Publishable Summary

Project website: http://tue.nl/imavalve

1.1 Summary of project objectives

Valve replacement with mechanical or bioprosthetic prostheses is the most common intervention for valvular disease, with almost 300,000 annual replacements worldwide. There are more and more patients with grown up congenital heart disease (GUCH) who have survived into adulthood thanks to surgical innovations and will require one or more heart valve replacements later in life. Although existing valve prostheses generally have resulted in enhanced survival and quality of life, prosthesis associated problems affect 30-35% of the patients within 10 years post operatively. In particular young recipients of current heart valve replacements have significantly reduced life expectancy (up to 50%) compared to age matched healthy individuals, and experience a high probability of serious valve-related morbidity throughout life. In patients younger than 18 years freedom from reoperation is only 58-68% at 15 years.

Heart valve prosthesis associated complications include thrombo-embolic events requiring lifelong anticoagulation in case of mechanical valves, and limited durability due to calcification and structural failure in case of biological valve substitutes. Prosthetic valves are non-viable structures and, therefore, do not have the ability to grow, repair or adjust to functional demand changes. Living, tissue engineered heart valves are expected to overcome these limitations.

In situ tissue engineering using a biodegradable synthetic scaffold that recruits endogenous cells from the bloodstream is emerging as a promising technology to create living heart valves inside the human body having the potential to last a lifetime. Compared to classical tissue engineered heart valves this new technology demonstrates off-the-shelf availability at substantially reduced cost, logistics and regulatory complexity.
The ImaValve project aims to develop intelligent materials needed for the in-situ engineering of heart valves, to process these materials into a functional heart valve scaffold that can be implanted via a minimally-invasive (transcatheter) implantation technique at the aortic position, and to take all necessary (pre-clinical) steps to enable a first-in-man clinical trial after completion of the project.

A novel approach to the biodegradable scaffold is pursued, that combines a relatively slow degrading elastomeric material with a fast degrading bioactive hydrogel material. These materials are processed into a fibrous heart valve scaffold by means of electrospinning. The elastomeric material ensures long term functionality of the valve while supporting in-vivo mature tissue formation, whereas the fast eroding hydrogel material modulates the early inflammatory host response to the scaffold and creates the necessary void space for cells and neo-tissue formation between the elastomeric fibers.

To meet our goals we will:

i. Develop supramolecular slow degrading (months), durable elastomeric materials and fast eroding (week) hydrogel materials that can be rendered bioactive. Combine these materials into an electrospun heart valve scaffold.

ii. Develop a stent-scaffold combination that is suitable for transcatheter delivery of the heart valve scaffold, and that has sufficient strength and durability to sustain the pulsatile hemodynamic loads.

iii. Develop a mechanistic understanding of the human host response to the scaffold, and the effect of selected bioactives (i.e. TGF-β, MCP-1, SDF-1α) on this host response and subsequent early tissue formation

iv. Achieve sufficient alignment and associated matrix anisotropy of the in-situ deposited collagen to prevent valve leaflet retraction and to attain long-term cell and tissue homeostasis.

v. Demonstrate that the implanted heart valve scaffold in vivo will transform into a functional, living stable heart valve.
1.2 Summary of the work

The achievements of the project (summarized in figure 1 at the end of this document) are described below. Overall the project is running smoothly. Collaborations are open and result-oriented. The results have been delivered on schedule. The consortium takes particular pride in the collaborative development of the first ImaValve prototypes, consisting of slow degrading elastomeric scaffolds sutured in a stent, and the testing thereof in a prototype delivery system, which is a good demonstration of the integrated nature of the consortium.

**Polymer synthesis, processing and testing:** SUPRA designed and synthesized biodegradable UPy polymers based on two biodegradable and biocompatible polymer backbones. Materials were tested on their mechanical and rheological behavior in order to assess their propensity for processing and their compliance to the mechanical restraints of the cardiovascular implant. Promising materials were electrospun into heart valve scaffolds, mounted on a stent from partner MEDIC, and subjected to fatigue testing to assess durability. One class of materials showed superior behavior in all tests. Subsequently, SUPRA further optimized the synthesis with the intention to simplify electrospinning, while keeping the excellent mechanical performance. Future work on the synthesis of UPy polymers will focus on scaling-up and optimizing the synthesis further to be fully applicable to GMP-production standards.

**Incorporation of bioactives into Upy polymers:** With the intention to stimulate M2 macrophage polarization upon monocyte recruitment to the scaffold, TU/e designed a method to bind IL-4 to UPy polymers via its heparin binding domain. Scaffolds spun from PCL-based polymers, functionalized with heparin binding peptide (UPy-HBP), demonstrated high porosity and a significant increase in heparin immobilization compared to non-functionalized controls. In the next period scaffolds will be combined with IL-4 to test macrophage polarization.
**Stent design and stent-scaffold combination:** Using their computer modeling of the stent-scaffolds interface, JENAV optimized their Nitinol stent design to meet all the required objectives for use in the project. In M11 JENAV left the consortium due to internal reorganizations. JENAV granted all access rights to the partners in order to proceed as if they had remained a party. MEDIC proceeds with the stent-related activities and is currently optimizing the mold of the stent.

**Design of delivery catheter:** MEDIC manufactured and successfully tested the Transapical 22Fr delivery system. It was decided however to perform the first in-vivo tests of the valve-sutured-stent with a 24Fr delivery device (which was also fabricated) to get direct input for potential adjustments. After testing, further optimization will be performed in close collaboration with TU/e and UZH.

**Assessment and modulation of early tissue formation:** To modulate and eventually harness the foreign body response to the scaffold, TU/e currently focuses on targeting IL-4 on the scaffold. In-vitro studies confirmed overexpression of M2a macrophages in IL-4 treated cells, favorable of regenerative tissue formation; as opposed to M1 overexpression in IL-10 treated cells. Next, macrophage recruitment and polarization will be studied in scaffolds with and without IL-4 and under physiologically relevant levels of cyclic stretch and flow using a modified mesofluidic system. TU/e further aims to target Notch signaling on the scaffolds to achieve layered tissue formation, mimicking native heart valve leaflets. For this, molecular tools and signalling probes have been developed to follow Notch signalling real time. Preliminary experiments addressing the influence of either flow or stretch on endothelial and vascular smooth muscle cells demonstrate that the Notch signalling pathway is stress sensitive.
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**Cell and tissue homeostasis:** TU/e developed experimental and computational (finite element) models to understand and predict the remodeling of neo-tissue into a mature, functional and stable valve. In particular the aim is to know which mechanical stimulus is driving cell and tissue homeostasis. An in-vitro engineered tissue model was designed and tested to quantify cell traction, tissue pre-stretch, and tissue morphometric and structural changes with time in a growing/remodeling tissue. Finite element modeling is used to guide and interpret experiments and translate data to heart valve morphology. The model includes remodeling behavior of the relevant structures for mechanoresponse at both the cellular (actin) and tissue (collagen) level. Computational simulations of tissue engineered heart valves implanted in either the pulmonary or the aortic position confirmed the experimentally-observed valvular insufficiency due to cellular contractility at the pulmonary side. In aortic position a native-like circumferential collagen organization and normal valve closure.

**Preclinical experiments:** To ensure safe and effective ImaValve delivery into the pulmonary / aortic position the entire procedure using an animal hybrid operation facility was established at UHZ. This includes efficient animal selection, anaesthetic and surgical protocols, and pre-operative scanning protocols suitable to the ImaValve stent sizes. The surgical access was further developed to allow a full minimally, transapical access to deliver the ImaValve for both pulmonary and aortic applications. To ensure the safe and effective delivery, positioning and functionality of the implanted ImaValve UHZ developed standardized, clinically-relevant imaging protocols. A stepwise protocol for the ImaValve delivery was developed adapted to the specific sheep anatomy.

**Regulatory plan:** APPLET developed a first Regulatory roadmap in close collaboration with all partners. It was decided by the consortium to opt for two potential end-products: a non-bioactive ImaValve and a bioactive ImaValve. The roadmap was
adjusted to accommodate both options and classification for both devices was judged as a class III medical device for the non-bioactive ImaValve; and class III medical device combination product in case of added medicinal product in a bioactive ImaValve. Notified body BSI confirmed this classification.

Dissemination and exploitation: Several scientific conferences were attended by the participants and the ImaValve concept was presented at scientific fairs and in public lectures. Of particular interest are the invited presentations at the Heart Valve Society meeting, May, 2015, which is very topical for this project. Because of the early stage of the project, the number of publication is limited. SUPRA presented a first plan of exploitation.
1.3 Expected final results and their potential impact and use.

When successful the ImaValve project will result in a novel off-the-shelf available synthetic heart valve scaffold – suitable for transcatheter delivery via a purposely-designed stent and delivery system – which in-vivo gradually transforms into a living, durable aortic heart valve that lasts a lifetime. In addition, the project aims to provide preclinical proof of concept and a regulatory strategy including preclinical safety evaluation, to receive approval of a clinical trial by the responsible medical ethical committee and competent authorities after the project. Next to this, the project will:

- enhance our knowledge of biodegradable scaffolds and their safety and behavior in-vivo; and will deliver a novel library of degradable elastomeric supramolecular materials of interest to the biomedical field,
- substantially increase our understanding of the (inflammatory) foreign body response to degrading, bioactive materials, relevant to harnessing this response via material design.
- provide novel insights into the relationship between early tissue formation and later stage tissue maturation and organization, in order to guide neo tissue formation and maintain valve mechanical function.
- provide important information about the compliance of novel in-situ tissue engineering solutions with current clinical regulations, guidelines and standards.

Because of the early stage of the project the impact of project outcomes so far is hard to estimate. The potential impacts and implications of ImaValve (end) results include:

**Novel biomaterials:** The materials and material processing technologies developed in ImaValve have wide applicability in the field of regenerative medicine. The biodegradable elastomers are exceptionally versatile; their biomechanical and degradation properties can be tuned, which makes these materials particularly attractive for many tissue regeneration applications. Moreover, these biodegradable elastomeric materials provide independent control of the biomechanical and bioactive properties of the scaffold. Applications include, but are not limited to: (small) diameter vascular substitutes, venous valve replacements to treat peripheral
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artery disease, articular cartilage repair, myocardial regeneration, intervertebral disc regeneration, muscle regeneration, etc. Each of these applications address unmet clinical needs and represent huge market opportunities.

**In-situ heart valve tissue engineering** using synthetic biodegradable materials circumvents the key obstacles of traditional heart valve tissue engineering. The in-situ methodology bypasses the labor intensive, complex and costly cell and bioreactor culture phases (which require a total throughput time of about 8-10 weeks), and has off-the-shelf availability. The intrinsic manufacturing cost of the proposed technology is low. As such, the approach is attractive for commercialization.

**Enhanced European collaboration:** ImaValve combines the unique expertise, experience and infrastructures necessary to achieve the ambitions of the project. This combination of expertise and experience is not available in a single European member state.

**Improved quality of life:** The use of a living valves is expected to drastically alleviate current prosthesis associated problems that affect 30-35% of the patients within 10 years post operatively. In particular young recipients of current heart valve replacements have significantly reduced life expectancy (up to 50%) compared to age matched healthy individuals, and experience a high probability of serious valve-related morbidity (including stroke) throughout life. In patients younger than 18 years freedom from reoperation is only 58-68% at 15 years. The prevalence of aortic stenosis in Europe is 2.5% at the age of 75 years and almost 8% at 85 years. Today, it is estimated that 600,000 individuals in the EU have severe symptomatic aortic stenosis, while only a fraction of these patients is treated. Many of these patients would benefit from a transcatheter implantation of an ImaValve heart valve substitute.
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Figure 1: an overview of work packages and progress

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