve tissue engineering concept including minimally invasive techniques for both autologous cell harvest and implantation of the living substitute would overcome these limitations. Therefore, the purpose of the present study was to evaluate the fabrication, minimally invasive implantation and functionality of living autologous tissue engineered heart valves based on sandwich-structured biodegradable scaffolds and adult stem cells in an ovine model.

Methods:
Sheets of non-woven mesh fabricated from biodegradable wet-spun P(L,DL)LA multifilament fibers were moulded into trileaflet heart valve scaffolds using a 3D valve-shaped cast and thermal fixation. The scaffolds were coated with electrospun nanofibers and integrated in self-expanding nitinol stents. Scaffolds were seeded with autologous stem cells derived from the bone marrow and the peripheral blood (n=4). For comparison, implants based on fully differentiated cells isolated from jugular veins were fabricated (n=8). After 6 days of culture time in bioreactors, constructs were minimally invasively implanted as pulmonary valve replacements using an anterolatero-thoracic access and antegrade approach, whereas control valves were analyzed regarding tissue composition, structure and mechanical properties. In-vivo functionality of the implanted constructs was assessed immediately after the procedure and then weekly up to 4 weeks post implantation using transthoracic echocardiography. After explantation, the heart valves were analyzed regarding tissue composition and architecture as well as mechanical properties. The results were compared to the in vitro counterparts and to native valves.

Results:
After seeding and the in vitro culture process, all valves demonstrated macroscopically excellent tissue formation. The tissue engineered heart valves were successfully implanted in all cases, using a minimally invasive approach. Post-implantation echocardiography demonstrated in-vivo valvular functionality, including leaflet movement and sufficient opening and closing behavior. Histology revealed layered neo-tissues, including endothelialized surfaces without indication of endogenous inflammatory reaction.
The amount of collagen and glycosaminoglycans was higher in stem cell-based than in vascular-based tissues while cell numbers were comparable in all valves. Mechanical profiles demonstrated physiological tissue strength but less elasticity independent of the cell source.
Importantly, the scaffold material could withstand the crimping and deployment processes without structural damage or detachment from the nitinol stent. Adult stem cells, which were isolated from the bone marrow as well as peripheral blood, demonstrated growth characteristics comparable to mature vascular cells, resulting in sufficient quantity for seeding at passage three. Bone marrow-derived cells cultured in fibroblast-inducing medium showed a myofibroblast-like phenotype expressing vimentin and α-SMA. Peripheral blood-derived endothelial progenitor cells exposed to endothelial medium formed cobblestone-like monolayers and showed endothelial characteristics similar to previous studies.

Conclusions:
These preliminary results demonstrate that the engineering and minimally invasive implantation of autologous living heart valve replacements is feasible. Furthermore, the use of autologous bone marrow- and blood-derived stem cells enabled a complete minimally invasive heart valve tissue engineering approach. This approach is further strengthened by the application of a new sandwich-structured scaffold that accelerated tissue formation in vitro and did not cause an endogenous granulomatous inflammatory reaction in vivo.
Therefore, the presented autologous tissue engineered concept might realize minimally invasive repair of heart valve defects also in young adults. However, further improvement of the scaffold design, particularly with respect for trans-catheter delivery, is required and has to be investigated in future animal studies.

References:
1. Hoerstrup SP. Circulation. 2000;7;102:III44-9.
2. Hoerstrup SP. Circulation. 2002;24;106:I143-50.
3. Mol A. Circulation. 2006;4;114:I152-8.