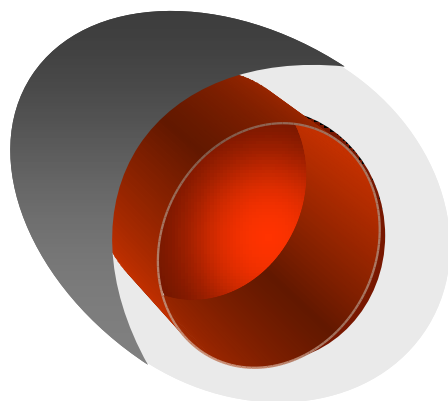


Grant Agreement number: 214402

ANGIOSCAFF – PUBLISHABLE SUMMARY



ANGIOSCAFF

1. PUBLISHABLE SUMMARY

1.1. Project Objectives

The **overall objective** of ANGIOSCAFF is to create bioresponsive, bioactive and injectable materials capable of carrying therapeutics, which can be used for tissue regeneration in humans. The new biomaterials will respond to cell-associated environmental signals, such as extracellular proteases and endoglycosidases and generate bioactivity by virtue of bound peptides or recombinant adhesion molecules and growth factors.

This requires:

1. Radical innovations in state-of-the-art biomaterials.
2. The design of high-performance biomaterials inspired by natural processes.
3. Biomaterials that control cell differentiation.
4. Injectable biomaterials that can induce angiogenesis in the body.
5. Bioresorbable, highly porous and structurally sound tissue-engineered scaffolds.
6. Functionalized biomaterials that have direct influence on cell behavior.
7. Bioactive scaffolds with broad applicability for complex tissues.
8. Advanced bioactive scaffolds enabling internal growth of tissue and site-specific delivery of bioactive signaling factors.
9. Effective delivery devices.

In line with these objectives, the work has been divided into six interlocking but distinct strategic areas. Individual partners bring in specific expertise and resources that are collectively leveraged.

The first area develops novel biomaterials platforms suitable for use in regenerative medicine. These biomaterials will possess *in situ* transformation, biospecific resorption and incorporation of biological ligands (referred to herein as *bioactives*, or *morphogens*) to induce tissue-specific differentiation and morphogenesis, with emphasis on angiogenesis.

The second area develops engineered morphogenetic biomolecules (peptides, proteins and the genes that encode them) to induce angiogenesis and other desirable angiogenesis-associated morphogenetic processes in the target tissues. These engineered morphogens will be combined with the developed biomaterial platforms so that all of the constructs developed will be biofunctionalized to be bioactive.

The third area functionally validates the biomaterials on the blood vessel itself by: (a) *in vitro* and *in vivo* characterization of their intrinsic angio- and lymph-angiogenic potential; (b) using them as a quantitative, designed and controllable platform to probe hypotheses on fundamentals of blood and lymph-angiogenesis.

Thus, Angioscaff's objectives are both translational (induction of blood and lymph-angiogenesis) and fundamental (development of material and molecular tools for the understanding of angiogenesis).

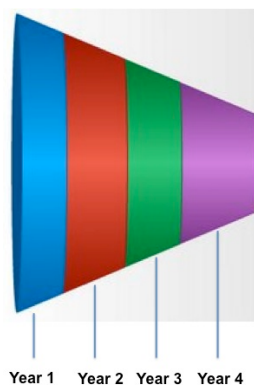
The fourth area assesses the functional activity of the biomaterials in bone repair by: (a) developing develops materials and biomolecular therapeutics for bone repair with and without transplanted stem and progenitor cells; (b) using these constructs as a quantitative, designed and controllable platform to test hypotheses on fundamentals of osteodifferentiation, osteogenesis and bone repair, with an emphasis on the role of angiogenesis in these processes.

The fifth area develops materials and biomolecular therapeutics for skin repair. These constructs will be used as a quantitative, designed and controllable platform to test hypotheses on fundamentals of skin (lymph)angiogenesis in normal and pathophysiological models (e.g., in diabetes) and their impact on skin healing in pathophysiological situations such as burns and chronic wounds.

Finally, **the sixth area** addresses a broader application of the materials for neuromuscular tissue by developing materials and biomolecular therapeutics for neuromuscular repair with and without transplanted cells in the context of skeletal muscle, cardiac muscle and associated motor and sensory nerve.

1.2 Work performed since beginning of the project

The Angioscaff project strategy is designed along a funnel approach in which over the duration of the project a series of biomaterial and bioactive morphogen combinations are developed with broad application in mind, and then fine tuned at a pre-clinical validation level according to the specific needs to the tissue that is being targeted for repair.



Year 1: Initiate development of materials and morphogens, including optimized novel screening systems

Year 2: Perform initial screening of combinations and/or materials alone

Year 3: Identify and initiate advanced screening and validation of combinations and/or materials alone

Year 4: Finalize early stage pre-clinical validation of materials +/- morphogens

Since the beginning of the project we have developed a series of seven novel biomaterials tuned to combine with bioactives, which possess and transmit various patterns of spatio-temporal biofunctionality. We have selected and developed materials during year 1 and year 2 into biofunctionalized scaffolds containing growth factors, ephrins, adhesion molecules and genes for delivery into specific target areas. These bioactive materials are being validated alone or in combination in systems representing the pertinent target tissues.

The first stage of characterization of functionalized materials, which incorporates 110 different projects, is now completed. The materials have been tested in degenerative disease models to assess their stimulation ability to regenerate damaged tissues. In the second year we initiated the validation studies *in vitro* in angiogenic, skin, bone, cardiac, neurological and skeletal muscle models to specifically assess the regeneration and repair stimulating capacities of the materials. This enabled us to address the important question as to whether different materials are able to produce similar angiogenic and regenerative responses *in vivo*, leading to the achievement of the major milestone (milestone 7.2), in which the initial 110 projects were filtered down to 30. These 30 projects had the highest impact on generation of new knowledge and on the opportunity to meet realistic therapeutics and market needs.

Below we indicate and describe the main results achieved since the beginning of the project, focusing on the 30 selected projects and on their impact.

1.3 Main results achieved so far

Biomaterials and morphogens

During the first two years we have developed and explored biomaterials in combination with morphogens. The results obtained with the first generation materials created the incentive to **extend the existing biomaterial platform** to better meet the requirements of the other research areas. The results obtained by testing new biomaterials in angiogenesis and tissue engineering in bone, skin and neuromuscular tissues have informed further research, promoting development and characterization to improve **mechanical properties** of injectable matrices, adjust **biofunctionalization**, develop injectable **hyaluronic acid-based** matrixes and conjugate **FN-GBD to starPEG-heparin** hydrogels to produce new generation scaffolds (*Figures 1 and 2*).

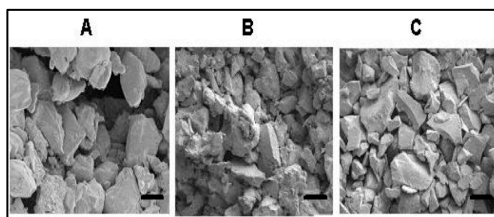


Figure 1. Microstructure of injectable, mechanically stable matrixes.
(A) PLGA/PEG; (B) PLGA/PEG + G5 glass (melt blend);
(C) PLGA/PEG + G5 glass (powder blend)

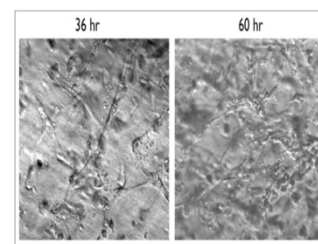


Figure 2. Endothelial cell morphology over time on new generation star-PEG-heparin gel. Cells attach to gels, have no sign of toxicity and start forming tubes.

To induce angiogenesis and desirable angiogenesis-associated morphogenetic processes in the target tissues we have generated growth factors-containing scaffolds such as: Fibrin-binding and cys-containing VEGF-A and VEGF-C and fibrin-binding PIGF; Fibrin-binding and cys-containing VEGF-syndecan; Wild-type, fibrin-binding and cys-containing TGF- β 1 and TGF- β 3; Fibrin-binding PDGF-AB; Wild-type, fibrin-binding and cys-containing IGF-1; Fibrin-binding BMP-2.

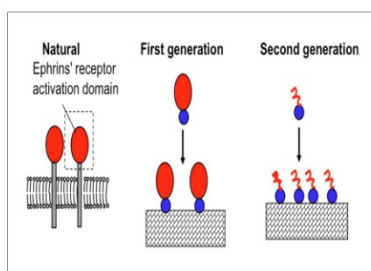


Figure 3. Schematic of ephrin-modified scaffolds. The whole recombinant ephrins activation domain is tagged for scaffold incorporation in the first generation scaffolds, whereas only small synthetic ephrin peptides are tagged in the second generation scaffolds (A.Zisch et al. Biomaterials 2004).

We generated second generation ephrin-modified scaffolds (TG-EMP peptides, *Figure 3*) by incorporating ephrin peptides into fibrin gels in the presence of serum components and cells. In particular, TG-EMP-A2 peptide activated EphA2 receptor signaling preferentially in fibrin matrix-bound form, whereas TG-EMP-B4 in free form retained inhibitory potential. TG-EMP-A2 could be efficiently incorporated in PEG-based gels and used for EphA2 receptor signaling activation in 2D and 3D cell cultures.

Controlled delivery of VEGF and Ephrin peptides improved the functionality of mesenchymal stem cells and endothelial progenitor cells in 3D culture, where cells also showed changes in gene expression, including chemokine receptor and growth factor genes.

Fibronectin fragments supported the isolation and expansion of human bone marrow-derived mesenchymal stem cells more efficiently compared to the full-length protein. The most efficient fragment comprises the CBD and the GBD.

In summary we have created, assessed and filtered our approaches to focus on the following specific areas.

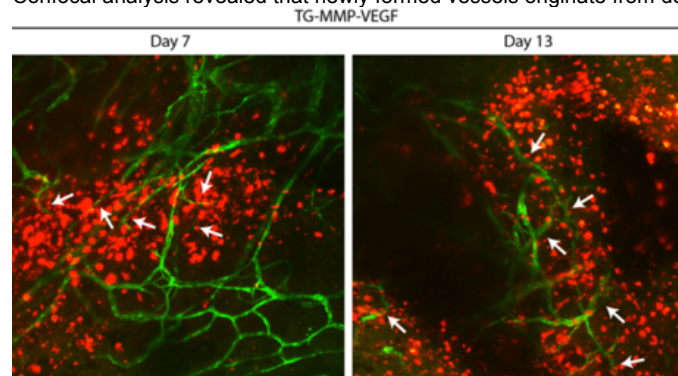
Material	Morphogens +/- Material	Tissue focus	Targetable Pathology
Fibrin	Fibronectin fragments, VEGF isoforms, PLGF, IGF1, NGF, NT3, BDNF, Ephrins, SDF	Skin, Bone, central and peripheral nervous tissue, Cardiac, blood vessels	Burns, skin wounds, diabetic ulcers, spinal cord injury, ischemic damage, cardiovascular disorders, osteoporosis, fracture, bone replacement, craniofacial and rare diseases of bone, muscle, skin
PEG Peptide	VEGF, Ephrins	Bone, blood vessels, cardiac	Ischemic damage, cardiovascular disorders, rare bone diseases, osteoporosis, fracture, bone replacement, craniofacial
Fibrinogen polymer	BMP2	Bone	rare bone diseases, osteoporosis, fracture, bone replacement, craniofacial
Hyaluronic acid	BMP2, nucleic acids,	Bone, cardiac, blood vessels (nucleic acid delivery to tissues)	Ischemic damage, cardiovascular disorders, rare bone diseases, osteoporosis, fracture, bone replacement, craniofacial
Porous scaffolds	BMP2, VEGF	Bone	rare bone diseases, osteoporosis, fracture, bone replacement, craniofacial
Calcium Phosphate	BMP2	Bone	rare bone diseases, osteoporosis, fracture, bone replacement, craniofacial

Blood vessel growth

We evaluated OECs for their vasculogenic potential in 3D cultures as compared to MVECs upon the addition of the growth factor VEGF. Angiogenesis could be readily monitored using immunofluorescence and flow cytometry.

Our results using zebrafish as a model for angiogenesis demonstrated that normal blood supply is restored 7 days after implantation of the biomaterials. Adding our engineered VEGF improved vessel growth (Fig 4). The stiffness of the gels added is an important variable to consider to achieve full vascularization.

Figure 4. Subcutaneous implantation of fibrin gels in zebrafish. Arrows point to new vessels (in green) growing within the fibrin gel (in red) in the presence of TG-MMP-VEGF at (left) 7 days or (right) 13 days post implantation. Confocal analysis revealed that newly formed vessels originate from deeper tissues.



We established a wound-healing model on the mouse ear to study lymphangiogenesis. High doses of VEGF-C caused lymphatic hyperplasia but lymphatics were still functional, and there was no effect on wound-associated macrophages or the wound healing process.

SDF-1a loaded hydrogels exhibited improved remodeling and cell infiltration as compared to control gels. Subcutaneous implantation of SDF-1a loaded starPEG-heparin hydrogels increased the number of EPCs homing to the tissue.

Bone repair

Bone repair could readily be modelled using in vivo bioluminescence imaging and CT analysis of bone density and corrosion casts aided by computer based modeling of vascular structures. A significant improvement in bone volume was generated when fibrin plus growth factors were used.

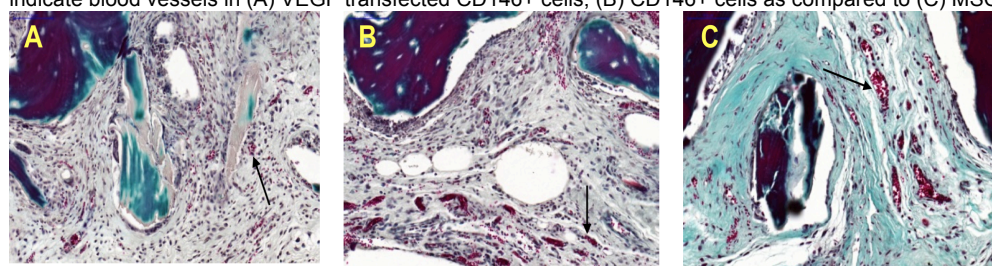
To first demonstrate the pattern of bone formation in hyaluronic acid gels with or without BMP-2, gels were modified by the addition of nanohydroxyapatite particles and analysed to determine which cells are responsible for osteogenesis. An increased migration of macrophages, endothelial cells and pluripotent cells in the implants containing BMP-2 was observed.

3D porous structures fabricated by both rapid prototyping (RP) and electrospinning techniques were evaluated for protein release and results indicated that for both types of scaffolds, BMP2 strongly bound to the surface. Thus, these materials can provide a slow release system for growth factors.

The influence of the insulating e-PTFE membrane to modify the segmental defects was evaluated in a rabbit ulna defect. Placing a non-resorbable GORE-TEX membrane around the radius resulted in a 3 fold increase in the amount of bone present in the defect as compared to the control group.

To examine bone formation and angiogenesis, we used MSC, CD146+ or VEGF transfected CD146+ cells that were implanted subcutaneously into nude mice at ectopic sites. Analysis of vascular density showed that VEGF transfected CD146+ and CD146+ cells had significantly higher densities as compared to the MSC cells (Fig. 4).

Figure 5. Masson's Trichrome staining of the retrieved scaffolds at two week after implantation. Arrows indicate blood vessels in (A) VEGF transfected CD146+ cells, (B) CD146+ cells as compared to (C) MSCs.



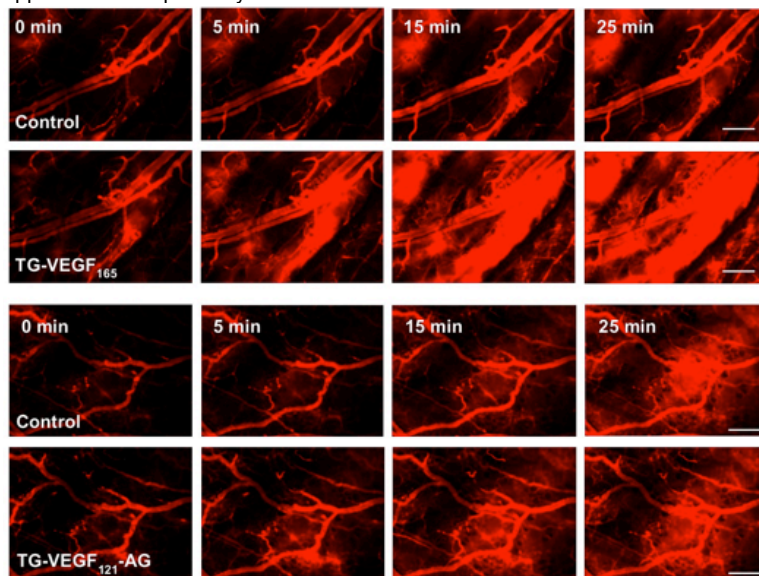
We sought to determine the relationship between the level of aldehyde modifications of HA and its effect on in vitro transfection efficiency. Effectiveness of HA derivatives for in vitro transfection of normal, adipose tissue mesenchymal or tumor U87 cells correlates positively with the degree of aldehyde modification.

The osteoinductivity of calcium phosphate foams loaded with the osteogenic morphogen BMP-2 was studied for bone repair. Following subcutaneous implantation of materials into athymic nude mice and analysis by histology, new bone formed subcutaneously after CDHA implantation.

Skin repair

We have tested Fibrin in combination with the developed VEGF-Syndecan and VEGF using our newly developed intravital vessel permeability model on the mouse ear dermis. Permeability was minimal until the VEGF variants were added, and the rate of leakage was much less when using VEGF-Syndecan.

Figure 6. The effect of TG-VEGF-Syndecan and TG-VEGF-165 on vascular permeability. Fluorescent stereomicroscope images of i.v. injected TRITC-dextran leakage from blood vessels to the extracellular tissue. Representative images of vessel permeability of (A) control and TG-VEGF₁₆₅, (B) control and TG-VEGF₁₂₁-AG applied fields respectively.



Using the rodent epigastric flap model in an ischemic setting, the amount of VEGF needed was tested for use with fibrin gels. Lower VEGF dosing lead to equal wound closure in the vital areas as compared with higher VEGF, but faster closure in the ischemic areas. These results indicate a positive effect of VEGF on wound healing in an ischemic area with appropriate dosing of therapeutic protein.

To substantiate the altered cell spreading and focal adhesion formation of cells adhering to FN fragments and FN-VEGF constructs, HUVECs were stained for integrin binding. Efficient cell adhesion to FN-VEGF promoted the re-distribution of integrin avb3 to focal contacts, ultimately resulting in increased cell spreading.

We investigated the involvement of focal adhesion kinase (FAK) signaling in response to PIGF stimulation. These results allow us to detail the critical role of PIGF-2/Nrp-1 interactions for FAK activation.

Neuromuscular repair

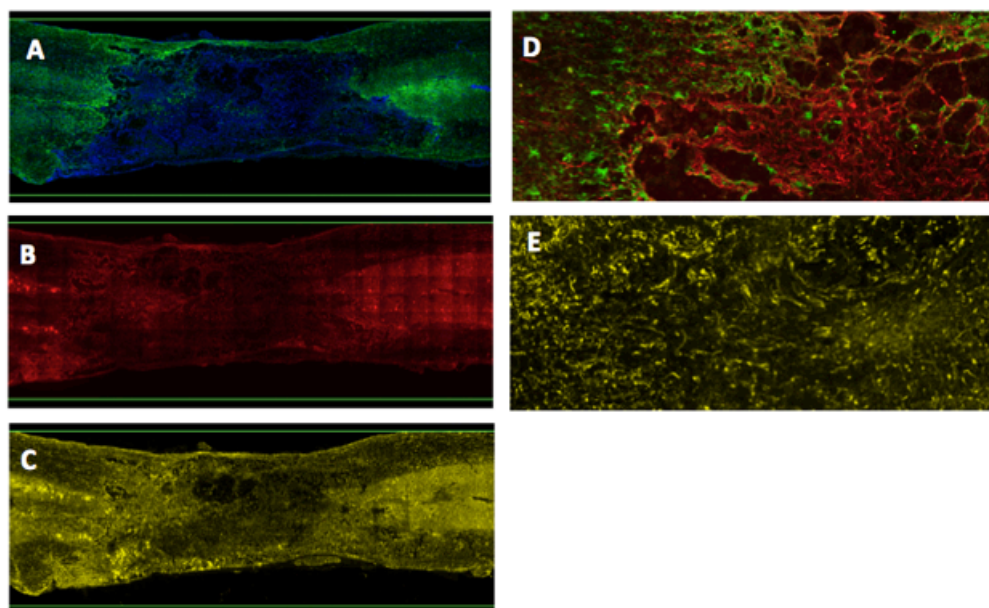
Work has now advanced to assess the effect of biomaterial-bioactive combinations in neurological, cardiac muscle and skeletal muscle repair.

Both fibrin with and without FN fragments strongly supported the growth of DRGs in vitro, as compared to other biomaterials and also allowed increased axon growth in a microchannel prototype in sciatic nerve injury model.

In a spinal cord contusion model, the recovery of rats receiving TG-PEG gels loaded with FN fragments and the growth factors NT-3 and BDNF had improved spinal cord recovery (Fig. 7).

Figure 7. Representative images of post spinal cord contusion and recovery of rats receiving TG-PEG gels loaded with FN fragments and the growth factors NT-3 and BDNF. (A) Reactive astrocytes (green) and DAPI

(blue). The astrocytes surround the lesion area and infiltrate at certain regions. (B) Chondroitin sulphates (red) were present throughout the lesion. (C) Neurofilament staining (yellow) clearly defined the white and grey matter in the spinal cord and was present throughout the lesion in a less organized manner. (D) Reactive astrocytes (green) and chondroitin sulphate (red) at the edge of the lesion. (E) Neurofilament stain in middle of lesion (n=5).



Combining iPS derived cardiac muscle stem cells with the biomaterial resulted in a significant amelioration in the anatomical parameters of the regenerating heart in an *in vivo* model.

Vessel associated progenitor cells (Mesoangioblast-Mabs) combined PEG-Fibrinogen and the growth factors VEGF, PlGF and IGF1 were used to study *in vitro* skeletal muscle tissue generation. Gels embedded Mabs that expressed PlGF had increased vessel recruitment in *de novo* generated artificial tissue compared to unmodified Mabs. Also, improved maturation of well-differentiated myofibers and an overall increase in the size of differentiated muscle fibers was detected in plugs with IGF1.

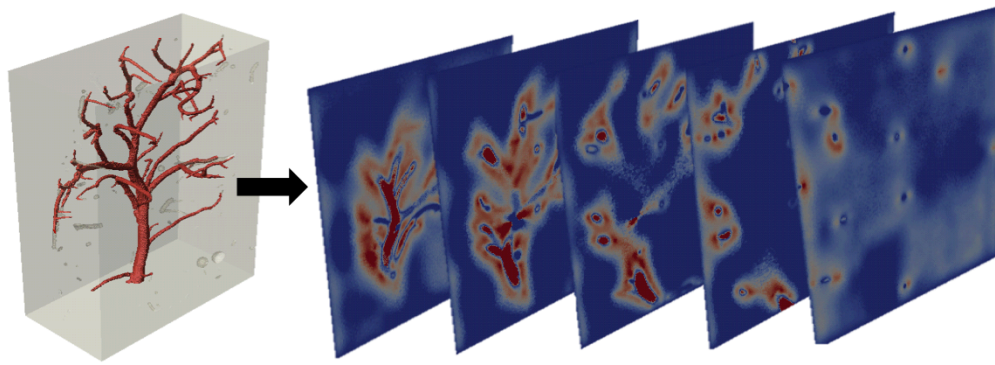
Imaging

The advances we have made in developing regenerative approaches need optimized tools for clear pre-clinical demonstration of effect in animal models. Imaging is vital to measure and assess tissue repair.

Our results using micro-CT imaging can be readily applied in preclinical models of tissue regeneration. We have also shown that dynamic imaging is a useful modality to assess the kinetics and perfusion of different tissues.

We were able to simulate the oxygen concentration brought by the blood flow rate diffusing into the surrounding tissue in order to nourish cells embedded in the surrounding extra cellular matrix (Fig. 8).

Figure 8. Oxygen distribution in the surrounding tissue of the vascular network (detailed of a branch showed in various cross sections).



1.4 Expected final results & their potential impact and use

The proposed projects capitalize on combined expertise in different areas of regenerative medicine. Collaborative interactions allow us to merge our unique and complementary expertise in the field. The expected final results will be the generation of new scaffolds that:

- Are 'smart', 're-adsorbable', can accommodate seeded cells and present correct signals
- Contain factors that stimulate specific cell differentiation *in vivo*
- Provide sufficient vascular supply

Moreover, we will:

- Precisely identify the type and numbers of cells (endogenous and/or exogenous) required to restore biological functions to regenerate tissues.
- Validate these bioactive biomaterials to the first step of pre-clinical development in relevant animal models.

Impact on science

Angioscaff includes teams working on specific areas of tissue engineering that are associated with clinical targets.

The clinical targets were selected because of their high societal and economic impact. By translating our knowledge in biomaterials and angiogenesis into clinical targets we put our science to the real test of usefulness. New biofunctional materials and angiogenic molecules with therapeutic application will be introduced to the clinic and to commercial development.

The impact of Angioscaff is best illustrated by the need for organ transplantation, the major motivation behind regenerative medicine: 25% of patients waiting for an organ donor die before one can be found. In 2001 there were only 12 607 donors available to help 81 528 patients in need. The promotion of tissue repair via the use of optimized scaffolds (porous, angiogenic, bioactive and resorbable) that stimulate endogenous cells to regenerate a fully functional tissue should address this problem and decrease related mortality.

Impact on society - socio economic benefits

Degenerative diseases create a life-altering experience for the person with injury, for their partner, parents, siblings, and children. The subsequent diminishment of body functions associated with the diseases can cause depression and loss of self-esteem. It has been considered essential, based on European policy consistent with human rights principles, that people with disabilities should be treated with dignity, encouraged to have independence, be given equality of opportunity, encouraged to have an active participation, a full citizenship and a high quality of life. Given the diversity of degenerative diseases indicated above, pathological manifestation can occur at any age: either as a child, during an individual's most productive years, or as an aged person. The trauma frequently results in morbidity, and as a result, patients typically require continuous physical and medical care depending on the disease, severity of manifestation, degree of disability, and location of injury.

The prevalence of degenerative diseases is on the rise because aging population is increasing and this has created the need for biomaterials. Over the past 50 years, average life expectancy at birth has increased globally by over 20 years, from 46.5 years in 1950-55 to 65.2 years in 2002. Today there are 600 million people in the world aged 60 years or over, and this will double by 2025 and reach 2 billion by 2050. While degenerative diseases are not the exclusive domain of the aged, they do impact this sector of society the highest with subsequent increased social and economic burdens on the health care systems on which they depend.

The direct healthcare costs of organ replacement are about € 240 billion globally (about 8 percent of global healthcare spending) arising from therapies that keep people alive (such as kidney dialysis), implanted replacement devices, and organ transplants. With a € 240 billion global industry already built on first generation tissue and organ therapy products and substitutes, regenerative medicine has a potential to exceed € 600 billion by 2030.

1.5 Project Contact Details and Logo

Project Coordinator Contact details:

Professor Jeffrey A. Hubbell, Ecole Polytechnique Federale de Lausanne
Tel: +41 21 693 9681
Fax: +41 21 693 9665
E-mail: jeffrey.hubbell@epfl.ch
Project website² address: www.Angioscaff.eu

