

PROJECT FINAL REPORT



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Name of the scientific representative of the project's co-ordinator¹, Title and Organisation:

Prof. Carlijn Bouten

Technische Universiteit Eindhoven

Department of Biomedical Engineering

Gebouw 15, Gemini-Zuid 4.117

Postbus 513, 5600 MB Eindhoven, The Netherlands

Tel : +31 40 247 3006
E-mail : C.V.C.Bouten@tue.nl
Project website address : <http://www.tue.nl/imavalve>

¹ Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

Part I: Final publishable summary report

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Part I: Final publishable summary report

Please note that a more detailed, confidential final report has been uploaded as Deliverable 8.4.

1. Executive Summary

Background: Heart valve disease is considered a substantial health problem worldwide. It can present itself at birth, or be acquired over the course of life in adults. Existing treatments for heart valve replacement have significant drawbacks with respect to durability. Importantly, re-operations are often required due to growth in children or valve failure. In situ tissue engineering has emerged as a promising technology to create living heart valves inside the human body by using a biodegradable synthetic material that has the potential to attract one's own cells. Such living heart valves could last a lifetime: one valve for life. This new technology enables the creation of heart valve prostheses with off-the-shelf availability at significantly reduced costs.

Objective: A European consortium consisting of academic and industrial partners based in the Netherlands, Switzerland and Germany was formed: ImaValve (Intelligent materials for in-situ heart valve tissue engineering). They worked over four years to create a living, human aortic heart valve that can last a lifetime. The valve was designed such that it can be delivered minimally invasively, is available off-the-shelf, has the capacity to grow, remodel and repair, and can serve many patients.

Results: The ImaValve project has resulted in a novel off-the-shelf available synthetic heart valve prosthesis. The material, or scaffold, has the potential to gradually transform *inside the body* into a living, durable aortic heart valve. Notably, this valve prosthesis can be delivered through a minimally invasive transcatheter procedure, eliminating the need for open heart surgery. To this end, a purposely-designed stent and delivery system was developed. The successful ImaValve prototype showed promising functionality as aortic valve replacement in sheep. The project obtained valuable information on the behavior of the scaffold material inside the body of the animals. This can further benefit strategies not only for heart valve regeneration, but for regenerative therapies of other body parts as well.

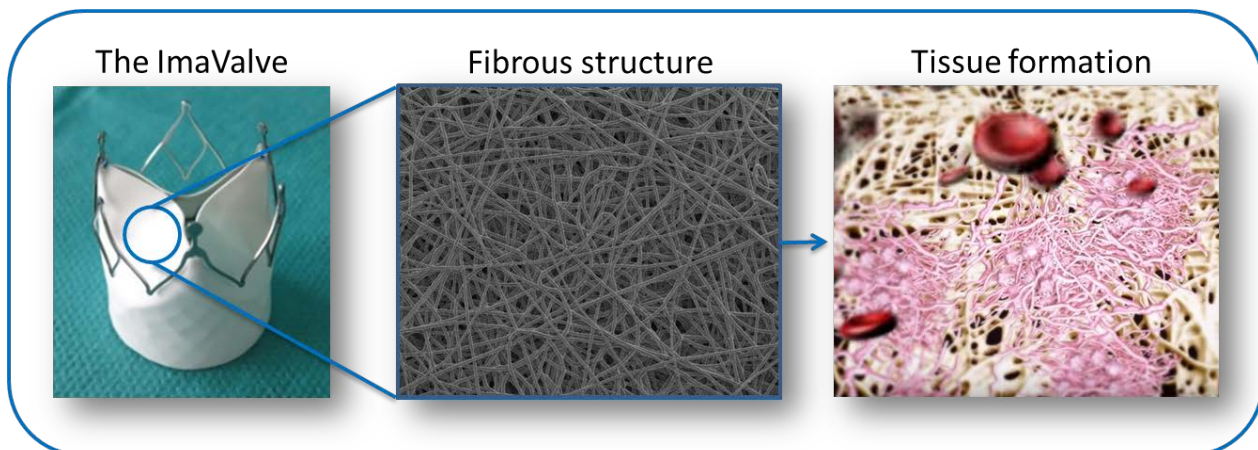
Impact: In addition to advancing scientific and medical knowledge on the topic, this research has brought us one step closer to a living, easily available and usable aortic heart valve. This is expected to improve quality of life and reduce the health burden of heart valve disease by minimizing the number of reoperations needed, as this valve would be implanted once and last for life. In such, the societal and economic benefits are significant.

2. Project context and objectives

Approximately 1% of newborn children have some form of congenital heart disease. All four cardiac valves can be affected by a defect present at birth. In the adult, the prevalence of heart valve disease increases with age. When surgical replacement of a malfunctioning valve is required, the options are generally a man-made mechanical valve, an animal valve (usually derived from pigs), or a human valve following a donor's death. All current solutions have some drawbacks, and require either multiple surgical reoperations or lifelong anticoagulant medication. Indicatively, 300.000 valve replacements are performed every year, and one third of the patients have prosthesis-related complications within ten years. In one third of the children receiving a heart valve prosthesis, a repeat surgery will be required within fifteen years. The ImaValve consortium aimed to address this pressing issue by using a relatively new technology, tissue engineering, that provides an

alternative to current standards by guiding the body to heal itself. In particular, in situ tissue engineering takes advantage of the properties of certain biomaterials to interact with the body and promote tissue healing, regeneration and growth. The aim of this project was to design synthetic materials, thus not naturally occurring, that facilitate the regeneration of a living heart valve. The following objectives were defined:

- i. Develop two types of scaffold materials: one slow resorbable, durable elastic polymer and one fast resorbable, highly hydrated polymer, that can both interact with components of the human body.
- ii. Develop a stent-scaffold combination that is suitable for transcatheter delivery.
- iii. Understand the mechanisms by which the human body responds to the scaffold material, and the effect of selected factors on this host response.
- iv. Achieve the formation of new tissue that closely resembles the original form.
- v. Demonstrate that the implanted scaffold will transform into a functional, living stable heart valve inside the body.
- vi. Indicate the necessary steps and prepare documentation related to safety and regulatory criteria for a first-in-man study after the completion of the project.



Form follows function: This image shows a heart valve prosthesis, for non-invasive surgery, made of a bio-resorbable supramolecular polymer. The polymer has been designed and processed in a way to mimic the architecture of the heart valve on microscopic and macroscopic levels. This way, it provides the ideal scaffold that more accurately recapitulates its function. The magnification reveals the fibrous structure that promotes cell-ingrowth and induces tissue formation.

3. Main S&T results

The consortium has followed two major research lines, namely:

1. The development line towards clinical evaluation of the proposed novel off-the-shelf available synthetic heart valve prosthesis for transcatheter implantation. This line includes the material development and in-vitro testing, the stent and delivery design and development, the preclinical testing, as well as the necessary steps and documentation related to safety and regulatory criteria for a first-in-man study after the completion of the project.
2. The fundamental research line to investigate material driven tissue formation and organization to provide further understanding of relevant processes. This knowledge can benefit further relevant research in tissue engineering.

i. Material development and in vitro testing

Synthetic polymer materials (based on ureido-pyrimidinone (UPy)) that display elastic properties, can be rendered bioactive and can be processed into fibers with diameters in the micrometer range through a technique called electrospinning, have been designed, produced, and characterized. Additionally, highly resorbable, hydrated gel-like materials were designed and characterized as a second material component. Three families of biodegradable and biocompatible UPy-polymers have successfully been developed. This also included the development of protocols for scaling-up the production process under good manufacturing practices (GMP) complying to ISO 13485 standards. Based on extensive mechanical testing and by investigating their electrospinning properties, several polymers have been identified that meet the necessary requirements to be used as a heart-valve material. Based on feedback from the project partners, one polymer was selected and this polymer has been successfully used for all further in-vivo testing. Laboratory testing ensured that both the polymer and the degradation products are biocompatible. Furthermore, a protocol to incorporate bioactive factors has been developed. Finally, a method to fabricate hybrid scaffolds with a tunable shell-core architecture has been developed. The hybrid scaffold has been obtained by coaxial electrospinning of a fast-degrading hydrogel (shell) and a slow degrading elastomer (core). The resulting fiber morphology has been characterized with various microscopic techniques and the mechanical properties and the chemical composition has been successfully determined.

ii. Development of transcatheter aortic valve implantation (TAVI) delivery system

The initial stent design was based on a proprietary design and was appropriately modified following feedback from the initial *in vitro* testing and later *in vivo* trials. The most important developments in the stent design were aimed at improving surgical feasibility of delivery. These included the addition of markers to guide the correct positioning of the valve in the aortic root and fine tuning of the stents' roughness in order to ensure compliance with the leaflet material. With regard to the valve delivery system, the starting prototype was updated in parallel with tuning of the stent, prosthesis and overall surgical procedure. The size of the delivery catheter was selected to ensure smooth pre-operational handling (such as easy and correct valve loading) with surgical requirements of controlled valve release and minimal invasiveness. In order to improve control over valve deployment in the aortic circulation, the inner release mechanism was adapted and surgical handling was taken into account by adding ergonomic features and markers.

iii. Assessment of the early host response to scaffold materials

The effect of the scaffold material and the mechanical loading on cell fate in the early stages after implantation and tissue homeostasis in later stages of tissue formation was evaluated. To this end, we studied the macrophages, a type of cell in the immune system, as a model for early infiltrating cells on the scaffold materials. This cell type could be made pro-regenerative by stimulating with the bioactive factor IL-4. This was achieved either by addition of IL-4 in solution to cell cultures, as well as by immobilizing it on scaffolds. It was found that there is an effect of mechanical loading on the macrophages, which can turn them towards an inflammatory state. Scaffold fiber diameter however did not have a profound effect on macrophage state. Additionally, in order to provide a method to control and understand later tissue formation and the interplay with wall stress, the response of the Notch cell signaling pathway to mechanical cues was studied, and incorporated in a computational model of cardiovascular cell signaling. This model allowed for the prediction of when homeostasis will be reached via phenotypic switching of a cardiovascular tissue under mechanical loading, as a function of the (chosen) tissue geometry. As this phenotypic switching is strain-dependent, this result indicates that the model will most likely be able to also predict the emergence of layer formation in cardiovascular tissues due to the presence of physiological strain gradients. To further study and confirm the underlying mechanisms and the predictions made by the computational model, we developed a heart-valve-on-a-chip microfluidic device. This device allows for the controlled coculture of two valvular cell types under

the hemodynamic loading that will be experienced by the tissue engineered heart valves. Therefore, it was possible to further test these effects in complex culture systems and to understand how cell fate affects and, eventually, could be controlled in tissue formation.

iv. Assessment of growth and remodeling into a heart valve

Experimental and computational models to understand and predict the growth and remodeling of neo-tissues into a mature, functional, and stable heart valve were developed. To ensure long-term functionality of the ImaValve, it is of key importance that it is capable of functional adaptation, similar to the native valve that it will replace. Therefore, 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. With this experimental model, we demonstrated that a geometrically and mechanically stable state could be reached in tissue-engineered heart valves, dependent on the initial scaffold design.

Additionally, numerical models were used to guide and interpret experiments and translate findings to relevant in vivo conditions for heart valves. The models included growth and remodeling behavior of the relevant structures 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. For the aortic position, a native-like circumferential collagen organization and normal valve closure were predicted. With an additional computational model, we demonstrated that growth and remodeling play different roles in preserving mechanical tissue homeostasis with age. Finally, we used our computational framework to improve the design of the ImaValve.

v. Preclinical testing

The ImaValve prototype was successfully loaded into the transcatheter aortic valve implantation (TAVI) delivery system, developed in this project. A proof-of-concept study was performed in sheep in order to assess the intra-operative handling and valve deployment into the pulmonary position. Subsequently, we have pursued a step-wise improvement of valve and stent design to account for the specific geometry of the sheep aortic root, to ensure sufficient leaflet coaptation and cusp mobility, and ensure its functionality immediately upon implantation. The intraoperative handling and valve deployment in the aortic position in a preclinical sheep model confirmed the stable valve functionality under aortic pressure conditions up to six hours. An additional study in a small animal model (rat) evaluated the effect of the inclusion of bioactivity in scaffold materials on the early tissue formation up to 3 months. Encouraging results were obtained which provide a positive outlook for biofunctionalization of the ImaValve in the future.

vi. Regulatory affairs

The regulatory focus was to develop a pure class III medical device. The respective strategy, including the essential requirements and applicable standards, was established. The strategy was continuously scrutinized and revised based on project evolution and changes in the regulatory environment. A template for a Technical File was developed, including a tracking tool and instructions for use, which will serve for clinical trial authorization and marketing authorization (CE marking). The pathway to the First-in-Man study after project completion was mapped out in detail, and a rough study outline was developed.

4. Impact

The scientific, economic, and societal impact of this work will be visible in the short-term as well as long-term. In the developmental line, the successful step-by-step approach that led to the achievement of a functional

design (valve, stent, and delivery system) was demonstrated. The remodelling potential of the material was confirmed in a small animal model, whereas safe, accurate, and repeatable implantation of the ImaValve prosthesis was demonstrated in larger animals. The regenerative potential of this valve may render it a suitable prosthesis for young adults and can become the next-generation valve replacement. The quality of life of valve recipients is anticipated to increase as a functional valve would be provided that does not necessitate reoperations or life-long anticoagulant medication. Additionally, the device is implantable by a minimally invasive procedure and eliminates the need for and potential implications of open heart surgery. Thus, risk and costs are minimized, resulting in economic and societal benefits.

From a scientific viewpoint, this work enhanced our knowledge of the ways materials and the body components can interact with each other. The combination of laboratory work with computational and animal studies resulted in important insights and discoveries that can be extrapolated into further models, and accelerate tissue engineering research. The material platform developed in this work can also be applied in other medical fields that need flexible, biodegradable and easy-to process materials for implants.

This work strengthened collaborations between partners that will result in further cooperation and advancement of goals beyond expiration of current project. Additional funding has been secured, among others with an ERC grant. Finally, this work introduced a novel template for a road map to clinical development and marketing authorization of in-situ tissue engineered products in the EU. As this field currently still lacks proper regulation, this is an important contribution to legislation of novel products that will come forward in the golden future of regenerative medicine. As such, it can provide an opportunity to establish European leadership in the emerging regenerative medicine markets.

The results obtained during this project were disseminated in over 100 presentations (poster & oral) at international scientific and clinical conferences. Additionally, twenty-five manuscripts have been submitted to peer-reviewed scientific journals for publication. Fourteen of these papers have already been accepted for publication in high standard journals, such as Nature's scientific reports, PNAS, Acta biomaterialia and a feature article about the ImaValve technology in International Innovations. Five manuscripts are still under review and some further publications as well as doctoral theses are in preparation. Additionally, the promising results of the project have been presented at, among others, the 50th anniversary of the first heart transplant in Cape Town, South Africa, the Heart Valve Society Scientific Meeting, the Transcatheter Cardiovascular Therapeutics Conference, and the Brandenburg School of Regenerative Therapies Symposium. Apart from the scientific community, the broader public was familiarized with the ImaValve project in over 20 interviews for popular media, from local radio to national television, the consortium's presence at several meetings of Dutch patient organizations, and a public movie (<https://www.youtube.com/watch?v=TWJ7lyZjC3U>).

5. Project website and contact details

The public website is accessible through: <http://www.tue.nl/imavalve>

6. Coordinator contact details:

Prof. Carlijn Bouten
 Eindhoven University of Technology
[Department of Biomedical Engineering](#)
 Building 15, Gemini-Zuid 4.117
 Postbus 513, 5600 MB Eindhoven
 The Netherlands
 Tel: +31 40 247 3006
 E-mail: C.V.C.Bouten@tue.nl

7. List of Beneficiaries

Table 1. The ImaValve project consortium

Beneficiary name	Short name	Principal investigator	Country	Contact details
Technische Universiteit Eindhoven	TUE	Carlijn Bouten	The Netherlands	c.v.c.bouten@tue.nl
Suprapolix BV	SUPRA	Tonny Bosman	The Netherlands	bosman@suprapolix.com
Universitaet Zurich	UZH	Maximilian Emmert	Switzerland	Maximilian.Emmert@usz.ch
JenaValve Technology GmbH	JENAV	n.a.	Germany	n.a.
MediCut	MEDIC	Aryan Fallahi	Germany	A.Fallahi@medicut.de
Appletree CI Group AG	APPLET	Georg Mathis	Switzerland	g.mathis@appletree-cig.com
Xeltis BV	XELTIS	Martijn Cox	The Netherlands	martijn.cox@xeltis.com

8. Project logo

