



Contract no.: NMP3-CT-2005-013633

Project acronym: BioSys

Project full title: Intelligent Biomaterial Systems for Cardiovascular Tissue  
Repair

Specific Targeted Research Project

Thematic Priority: 3 – NMP - Nanotechnologies and nano-science, knowledge-based  
multifunctional materials and new production processes and devices

### **Publishable Summary**

**Period covered:** from 1<sup>st</sup> January 2005 to 30<sup>st</sup> June 2008  
**Date of preparation:** 30. June 2008

**Start date of project:** 1<sup>st</sup> January 2005  
**Duration:** 42 months

**Project coordinator name:** Rheinisch-Westfälische Technische Hochschule Aachen, RWTH Aachen University  
**Revision:**



---

## Table of Contents

1. SUMMARY ..... 2





## 1. Summary

The work contents of workpackage 1, fibre production and optimisation, are mainly carried out at Tampere University of Technology (TUT) at the Institute of Fibre Materials Science (TUT-FMS) and the Institute for Biomaterials (TUT-BIOM). TUT-FMS produced wet-spun and electro-spun fibres and TUT-BIOM produced melt-spun fibres from PLA. The medical, mechanical and structural properties provided the key requirements for the fibres produced by TUT-FMS and TUT-BIOM.

At TUT-BIOM the aim of the study was to produce as fine fibres as possible to be used in the textile and device manufacturing in this project. Fairly fine fibres were produced, characterised and analysed. Due to their greater strength and strength retention ability compared to the wet spun fibres they were used as constructive fibres for the cardiovascular graft.

At TUT-FMS, the study of wet-spun fibres started by solubility experiments of PLA. The solvents and coagulants were selected for the spinning experiments. Wet-spun fibres with different fibre properties were produced. Fibres were analysed and characterised. Fibre modification by adding bio molecule into the fibres has been studied as well.

Electrospinning was used to coat moulded 3D heart valve implants made from non-woven mesh. Electrospun nanofibre structure contains interconnected pores, which are expected to initiate cell in-growth. Implants were sterilized with gamma irradiation.

The scope of Workpackage 2 was the development and design of textile structures for vascular graft scaffolds as well as heart valve. Warp knitting technology was applied for the development of three dimensional scaffolds for tissue engineering of vascular grafts and heart valves. Nonwoven manufacturing technologies (for micro and nano fibre) were applied for producing composite scaffolds for heart valves. For selection of the most suitable textile structure for further processing into tissue engineered implants, characterisation of the textile structures was essential. By characterizing the textile scaffolds, the mechanical performance (stress–strain behaviour) and the tissue engineering performance (porosity) of the structures were tested and compared.



Workpackage 2 was led by RWTH Aachen University - Institut für Textiltechnik (RWTH-ITA). 20 textile scaffold prototypes with different designs were manufactured and tested during the project. 8 prototypes were warp-knitted textile structures for the vascular graft scaffold, 10 different types of non woven structures were made for the heart valve scaffold and 2 prototypes by using the warp-knitting technology. The structuring tests showed the melt spun fibres to be the most suitable material for designing the warp-knitted textile scaffolds and the wet spun fibres for the non-woven scaffolds. In order to process the melt spun fibres to suitable warp knitted structures, several machine modifications have been accomplished.

Characterization of the non woven structures was accomplished regarding fibre orientation pore area, pore area distribution as well as mechanical characteristics. Characterization of the warp knitted structures was accomplished regarding pore diameter, pore diameter distribution and mechanical characteristics.

One aspect of the fibre optimisation involved examination of the cell response. To this end, the cytotoxicity of both the melt-spun and solution-spun fibres, created by TUT, was examined in work package 3 by the team at the NCBES, where it was revealed that neither fabrication method affected cell metabolic activity or cell proliferation. Thereafter, the same methodology was employed to examine the woven and non-woven textiles produced by the team at RWTH in Aachen. As seen in the case of the fibres, the textiles did not elicit a cytotoxic response either. Subsequently, cell-seeded PLA textile/fibrin gel composite scaffolds were produced. Using a range of molecular biology techniques including RT-PCR and microarray technology, it was revealed that the presence of the textiles did not adversely affect the gene expression of smooth muscle cells and promoted the formation of collagen and elastin, key components of blood vessel formation. Furthermore, when the composite was seeded with human endothelial cells, there was evidence to suggest that the composite material promoted angiogenesis. Taken together, the results obtained in WP 3 regarding the cell response, mechanical properties and degradation profile suggest that the BioSys composite has tremendous potential for tissue engineered cardiovascular applications.

The main aim of WP 4 was to examine the drug elution profile of a range of model bio molecules from the fibrin gel and PLA fibre materials. Initially, a methodology was developed for loading the drugs and examining the drug distribution profile in both materials.



Upon optimisation of the drug distribution in the fibres, a novel method of drug loading was developed for wet-spun fibres. The team at the NCBES developed the method and the drug-loaded fibres were fabricated by solution spinning at TUT. Subsequently, the drug elution profile was examined, where it was demonstrated that drugs could be homogeneously distributed in solution-spun fibres and released in a controlled manner in a simulated physiological environment. The results of which have resulted in a patent application for the NCBES and TUT.

Workpackage 5 dealt with the implementation of the novel developed scaffold systems into cardiovascular implants. ACT-Lab followed the development of a small-calibre vascular graft, whereas USZ was responsible for the implementation into a heart valve prosthesis. Both partners, ACT-Lab and USZ, demonstrated the feasibility of tissue engineered cardiovascular implants based on the novel developed scaffold structures and have proved the feasible use in the large animal model sheep by the implantation of the small calibre vascular graft into the carotid artery and in the tissue engineered heart valve into the pulmonary position.

Based on the requirements for a heart valve scaffold design including sandwich-like structured heart valve leaflets in the first year a new scaffold material based on PLDL nanofibers for cardiovascular application was designed. In order to implement the newly developed structure into the heart valve tissue engineering process in vitro experiments were performed.

As a first step the different components of the sandwich-like structured heart valve leaflets, namely a cell carrier and a mechanical support were investigated. Therefore, the new textile material HV-NW-1 that was fabricated at RWTH-ITA from electrospun fibers (w024c150f19) produced by TUT was modified resulting in four versions of a non-woven textile material HV-NW-5 differing in porosity. Cell carriers were fabricated from all four versions and seeded with human saphenous vein-derived myofibroblasts and cultured for 6 days. When analyzing the samples with particular respect to cell ingrowth, cell distribution and cell proliferation capabilities as well as to the production of extracellular matrix elements results suggested HV-NW-5 version B as the most appropriate scaffold material for heart valve tissue engineering (see first and second year's reports).



As a next step for the implementation of the newly developed material into the heart valve tissue engineering process and with respect to following in vivo studies the impact of different sterilization methods including H<sub>2</sub>O<sub>2</sub> based plasma sterilization, ethanol sterilization and ethylenoxide sterilization was investigated. All applied sterilization methods resulted in shrinkage or changes of the material. In contrast, after gamma sterilization (evaluated by TUT) materials preserved structure and shape. Therefore, in all following studies materials were sterilized using gamma-irradiation (see midterm report).

Based on these obtained results complete sandwich-like structured heart valve scaffolds were fabricated from non-woven meshes (RWTH-ITA) coated with electrospun nanofibers (TUT). For evaluation of these prototypes in vitro ovine jugular vein-derived cells were seeded onto the scaffolds and constructs were cultured in a biomimetic system or statically. After 14 days in vitro culture time, heart valves were analyzed as to tissue formation and tissue composition. Results demonstrated better cell ingrowth and increased tissue formation after conditioning in the biomimetic system. Moreover, when leaflets were coated with electrospun fibers higher extracellular matrix production could be observed compared to non-coated valves.

As the last step sandwich-like structured heart valve scaffolds were investigated as to their functionality in vivo. Therefore, cells were harvested from sheep, isolated and proliferated. Scaffolds were seeded with autologous ovine cells and cultured in a biomimetic flow system. After 9 days, valves were removed from the biomimetic system and coated with autologous endothelial cells. After an addition of 3 days, a part of the generated heart valves was analyzed directly, whereas the other part was implanted. The in vivo functionality was monitored by echocardiography up to 4 weeks. Initially good performance could be observed. However, some of the valves demonstrated thickening of the leaflets over time resulting in a limited functionality. Analysis of the explanted valves and control valves revealed cell attachment and ingrowth into the scaffold material resulting in layered tissues with endothelialized, eNOS positive surfaces. Furthermore, good extracellular matrix production with amounts of glycoaminoglycans and cell number comparable to native tissues could be detected. When evaluating the mechanical properties by tensile tests mechanical profiles demonstrated physiological tissue strength but less elasticity. Moreover, the tensile strain at max. tensile stress and the max. tensile stress demonstrated higher values for tissue



engineered heart valves compared to the pure scaffold material indicating that the mechanical properties are mainly from the newly produced tissues.

In conclusions, these experiments demonstrate that the fabrication of a sandwich-like structured heart valve scaffold based on PLDL is feasible and indicate the potential of the newly developed material. Combining the new composite scaffold with cells and biomimetic culture conditions living heart valves can be generated. Thus, the sandwich-like structured biomaterial developed in this project might be an attractive alternative to the currently used heart valve scaffolds. However, as some of the tissue engineered heart valves demonstrated limited functionality in vivo further improvements are required before heart valves based on this technology could be implemented as functional prosthesis into clinical routine.

The work performed in Workpackages 1 – 5 was supported by the project management workpackage (WP 0). This workpackage is mainly carried out by 3T TextilTechnologieTransfer GmbH (3T). The objectives of project management include the intellectual property management, the establishment of a consortium agreement, the responsibility for communication within the consortium, the controlling of project advance and time schedules, the review of milestones and deliverables and the organization of regular reports and generation of special reports such as mid-term report, summary report or exploitation report.