

# PROJECT FINAL REPORT

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## 4.1 Final publishable summary report

### Executive summary

Congenital and acquired diseases of the heart, such as heart valve deterioration, large artery dysfunction and coronary artery blockage, are the leading causes of morbidity and mortality in Europe today, being the first cause of death in people above 60 years old. Successful treatment of cardiovascular disease is limited in many situations by the lack of suitable autologous tissue to restore injured cardiac muscle and heart valves or to serve as vascular conduits to replace or bypass diseased vessels. In cases in which autologous material is lacking, synthetic grafts may be used. However, when compared to native tissues, available prostheses still present disadvantages. Considering that by 2035 one person over two will be over 65 years old, it is obvious to understand that there is a great clinical and market interest in developing new therapies to regenerate cardiovascular tissues.

The development of new therapies for treating cardiovascular diseases was the topic of BIOSCENT, an European research project conducted by a Consortium of regenerative medicine and tissue engineering experts, which aimed to develop “*BIOactive highly porous and injectable Scaffolds controlling stem cell recruitment, proliferation and differentiation and enabling angiogenesis for Cardiovascular Engineered Tissues*”.

BIOSCENT was a 5-years large-scale collaborative project, co-funded under the EU Seventh Framework Programme, that involved both industrial and research institutions, to ensure that the projects objectives were met. The Consortium was composed by 15 partners (11 research institutions, 1 LE, 3 SME), including: University of Pisa (UniPI, IT), coordinator of the project in the person of Dr. Elisabetta Rosellini ([elisabetta.rosellini@diccism.unipi.it](mailto:elisabetta.rosellini@diccism.unipi.it)); Czech Academy of Experimental Medicine (CzAS, CZ); Petru Poni Institute of Macromolecular Chemistry (PPI, RO); Pera Innovation (PERA, UK)/Technologia Avanzadas Inspralia (ITAV, ES); University of Manchester (UniMA, UK); Politecnico of Torino (PoliTO, IT); Hannover Medical School (MHH, DE); Hubrecht Laboratory (HubL, NL); University of Parma (UniPAR, IT); IMM Research (IMM, FR); University of Erlangen-Nuremberg (UniEN, DE); Chempilots (CHEM, DK); Cambridge Research Biochemicals (CRB, UK), Immunological and Biochemical test systems (IBT, DE); Sorin Group Italia (SORIN, IT).

The overall objective of the project was the development of innovative multifunctional polymeric scaffolds that thanks to their bioactivity are able to guide cardiovascular tissue formation from adult stem cells.

Two different strategies were investigated:

1. the more traditional *in vitro* tissue engineering approach, in which cells are cultivated on a polymeric scaffold inside a bioreactor and the matured tissue is then implanted as a prosthesis;
2. the more industrial appealing *in situ* tissue engineering approach, in which unseeded bioactive scaffolds are implanted, to recruit patient own stem cells and guide tissue regeneration.

The *in situ* regeneration and repair of specific cardiovascular tissues is an exciting possibility, since technologies attractive to the cardiovascular industries are those that do not involve handling of patient cells.

In particular, the BIOSCENT project aimed to the regeneration of all the three different cardiovascular tissues: myocardium, blood vessels and heart valves. For the treatment of ischemic myocardium, two different solution were proposed: a cardiac patch, made of cells cultured on a 3D highly porous scaffold, to be implanted on the fibrous scar and a cardiac injectable system, to be implanted epicardially for promoting *in situ* tissue repair. In addition, functionalized tubular scaffolds were produced for the *in situ* tissue engineering of blood vessels, while bioactive scaffolds in the shape of three leaflets valves were studied for the *in vitro* and eventually *in vivo* tissue engineering of heart valves.

To achieve such results, the multidisciplinary of the Consortium has been of fundamental importance.

During the first two years of the project, after an extensive screening work, carried out by materialists and biologists, we identified for each end product: polymers for scaffold fabrication, stem cell sources for tissue regeneration and signals for guiding tissue growth by scaffold functionalization. Computer aided design (CAD) for polymer processing and final product prototyping were also initially addressed, particularly for the HV subtopic, where understandings of final product behaviour was critical to ensure the development of functional devices. Then, such results were integrated with innovative fabrication techniques and advanced functionalization strategies, for the development of several prototypes for each end product. Moreover, with the perspective of regenerating the engineering tissue *in vitro*, cell culture devices were designed and fabricated, in order to satisfy the four distinguished BIOSCENT end product. Bioactive scaffold prototypes underwent *in vitro* morphological, physicochemical, mechanical and functional characterizations and, based on the positive outcome of such tests, they underwent biological characterization by cell culture tests in static and in dynamic conditions.

The results of the *in vitro* characterization allowed the selection of prototypes for *in vivo* tests in animal models. The results of *in vivo* tests were very promising, especially for the cardiac patch and cardiac injectables.

### **Summary description of project context and objectives**

Congenital and acquired diseases of the heart such as heart valve degradation, great artery dysfunction and coronary artery blockage are the leading causes of morbidity and mortality in developed countries today (European Cardiovascular Disease Statistics, 2008 Edition. British Heart Foundation Health Promotion Research Group. Department of Public Health, University of Oxford, Statistical Fact Sheet - Populations, 2009 Update. International Cardiovascular Disease Statistics. American Heart Association). A large fraction of the total costs that the EU spends on health care can be attributed to tissue loss or organ failure, especially with accumulating costs from heart damage and heart failure. These costs are not only of monetary value, but more importantly, of value in human life and quality of life.

Successful treatment of cardiovascular disease is limited in many situations by the lack of suitable autologous tissue to restore injured cardiac muscle and heart valves or to serve as vascular conduits to replace or bypass diseased vessel. In cases in which autologous material is lacking, synthetic graft material may be used. However, compared with native tissue, the performance of synthetic material often pales as a tissue replacement.

Surgical replacement of diseased human heart valves by mechanical and biological prostheses is commonly used, with approximately 275.000 valve replacements done each year worldwide (FJ Schoen, Pathology of heart valve substitution with mechanical and tissue prostheses. In: MD Silver, AI Gotlieb and FJ Schoen, Editors, *Cardiovascular pathology* (3<sup>rd</sup> ed.), WB Saunders, New York (2001), pp. 629–677). Although survival and quality of life are enhanced for many patients, prosthesis associated complications and failure are frequent and have considerable impact on patient outcome. The problem is emphasised by pediatric applications, where growth of the recipient often necessitates one or more reoperations, since all of the treatments currently available are unable to grow and remodel with the surrounding tissue.

Vascular bypass can be accomplished with autologous veins or arteries or with synthetic grafts composed of materials such as polyethylene terephthalate (PET, Dacron) or expanded polytetrafluoroethylene (ePTFE, Gore-Tex). Although both native and synthetic grafts can be used in the high-flow environments of large diameter grafts, only the former is acceptable in the smaller-diameter low-flow vessels. Unlike the 85% to 95% long-term patency in large diameter vessels, small diameters (<5 mm) synthetic grafts have met with early thrombotic complications and late intimal hyperplasia, often leading to total graft occlusion (Bos GW, Poot AA, Geugeling T, van Aken WG, Feijen J. *Small-diameter vascular graft prostheses: current status*. Arch Physiol Biochem. 1998; 106: 100–115). Less than 50% of the small-diameter grafts remain patent 5 years after implantation.

Despite recent advances in the treatment of acute myocardial infarction, the ability to repair extensive myocardial damage is limited. The adult heart is incapable of effective cardiomyocytes regeneration after injury or infarction. Cardiomyocytes do not regenerate after birth; for this reason, loss of cardiomyocytes leads to regional contractile dysfunction, with the injured myocardium becoming a noncontracting fibrous scar that alters the workload of the surrounding tissue (St. John Sutton MG and Sharpe N (2000) *Left Ventricular Remodeling After Myocardial Infarction: Pathophysiology and Therapy*. Circulation 101:2981-2988). If the injured area is large, the remaining myocardium will ultimately deteriorate, leading to congestive heart failure. Unlike heart valves or blood vessels, heart muscle has no replacement alternatives. Current treatments for acute myocardial infarction and subsequent heart failure include mechanical support using left ventricular assist devices and cardiac transplantation. But both of them present serious problems, such as the lack of organ donors and complications associated with rejection and infection.

For all these reasons, there has been in recent years an urgent demand for new methods to repair and replace damaged cardiovascular tissues. Tissue engineering has been proposed as a solution to these problems by replacing tissue or organ function with constructs that contain specific populations of living cells (Rabkin E., *Cardiovascular Tissue Engineering*, Cardiovascular Pathology 2002, 11: 305-317; Nugent HM, *Tissue Engineering Therapy for Cardiovascular Disease*, Cardiovascular Tissue Engineering 2003, 92: 1068-1078). The progress toward development of individual cardiac components to date has been quite encouraging, although significant advances must be made before these will achieve routine clinical use.

The overall objective of the BIOSCENT project was the development of innovative bioactive polymeric scaffolds able to guide tissue formation from dissociated stem cells, for engineering autologous cardiovascular replacements, namely vascular tissues, heart valves and cardiac muscle. Two different strategies were followed to approach creating new engineered tissue:

1. *In vitro* tissue engineering: according to the most frequent tissue engineering paradigm, autologous stem cells were seeded on scaffolds composed of synthetic and/or natural materials and the tissue was matured *in vitro* in a bioreactor, in order to obtain a functional construct that can be implanted in the appropriate anatomic location as a prosthesis.
2. *In situ* tissue engineering: unseeded scaffolds that attract endogenous cells and control cell proliferation and differentiation were implanted to repopulate and remodel an altered cardiovascular tissue.

The *in situ* regeneration and repair of specific cardiovascular tissues is an exciting possibility, since technologies attractive to the biomedical industries are those that do not involve handling of patient cells. It is the most scientific-challenging option but most global-market attractive. *In situ* tissue engineering can overcome some of the major difficulties of the traditional *in vitro* approach of tissue engineering. The *in situ* approach can promote successful generation of an engineered construct in the patient body as a “natural bioreactor” and avoid the risk and difficulties associated with cell expansion, differentiation, organization and biograft implantation. However, the success of this approach depends on the ability of the scaffold to recruit enough cells to replace the damaged extracellular matrix and parenchyma and to control stem cells proliferation and differentiation. Moreover, this approach might prove difficult in elderly and sick patients, with impaired stem cell function and regenerative capacity, for which an optimized *in vitro* approach seems to be the most convenient and promising therapy. Consequently, the two-folded strategy proposed in the project is the most wise in terms of risk-balance and market penetration.

The success of tissue engineered replacements is dependent on three main issues: (a) the scaffold, which serves as a guiding structure for tissue development; (b) the cell source from which a living tissue is grown; (c) the *in vitro* culture conditions of the living construct before implantation. As a consequence, the BIOSCENT project took in consideration all the three components of the “cell-scaffold-bioreactor system”.

The specific objectives of the BIOSCENT project were the following:

- 1) realisation and optimisation of bioactive scaffolds, to be achieved in six steps:
  - synthesis/preparation of new polymeric systems;
  - matrix functionalization using traditional methods and advanced functionalization techniques, such as molecular imprinting;
  - controlled release of growth factors and angiogenic factors from the scaffolds;
  - Finite Element Modelisation for heart valve material selection, mould production and device prototyping with respect to material processing requisites, namely, electrospinning, free-drying, solvent casting and melt pressing.
  - fabrication of injectable scaffolds;
  - fabrication of highly porous three dimensional scaffolds, through: traditional techniques, micro-fabrication techniques, nanofiber-based techniques;
- 2) identification of the best cell source, in relation to the tissue that has to be regenerated; optimisation of isolation/culture techniques and identification of the factors involved in cellular signalling during tissue development (e.g. biochemical factors, growth factors, mechanical and electrical stimuli...);
- 3) development of suitable dynamic culture environments;
- 4) development of integrated experimental protocols for guiding stem cell plasticity.

For delivering the project objectives, the biomaterial represent the “heart of the matter”, since the scaffold can function for guiding the formation of a tissue from dissociated implanted cells *in vitro* and for the regeneration *in situ* of remaining healthy cells. The biomaterials developed within this project are able to stimulate highly precise reactions with proteins and cells at the molecular level. Such materials provide the scientific foundation for molecular design of scaffolds that can be seeded with cells *in vitro* for subsequent implantation or specifically attract endogenous functional cells *in situ*. The key concept is that the scaffold contains specific chemical and structural information able to control tissue formation, in a manner analogous to cell-cell and cell-extracellular matrix communication and patterning during embryological development.

Besides the design of bioactive scaffolds with smart properties, it has been necessary to make an optimal selection of stem cells and culture environments, which could appropriately confer biochemical, morphological, electrical and mechanical stimuli to a developing tissue.

The final aim was to design and develop four different end products:

- 1) CARDIAC PATCH: highly porous three-dimensional scaffolds for *in vitro/in situ* tissue engineering of myocardium;
- 2) CARDIAC INJECTABLES: injectable scaffolds for *in vitro/in situ* tissue engineering of myocardium;
- 3) VESSELS: tubular scaffolds for *in situ* tissue engineering of blood vessels;
- 4) HEART VALVES: three-dimensional scaffolds in the shape of a tri-leaflets valve, for *in vitro* and *in situ* tissue engineering of heart valves.

## Description of the main S&T results/foregrounds

The overall objective of BIOSCENT project has been the development of innovative bioactive polymeric scaffolds able to guide tissue formation from autologous adult stem cells, for engineering autologous cardiovascular replacements (cardiac muscle, blood vessels and heart valves). For this reason BIOSCENT focused in the study of the 3 main components of tissue engineering:

- **Biomaterials** that serve as a guiding structure for tissue development.
- **Stem cells** to ensure the correct differentiation to the desired cell type from which a living tissue is grown.
- **Bioactive molecules** that ensure cell proliferation and survival within the designed constructs.

Tissue engineering solutions that have been developed in BIOSCENT project are intended to address the majority of cardiovascular diseases. Therefore, the tissues targeted by BIOSCENT solutions are cardiac muscle, vascular tissue and valve tissue, resulting in the 4 final products which have been developed during the project: cardiac patch and cardiac injectable (for cardiac muscle replacement), tubular scaffolds (for vascular tissue regeneration) and three leaflets scaffolds (for heart valve replacement).

In order to respect the multidisciplinary nature of the BIOSCENT project, the project progressed over the first two years of activities along three folds: polymer, cells and signals.

48 biodegradable and biocompatible polymeric systems were developed and fully characterized *in vitro*, in order to select the most promising material for the fabrication of the different end products. Specifically, 5 materials were selected for the cardiac patch, 3 for cardiac injectables, 5 for heart valves and 4 for blood vessels.

In parallel, stem cell sources were identified for the regeneration of the different tissues and appropriate culture conditions were defined. At the same time, cell signalling study for downstream scaffold functionalisation was also performed, in view of developing tissue engineering tools capable of cell recruitment and favouring cellular adhesion/proliferation/differentiation and promoting angiogenesis.

Major achievements with reference to stem cell source selection and signals identification are illustrated below.

### **1) Cardiac Progenitor Cells (rCPCs) as the best source of adult stem cells for myocardial regeneration**

To accomplish the goal proposed by the BIOSCENT project, UniPAR has selected Cardiac Progenitor Cells (CPCs) as the best source of adult stem cells to generate new myocardium. We have optimized isolation and culture techniques in order to obtain a large number of cells to test the *in vitro* and *in vivo* ability of several cardiac injectables and cardiac patches to sustain cardiac regeneration.

### **2) Growth factors that stimulate cardiomyocyte differentiation**

HubL used zebrafish genetics and embryology to identify growth factors that stimulate cardiomyocyte proliferation, migration or differentiation. As a result we have identified the growth factor bone morphogenetic protein (BMP) as a regulator of cardiomyocyte differentiation during embryonic heart development. Using zebrafish mutants defective in BMP signalling and transgenic zebrafish lines that have either down- or upregulated BMP signalling we showed that BMP signalling plays a dual role during cardiomyocyte differentiation. While early BMP stimulation promotes cardiomyocyte formation, late BMP stimulation of BMP signalling inhibits further differentiation of the cardiomyocytes in the embryo. Thus, our results contribute to a better understanding of how cardiogenic differentiation is regulated *in vivo*. A good understanding of the *in vivo* regulation will allow optimizing this process *in vitro* and in the in the process of cardiac regeneration.

### **3) Signals that promote angiogenesis**

To identify signals that regulate angiogenesis HubL used zebrafish genetics. We identified a novel gene (*ubiad*) that is required to maintain blood vessel integrity. Zebrafish mutant embryos defective in this gene initially form blood vessels but these are lost due death of the endothelial cells. We found that the protein encoded by this gene is required to produce the antioxidant CoQ10. Our study places UBIAD1 in a pathway with important therapeutic implications for cardiovascular failure,

such as the opportunity to decrease oxidative damage and counteract some of the side effects of statins. In addition, pharmacological or genetic stimulation of UBIAD1, as a CoQ10 biosynthetic enzyme, represents a promising therapeutic approach for antioxidant-related diseases such as aging and cancer.

#### 4) Biotinylation technology

In parallel to the identification of signals, new strategies for their inclusion in the scaffolds were investigated. IBT (which is one of the SMEs of the Consortium) has expanded the applications of the company's biotinylation technology during the BIOSCENT project. The technology has initially been developed to prepare reagents for immunoassays for growth factors, cytokines and other molecules. Biotinylation is an alternative to radioactive labelling of molecules. The Biotin labelled molecules do not emit radiation and are much more stable, than radioactive labelled molecules. The disadvantage of conventional biotinylation methods is that the biotinylated molecules can lose their biological activity. The method, that has been developed by IBT results in Biotin labelled molecules with full biological activity *in-vitro* and *in-vivo*. In the BIOSCENT project the applications in tissue therapy and tissue engineering have been expanded and biotinylated molecules prepared by IBT are used in some of the devices developed by the Consortium members. In addition IBT has developed a fully validated immunoassay for the quantitative measurement of SDF-1 alpha, one of the key molecules in tissue repair after cardiac infarction.

#### 5) Design of a coarse capillary structure to promote *in vitro* capillary sprouting

In collaboration with MHH, CHEM (one of the SMEs of the Consortium) explored the possibility of creating a coarse capillary structure, which could induce and support spontaneous capillary sprouting in order to obtain 3D vascularised tissue. Work concerning the realization of a polymer 3D capillary structure is attributed to CHEM, while MHH contributed with biocompatibility (*in-vitro* cultures) and bioreactor development and testing.

The 3D capillary structures were designed and manufactured according to the following steps:

- 1) Manufacture of injection moulded water-soluble polymer templates using commercial polyvinylalcohol (PVA), which could be assembled to create 3D structures
- 2) Coating of the 2D/3D structures by spray coating and/or dip coating with water-insoluble polyurethanes (PU) solvated in Tetrahydrofurane – 3 commercial medical grade PUs were investigated. An ideal material would yield good structural integrity and form stability for the final structure as well as be biocompatible enabling cell seeding and proliferation. Feasibility was proven of sandwiching different types of PUs in order to combine superior biocompatibility of one type with the superior mechanical performance of another if/when needed.
- 3) Inducing of controlled reproducible microporosity in the PU coated structures of pore diameters 40, 60 and 80µm by precision laser ablation with a 355nm laser.
- 4) Removing inner PVA template by water leaching and lumen rinsing.

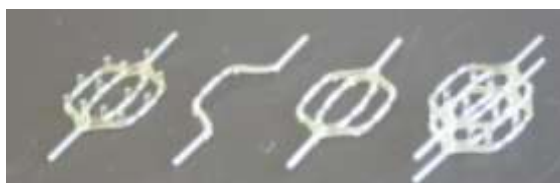


Figure 1: (from left to right) Injection moulded PVA template, cut linear strand, cut planar 2D strand and assembled 3D network ready for PU coating

*In-vitro* biocompatibility of the 3 different PUs was determined using cultured Human umbilical cord vein-derived endothelial cells. Planar polyurethane membranes were manufactured and seeded with trypsinized endothelial cells at a density of  $1 \times 10^5$  cells/cm<sup>2</sup> and placed in cell culture media.

Adhesion and growth was monitored over 14 days by phase contrast imaging combined with a final rhodamine fluorescent dye evaluation. Developed coarse vessel structure could be embedded in a pro-angiogenic 3D matrix to promote in vitro angiogenesis.

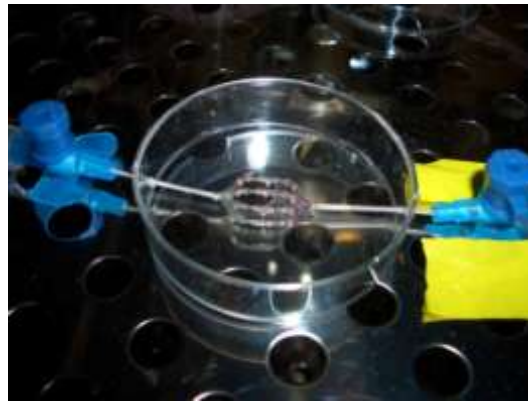


Figure 2: Incubation chamber with a mounted and canulated PU capillary structure of the custom perfusion bioreactor system

### 6) The aortic valve behavioural study and model design for manufacturing

Mathematical modelisation of prosthetic medical device is the necessary path to analyze the physical and dynamic behaviour, for the system to be designed so as to comply the human's identical functioning, for diseased organ replacement. This system characterization depends on factors like geometry shape and dimensions, material selection and mould creation for roll to roll processing technology.

Pera Innovation technology and Technologias Avanzadas Inspiralia S.L., developed FEM model of aortic human heart valve to provide BIOSCENT consortium further understanding of the work function of the device. The model was of great use to ascertain the material specifications that must be met, selection of polymeric scaffolds, as well as product requirements in the perspective of mould tooling and end product development. The interest of this work was presented in TE conferences and reported in peer reviewed articles.

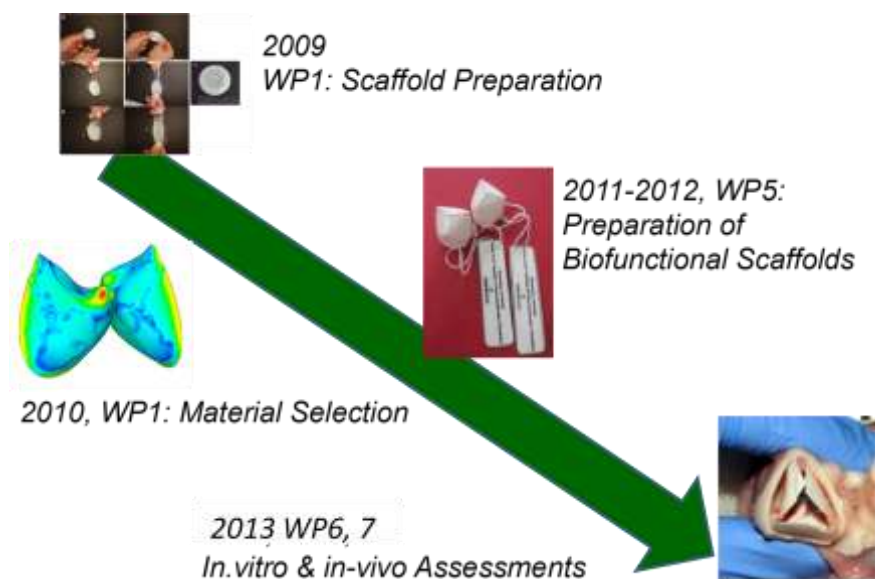


Figure 3: BIOSCENT heart valve development, from user needs and design definition to design output and device prototyping.

The results obtained in the identification, for each end product, of polymers for scaffold fabrication, stem cell sources and relative culture conditions, and signals able to guide tissue regeneration, were



then integrated with innovative fabrication strategies and advanced functionalization techniques, for the development of bioactive scaffolds prototypes for all the four different BIOSCENT end products.

### **Cardiac Patch (CP)**

The BIOSCENT cardiac patch, together with the cardiac injectable, is aimed to regenerate infarcted myocardium. Specifically, the cardiac patch is suitable for being applied epicardially (external surface of heart) or intramurally (heart's wall after excision of a ventricular aneurysm).

Heart attacks are the main cause of deaths in patients suffering from a cardiovascular disease. It is estimated that 32 million heart attacks occur globally each year, from which about 12.5 million are fatal. Myocardial infarction or acute myocardial infarction, commonly known as a heart attack, results from the partial interruption of blood supply to a part of the heart muscle, causing the heart cells (cardiomyocytes) to be damaged or die.

If impaired blood flow to the heart lasts long enough, it triggers a process called the ischemic cascade; the heart cells in the territory of the insufficient blood supply die. Mature cardiomyocyte cells are unable to divide, thus having no possibility for regenerating the damaged cardiac tissue. It results in that a collagen scar is formed in place of cardiac cells, having clearly different structural, contractile and electrical properties. This myocardial scarring puts the patient at risk for potentially life threatening arrhythmias, and may result in the formation of a ventricular aneurysm that can rupture with catastrophic consequences. Injured heart tissue conducts electrical impulses more slowly than normal heart tissue. The difference in conduction velocity between injured and uninjured tissue can trigger re-entry or a feedback loop that is believed to be the cause of the lethal arrhythmias.

A solution capable of achieving regeneration of cardiac muscle cells could prevent these lethal arrhythmias to happen. This is exactly what the BIOSCENT cardiac patch seeks: using stem cell therapy for regeneration of infarcted cardiac tissue.

Stem cell have the unique capability of dividing and differentiating into diverse specialized cell types. Therefore, if they are successfully introduced within the damaged cardiac muscle, they can replace the scared tissue. Initial approaches were based in introducing these cells by means of injection of cells in suspension either into the bloodstream or directly into the heart. However, this strategy has not proved efficiency since most of cells result "non-usable", as they move away from the damage area.

Tissue engineering aims a similar objective, but in this case using a degradable biomaterial as scaffold for guiding tissue generation. The scaffold allows for the stem cells to be placed in the desired damaged cardiac muscle area, thus highly improving stem cell therapy efficiency.

The concept of the cardiac patch developed in the framework of BIOSCENT project is to use a three-dimensional polymeric scaffold, as a vehicle for delivering stem cells to the damaged area. These scaffolds are functionalized with growth factors or other bioactive agents capable of stimulating cellular growth, proliferation and differentiation.

The polymeric scaffolds have been selected among synthetic and natural polymers, having the singularity of been simultaneously biocompatible and biodegradable. Biocompatibility is required in order to avoid rejection by the host human body. Moreover, the polymer have to be biodegradable in a way that the degradation rate have to be similar to the rate of tissue formation,

without producing toxic substances during degradation. In this way, the scaffold itself will disappear once the new cardiac tissue is definitively formed, leaving a repaired cardiac tissue in its place with similar contractile properties, electro-physiological stability and vascularization ability than autologous tissue.

Other main function of the cardiac patch is to serve as mechanical support while tissue is regenerating. For this reason, another important feature that the polymeric scaffolds of the cardiac patch has to comply is having similar, or even improved mechanical properties than the human myocardium. Beside these essential features, there are other important requirements for the polymeric scaffolds that have been considered for cardiac patch development:

- **Porosity:** as the main function of the polymeric scaffold is to deliver the stem cells to the damaged area and therefore to guide tissue formation, porosity is a key factor in two senses: a) pores must have appropriate dimensions for hosting the cells and b) pores have to allow for optimal cell rooting.
- **Thickness:** it is important as it influences the operability during implanting.
- **Suturability:** this factor is intrinsically linked to the mechanical properties of the polymeric material. For implantation of the cardiac patch in a human heart, it has to be sutured to the hosting organ. It is important that the material resists the suture without compromising its mechanical properties during and after being sutured. In order to guarantee the cardiac patch suturability, one strategy that has been followed in BIOSCENT project is to develop a multilayer polymeric material integrated by a stitchable support (made of a biodegradable polyurethane, with low porosity but high suturability) that serves as the base for suture, and a porous scaffold (made of a biodegradable polycaprolactone, with optimal porosity for fitting the cells) that is in essence the responsible for tissue regeneration.
- **Vascularization:** for an optimal tissue formation it has to receive the sufficient blood supply, therefore is essential that new blood vessels and capillaries are formed while the new tissue is being generated. The scaffolds have to allow this vascularization process to take place.

Overall, 25 cardiac patch prototypes were developed and fully characterized *in vitro* and *in vivo*, producing the selection of 4 most promising prototypes. A brief overview is provided below:

### **CP1) Cardiac patches based on polyglycerol sebacate (PGS)**

For the development of a Tissue Engineered Cardiac Patch end product, two polymeric systems were selected at UniEN based on results of preliminary investigations: (1) polyglycerol sebacate, PGS, and PGS blend system with poly( $\epsilon$ -caprolactone) (PGS/PCL).

The aim of the research was achieved by the following objectives:

1. Fabrication and characterization of two dimensional PGS dense films.
2. Fabrication of porous PGS scaffolds.
3. Fabrication of PGS/PCL fibers with anisotropic properties.
4. Fabrication of scaffolds with topographical cues for cell guidance.
5. Incorporation of biomimetic functionality in the fabricated PGS and PGS/PCL scaffolds
6. Functionalization with vascular endothelial growth factor (VEGF) to promote vascularization

The synthesis of PGS involved two steps: (1) pre polycondensation step and (2) crosslinking. The PGS films were assessed in terms of microstructural, mechanical, thermal properties, *in vitro*

degradation, and cell culture studies in order to assess their suitability as scaffold material for cardiac patch application. Assessment of the sterilization suitability of PGS was also conducted. Porous PGS scaffolds were fabricated by the salt leaching method. Structured porous 3D scaffold were also produced by laser microablation technique and electrospun PGS/PCL based scaffolds were also developed. Contact guidance was achieved by introducing topographical patterns at micro and nanoscales both on PGS films and on PGS/PCL fibers mats. Two different strategies were further developed for functionalizing the PGS matrices: i) covalent binding of GRGDSP and YIGSR peptide sequences onto the PGS surface and ii) development of biomimetic PGS membranes via molecular imprinting (MIP). In addition, functionalization with vascular endothelial growth factor (VEGF) to promote vascularization was investigated.

### **CP2) Cardiac patch based on a blend of natural polymers**

These prototypes, developed by UniPI, were based on a blend of natural polymers, mimicking the composition and the interactions among components in native cardiac ECM. Scaffolds were prepared in two forms: as highly porous sponges, by freeze-drying, and as microfabricated structures, to reproduce a simplified model of the cardiac ECM microarchitecture, by Soft Lithography. Functionalization was performed through a highly innovative strategy, by deposition of molecularly imprinted particles, with recognition properties towards two peptide sequences or a chemoattracting agent. The effect of scaffold functionalization by Molecular Imprinting on cardiac progenitor cells was tested for the first time in this project.

### **CP3) Cardiac patch based on reinforced natural polymers**

The second prototype developed by UniPI had a “sandwich” configuration, with two external layers of highly porous sponge based on a blend of natural polymers and an internal reinforcement membrane, made with a synthetic polymer, to provide appropriate suturability.

The scaffold material used for the sponge mimics the chemical composition and the interactions among components in native cardiac ECM.

Scaffold functionalization was based on an innovative application of the avidin-biotin binding system, in which avidin was used as a bridge between a biotinylated polymer (on the scaffold) and a biotinylated growth factor.

### **CP4) Composite cardiac patch**

The fourth prototype, developed by ITAV/PPI/UniMA/CzAS, is based on a laminated polymeric architecture so as to provide primarily a structural reinforcement of the heart pericardium. The polymers arranged as follows:

- a stitchable support for suturability and cardiac patch structure resilience to heart muscle function (PPI).

- a porous structure made by a bioartificial blend with a pore sizes varying from 50 nm to 200µm to permit nutrient transport, angiogenesis and cell homing (PERA/ITAV).

- gel and gel/electrospun fibers additional layers (gels: UniMa/PPI; electrospun fibers: CzAs) complement the architecture to provide two non competitive drug release systems, for short and long term cell signaling release. Another asset of such strategy is to provide the device with an extracellular-matrix like structure, favoring cell seeding and device biointegration.

BMP-4 have been encapsulated in the fibers, the latter embedded in self-assembly peptidic hydrogel. The system was designed to obtain maximal release within 2-3 days post seeding.

One important advantage of this patch is that its shape and size could be easily adapted to the patient's needs. The polymeric materials can be cut at the time the surgery takes place according to the size and shape of the damaged myocardium area. The whole assembly is mounted up without any glue, an asset from a regulatory perspective.

## **Cardiac Injectable (CI)**

The cardiac injectable is also aimed to treat damaged myocardial tissue, but in this case the treatment is more suited for endocardial treatment. The cardiac injectables have the advantage of that they can be injected directly in the damaged area, even if this area is hardly accessible. For this reason, they are the perfect solution for being applied in the inner surface of the heart after a coronary angioplasty to revascularize areas suffering an acute myocardial infarction.

Conversely than for the cardiac patch, the injectables are not intended to provide any mechanical support, thus their mechanical properties dealing with contractile behavior and suturability are not aspects that have been considered during their development. However, rheological properties are crucial aspects in terms of achieving an optimal injectability, which highly influence the operability during injection. Cardiac injectables are in fact delivered through a narrow needle.

The cardiac injectable is basically a polymeric scaffold that is injected in a liquid form and that once placed in the desired area changes its physical state from liquid to solid through gelification, without harming the cells of the surrounding myocardium. Therefore, the gelation time is a key aspect. Other features, such as biodegradability, biocompatibility and rapid vascularization after implantation, have been taken into consideration during CI development, as well as for CP.

Taking all of these features into account, 13 different prototypes were developed and characterized and 3 of them were finally selected as best performing:

### **CI1) Injectable microspheres**

Microspheres represent an innovative solution in the development of injectable scaffolds, which are usually obtained by using strategies such as thermoreversibility, in situ polymerization and in situ cross-linking.

Injectable microspheres, based on a blend of natural polymers, were prepared by UniPI through a water in oil emulsion, using a phospholipid as surfactant. The phospholipid not only avoids microspheres coalescence, but it also produces a sort of surface imprinting on the microspheres, that could promote cell adhesion.

Microspheres were also functionalized by loading with a growth factor, to promote cell recruitment and tissue regeneration.

### **CI2) Responsive peptide based hydrogel**

The prototype concept developed by UniMA was based on the use of  $\beta$ -sheet self-assembling peptides to design injectable hydrogels. These hydrogels can be used in a variety of way depending on end-user requirement. They can be functionalized, loaded with cells and made responsive depending on the requirements. Self-assembling can be triggered using a variety of stimuli such as pH, ionic strength, temperature and enzymes. These peptides self-assemble and form  $\beta$ -sheet rich fibers that entangle and form hydrogels. The functionalization of these systems simply requires the synthesis of functionalized peptides (functional group usually covalently linked to the end of the octa-peptide). The functional peptide can then be dosed by simple mixing and the self-assembly process ensures that the functional peptide is incorporated in the network. The simplicity of the structure formed, cross  $\beta$ -sheet, ensures that the functional group is placed on the surface of the fiber and is therefore available/accessible. Through this project we developed a number of systems that fulfill the requirement of the biologist. These technology and material were then transferred for biological testing.

### **CI3) Injectable thermoreversible polyurethanes**

Injectable thermoreversible hydrogels are a new series of amphiphilic polyurethanes which have been synthesized by PPI using synthetic and natural oligomers and aliphatic diisocyanate. The most suitable diisocyanates are: methyl ester L-lysine diisocyanate, 1,4-butane diisocyanate, hexamethylene diisocyanate, etc. The release of degradation products from the urethane segments of this polyurethane is harmless. The gel point (35-37°C), gel time (<60 seconds), stability and crystallization are controlled by polyurethane chemical structure. The functionalisation of these polymers can be done during synthesis and the scaffold preparation is very easy.

As a general comment, it is interesting to underline that these prototypes use three different strategies to achieve injectability.

### **Blood Vessels (BV)**

The BIOSCENT blood vessels are aimed to treat coronary artery disease. Coronary artery disease, also named atherosclerotic heart disease, is the most common type of heart disease and cause of majority of heart attacks. The disease is caused by plaque building up along the inner walls of the arteries of the heart, which narrows the arteries and restricts blood flow to the heart. It is the leading cause of death worldwide.

The most widely extended treatment for coronary artery disease is called “coronary artery bypass surgery”. In this type of surgery, arteries or veins from elsewhere in the patient's body are grafted to the coronary arteries to bypass atherosclerotic narrowings and improve the blood supply to the coronary circulation supplying the myocardium (heart muscle). However, the main drawback of this surgical procedure is that is highly invasive, since an artery or a vein of another part of the patient has to be taken and grafted to the coronary artery. Moreover, that grafts can become diseased and may occlude in the months to years after bypass surgery is performed.

A partial solution to this problem is the use of synthetic grafts to substitute the occluded coronary artery or stents to help the artery to remain open. Synthetic grafts and stents can be implanted through a less invasive surgery. Nevertheless, their limitations include: thrombogenicity, anastomotic intimal hyperplasia, aneurism formation, infection and occlusion. Tissue engineering has emerged as an attractive alternative to overcome these limitations.

The concept of BIOSCENT blood vessel is a tissue engineered graft consisting in a tubular polymeric scaffold functionalized with growth factors capable of promoting cell differentiation and proliferation, thus blood vessel tissue regeneration. Its main advantage is that, as being an active vascular conduit, it is capable to react to biological stimuli and adapt its caliber to the metabolic needs of the re-vascularized myocardium. This helps overcoming occlusion issues. Other important advantage is the reduced invasiveness and the subsequent improved rehabilitation.

Together with the universal requirements of biocompatibility and biodegradability for tissue engineered solutions, the suturability and the mechanical properties have been the key factors considered during BV development, as for the CP. The elasticity of the grafts needs to be similar to the viscoelastic nature of an artery in order to provide a proper blood supply.

In the scope of the blood vessels subtopic, 11 prototypes were prepared and tested *in vitro* and two of them were selected for implantation in animal models:

#### **BV1) Blood vessels scaffold based on reinforced natural polymers**

The blood vessel scaffold developed by UniPI was based on a blend of natural polymers, mimicking native blood vessels (in terms of chemical composition and interactions among components). It was reinforced with a synthetic material, in order to provide adequate resistance to sutures.

Tubular structures were produced using a mould, design and developed in the scope of the project. No functionalization was performed, since cell culture tests demonstrated that the material itself was able to promote excellent cell adhesion and proliferation. This represents a great advantage in terms of production time and price of the device.

### **BV2) Blood vessel scaffold based on synthetic material**

Polyurethanes are a class of materials frequently used in a broad range of biomedical applications. The surface functionalization of these polymers is vital for tissue engineering.

PPI developed highly micro-porous tubular scaffolds by phase inversion techniques, using water vapours. Moreover, a mild, non-destructive method for activation of polyurethane surface and subsequent heparin binding was developed.

### **Heart Valve (HV)**

The BIOSCENT heart valve is aimed to treat heart valve dysfunction. It is estimated that only in USA, more than five million Americans are diagnosed with heart valve disease each year. Without an aortic valve replacement, 50% of diagnosed patients will not survive more than an average of two years after the onset of symptoms.

Valvular heart disease is any disease process involving one or more of the valves of the heart (the aortic and mitral valves on the left and the pulmonary and tricuspid valves on the right). The heart consists of four chambers, two atria (upper chambers) and two ventricles (lower chambers). There is a valve through which blood passes before leaving each chamber of the heart. The valves prevent the backward flow of blood. They act as one-way inlets of blood on one side of a ventricle and one-way outlets of blood on the other side of a ventricle. As the heart muscle contracts and relaxes, the valves open and close, letting blood flow into the ventricles and out to the body at alternate times.

Heart valve disorders can arise from two main types of malfunctions:

- **Regurgitation:** The valve does not close completely, causing some of the blood to leak backward through the valve. The heart is forced to pump more blood on the next beat, making it work harder.
- **Stenosis:** The valve opening becomes narrowed, limiting the flow of blood out of the ventricles or atria. The heart is forced to pump blood with increased force in order to move blood through the narrowed or stiff (stenotic) valve(s).

Heart valves can develop both malfunctions at the same time (regurgitation and stenosis). When heart valves fail to open and close properly, the implications for the heart can be serious, possibly hampering the heart's ability to pump blood adequately through the body. Heart valve problems are one cause of heart failure. Valve problems may be congenital (inborn) or acquired (due to another cause later in life). In some cases, the only treatment for heart valve disease may be careful medical supervision. However, other treatment options may include medication, surgery to repair the valve by a cardiac surgeon, or surgery to replace the valve. Treatment varies, depending on the type of heart valve disease, and may include one, or a combination of, the following:

- **Medication:** Medications are not a cure for heart valve disease, but in many cases are successful in the treatment of symptoms caused by heart valve disease. These medications may include:

- Beta-blockers, digoxin, and calcium channel blockers, to reduce symptoms of heart valve disease by controlling the heart rate and helping to prevent abnormal heart rhythms.
- Medications to control blood pressure, such as diuretics or vasodilators that ease the work of the heart.
- **Surgery:** Cardiac surgery may be necessary to repair or replace the malfunctioning valve(s). Cardiac surgery may include:
  - **Heart valve repair:** In some cases, cardiac surgery on the malfunctioning valve can help alleviate symptoms. Examples of heart valve repair surgery done by cardiac surgeons include cutting scarred flaps so they open more easily; remodeling abnormal valve tissue so that the valve functions properly; or inserting prosthetic rings to help narrow a dilated valve. In many cases, heart valve repair is preferable, because a person's own tissues are used.
  - **Heart valve replacement:** When heart valves are severely malformed or destroyed, they may need to be replaced with an entirely new replacement valve. Replacement valve mechanisms fall into two categories: tissue (biologic) valves, which include animal valves and donated human aortic valves, and mechanical valves, which can consist of metal, plastic, or another artificial mechanism.

The heart valves developed in BIOSCENT are to be used in all cases when medication and heart valve repair cannot alleviate the symptoms and/or solve the valve malfunction through repairing surgery. These cases account for almost 50% of the total diagnosed heart valve malfunctions. The use of biological valves or mechanical artificial valves have proven to be clinically successfully. However, they still have some important limitations such as the need of long-term anticoagulation therapies and the risks of calcification that leads to re-intervention. Tissue engineering offers the potential advantages of being a non-obstructive and non-thrombogenic therapy, thus overcoming the limitation of current heart valve replacement surgical treatments.

The BIOSCENT heart valves are tissue engineered solutions, meaning that valves are biological active implants capable of growing with the patient. This offers the advantage of preventing the need of re-interventions, being therefore long-term solutions. As in the other three BIOSCENT products, the HV is basically composed by a biodegradable and biocompatible polymeric scaffold. In terms of heart valve scaffold bioactivity, while functionalization with CCN1 as bioactive agents to promote tissue formation was studied *in-vitro*, polyurethane based polymers with a lysine bearing hard segment have also been demonstrated to be cell attractive.

Another point of consideration was the design of the device. Indeed, initial device configuration was based on a stent approach so as to minimize physical constraints on polymeric leaflet where part of the load effort would be born by the stent. Nevertheless, due to the incapacity to obtain within the Consortium or externally such element for competitive reasons, the work done during the first two years of the project had to be furthered so as to provide a second generation of prototypes in a stentless configuration. As such, PERA/ITAV developed a corrugated root model where the first generation of BIOSCENT heart valves would be integrated into a flexible conduct. The latter has been engineered to optimize leaflet closure by the introduction of sinus obtained from echocardiographic data.

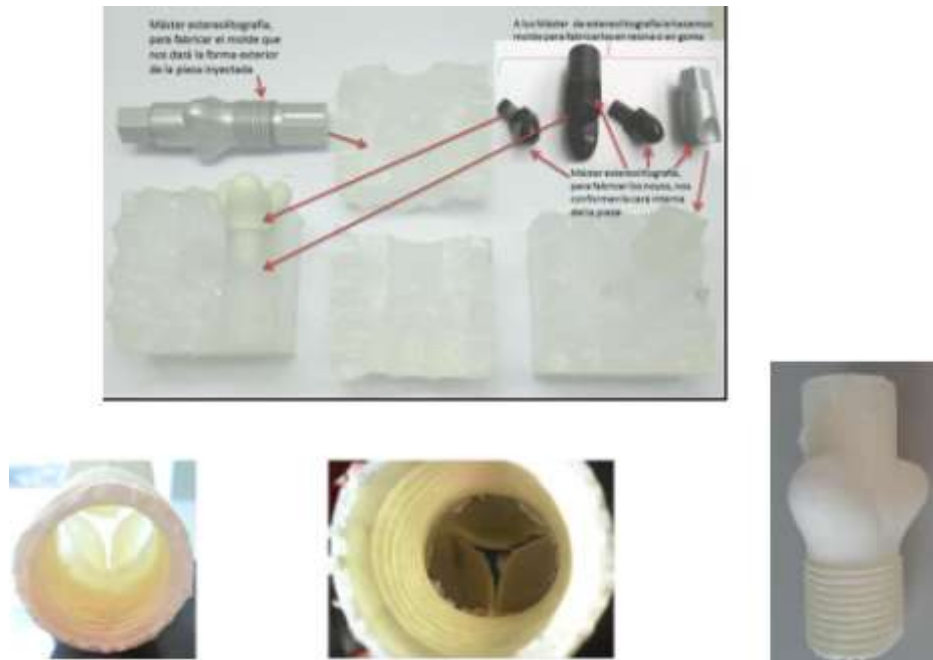


Figure 4: BIOSCENT second generation HV prototypes

Overall, 18 different prototypes were developed and tested as heart valve scaffolds. Results of *in vitro* characterization and suturability tests allowed to select the two most promising ones, that were admitted to the *in vivo* evaluation. A brief overview is provided below:

#### **HV1) Heart valve scaffolds based on reinforced natural polymers**

The heart valve scaffold developed by UniPI was based on a blend of natural polymers, mimicking the three layers morphology of natural heart valves. It was reinforced with a synthetic material, in order to provide adequate resistance to sutures.

Scaffolds were produced through an innovative fabrication strategy, defined during the project, by using an appropriate mould, designed and developed in the scope of BIOSCENT.

No functionalization was performed, since cell culture tests demonstrated that the material itself was able to promote excellent cell adhesion and proliferation. This represents a great advantage in terms of production time and price of the device.

#### **HV2) Heart valve scaffolds based on synthetic material**

PPI developed in collaboration with PERA/ITAV heart valve scaffolds based on a synthetic material, synthesised in PPI lab.

The fabrication procedure comprised two stages: the first stage was the preparation of the polyurethane solution and the second stage was the fabrication of the scaffold by casting.

Apart from bioactive scaffolds, other important products have been developed in the framework of this research project. Specifically we are referring to the Dynamic Culture Devices (the bioreactors) for stem cell culture within the scaffolds and *in vitro* testing of tissue engineering solutions under dynamic conditions. *In vitro* testing is commonly accomplished under static conditions, meaning that stem cells are seeded within the functionalized scaffold and the cell differentiation and proliferation rates are measured at specific and predefined times. This testing method provides valuable information in terms of cell aggregation and rate of tissue formation. However, the information obtained through this method is not fully translatable to a “life tissue”, since this last one will be dynamically changing along time. Therefore, a method that allows to carry out tests under dynamic conditions similar to those of the native tissue, can provide more accurate information on how the scaffolds will guide tissue regeneration “in vivo”.



PoliTO and SORIN developed the following DCDs:

1. Cardiac Patch DCD (CPDCD, PoliTO)
2. Hydrogel DCD (HDCD, PoliTO)
3. Blood Vessel DCD (BVDCD, SORIN)
4. Heart Valve DCD (HVDCD, SORIN)

The DCDs were designed and manufactured for delivering custom parameters, that simulate physiological conditions, in order to (1) test the BIOSCENT scaffolds, (2) to study and promote proliferation/differentiation of cells seeded on scaffolds through specific chemical/physical stimulations (to be applied within the DCD), and (3) to monitor parameters of relevant significance for each in vitro cell culture system.

### **1. CPDCD (Cardiac Patch DCD)**

The Cardiac Patch Dynamic Culture Device (CPDCD, Fig.5) has been designed and developed for testing cardiac constructs based on cells seeded on cardiac patches developed in BIOSCENT project. The aim of the CPDCD is the generation of a biochemical and physical environment suitable for growth and differentiation of cells cultured on BIOSCENT cardiac patches, by the control and the delivery of concurrent defined stretching and electrical patterns, according to the technical specification provided by BIOSCENT partners. Moreover, the CPDCD is characterized by a high versatility of the mechanical and electrical stimulation systems, allowing the device to be employed with several constructs and for different applications, and it is valuable to assure full compatibility with GLP procedures.

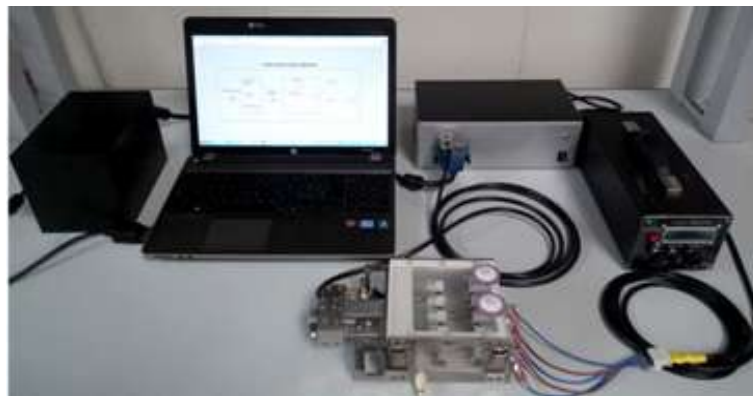


Fig.5. CPDCD setup.

The factory tests allowed to assess the suitable working volume (70 ml), and the sealing and the sterility maintenance of the culture chamber. Moreover, the tests demonstrated that the motor and the controller are able to provide physiologic-like uniaxial tensile cyclic loading for prolonged time intervals.

Finally, the CPDCD user's manual has been drawn. It provides information for the installation and daily use of the CPDCD.

### **2. HDCD (Hydrogel DCD)**

The Hydrogel Dynamic Culture Device (HDCD, Fig.6) has been designed and developed to produce a suitable biochemical environment and microgravity condition for testing viability, growth and differentiation of cells cultured on cardiac injectable gel microspheres (hydrogel microspheres) developed in BIOSCENT project.



Fig.6. HDCD setup.

Microgravity condition is obtained by establishing mixing slow vortices of the culture medium within the culture chamber, that allow to maintain adequate cell-seeded hydrogel suspension and nutrient/oxygen transport without using rotating components.

Moreover, the HDCD is characterized by a high versatility, allowing the device to be employed with several constructs and for different applications (model system, expansion system), and it is valuable to assure full compatibility with GLP procedures.

Findings of both factory tests and CFD studies demonstrated the suitability of the HDCD: the combination of operating conditions and properly shaped chamber walls give rise to flow separation, with the ensuing formation of stationary vortices within the culture chamber, without using rotating components. The balance between hydrodynamic and gravitational forces guarantees microgravity condition, avoiding both specimen sedimentation and shear stress values critical for cells.

Finally, the HDCD user's manual has been drawn. It provides information for the installation and daily use of the HDCD. Due to its innovative content, a patent application for the HDCD bioreactor was submitted at the Italian Patent and Trademark Office on 16<sup>th</sup> May 2011 (TO2011A000432, "Microgravity generator device").

### 3. BVDCD (Blood Vessel DCD)

In order to test the BIOSCENT vascular constructs before their clinical use, a device called Blood Vessel Dynamic Culture Device (BVDCD, Fig.7) was developed.

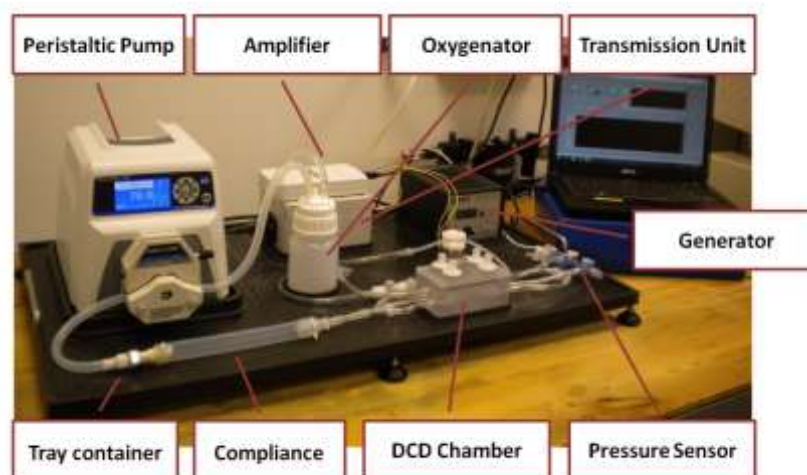


Fig.7. BVDCD setup.

The BVDCD allows to simulate the physiological conditions of the human body through pulsatile-like regimens. Moreover, with this device the parameters of vascular function (pressure, flow, resistance) can be monitored on-line and modified according to different pathological conditions.

The aim of the BVDCD is to investigate novel vascular artificial grafts to be used as scaffolds for replacement, before clinical acceptance. BVDCD allows the testing of unseeded as well as cell-seeded scaffolds in respect to strength and mechanical behavior of their polymer structures as well as cell viability, growth, phenotype, cell retention under shear-stress conditions and vasoactive function under physiological conditions and over a period of several days.

The BVDCD is designed to test blood vessel constructs by providing fluidic stimulation through pulsatile physiological-like pressure regimens. In the BVDCD a roller pump constantly pumps culture medium within the circuit while providing a continuous feeding of the construct. In order to generate a correct pressure wave able to feed the scaffold throughout, the system is implemented with a pinch valve.

Findings of factory tests demonstrated the suitability of the BVCD in delivering physiological-like stimuli, with a chamber working volume of around 80 mL, and that the chamber is perfectly isolated.

The BVDCD user's manual, containing instructions and warnings for BVDCD handling, maintenance and use, has been drawn.

#### 4. HVDCD (Heart Valve DCD)

The Heart Valve Dynamic Culture Device (HVDCD, Fig.8) was specifically designed to test stem cell recruitment, proliferation and differentiation towards cardiac cell phenotypes on heart valve constructs of reduced area.

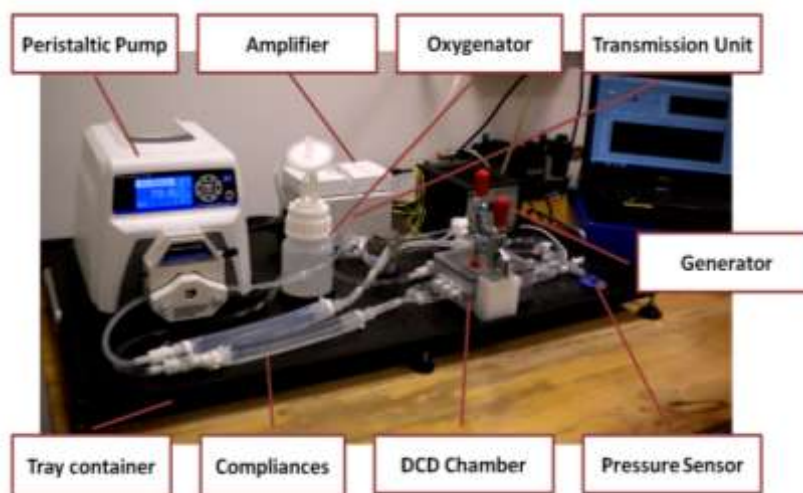


Fig.8. HVDCD setup.

HVDCD is able to reproduce physiological-like biomechanical environments on a piece of heart valve scaffold (leaflet or leaflet portion) in order to test its biological-related properties. In order to overcome the current limits of the existing bioreactors, the HVDCD is designed to work on heart valve constructs of smaller scale than that of a whole heart valve, thus allowing the use of a reduced-scale apparatus through an overall reduction of all the fluid-dynamic parameters and of the circuitual priming volumes.

Findings of factory tests demonstrated the suitability of the HVCD, after the optimal dimensioning of the components of the hydraulic circuit, of reaching near-physiological hydrodynamic conditions. The HVDCD user's manual, containing instructions and warnings for HVDCD handling, maintenance and use, has been drawn.

All the developed bioactive scaffold prototypes (for a total of 67 devices) underwent a complete *in vitro* morphological, physicochemical, mechanical and functional characterizations. Based on the positive outcome of such tests, biological characterization by cell culture tests in static and in dynamic conditions, using the developed bioreactors, was also carried out. The results of the *in vitro* characterization allowed the selection of the most promising prototypes for *in vivo* tests in animal models.

A brief overview of the main results obtained during biological characterization and *in vivo* tests is reported below.

## **In vitro and in vivo evaluation of cardiac patch and cardiac injectables**

### In vitro test of prototypes

UniPAR defined protocols for the *in vitro* characterization of bioactive scaffolds for cardiac patch and cardiac injectable.

The biological characterization through cell culture tests has been performed first in static condition, to evaluate the effects on cell morphology and survival, and then in dynamic condition, to determine the role of mechanical and electrical stimuli on CPC differentiation. The properties of statically and dynamically cultured samples have been investigated by means of immunocytochemistry techniques and advanced image analysis. We have documented the impact of bioactive scaffolds on cell adhesion, survival, proliferation and differentiation also evaluating the effect of scaffold functionalization with growth factors or epitopes involved in cell binding to extracellular matrix (laminin, fibronectin).

We have proposed an overall evaluation based on a scoring system for the *in vitro* assessment of cardiac prototypes regarding:

- Physical stability in medium
- Cell Survival
- Cell adhesion
- Cell Differentiation
- Cell detectability

### In vivo test of prototypes

UniPAR defined the injured rat heart as the best experimental model to evaluate the *in vivo* performance of bioactive scaffolds. Our purpose was to provide preclinical *in vivo* safety and performance data for the prototypes of implantable (Cardiac Patch, CP) or injectable (Cardiac Injectables, CI) scaffolds produced by BIOSCENT partners both in the cryoinjury and in the coronary artery ligation models.

Pre-surgical health, surgical procedure and post-operative observations have been performed according to the animal welfare requirements, as prescribed by the International Standards and Good Laboratory Practice for Non-Clinical Laboratory Studies. Animal sacrifice has been performed at scheduled times through ethical procedure (euthanasia). Cardiac prototypes were sutured or injected with (seeded configuration) or without (unseeded configuration) the addition of rCPCs isolated from Enhanced Green Fluorescent Protein (EGFP<sup>POS</sup>) transgenic rat heart.

Formal necropsies have been made and data explant analyses have been performed.

In particular, after the excision procedure the hearts have been processed for the morphological and immunohistochemical evaluation that has been carried out according to the following steps:

- Macroscopic examination;
- Microscopic evaluation (light microscopy);
- Immunohistochemical analysis.

Moreover, for the *in vivo* characterization of cardiac prototypes we have pointed our attention on their efficacy to repair the damaged heart and to their ability to encourage progenitor cells mobilization, homing and engraftment, without modify the biocompatibility and biodegradability of

the scaffolds. We have also evaluated the effect on these parameters of functionalization with growth factors or epitopes involved in cell binding to extracellular matrix (laminin, fibronectin).

A score has been assigned to each material according to the following parameters:

- Cardiac Patches: suturability, applicability to small animals, biodegradability, and biocompatibility.
- Cardiac Injectables: injectability, detectability, biodegradability, and biocompatibility

## **In vitro and in vivo evaluation of blood vessel and heart valve scaffolds**

### In vitro test of prototypes

MHH was provided with a variety of prototype scaffolds from UniPI, PPI and PERA/ITAV. Every scaffold type varied in the type of basic substance, modification and its mechanical properties. MHH's aim was to test these different materials in a large animal model. As a first step we tested the various prototypes under static cell culture conditions, regarding initial cell adhesion, cell proliferation and migration.

Scaffolds based on natural polymers (provided by UniPI) proved to be highly successful and therefore were accepted for the next phase (dynamic cell culture testing).

Scaffolds based on synthetic polymers (from PPI and PERA) were much more challenging and needed further improvement to obtain approval for further testing.

In the next phase MHH was provided with 3D prototypes, resembling either a blood vessel or a heart valve construct. Again, we tested for cell attachment, proliferation and migration under dynamic cell culture condition (pulsatile medium flow). This was necessary to test the cell behavior in an *in-vivo*-like, controlled environment. Since we only used scaffold types that passed the first static tests we could show promising data regarding the scaffold's cell properties in a dynamic 3D situation. However, tests under dynamic conditions pointed out that the mechanical properties of these scaffolds did not mimic at all their native counterparts. First blood vessel did not show adequate suture resistance, but such limitation was successfully solved by UniPI via reinforcement of the scaffolds. For heart valve prototypes, some concerns about a proper functionality in the upcoming *in-vivo* experiments were raised.

### In vivo test of blood vessel prototype

Prototypes were implanted in native carotid artery in sheep (n=16). The best survival rate and best patency results were found for the grafts based on natural polymers, even though some of these grafts had to be reoperated due to bleeding. At explantation, occlusions observed in some grafts sonographically had to be verified macroscopically. Especially prominent on synthetic scaffolds, a tissue layer on the luminal surface of the grafts (neointima) was observed. Since this neointima was only loosely attached to the graft material, the tissue layer was not maintained in the preparation process for Raster Electronic Microscopy (REM) investigation. REM revealed erythrocytes and fibrin-like filaments on the grafts' surface and in one case only a confluent cell monolayer occupying part of the surface.

Histological stainings (H&E, Movat's Pentachrome, von Kossa) of longitudinal sections of the grafts gave no evidence of cells located within the natural polymer scaffold. Likewise, in the synthetic scaffolds cells were only found in the preformed niches at the adventitial side of the grafts. Apparently these cells produced additional extra cellular matrix, but no cells were found within the material itself. The thickness of the grafts was consistent throughout the grafts. Immunochemical stains showed that no vWF+ positive cells (endothelial cells) were found within the grafts, but only on the neointima on the grafts, in the host tissue surrounding the grafts and occasionally on the grafts' surface. A large number of CD45+ cells (leukocytes) were found in the native tissue adjacent to the grafts.

All in all the grafts function could be described as suboptimal: prototypes developed in the scope of the BIOSCENT project are promising but they need further improvements.

#### *In vivo* test of heart valve prototypes (seeded approach)

Valvular grafts made out of synthetic (n=6) and natural polymers (n=6) were seeded with one million autologous endothelial cells and then successfully implanted into the pulmonary valve position. The surviving rate was good (only one loss in each group) so five animals of each group reaching the planned explantation date. While the function was satisfactory at implantation, 2 natural polymer valves had developed an insufficiency, another 2 a stenosis and 4 synthetic polymer valves were stenotic shortly before explantation. The morphological analysis showed deterioration of the natural scaffolds, while synthetic scaffolds showed no changes besides a thick neointima layer on the surface of the cusps.

Analysis by REM revealed only fibrin filaments and erythrocytes on the surface after the neointima layer detached. Microscopically no cells were found within the implanted material besides in the preformed niches of the synthetic scaffold cusps, where newly produced extra cellular matrix surrounded the cells. A thick neointima layer was found in a number of synthetic scaffold, which was more prominent on grafts implanted for the longer period of time. vWF+ cells were found in the host tissue in form of vessel-like structures and on the neointima on the cusps. Similar to the vascular grafts a large number of CD45+ cells were found in the host tissue adjacent to the grafts. Overall, seeded synthetic and natural heart valve scaffolds exhibited similar suboptimal functional results and indications of an immunological reaction were detected.

#### *In vivo* test of heart valve prototypes (unseeded approach)

During this phase IMM took charge of housing and care of sheep, providing the animals and the facility and basic surgical tools for surgery, implanting the valves, performing follow-up evaluations and performing harvesting at explant procedure.

Six animals have been implant in total, divided into 2 groups of 3 animals respectively implanted with either the UniPI valve (based on natural polymers) or the ITAV/PPI valve (based on synthetic polymer). All animals completed the scheduled 2 months follow-up. Animals underwent cardiopulmonary bypass and were implanted with the test article BIOSCENT valves in the pulmonary position using a beating heart technique. The follow-up lasted 2 months during which, blood samples and echographic evaluation were regularly scheduled. At explantation time, full clinical, biological, functional, macroscopic and microscopic evaluation of the valves was performed.

Animals of the two groups did not show clinical adverse events and all completed their scheduled follow-up. Functional echographic evaluation at the early phase of the follow-up showed that animals in the 2 groups presented with valvular dysfunction consisting mainly of regurgitation and were therefore preventively treated with lasix. Over the course of the study the results of the biological evaluation did not show abnormal findings. The functional evaluation by echographic assessment demonstrated that the valvular leaks observed in the early follow-up worsened over the follow-up. The transvalvular gradients were mild to moderate and increased slightly during follow-up in the 2 groups. The gradients were slightly lower in the ITAV/PPI valves (already after implant), which was most probably related to the difference in the design (shape) of the valve.

At explant time, the function of the valves of both groups seemed suboptimal as mild to moderate gradients were present but mostly because moderate to severe regurgitations were observed. No valve migration or suture tear were observed, and only slight structural alterations were noted as small tears or detachment at the commissures of both types of valves.

As final considerations about project outcome, it is necessary to underline the large amount of scientific data produced during the BIOSCENT project.

For each end product, a large number of materials (around 50 in total) was initially proposed; all of them were fully characterized in vitro, including an in deep biological investigation.



Based on the outcome of this screening, a smaller number of materials were selected for bioactive scaffolds development.

By combining selected materials with different scaffold fabrication techniques and functionalization strategies, a huge amount of prototypes (about 70 in total) was developed and again a complete *in vitro* and *in vivo* characterization was performed.

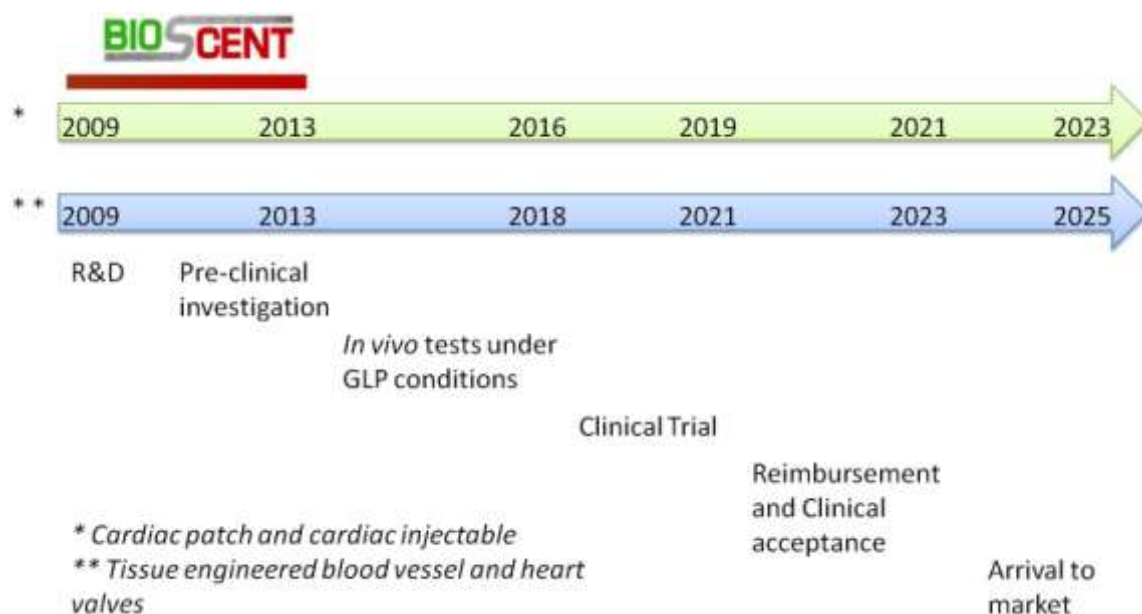
It is also worth to underline that, despite the objectives set in the project proposal were always recognized as highly ambitious, the BIOSCENT Consortium has been able to complete all project tasks for all the four end products.

In the end, different level of advancement were reached for the different end products:

- for cardiac injectables, prototypes are already optimized and we are ready to perform *in vivo* studies on large animals;
- for cardiac patch, prototypes need only minor fine-tuning, before moving to large animals;
- for blood vessel and heart valve, prototypes revision is necessary, but developed scaffold are overall promising.

From a research point of view, the Consortium has already demonstrated its commitment toward the application for one or more future projects, in which performing prototype fine tuning and *in vivo* tests on large animals under GLP conditions, in order to be ready, in 3-5 years, for the first implant in man. Such project(s) would have a strong impact on all the three pillars of the next European framework programme, Horizon2020: excellent science, industrial leadership, societal challenges.

From a market point of view, the time to market for the different BIOSCENT end products is summarized in the scheme below:



The different time to market for the BIOSCENT products is explained by the different readiness level of the prototypes at the end of BIOSCENT project:

- The Cardiac Patch and Cardiac Injectable prototypes that have been developed during the project are by far the most advanced prototypes in terms of readiness to enter clinical trials, thus being closer to market products. Only minor prototype fine-tuning will be required for their optimization.
- The Heart Valves and Blood Vessels prototypes developed during the project still require an optimization stage in order to be fully functional for being securely implanted in large

animals and in a later stage in humans. Therefore, these prototypes will need longer development times and funds before entering the market.

In terms of the possible exploitation pathways, given the current stage of development of the four BIOSCENT product prototypes, the market readiness of each of them and also nature of the consortium partners-involved in their development (IPR owners are mainly Universities and non-profit Research Institutions), the following two strategies have been defined as the most suitable for exploitation of BIOSCENT products:

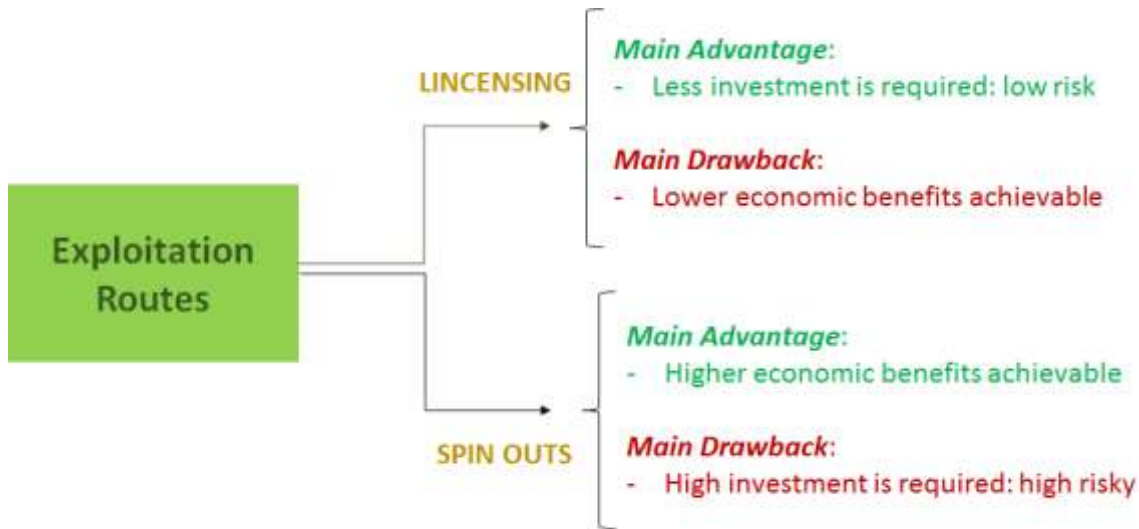
- Creation of *spin-out companies* from the Institutions currently owning the IP (patents). In this case, the objective for the creation of a spin-out company would be serving as a link between the “research institutions” with no industrial activity and the industrial partners that, as licensees of the IP, will be the key channels to bring the BIOSCENT products to the market. If being this the strategy of choice, once the spin-outs are created, their first objective will be to search for funds (public or private funds such as venture Capital or Business Angels). These funds will be dedicated for further prototype optimization, for accomplishing testing in large animals and entering clinical trials in humans. Once in Phase 2 of the clinical trials process, the spin-out would be in a very lucrative position to extend licenses to the “big players” of the cardiovascular devices market, such as the project partner SORIN. At this stage these big players could afford the remaining clinical trials costs and would bring the BIOSCENT products to the market, through a “win-to-win” situation, where both the spin-out company and the “big player” would receive interesting revenues from product commercialization.
- Directly *licensing the IP* (patents) to “big players of the cardiovascular devices market. The direct licensing strategy implies that after the BIOSCENT project, the IP owners will have to actively (through their technology transfer offices) search for IP licenses within the major Global Players of the cardiovascular medical device market. The project partner SORIN would be the first global player to be offered as exclusive licensee of the IP, so they could take advantage of being the only one with possibility of exploit these BIOSCENT results and benefit from the highly lucrative economic benefits of bringing these products to the cardiac surgery market. Once the license agreements are signed with an industrial partner, this partner will assume the further costs required for bringing the BIOSCENT products to the market, namely costs of the prototype optimization (in case it is decided not to be done by the IP owners before licensing, due to a strategic decision or lack of enough financing) and the costs of pre-clinical and clinical trial processes. Following this strategy, once the products enter the market, both the Industrial Partner responsible for selling the products in form of direct benefits, and the original IP owners in the form of royalties, will receive economic benefits from the commercialization of BIOSCENT products.

The selection of the preferred exploitation pathway from the two ones described above is a strategic decision that will be taken in a post project stage by the institutions implied in the IP generation. Some of these factors that deserve to be evaluated while deciding the exploitation strategy to be followed could be the following:

- The main features of the targeted market
- The nature of the IP holding entities
- The degree of technology development
- The particular interests of the IP holders
- The availability of financial resources (public or private financing such as Venture Capital or Business Angels would be required for the creation of a spin-out company)



It deserves to be highlighted that both strategies are equally effective in terms of ensuring the entrance of BIOSCENT products to the cardiovascular market, in the timing shown in the above figure. Nevertheless, the cost/benefit and economic risk is quite different for each of them. As a general approach, the following figure shows which are the major risks and advantages of each “route to the market”:



**The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results**

**Socio-economic impact or BIOSCENT project**

Cardiovascular diseases (CVD) are considered as one of the major causes of death and disability all over the world. This disease category encompasses all health problems affecting the heart and surrounding vascular system such as high blood pressure, valvular heart disease, stroke and rheumatic heart disease.

Diseases of the heart and circulatory system (cardiovascular disease or CVD) are the main cause of death worldwide, accounting for more than 17.3 million. In Europe, it is estimated up to 4 million persons die each year due to cardiovascular associated diseases. Nearly half (47%) of all deaths are from CVD (52% of deaths in women and 42% of deaths in men). The main forms of CVD are coronary heart disease (CHD) and stroke. Just under half of all deaths from CVD in both men and women are from CHD, with stroke accounting for nearly a third of deaths in women and a quarter of deaths in men. Apart from the societal problem, which is the most dramatic aspect, these frightening figures have also a great impact in the European economy. Overall CVD is estimated to cost the EU economy almost €196 billion a year. Of the total cost of CVD in the EU, around 54% is due to direct health care costs, 24% to productivity losses and 22% to the informal care of people with CVD.

Given the European and International significance of the CVD problem, there is a great interest in new methods for preventing and treating health problems associated with CVD. However, a successful and fully satisfactory treatment of CVD is in many cases limited due to the lack of autologous tissue to restore injured tissue. This means that heart cannot repair itself once damaged, resulting in the formation of scar tissue with different contractile, mechanical and electrical properties. At the end, this leads to malfunction of the whole circulatory system due to the inability of the heart to supply enough blood to the body. The most extended solution, when autologous

tissue is damaged is to either its replacement using synthetic graft material, or cardiac transplantation. However, these solutions have important limitations such as insufficient performance of synthetic materials compared with native tissue or complications associated with rejection and infection if dealing with organ transplantation. Of course, the cardiac transplantation has the additional limitation of insufficient organ donors.

In this context, the development of new treatment therapies capable of repairing and regenerating infarcted cardiac tissue, such the solutions developed in BIOSCENT project, have awaked a great interest for the healthcare community. This type of solutions will avoid underperformance issues of synthetic grafts as well as rejection, infection and “lack of donors” issues of organ transplantation. Tissue engineering-type surgeries have gained popularity in the last decade. These repairs offer faster recovery times for the patient and have shown high recurrence rates. As more successful cases of surgery are reported, people will be more prone to opt for tissue engineered options, thus increasing their market uptake.

It is quite clear that the societal impact of TE cardiac solutions could be enormous, as patients life quality would be improved significantly. Nevertheless, from an economic perspective, there is still an open debate on the cost-efficiency of tissue engineering-type surgeries, compared with conventional cardiac surgery. Tissue engineered products typically are more expensive than conventional solutions due the high regulatory compliance costs and the challenging manufacturing process. This represents a key challenge to the adoption rate in the market. On one hand, estimations show that tissue engineered products will be more expensive than conventional treatments. On the other hand, the global crisis is forcing the reduction of the healthcare expenditure by the global healthcare systems, which are the buying decision makers. The trend is therefore moving towards more cost-effective solutions.

Notwithstanding, the healthcare cost associated with interventional cardiology are due to the costs of the surgical processes, the hospitalization time and the post-interventional therapies (medication), while the implant cost represents only a small proportion of the total costs. The hospitalization time and post-interventional therapies (medication) are the responsible of the majority of the total costs. In addition, in several cases the implants can lose their functionality along time due to degradation, obstruction, etc. This results in the need of a second re-intervention, with the consequent costs it represents to the healthcare system. Therefore the most effective way to significantly reduce the direct healthcare costs would be to reduce the hospitalization time and to avoid/reduce the post-interventional medication and the likelihood of re-interventions. In this sense tissue engineered cardiac implants, such as the BIOSCENT products, offer the following advantages compared with conventional surgery process:

- The ***Cardiac Patch and Cardiac Injectable***, by enabling the recovery of the heart muscle, will allow a lower level of re-infarction and the need of less medications and less hospitalizations.
- The ***engineered blood vessel*** will reduce the likelihood of a second re-intervention and will avoid the harvesting of an autologous conduit, thus being less invasive. In particular, this second characteristic allows a shorter hospitalization time of the patient and avoids the typical complications of conduit harvesting (e.g. edema, infections, linfhorrea).
- The ***Tissue Engineered Heart Valve***, in terms of improved quality of life, puts together the main advantages of mechanical bioprosthesis (durability) and the biological bioprosthesis (freedom from anticoagulant drug therapy). Patients receiving a tissue

engineered HV have lower likelihood of a second intervention and are not exposed to the anticoagulation therapy dose-related risk of bleeding complications.

Therefore, we can conclude that even being more expensive, tissue engineered implants have a great market potential since the overall healthcare costs associated with CVD can be greatly reduced in the medium term. This is in line with the current trend of the healthcare market of moving towards more cost-effective solutions. Therefore we can affirm that the BIOSCENT products will have a place in the cardiovascular market.

As more quantitative data will be available on economics of successful TE treatments, the healthcare systems will increase their interest in purchasing this type of solutions. In the meanwhile, it is important to increase the awareness of patients and doctors through active dissemination actions aimed to patient organizations and healthcare associations. These activities have been important during BIOSCENT project and will be continued after the project.

### **Main Dissemination & Exploitation activities in BIOSCENT project**

Dissemination and Exploitation related activities are of high relevance in the framework of a collaborative research project. These activities are the only realistic way to turn innovation into products in the market, thus into economic benefits.

In BIOSCENT project we have been able to develop highly innovative products with huge potential to change the paradigm of cardiovascular disease treatment. Therefore, our next target is to bring our technology to the market.

Dissemination activities have been performed with the aim of promoting the widest dissemination of BIOSCENT results among the industrial and scientific communities. These activities have been of great importance for BIOSCENT consortium partners since the beginning of the project as they are understood as the most effective way for communication of project results among the potential stakeholders. The primary objective of BIOSCENT dissemination is to achieve a high impact not only within the healthcare community and the patients as end user of the BIOSCENT results, but also within the scientific community given the scientific significance of the project outcomes.

The products developed in BIOSCENT project are aimed to provide effective tissue engineering solutions for the treatment of cardiovascular diseases, which are one of the major causes of mortality in the developed world. These cutting edge solutions will surely have a great impact in the healthcare sector in the near future, since they have a great potential to improve the patients quality of life as well as reducing costs for the health care systems all over the world. This is why dissemination has been essential for ensuring a widespread diffusion of the benefits achievable by BIOSCENT tissue engineering solutions.

The main dissemination activities carried out in BIOSCENT project can be summarized as following:

#### ***BIOSCENT Website:***

The BIOSCENT web site has been an active instrument for the promotion of the BIOSCENT results and also for the interchange of information. The web contains latest information about the project; as well as the list of partners, results, contact information, etc.

The web site is structured into a Public and a Private section. The Public section is accessible to the general public, while the Private section is password protected. The Public section includes the following information:

- The Home section, designed to attract the attention of the visitors. Includes a short summary presentation as well as most recent news about the project.
- The BIOSCENT Insight section, where a project summary, the societal justification, the scientific and technological objectives and the state of the art of the project are presented.
- The Partners section, including contact information for all the members of the BIOSCENT Consortium: industrial partners, academic partners and the European Commission. A link to the webpage of all project participants is included in the partner section.
- The Events section, where a calendar of all the dissemination events that have taken place and that are foreseen is presented.
- The News: this section is a repository of news related to the project and also of major announcement of the project results.
- The Links section: this section is updated periodically with links to web sites and publications related to the project; including standardization committees, EC web sites, main conferences and events, etc.

The Private section is designed as a tool for information exchange between the partners. It includes a partner's forum, a private news section and a repository of documentation about the project; including deliverables, meetings and presentations, mini projects, technology watch reports and bulletins, Related Publications, Contractual documents, project management templates, etc. The BIOSCENT web site is W3C 2.0 compliant and follows standard accessibility rules.

As important as a well-designed website is the dissemination of the site address itself. For that reason it is determinant to include the web site address in a relevant position in the main web searchers. It is also very important to include links to the web site the partner's own web sites and in some of the main web pages dealing with biomaterials, healthcare efficiency as well as any other related field that might be considered interesting for the project. This activity have been done proactively during the complete development of the exploitation phase of the project.

#### ***Conferences, seminars, workshops, trade fairs:***

One important activity in the BIOSCENT dissemination plan has been attendance to conferences, seminars and workshops. The BIOSCENT project has been present in the leading events dealing with biomaterials, stem cells, tissue engineering, cardiovascular and regenerative medicine. These events are not only relevant from the point of view of promotion and dissemination, but may become the only reasonable way to demonstrate the BIOSCENT products in its full potential to a massive audience. Many professionals attend these types of events in search for new technologies and ideas. Conferences and seminars also reinforce the innovative image of the BIOSCENT products and may lead the way to future improvements of the technologies. Presence in these types of events has been not only implemented by means of demonstrations or talks but also by writing papers, reports and benchmarks which have been later published in the conference proceedings.

### ***Publications:***

Scientific publications are the most effective means for dissemination of scientific results within the scientific community. This is why, in the course of the project we prepared several publications that have been published in Scientific Journals with high impact factors.

### ***Technology watch:***

Technology watch (TW) basically consists of an “organized and structured system for the searching, detection and analysis of the environment, aimed at disseminating and transmitting information and knowledge in a continuous manner, at the required time, so that the target group can be made aware of the main activities in their sector, within the technology field”.

BIOSCENT has been an ambitious project, being at the development of highly innovative tissue engineering solutions for treating cardiovascular diseases. Tissue engineering solutions have a huge market potential that will open up novel approaches in the treatment of cardiovascular diseases. For these reason BIOSCENT objectives arouse a great interest, not only for the scientific community, but also for the healthcare market. Many companies and research centers are interested in developing similar solutions. Therefore, keeping in mind the significance of BIOSCENT innovation, TW has been a very relevant activity during the course of the project.

The objective of technology watch is to alert of any scientific or technical innovation that could represent either a threat or an opportunity for the BIOSCENT consortium aims. The TW strategy was organized as a 5 step cyclic process:

- Identification of consortium needs, both technical information (scientific publications, events, specialized websites, projects, founding, grants, research groups, patents...) and commercial information (technological partners, competitors, companies, patented products, potential clients,...).
- Search for relevant information according to the identified consortium needs: identification of the appropriate information sources, identification of the keywords, broadening the search scope.
- Record the information: publication record, patents record, commercial information record, events record, informal contacts record.
- Analyze the information: determine the reliability of the information source; elaborate a database; evaluate the information against the following criteria: Who? What? When? How? And How relevant?; comparing/understanding the information and finally validate the results.
- Validate the information by experts and specific surveys.
- Diffusion of the information through technology watch reports, BIOSCENT website, Technology watch bulletins and press notes.

According to the above described TW protocol, the BIOSCENT consortium partners under the leadership of PERA/ITAV have conducted an active and exhaustive technology watch since the beginning of the project. The following TW activities have been conducted:

- Preparation of Technology Watch Reports. TW reports have been prepared each 6 months, coinciding with the Management Board meetings and milestones. The main objective of the reports has been to ensure that the decisions on how to organize the future work were made within the appropriate technical and market scenario. The structure of the TW reports has been almost standard for each report, as defined in the

- “protocol for technology watch procedure” at month 2, consisting in: a) scope of the TW report, b) background, c) analysis of the state of the Art, d) conclusions and e) annexes.
- Electronic dissemination of the most relevant information encountered during technology watch: publications, patents, press releases and other documents. BIOSCENT website has been the main dissemination channel for this purpose.
  - Technology Watch Bulletin. This bulletin has been prepared in a three month basis, including information about recent publication, patents, research, market news, EC initiatives, funded projects in related scientific and technological areas...
  - BIOSCENT Press notes that have been included in the website in the public section in order to give BIOSCENT the maximum dissemination level.

### ***Communication with stakeholders***

Since the beginning of the project, the BIOSCENT consortium partners have worked actively in identifying the potential stakeholder’s communities, which could be interested in BIOSCENT results. Given that BIOSCENT objectives are the development of novel therapies for treating cardiovascular diseases, the communities targeted have been: the healthcare community, medical devices/advanced therapies associations, regulatory organisms and patient associations.

Through communication with stakeholder groups, we have confirmed that there is a high demand in the healthcare market for the kind of solutions offered by BIOSCENT. Moreover, we have been able to understand the regulatory scenario affecting the medical devices and advanced therapy products sector. This information is of high relevance for future exploitation of BIOSCENT results.

### ***Communication with Policy makers***

Policy makers have been one of the main target groups for BIOSCENT project communication. Project outcomes and future perspectives have been presented.

### ***Other dissemination activities***

Other dissemination activities apart from the previous described ones, has been also important channels for communicating BIOSCENT results within the end user community, the scientific community and the general public. Some of the activities are detailed below:

- Web presence: As previously described, BIOSCENT website has been one of the major dissemination channels. In addition, BIOSCENT results have been promoted in different web pages.
- Training material. Some video clips have been prepared as training material for providing a visual support on how the new technology has to be used for professionals within the healthcare sector. Moreover, methodological and good practice guidelines have been also prepared with training purposes.
- Activities aimed at informing the general public, media coverage: The research activities carried out in the scope of the BIOSCENT project have received a great attention from media, especially in Italy (country of the project coordinator). On July 2011, an article presenting the BIOSCENT project was published on Stil’è, a magazine distributed by *IL SOLE 24-ORE*, the major Italian journal on Economy. During the last conference of the European Society of Biomaterials, held in Dublin on 4-8 September 2011, Dr. Elisabetta Rosellini presented results concerning the cardiac patch subtopic. An article on this was

published on the first page of the Irish Times, on September 6, 2011. After that, local media in Pisa and Italian national journals dedicated a strong attention to the project.

With regard to the main exploitation activities carried out during BIOSCENT project, a “Dissemination and Exploitation Board” (SDE board) has been supervising and leading all the exploitation related tasks they could be summarized as following:

- Identification of exploitable results arising from the project. Definition of the contribution of each partner to each exploitable result. BIOSCENT is a big collaborative project, in which up to 15 partners have been engaged in the development of 4 different end products, namely Heart Valve, Cardiac Patch, Blood Vessel and Cardiac Injectable. For the development of these 4 different end products, an interdisciplinary collaboration between material scientist, biologist and surgeons has been the key aspect for succeeding. Is therefore easily understandable that the collaboration network has been highly complex. Therefore, the SDE board has been the last responsible for defining the level of contribution of each partner to each result in order to achieve a final agreement on distribution of the Intellectual Property Rights.
- Monitoring of progresses in competitive technologies through Technology Watch activities. The technologies watch activities that have been carried out in the course of the project are detailed in the dissemination section of this documents.
- Checking regulatory issues dealing with exploitation of different BIOSCENT product types: Medical Devices (MDs) and Advance Therapy Medicinal Products (ATMPs). The regulatory framework is of high relevance for the healthcare market, since it highly influences the road to commercialization. For this reason, big efforts have been made during BIOSCENT project in order to understand the European and International regulations affecting the BIOSCENT products. Especially important has been to define which results can be classified as Medical Devices and which fall under the category of Advanced Therapy Medicinal Products (ATMPs).

**The address of the project public website, if applicable as well as relevant contact details.**

Project website: [http://inspiralia-delivery.eu/fp7bioscent\\_eu/](http://inspiralia-delivery.eu/fp7bioscent_eu/)

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