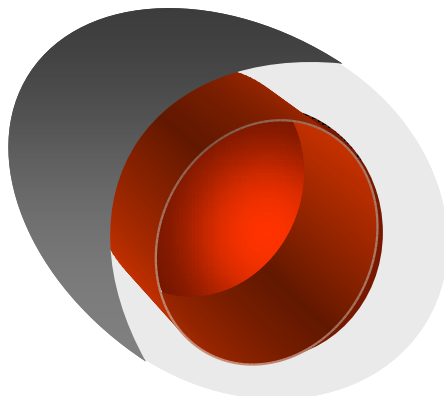


## **Final report**

**Grant Agreement number: 214402**

**Project acronym: ANGIOSCAFF**



**ANGIOSCAFF**

**Project title: Angiogenesis inducing bioactive and bioresponsive scaffolds in tissue engineering**

## Executive summary

Angioscaff was a partnership of 33 different opinion leaders in regenerative medicine that collaborated from December 2008 to November 2012 on a portfolio of 110 portfolio projects. These projects focused on early stage preclinical development of cutting edge regenerative therapies for the eventual treatment of the major age related and genetic degenerative diseases that result in tissue dysfunctionality and decline in contribution to our society.

The whole project concept was based on combining biomaterials composed of various chemical structures with bioactive molecules known to have influence on specific tissues and then validated either with or without exogenous cells in preclinically relevant animal models, as part of a tailoring towards tissue need. Seven different biomaterials were developed to be potentially combined with upto twenty different tissue stimulating factors.

The partnership consisted of globally renowned academic scientists and companies in biomaterials, biomechanics, imaging, developmental biology, preclinical development and clinical application who leveraged significant prior know-how and intellectual property to generate:

- 4 clear novel therapies and approaches for treating tissue damage in skin, muscle, heart or bone tissue which merit clinical translation.
- 89 publications which included approved publications in the highly cited journals; Science Translational Medicine, Nature Communications, Nature Reviews Neuroscience, Science, EMBO Molecular Medicine, Developmental Cell and Cell.
- 4 new patent application submissions (3 for bone and 1 for drug delivery) and significant value added to the intellectual property owned by the partners prior to the start of the project and assessed within the strategies of Angioscaff.
- 2 start up companies (Promimetic Limited focused on muscle repair and Delivery Limited which develops drug delivery technologies based on Hyaluronic acid).

These tangible outcomes were all focused on addressing traumatic and degenerative diseases which represent a significant proportion of chronic, progressive and often fatal diseases with profound human and societal costs. Effective, applicable and reimbursable therapeutic approaches are few despite the known diseases associations with a progressive decline in tissue function that prohibit a healthy ageing.

These diseases create a life-altering experience for the afflicted person, for their partner, parents, siblings, and children. The progressive diminishment of body functions associated with the diseases can cause depression and loss of self-esteem while the diversity of the diseases can give rise pathological manifestation at any age: either as a child, during an individual's most productive years, or more frequently as an aged person. The increased prevalence of degenerative diseases with the growing aged population, is well documented with significant socio economic impact which has precipitated the need for effective, affordable and widely applicable therapies. 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. 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. There is a great disparity between transplantable organs available and patient need with the present market built on first generation tissue and organ therapy products and substitutes; with the development of affordable and reproducible therapeutics regenerative medicine has a potential to exceed € 600 billion by 2030 and be of great benefit to all people suffering from what will be curable disease.

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## Project context and main objectives

### The context of regenerative medicine

Degenerative diseases represent a significant proportion of chronic, progressive and often fatal diseases with profound human and societal costs for which there are no effective therapeutic approaches and are associated with a progressive decline in tissue function that share many hallmarks of ageing. They create a life-altering experience for the afflicted person, for their partner, parents, siblings, and children. The progressive diminishment of body functions associated with the diseases can cause depression and loss of self-esteem. Given the diversity of degenerative diseases, pathological manifestation can occur at any age: either as a child, during an individual's most productive years, or more frequently as an aged person. There has been an increased prevalence of degenerative diseases with the growing aged population, which has stimulated a growth in the need for effective, affordable and widely applicable therapies. 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. The economic impact of morbidity in this population represents a significant burden, which requires effective and rapid solutions.

Solutions are being developed representing combinations of biomaterials, cells, and bioactive growth factors which can be tailored to treat these diseases, however in the context of the real world these patient focused therapies must have the following characteristics:

- **Cost effective** (resolve direct and indirect costs)
- **Reimbursable** (it is affordable for customers; governments and HMOs)
- **Reproducible** (so its worth reimbursing)
- **Broadly applicable** (a platform to be tailored and expanded)
- **Exportable** (it works for everyone, everywhere)
- **Generating a Return on Investment greater than 3%** (so its worth it)

This 'must have' list to some extent represents the holy grail of all medical development, with the exception that this grail can be achieved. To be clear what is being referred to above is an apothecary, which contains on one-side materials, in the middle factors or drugs and on the other side the patients cells. They are clinically approved products in their own right, but more importantly have been approved to be used in any number of combinations based on the patients need as determined and tailored by the doctor following diagnosis.

### The material

Biomaterials are an important part of the medical device industry, and now they are becoming more prevalent as scaffolds in the development of sophisticated therapeutic products, such as sustained drug delivery therapeutics. To date, the choice of scaffold in these applications has largely been dependent on predicated biomaterials, but increasingly, with advancement in biomaterial science, custom scaffolds are being developed with specific properties needed for a particular application. Emerging strategies employ the design of materials that allow release of active factors "on demand" e.g. enzymatically-triggered release of active factors to optimally meet therapeutic requirements. Design of new materials that meet specific performance criteria is, however, still a challenge. It is currently not possible to freely choose among components that should be assembled/connected into materials. Equally challenging is the development of design principles for new materials based on understanding and quantification of the relationship between the scaffold characteristics – such as molecular composition, morphology and physical properties – and the in vivo outcome.

It has become clear that the native extracellular matrix supplies critical chemical, biological and physical signals to initiate or sustain cellular functions within the tissue that are favourable to tissue repair. It is not surprising, therefore, that many of the novel matrix materials in use or under development are designed to mimic the characteristics of the extracellular matrix.

One such characteristic is bioadhesion, which is an important property that allows cells and tissues to adhere to biomaterials and has enabled their use as tissue adhesives in surgical repair or as inductive scaffolds for tissue regeneration. Bioadhesive features can be engineered into a biomaterial in order to facilitate interactions between the implant and its surroundings. Cell-adhesion modifications to scaffolds have also been used effectively to promote enhanced bone repair, to provide an essential foothold for neurite outgrowth in axonal regeneration, and to understand the regulatory role of mechanotransduction in stem cell fate determination.

Engineering this type of bioactivity is instrumental for materials that are called on to mediate specific biological events in the body based on endogenous cell recruitment, local morphogenesis, and controlled cell differentiation. Many of these events can be induced by using exogenous growth factors that are delivered with

spatiotemporal control. However, most materials do not inherently sequester these growth factors and thus fall short of precisely controlling their sustained or localized bioavailability. In vivo, growth factor bioavailability is tightly regulated by nonspecific associations between the factor and extra cellular matrix. Using strategies premised on such interactions with scaffolds, design modifications to materials have recently been used to improve the localized growth factor availability, with remarkable results in their ability to mediate tissue repair.

### **Defining the bioactive factor**

To a large and increasing extent, defining the optimal delivery of bioactive factors is premised on the early processes that occur during development. The reason is that this is when growth, spatial and structural characteristics are defined in the tissue, and a potent mix of factors (materials, cells and growth factors) lays the foundation for a functional tissue during a 9 month gestation, that has to fully functional at birth and remain this way for another 70+ years.

In mammals, once development and growth are completed, many organs such as the brain and the heart, have limited ability to regenerate following acute or chronic damage from trauma or disease. This is despite the fact that these organs have grown in size from birth to adulthood due to the presence of residual stem cells which divide very slowly to permit growth. Such stem cells are found in each organ: the lung, the brain, the skin etc., but they are clearly not normally capable of permitting extensive regeneration. This is because the developmental signalling pathways that were involved in initial building of the prenatal tissues have been switched off or down-regulated to a lower level in cells at the completion of development which has been matched with a number of inhibitory factors (inflammation and scarring) which are required in the adult to ensure adequate protection from the environment following damage and thus the survival of the individual.

The best evidence that developmental signalling pathways are down-regulated once organogenesis has been completed is the fact that many organs can regenerate when still in a developmental state. For instance, the prenatal spinal cord will regenerate perfectly after severing and there is a precise stage in late development when regeneration no longer occurs. Similarly prenatal mouse digits regenerate perfectly if amputated when in utero and skin wounds heal perfectly without scarring if the damage is similarly performed in utero. This loss of ability is likely due to changes within the cells of the organ themselves.

There is still significant support needed for and performed within fundamental research which continues to identify the best possible bioactive factors and other environmental stimuli that can be combined with the biomaterials to provide the correct information and environment to the target cell.

### **The cell**

Human tissues can self-repair in response to moderate injuries, but are not able to regenerate when significant loss of tissue occurs in extensive trauma or surgery. Similarly, they cannot sustain repeated cycles of degeneration/regeneration. Reconstructive strategies, such as autologous cell transplantation and injection of progenitor cells yield only modest therapeutic outcomes, mainly because the tissue often presents an inflamed or sclerotic environment that results in poor survival and only modest integration of engrafted cells that are also targets of an immune reaction. Moreover, the in vitro cultivation history of the grafted cells can also negatively affect the efficacy of cell transplantation, although this may be prevented by culturing cells on biomaterials. Among the new therapeutic strategies for several age related and degenerative diseases, stem-cell transplantation is becoming a promising clinical option.

Presently, there is a focus on the use of engrafted stem cells as therapeutics which are best complemented by advancing our understanding of the basic biology of stem cell activation. The potential successes and applications of engrafted stem cells, need to be matched with those aimed at mobilizing endogenous stem cells whose limited regenerative potential, can probably be restored with the correct stimuli. As stated above, injury or disease often produces inappropriate re-patterning of the tissue culminating in scar tissue formation (fibrosis), inadequate blood vessel creation, or chronic inflammation; none of which are beneficial. Therefore we anticipate that the cell of choice for regenerative medicine may be exogenous and/or endogenous, depending upon the specific pathological situation. For example, a cell which is transplanted at low numbers (isolated from the patient, developed as an iPS, or obtained from potential donors) in a bioactivated scaffold, that upon integration activates the endogenous cells to hybridise with the implant and restore function in the degenerating tissue.

## Angioscaff

The design of bio-interactive therapies is therefore critically dependent on the understanding of how relevant cells interact with natural materials as tissues form and remodel in-vivo, in response to corrective stimuli. The aim is to fabricate the basics of each tissue (then let cells take over), as opposed to hoping that cells will start the process themselves, with the intention to speed them along later, and restore tissue functionality. This requires that sufficient nutrient is provided to the tissue via angiogenesis to enable growth matched with providing tailored stimuli to the tissue itself to enable a complete functional restoration.

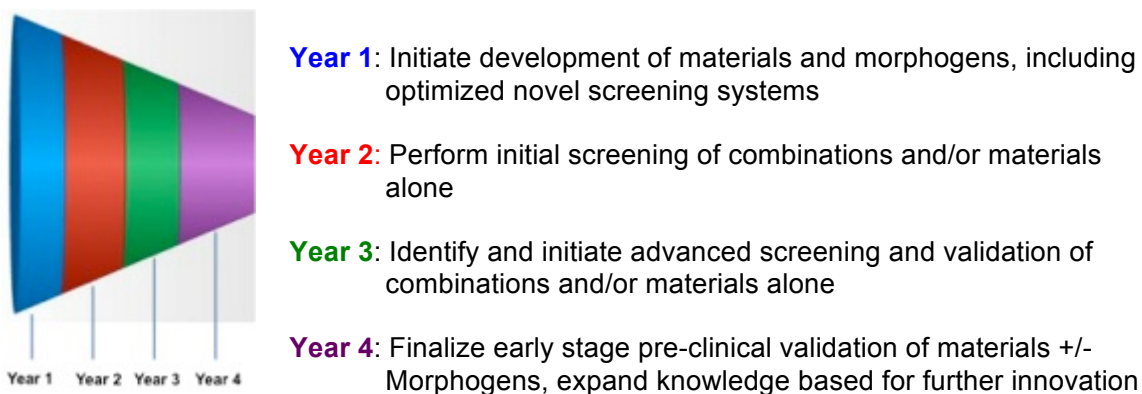
We therefore aimed to create bioresponsive, bioactive and injectable materials capable of carrying therapeutics, which could be used for tissue regeneration in humans. The new biomaterials were to respond to cell-associated environmental signals, such as extracellular proteases and endoglycosidases and generate bioactivity by virtue of bound peptides or recombinant adhesion molecules and growth factors.

To achieve this we assembled a partnership of 33 globally renowned specialists in the regenerative medicine field who were to design and develop therapies that represented:

1. Radical innovations in state-of-the-art biomaterials, that could control cell differentiation in a structural or delivery type system.
2. High-performance biomaterials inspired by natural processes which would enable internal growth of tissue and site-specific delivery of bioactive signaling factors
3. Injectable biomaterials that could induce angiogenesis in the body.
4. Bioresorbable, highly porous and structurally sound tissue-engineered scaffolds.
5. Functionalized biomaterials that would have direct influence on cell behavior in a broad range of tissues.

To achieve this the work was divided into six interlocking but distinct **strategic focuses** (as illustrated in table 1) in which individual partners would bring in specific expertise and resources that were collectively leveraged and funnelled over the 4 year duration (Figure 1).

Figure 1: Funnelling strategy of Angioscaff in which the large number of high impact innovations generated in the first year would be slowly filtered to a lower number of innovations by year 4, to focus on those studies which would generate fundamental knowledge advances for long term research and applicable game changing innovations which could be translated into humans in the short term.



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

**The second focus** would develop 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 would be combined with the developed biomaterial platforms so that all of the constructs developed would be biofunctionalized to be bioactive.

**The third focus** functionally validated 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.

**Table 1: Angioscaff's material and morphogen components with original tissue and disease applicability**

Material	Material +/- Morphogens	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

**The fourth focus** assessed the functional activity of the biomaterials in bone repair by: (a) developing 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 focus** developed materials and biomolecular therapeutics for skin repair. These constructs were used as a quantitative 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 burns and chronic wounds.

Finally, **the sixth focus** addressed the 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 tissues.

## High impact results

Portfolio project design and implementation in which 3 to 4 teams were working together on each project within the total project map resulted in 110 different projects ongoing between the partners which generated significant innovations with high impact in the regenerative medicine, degenerative disease field of endeavour. Below we indicate those innovations which will have game changing impact in the short term development of state of the art therapeutics; while we do not report on the extensive amount of insights and fundamental knowledge generated by the partnership which will serve as a long term reinforcement for our continued innovation in this sector.

### 1 - Biomaterial design

6 biomaterial platforms were generated based on Fibrin, PEG Peptide, Fibrinogen Polymer, Hyaluronic acid, Porous scaffolds or Calcium phosphate. Each material had specific nascent properties which lent itself to application to the different tissues we were aiming to repair. Leveraging the expertise of the biomaterial teams, all of whom had extensive prior knowhow as illustrated in Table 2 we set about optimising their preparation, integrating in biofunctionality and design directly related to the known needs to achieve a tissue functional restoration.

**Table 2:** Biomaterials present and state of development at the beginning of the project

<b>Biomaterial</b>	<b>Team in which expertise was resident and state of development at time of the initiation of Angioscaff</b>
Fibrin with bound morphogens	Invented by Jeffrey Hubbell from the EPFL, the first generation materials had been taken into Phase II clinical trials in man
Fibrinogen-polymer hybrids	Dror Seliktar from the Technion developed this very promising approach; initial structure-(bio)function relationships have been published in high impact journals and the material had entered phase 1 clinical trials for bone repair
Hyaluronic acid hybrids	Jons Hilborn from Uppsala developed the initial chemical schemes; first results had been published with preclinical modelling
PEG-peptide hybrids	Invented by Jeffrey Hubbell and Matthias Lutolf at the EPFL and next generation modifications made by Carsten Werner from Dresden, the first generation materials had been taken to large animal preclinical studies
Porous biofunctional scaffolds	Invented by Kevin Shakesheff in Nottingham, initial characterization of feasibility was complete with refinement and mechanistic studies under way.
Porous calcium phosphate scaffolds	Invented by Partner Josep Plannell from IBEC in Barcelona for bone repair; mechanistic understanding supported by initial mathematical and experimental models had been performed

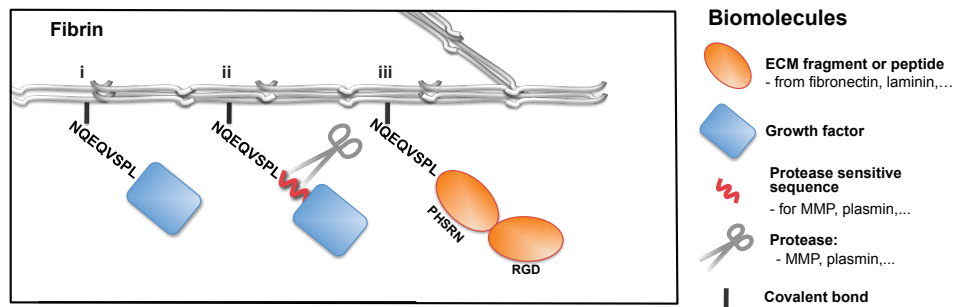
All the underlying design of the materials were already patent protected and owned by the teams themselves; extension of use of the materials and optimisation of their design was defined as the optimal approach for both adding value and generating potential portfolio's of intellectual property around each material; secondary IP generated as part of Angioscaff would be dependent on the underlying IP owned by the scientists and therefore further protect the inventions.

#### ***j) Development of the Fibrin scaffolds***

We developed a technology to customize fibrin which incorporated bound morphogens within the fibrin meshwork and permitted a controlled release to direct cellular behaviour through a target-specific biofunctionality. This involved generating a transglutaminase sequence bridge that could link the morphogen to the fibrin which would be cleaved in situ by endogenous enzymes (Figure 2).

**Figure 2: Coupling scheme with fibrin gel.** Target morphogens such as ECM derived proteins, peptides, and growth factors (i-iii) are produced with trans-glutaminase (TG) sequence (NQEQVSPL). TG-morphogen will be mixed with fibrin gel in the presence of Factor XIIIa, covalently coupled immediately to the gel.

The TG-morphogen (i) can retain in the gel until fibrin degradation. By introducing protease sensitive sequence between TG and morphogen (ii), morphogen will be cleaved off by MMP and plasmin enzymatic reaction and released from fibrin gel.

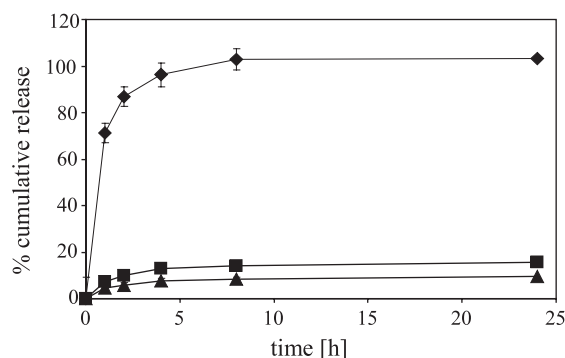


We could link synthetic peptide or recombinant protein morphogens with the trans-glutaminase sequence (NQEQVSPL: TG sequence) that bound to the fibrin during coagulation under the enzymatic influence of the coagulation transglutaminase factor XIIIa (Figure 2).

Every component of the technology (Fibrin, morphogens, and other parameters such as thrombin, factor XIIIa) were designed to be modifiable depending on target tissues, assay conditions. For example neural cells prefer soft gel, while myoblasts prefer a slightly harder gel.

Functionality was confirmed using a variant of VEGF (VEGF121) in incorporation and release assays. TG-VEGF121s could be retained within the fibrin gel 24hrs after coupling whereas free native VEGF121 was released by diffusive burst completely after 24hrs thus permitting a longer and better tuned morphogen presentation (Figure 3).

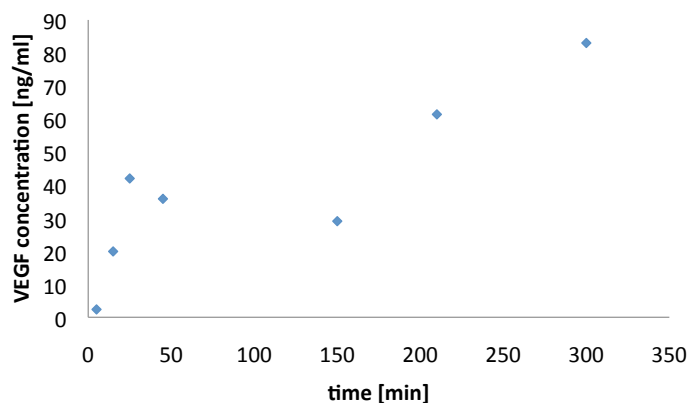
**Figure 3. Cumulative release profile of VEGF121 variants (♦VEGF121 , ▲TG-VEGF121, and ■TG-plasmin cleavage site-VEGF121) from customised fibrin gels.**



## ii) Development of the Fibrinogen polymer hybrids

The Fibrinogen polymer (composed of a hybrid of Fibrinogen and PEG molecules which are activated by UV light stimulation) had originally been designed as a stand alone material (no morphogens) to be used to enable bone repair; in line with the project strategy, modifications were performed to enable application in other tissues and potentially incorporate morphogens and other cell stimulating factors (chemical entities and hormones). After confirming that the Fibrinogen polymer system permitted extra factors to be included and that these factors could be sustainably released over a 6 hour period we optimised the system by editing the weight of the PEG molecules, and therefore density in the hybrid composition and confirmed biomorphogen release by integrating in VEGF, where we demonstrated that modifications to the hydrogel network structure which alters its density permitted a gradual release from the Fibrinogen Polymer (Figure 4).

**Figure 4: Sustained release of VEGF from the tailored Fibrinogen-Polymer**

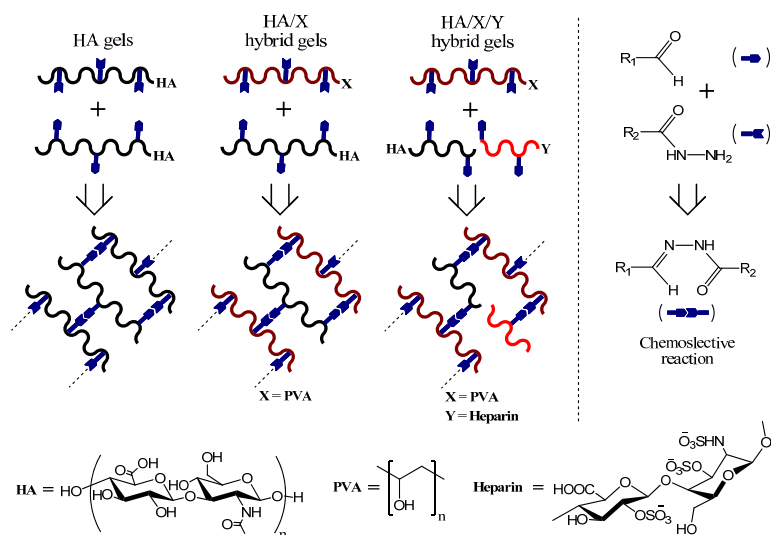


These tunable Fibrinogen Polymer systems were confirmed in vitro before translation to tissue specific modelling demonstrating that by changing biomechanical properties we could better regulate the rate of cell invasion, morphogen release and support controllable cellular outgrowth in various tissues by modulating relative amounts of fibrinogen and PEG, the Fibrinogen Polymers.

### ***iii) Development of the Hyaluronic acid systems***

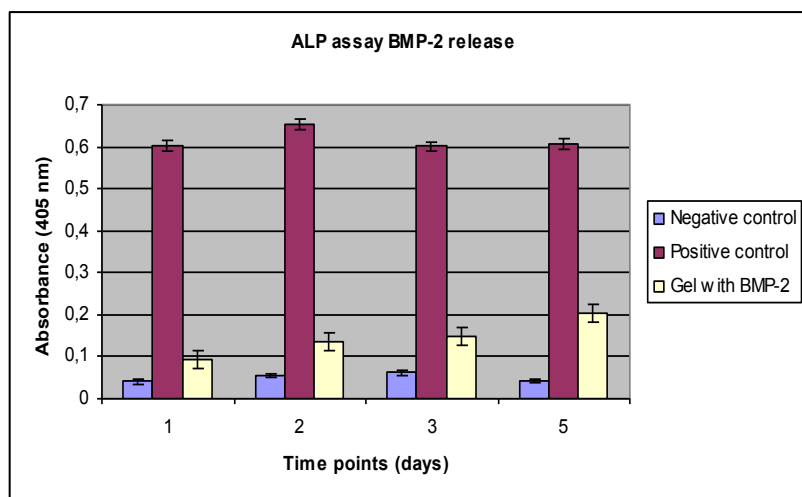
Hyaluronic acid (HA) is a highly versatile naturally occurring extra cellular matrix protein which can be extensively edited for tissue targeting and repair. We developed a novel system consisting of cross-linkable multifunctional HA derivatives, capable of forming a gel in situ in less than 5 min simply by mixing of the two HA solutions (Figure 5). This system could be tailored to address the specific characteristics of the tissue to be targeted.

**Figure 5. Schematic illustration of click chemistry system for in situ HA hydrogel formation.** Cross-linking occurs by mixing hyaluronic acid derivatives having complementary reactive functionalities in the form of electrophilic aldehydes and nucleophilic groups (i.e. hydrazide) respectively that react to form covalent hydrazone bonds in aqueous solutions. With this strategy, other components, shown as X and Y (i.e. poly-vinyl alcohol (PVA) or heparin), are able to be combined to HA hydrogel.

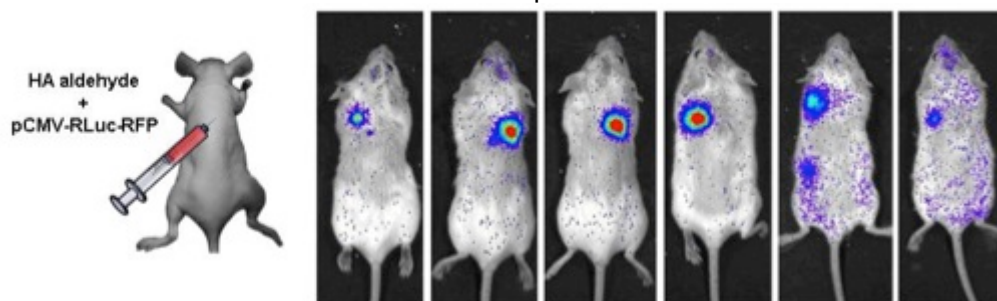


To demonstrate this we successfully developed the system for the delivery of two morphogens: growth factors and nucleic acid. For the morphogens we focussed on using HA as a bone repair material, tailored to deliver Bone Morphogenic Protein-2 (BMP2) directly, while for the nucleic acid an encapsulating system was developed for systemic application. In the context of BMP2, the HA gels could sustainably deliver BMP2 over 5 days (Figure 6), while in the context of nucleic acid delivery, we generated a system which delivered nucleic acid (in this instance a fluorescent probe) to living tissues (Figure 7).

**Figure 6. The release of BMP-2 from the hyaluronan gel.** The absorbance of alkaline phosphatase activity was measured at four different time points with a positive control (BMP-2 100 ng/ml), a negative control (no BMP-2) and medium from gels (BMP-2 100 ng/ml). The time points were taken at 1, 2, 3, and 5 days

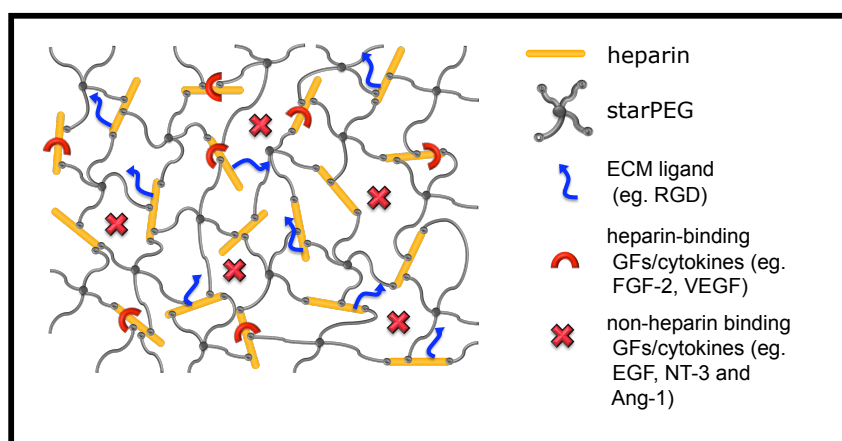


**Figure 7. HA based material cell transduction in vivo.** The images show light production by mouse cells at the site of inoculation with a HA/luciferase reporter DNA-construct mixture.



#### iv) Development of the PEG-Peptide hybrids

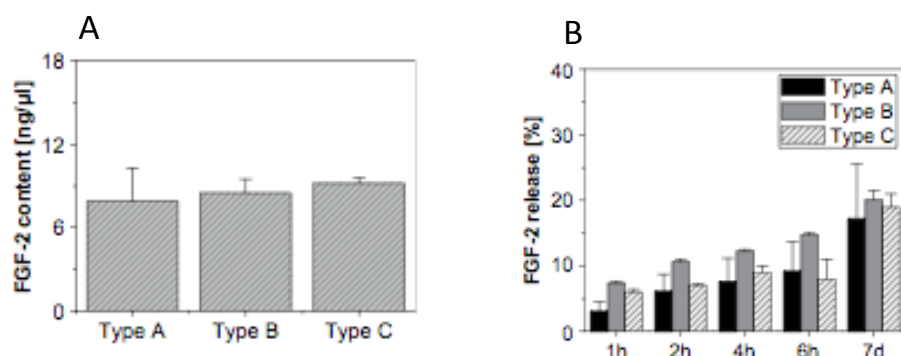
The PEG gel with heparin sites that was already developed (Hybrid materials consisting of heparin and star-shaped PEG) were tailored so that variations in physical characteristics and biomolecular functionalization formed by cross-linking of the amino end-functionalized star-PEG with EDC/sulfo-NHS meant that the heparin component could be functionalized through covalent attachment of cysteine-containing peptide such as cell adhesive RGD peptides, and non-covalent heparin



**Figure 8: Reaction of EDC/sulfo-NHS activated heparin with amine endo-functionalized star-PEG is used to form biohybrid gels.** Gel materials are additionally modified with adhesion ligands such as RGD peptides, covalently attached to EDC/sulfo-NHS activated heparin carboxy groups, and loaded with soluble signalling molecules growth factor eg. FGF-2, mediated by electrostatic interactions between the highly negative charged heparin and positively charged growth factors binding proteins (Figure 8). The degree of cross-linking upon formation of the meshwork structure could be systematically varied by varying heparin to star-PEG molar ratios,

(A-0.5, B-0.25, and C-0.17 respectively) resulting in different physical properties, including storage moduli, permeability and swelling, while the biomolecular composition remains invariant.

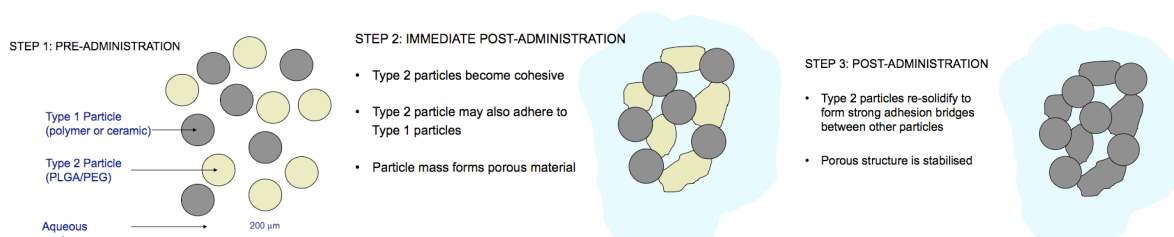
Biofunctionalisation was confirmed using FGF2, which was loaded into the different gels, and the capacity of the gel to release the FGF-2 was confirmed (Figure 9). These tailored morphogen releasing gels were then transferred to the Angioscaff validation teams for testing.



**Figure 9. Biomolecular functionalization of the different type of gels** (type A, B, and C). A) with FGF2. FGF2 could be reproducibly loaded into the gels (A) and could be effectively released (B).

#### v) Development of the Porous scaffolds

Controllable Porous scaffolds based on a slurry to solid transition phase of PLGA/PEG was components was created. Type 1 particle (polymer or ceramic) and Type2 particle (PLGA/PEG) can be mixed with aqueous carrier, making the type 2 adhere to type 1 particles which following resolidification form strong adhesion bridges between other particles, then the porous structure is stabilized (Figure 10).

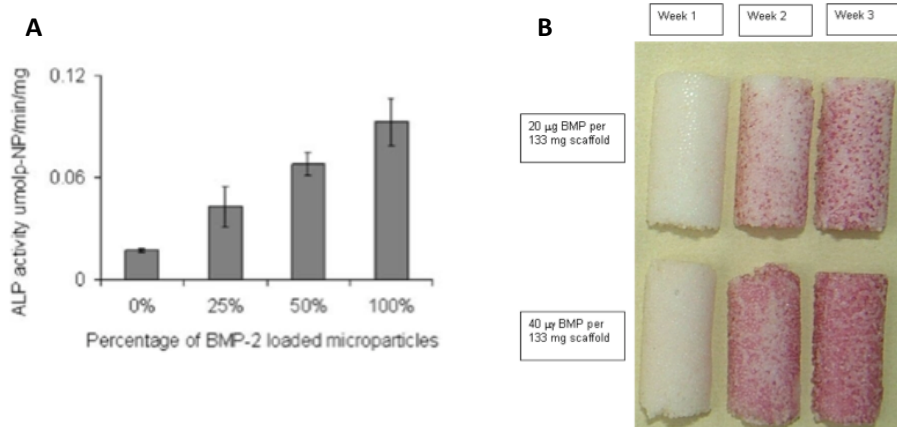


**Figure 10: Concept of PEG/PLGA scaffolds**

We redesigned these materials by substituting the PLGA-PEG with a triblock polymer PEG-PLGA-PEG which could be engineered to perform defined release of morphogens. For the purposes of bone repair, we biofunctionalised these with BMP2 and confirmed the capacity of the material to initiate bone generation (Figure X), prior to sending to the Angioscaff bone repair teams.

in vitro morphogen activity and release from the particles was confirmed by loading different amounts of BMP-2 in the scaffolds and culturing with murine myoblast (C2C12) cells. Cells grown on the particles loaded with BMP-2 differentiated into bone based on ALP staining in a dose dependent manner (Figure 11).

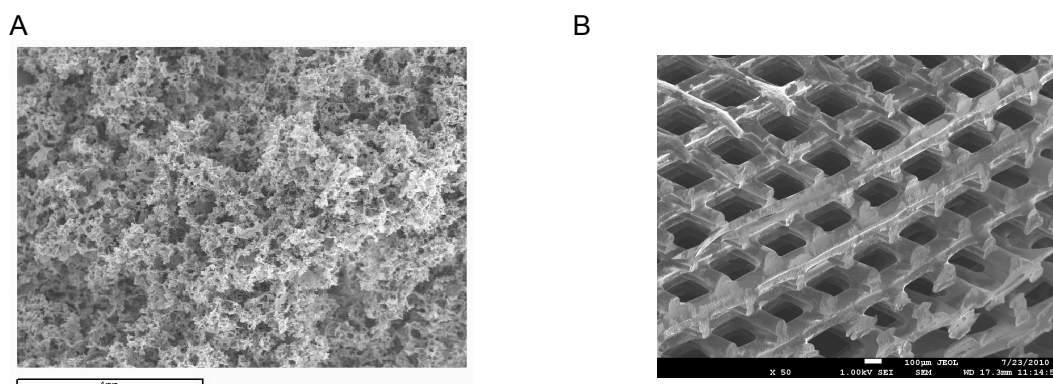
**Figure 11. in vitro BMP-2 activity released from the porous scaffold particles.** A) C2C12 cell ALP activity with different amounts of loaded BMP-2 to the particles. B) ALP staining of the particles at different time points.



## vi) Development of the Calcium phosphate scaffolds

First generation calcium phosphate(CaP) ceramics and biodegradable polyactic acid/CaP glass porous composite scaffold (PLA/glass) were engineered by rapid prototyping which produced mechanically resistant and geometrically well defined scaffolds and extracellular matrix like fibred scaffolds. Rapid prototyping consists in the layer by layer deposition of the material in order to fabricate 3D structures according to a predefined design (Figure 12).

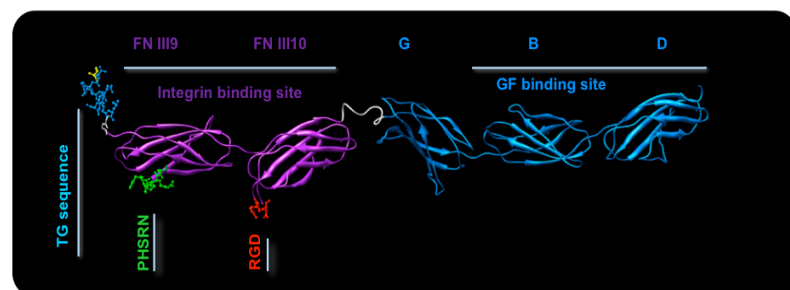
**Figure 12: PLA/Glass scaffolds generated in bioreactors (A) versus rapid prototyping (B)**



These materials, exclusively designed for bone repair were transferred to the bone validation teams for evaluation.

## 2 - Engineered morphogenic biomolecules

To enable the biomaterials developed to be effectively biofunctionalised with the broadest possible spectrum of growth factors (both commercially and non-commercially available) we developed a linker protein which was based on the TG domain linked to recombinant fibronectin (FN) fragments corresponding to the FN 910 domains, wild type FN 910, the structurally stabilized FN 9\*10 (mutation at Leu1408 to Pro), wild type FN 10 containing fibrin binding sequence (transglutaminase substrate sequence NQEQVSPL), and a FN fragment with a promiscuous growth factor binding domain (GBD) (Figure 13).



**Figure 13. Bi-functional FN fragment.**

These linker peptides were promiscuous to binding to the Fibrin, Fibrinogen Polymer and PEG-Peptide materials, permitting their biofunctionalisation. The linker protein had confirmed morphogen binding and release characteristics and could bind to a broad spectrum of growth factors (Figure 14)

**Figure 14. List of growth factors that can bind to the fibronectin fragments**

<b>VEGFs</b>	VEGF-165 VEGF-B VEGF-C PLGF-2 PLGF-3 EG-VEGF	<b>PDGFs</b>	PDGF-AB PDGF-AA PDGF-BB PDGF-CC PDGF-DD	<b>TGF-b</b>	TGF-b1
<b>FGFs</b>	FGF-2 FGF-4 FGF-5 FGF-7 FGF-8 FGF-9 FGF-10 FGF-17 FGF-18 FGF-21	<b>EGF</b>	HB-EGF	<b>BMPs</b>	BMP-2 BMP-7
		<b>HGF</b>	HGF	<b>Neurotrophin</b>	NGF NT-3 BDNF

We also generated the following specifically engineered morphogens for use within the partnership

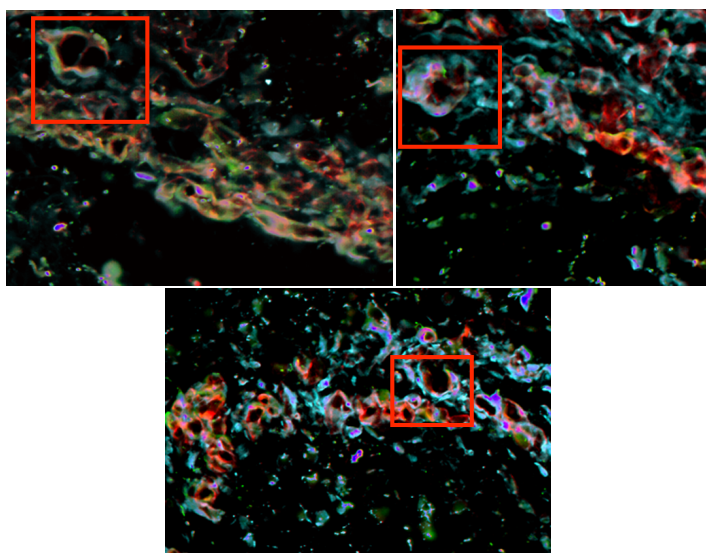
**Table 3: Morphogens and their characteristics**

Morphogen	Description
Fibrin binding VEGFA 165mut	Pro angiogenic, TG modified mutant form of VEGFA
Fibrin binding VEGFC	Pro angiogenic, TG modified form of VEGFC
Fibrin binding PIGF	Pro angiogenic, TG modified form of PIGF
Fibrin binding VEGF121-Syndecan (2 versions)	Pro angiogenic, TG modified mutant form of VEGFA fused with 15 or 50 amino acids (named VEGF-AG) from laminin that contains the cell surface syndecan-binding angiogenic sequence
Fibrin binding PDGF (3 versions)	Pro morphogenic, TG modified forms of PDGF AA, AB and BB
Fibrin binding IGF	Pro proliferation, TG modified form of IGF
Fibrin binding BMP2	Pro bone growth, TG modified form of BMP2
TG-YSA	Pleiotropic factor/development regulation, Ephrin A1 mimetic modified with TG
TG-SNEW	Pleiotropic factor/development regulation, Ephrin B1 mimetic modified with TG
TG-TNYL-RAW	Pleiotropic factor/development regulation, Ephrin B2 mimetic modified with TG
FITC-TG-YSA	Pleiotropic factor/development regulation, Ephrin A1 mimetic modified with TG and labelled with fluorochrome
FITC-TG-SNEW	Pleiotropic factor/development regulation, Ephrin B1 mimetic modified with TG and labelled with fluorochrome
FITC-TG-TNYL-RAW	Pleiotropic factor/development regulation, Ephrin B2 mimetic modified with TG and labelled with fluorochrome

All proteins were confirmed for in vitro bioactivity and biochemical characteristics in protein assays before being made available to the partners.

### 3 - Engineered blood vessel growth

Induction of blood and lympho-angiogenesis are critical for obtaining complete functional restoration of damaged tissue. During the course of the project we were able to obtain fundamental knowledge about blood vessels development, develop translational approaches and novel screening platforms to accelerate the design of biomaterial-morphogen combinations.



**Figure 15. In vivo effect of TG-VEGFA (100 ug/ml) on angiogenesis**

Immunofluorescent staining of matrix and tissue 9 days after implantation. Red: endothelium, green pericytes, blue: smooth muscle actin.

Biomaterial-morphogen combination approaches developed as part of the biomaterial engineering and

morphogen design was able to induce angiogenesis. In vitro assays confirmed the bioactivity of the material+TG VEGF while in vivo slow degradation of the material with high dose angiogenic factor confirmed the functionality of the system inducing growth of new blood vessels which elicited characteristic typically associated with normal vessels: high density smooth muscle and the presence of pericytes (Figure 15).

Validatory in vivo demonstrations of the efficacy of the biofunctionalised materials to induce angiogenesis, with the various morphogens and their impact on angiogenesis was illustrated in the soft tissue repair therapy development experiments (see later sections in this report). The in vivo angiogenic event in response to tissue damage is more complex and involves the infiltration of inflammatory cells, such as macrophages which play a critical role in tissue homeostasis as they secrete a spectrum of growth factors in response to selected stimuli. Using the lympho-angiogenic systems as a model we generated fundamental insights indicating that macrophage subtypes and their associated cytokines have different effect on lymphangiogenesis that is important to understand the role of lymphatics in immune cascade. This is especially important for successful lymphangiogenic therapy, as inflammatory signals need to be suppressed before VEGF-C treatment, otherwise continued high dose and expense will be needed.

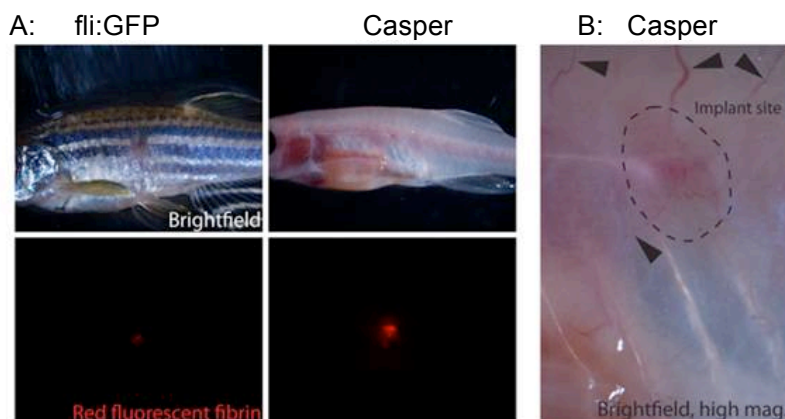
We successfully addressed the VEGF dosage issue through the generation of the novel morphogens described in the previous section. Of particular high impact was the VEGF-Syndecan fusion protein VEGF-AG. In vitro assays demonstrated that we could dramatically reduce the concentration of the proteins by as much as 200 fold and induced angiogenic events. Final validation of these outcomes were performed in the tissue repair models. While useful this illustrated that a significant and expensive bottleneck had to be addressed for future functionalised biomaterials as we could not be constantly dependent on the expensive murine models; in brief interim assays which would be low cost but enable a filtering and refinement of the functionalised materials was necessary so that only the best possible candidates would be selected for animal studies. We estimated that establishment of such assays would reduce times of development by at least 6 months and reduce costs by upto 30000 euros per screening.

Zebra fish had been previously assessed as an excellent tool to study angiogenesis during larvae development as well as in the adulthood. Upon external fertilization Zebra fish eggs develop rapidly (24-48 hours) into transparent larvae.

The use of zebrafish to study angiogenesis provides many advantages. First, there is a vast reduction of the use of mice and many experiments can be done in parallel as there is the ability to rapidly expand the fish colonies. The imaging procedure does not require histology or sectioning and thus can be adapted for time-course analysis in vivo on a single fish. When optimised for biomaterial screening this powerful model permitted fast and minimally invasive tests to screen and compare different biomaterials and morphogen combinations before moving to larger animals.

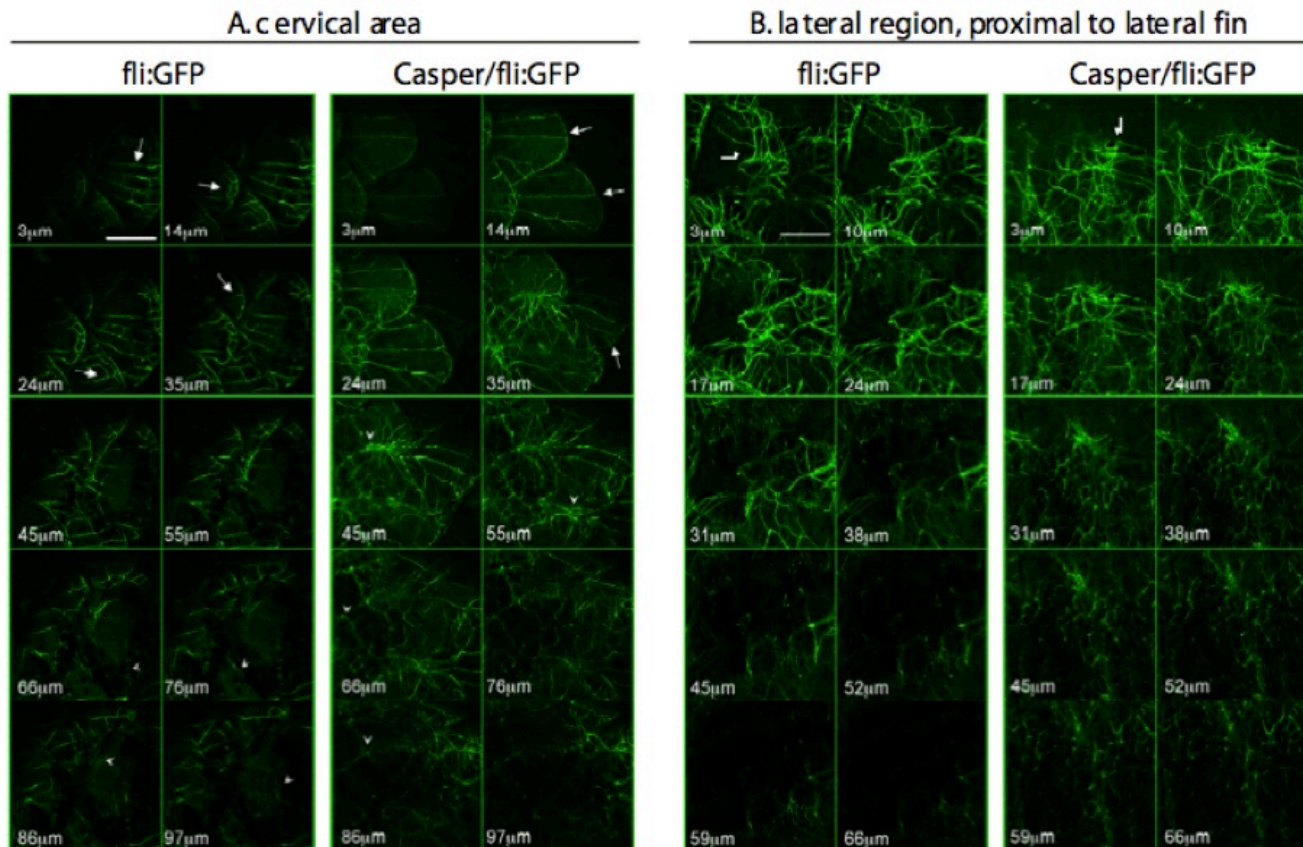
Transgenic fish models (fli:GFP) were available where endothelial cells express GFP protein under an endothelial specific promoter allowing the analysis of vascular development in the larvae or, with some limitations due to skin transparency, in the adult fish. However, it was the adult fish that represented the ideal model to obtain quality analytics as we had to generate subcutaneous pockets to address the activity of the materials, therefore an easier visualisation system was developed. This involved crossing the fli:GFP fish with the Casper fish, which lacks melanocytes, rendering their skin transparent, to obtain transparent adult fish with fluorescent vessels, which permitted an easier visualisation of the material implant site (Figure 16).

**Figure 16: fli:GFP vs Casper fish.** Panel A on the left is the fli:GFP, on the right the Casper fish, with the site of material implant clearly visible. Panel B is a higher magnification in bright field of the Casper fish.



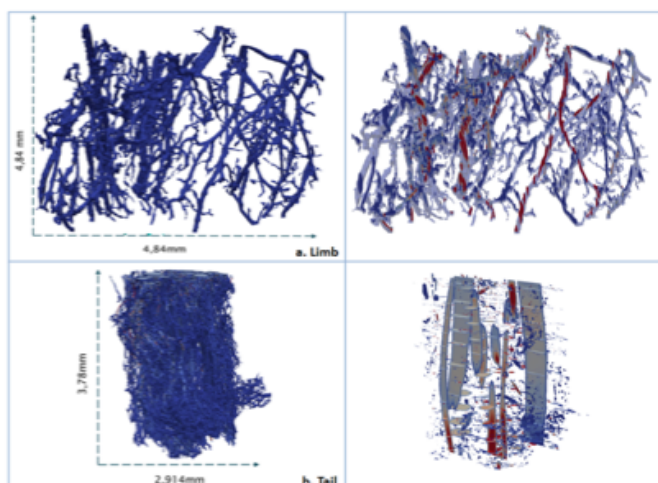
Utilising this system we could increase the depth of analytics to 60 microns, representing a 50% increase in visualisation of angiogenesis and demonstrate that the application of the Fibrin-TG VEGF could induce extensive angiogenesis (Figure 17).

**Figure 17. Confocal imaging comparison in fli:GFP vs. Casper/fli:GFP of fibrin-TG VEGF injected gels (representative images shown).** A) In the cervical area, scale vessels (from 3 to 35 $\mu$ m, arrows) are visible in both fish strains. Skin vessels (for 35 to 97 $\mu$ m, arrowheads) are instead more visible in Casper/fli:GFP. B) Imaging of the lateral region proximal to the lateral fin. Scales have been removed before imaging. Superficial skin vessels (from 3 to 38 $\mu$ m, arrows) are visible in both fish strains. Deeper vessels (for 38 to 66 $\mu$ m) are instead only visible in Casper/fli:GFP. Scale bar 500 $\mu$ m.



#### 4 - Innovative imaging

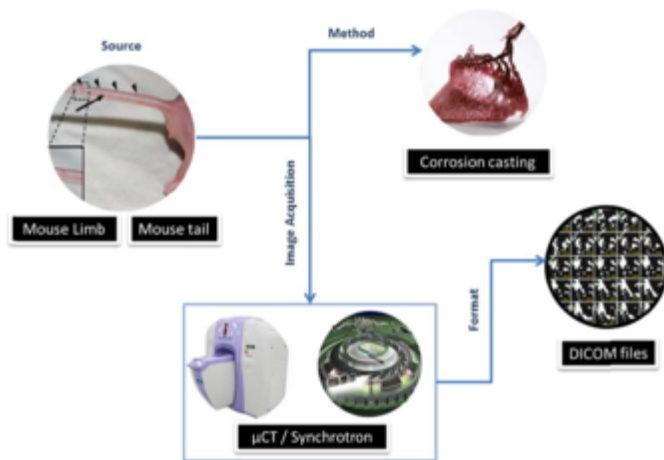
The advances made in developing regenerative approaches necessitated optimized tools for clear pre-clinical demonstration of effect in animal models. Imaging in the preclinical setting is vital to measure and assess tissue repair and has to be comparable to those used in the human clinical setting which are routinely based on using X-rays via a technique termed Microtomography, abbreviated to micro CT, which is used to generate 3D images of the tissue being analysed. An example of a micro CT image is indicated in below (Figure 18).



**Figure 18: example of an anatomical micro-CT indicating density and development of blood vessels, as well as blood flowing through them.**

One of the most important factors to control in the development of tissue engineering scaffolds is the growth of new vessels and the posterior formation of vascular networks due to their critical function to the transport of nutrients and oxygen to the cells. The procedure from preclinical experimentation to image creation is illustrated in Figure 19.

**Figure 19. Overview of image acquisition process.**



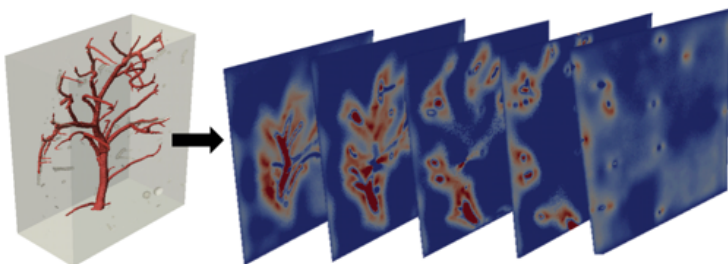
New blood vessel formation and their functional confirmation are some of the main challenges for the reconstruction of a functional tissue. Computational modeling offered the prospect of providing a better insight to assess the functionality of such networks. We developed a methodology to perform quantitative assessment of the functionality of micro-vascular networks and demonstrated that mechanical stimuli promote the angiogenesis process in tissue engineering constructs and therefore drive the functionality of the tissues.

Optimal contrast agents to use for assessing growth rate and density of the growing blood vessels were identified and evaluated with dynamic and anatomical *in vivo* micro-CT, which permitting a functional evaluation of the developed blood vessels based on the flow of blood through them.

We were also able to quantitatively assess the functionality of the micro-vascular networks in mice following treatment. 3D reconstruction of the vascular network was performed using a computational fluid dynamic analysis, which allowed for calculations of fluid flow and therefore demonstrate that not only were the developed blood vessels structurally correct but were also functional and able to deliver the critical nutrients necessary for growing tissues.

This demonstrated that dynamic imaging is a useful modality to assess the kinetics and perfusion of different tissues which we could use 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 (Figure 20).

**Figure 20. Oxygen distribution in the surrounding tissue of the vascular network (detailed of a branch showed in various cross sections).**

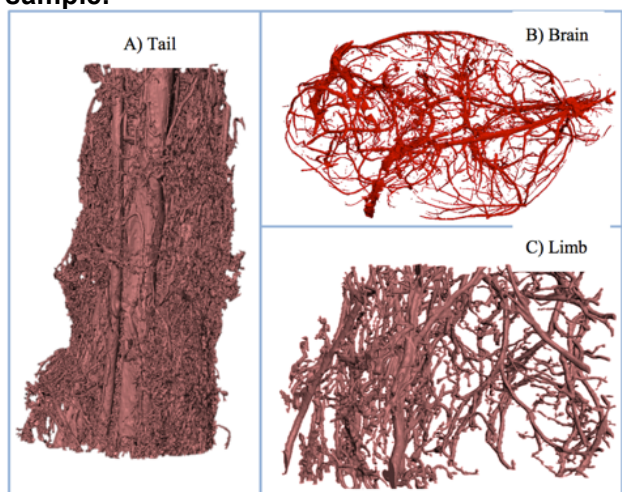


A methodology to import micro-CT data, reconstruct in 3D the vascular network and perform a computational fluid dynamic analysis was defined. These results allowed us to interpret the mechanical phenomenon involved in the angiogenesis process and the importance of cellular responses to mechanical stimuli in tissue engineering applications.

The newly developed method was further tested in an application for tissue engineering using an established model for muscle regeneration testing the degradable fibrin-based gel loaded with different concentrations of

VEGF (Vascular Endothelial Growth Factor) which was injected intramuscularly in the lower hind limbs of mice. To confirm the applicability of the newly created system analysis was performed in three complex networks (tail, brain, and limb) in order to test the method versatility, which was confirmed as illustrated in Figure 21.

**Figure 21. Structures of complex vascular networks in (A) Tail sample, (B) Brain sample, (C) Limb sample.**



The innovating imaging tools generated confirmed that we could import data from Micro-CT scans and reconstruct them into 3D complex vascular networks. This will be very useful for studying vessel formation in patients suffering from cardiovascular disease, tumors, and brain aneurysms, from which the images generated can be used to design new therapeutic strategies or used to monitor current ongoing treatments.

## 5 - Soft tissue repair therapy development

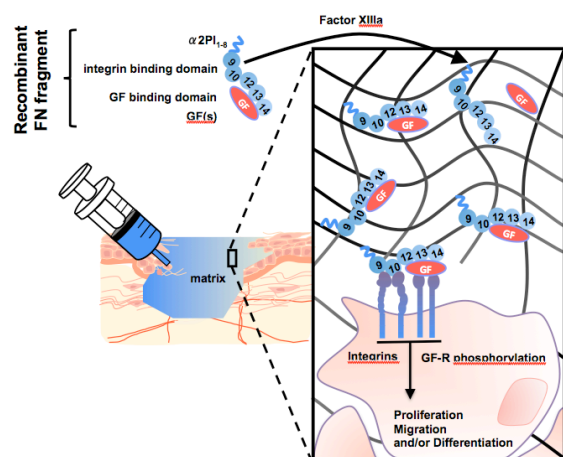
From the outset, we aimed to create new soft tissue repair strategies that would address the the shortfalls of existing therapies designed to treat skin burns, trauma, venous insufficiencies and potentially diabetic ulcers (arguably the most difficult to treat of the soft tissue diseases). This predisposed that effort be focused on addressing the correct signalling required via matrix-displayed bioactive factors which would create such advanced therapies.

The nature of the tissue itself automatically generated the short list of materials that could be potentially applied, limiting them to Fibrin, PEG and Fibrinogen systems, while the application of ‘hard’ materials would have been illogical.

The soft material+morphogen systems developed as part of the angiogenic and morphogen development components of Angioscaff (figure 22) , were quickly translated into the preclinical soft tissue repair assessment.

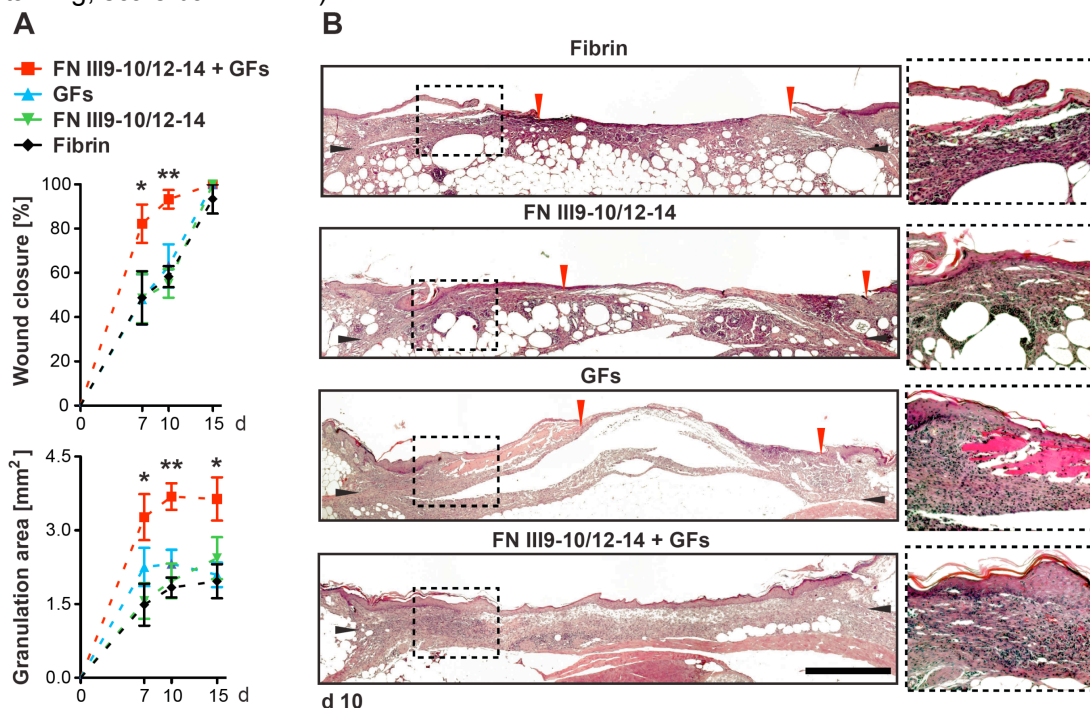
Increasing the level angiogenesis via longer term presentation of pro-angiogenic morphogens was determined to be a rate limiting step for effective tissue repair which we could achieve via protein engineering. PEGylated fibrinogen which can be used as a morphogen slow release matrix was effectively engineered to combine with the fibronectin fragment and the growth factor binding domain described above.

**Figure 22. Schematic of recombinant fibronectin (FN) fragment used to enhance regenerative effects by binding integrins and growth factors (GF).**

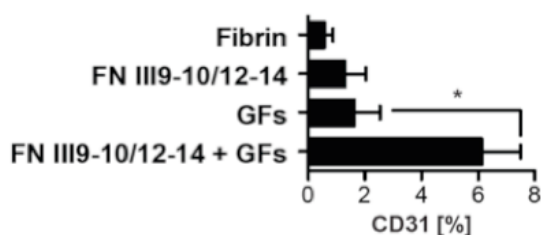


Through this engineering we demonstrated that growth factor signalling was enhanced via the sustained stimulation of the targeted growth factor receptors, which when tested in diabetic disease models revealed a significantly greater skin regeneration, which could be attributed to increased endothelial cell recruitment (Figures 23 and 24)

**Figure 23. Delivering GFs within the engineered fibronectin fragment greatly enhances skin wound healing and angiogenesis in diabetic mice.** (A) After 7, 10 and 15 d, (top) wound closure and (bottom) granulation tissue area were measured by histology (n = 6). Compared to all other conditions, wounds where GFs were delivered using FN III9-10/12-14 closed significantly more rapidly ( $*p<0.05$ ,  $**p<0.01$ , Student t-test) and contained more granulation tissue ( $*p<0.05$ ,  $**p<0.01$  Student's t-test). (B) Representative histology at 10 d. Black arrows indicate wound edges, while red arrows indicate tips of epithelium tongue (hematoxylin and eosin staining, scale bar = 1 mm).



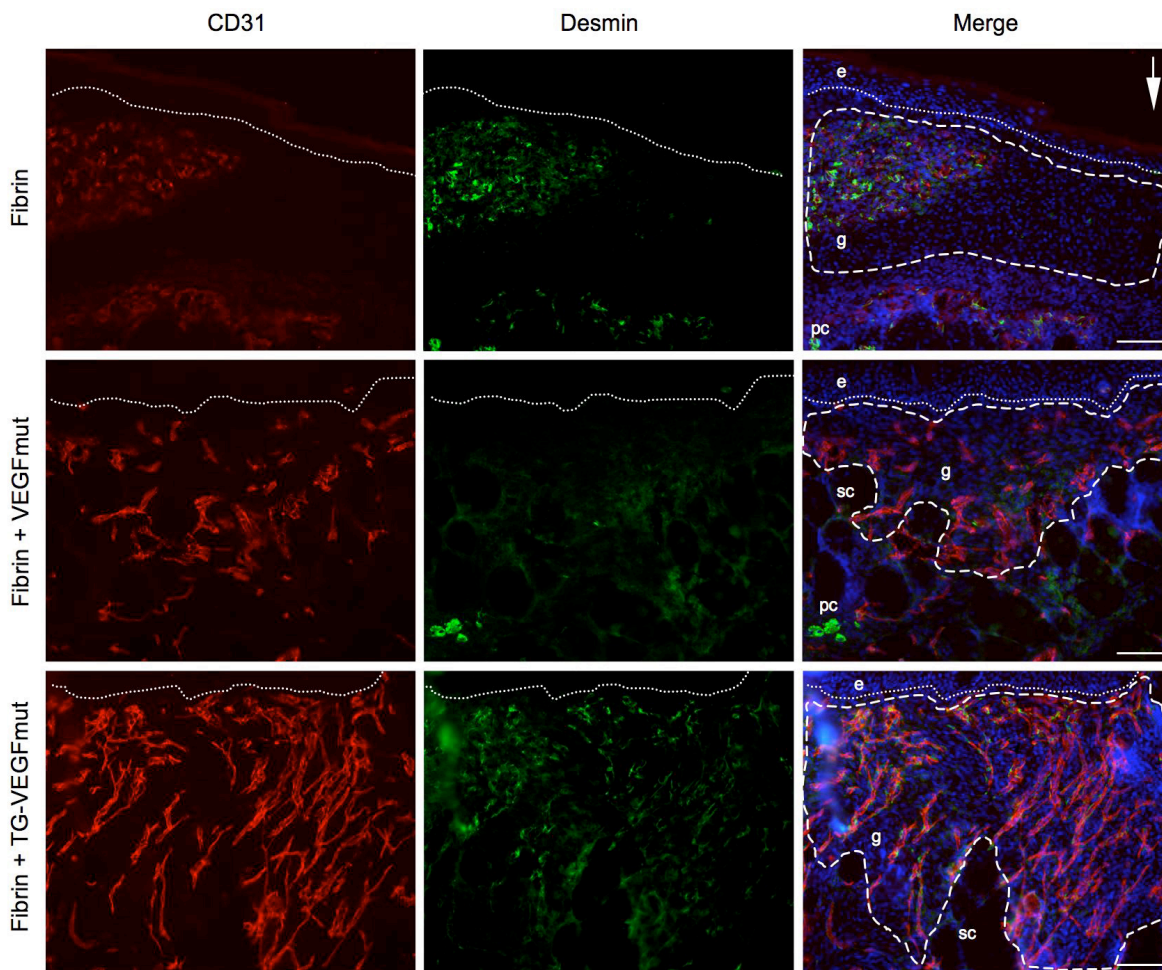
**Figure 24. Higher numbers of endothelial cells (ECs) were recruited when GFs were delivered within the engineered fibronectin fragment.** After 5 d, the percentage of endothelial cells present in the wound was determined by flow cytometry. ( $*p<0.05$ , Student's t-test, n = 6).



The significant success of this work and its potential application to heal chronic wounds and to treat diabetic ulcers, burns, soft tissue damages led us to further develop the morphogens to be delivered as either an augmented version of VEGF and a VEGF-Syndecan combination.

The augmented version of the VEGF was based on a VEGF<sub>165</sub> mutant (VEGFmut) which rendered the morphogen insensitive to plasmin processing while maintaining its capacity to be covalently incorporated into a biomaterial matrix (TG-VEGF<sub>165</sub>mut). Prior to in vivo testing its structural and functional integrity was confirmed by multiple in vitro assays. Subsequently, gels were formulated in full-thickness punch biopsy wounds created on the back of db/db mice containing either the VEGF<sub>165</sub>mut or the TG-VEGF<sub>165</sub>mut. Histological analysis at day 10 indicated a robust angiogenic response upon delivery of matrix-bound isoforms that was confirmed by immunohistochemical analysis and proved to be significantly more potent in inducing blood vessel growth into the wound area (Figure 25).

**Figure 25. CD31/desmin stained cyrosections of day 10 wounds post injury treated of mice treated with fibrin only, fibrin/VEGFmut, or fibrin/TG-VEGFmut.** Sections were stained with (Red) CD31 stain and (green) desmin stain. Abbreviations: e, epithelium; gr, granulation tissue; sc, subcutaneous fat tissue; pc, panniculus carnosus; F – fibrin gel; arrow indicates direction of wound edge; scale bar 100 mm



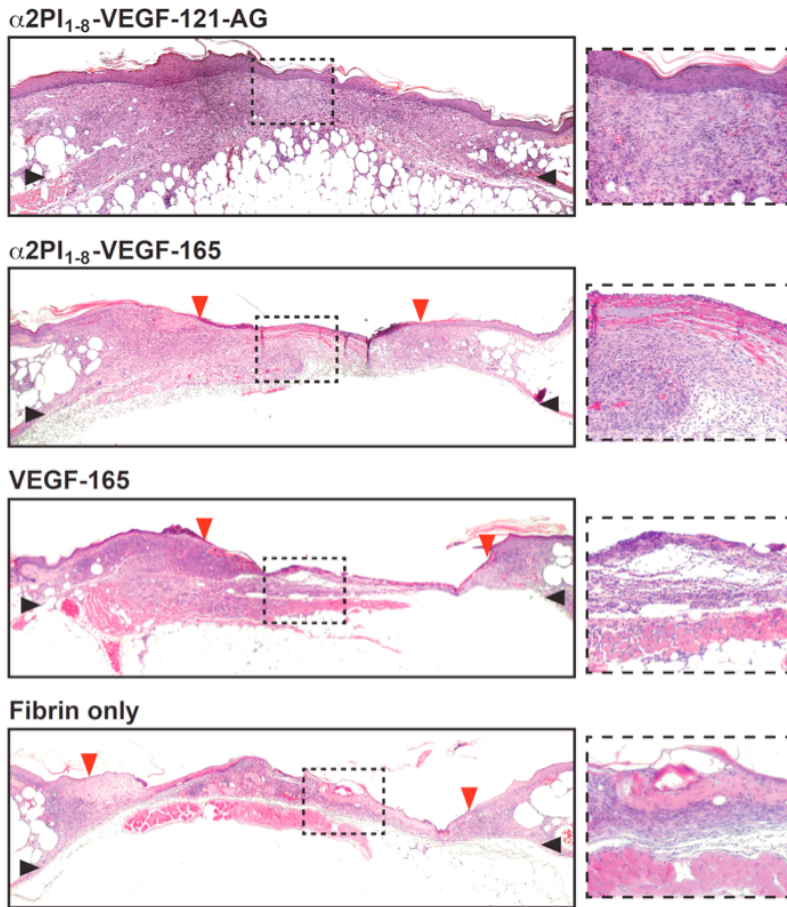
This revealed that wound closure kinetic was significantly increased in diabetic wounds treated with fibrin gels containing TG-VEGFmut as compared to fibrin-only treated wounds.

The VEGF-Syndecan (TG-VEGF-AG) was generated as part of the engineering of morphogenic biomolecules. Similar to the previous work, diabetic mouse (db/db) were used as a model for impaired wound healing to mimic chronic wounds. Following the formation of 4 full-thickness punch-biopsy wounds in the back skin of db/db mice, fibrin gels with matrix binding VEGF variants were implanted. Mice were monitored for skin regeneration after 10 days with focus placed on indicators such as wound closure, granulation tissue area and vascularization.

Several conditions were evaluated to compare a high vs low dose of VEGF protein and VEGF variants which included TG-VEGF-AG ( $\alpha 2PI1-8-VEGA121-AG$ ), TG-VEGF-165 ( $\alpha 2PI1-8-VEGA165$ ) and soluble VEGF-165, which was not matrix bound.

The work demonstrated that at high doses, wound closure was faster with TG-VEGF-AG, but no significant differences were seen between the VEGF variants. However, significantly more granulation tissue was present when wounds were treated with TG-VEGF-AG. At low doses, wound closure was faster with TG-VEGF-AG and TG-VEGF-165, and more granulation tissue was present with TG-VEGF-AG. Histological examination of the wounds also indicated that TG-VEGF-AG appeared to allow for the best wound closure and tissue structure (Figure 26).

**Figure 26. Delivering VEGF variants within fibrin gels enhances skin wound healing in diabetic mice (n=4).** Representative histology 10 days after treatment with TG-VEGF-AG ( $\alpha 2PI1-8$ -VEGA121-AG), TG-VEGF165 ( $\alpha 2PI1-8$ -VEGA165), soluble, wild type VEGF-165 or fibrin gels alone. Black arrows indicate wound edges, while red arrows indicate tips of epithelium tongue (hematoxylin and eosin staining, scale bar = 1 mm).



The multifunctional TG-VEGF-AG enhanced angiogenic potential using signaling pathways that are different from those used by natural VEGFs to promote its biological activities. We proved that TG-VEGF-AG improved the regenerative effects of fibrin gels *in vivo* in a diabetic mouse model of chronic skin wounds at doses where soluble VEGF delivered within fibrin had no significant effects.

The applications for using biomaterial enhanced VEGF variants are many: to heal chronic wounds, to treat diabetic ulcers, burns, and soft tissue damage. There are clear motivations to improve VEGF efficacy for wound healing since wild type VEGF-165 has recently failed the second phase of clinical trials for skin wound healing, and PDGF-BB in Regranex, which is used for the treatment of chronic diabetic foot ulcers, bears a warning on its product insert from the FDA about potential cancer risks. Therefore, our engineered TG-VEGF-AG could allow for a lowering of the effective VEGF dose to potentially improve safety and effectiveness which now needs to be tested in large animal models with a comprehensive examination of the mechanisms of action.

## 6 - Bone repair therapy development

We developed practical therapeutic materials for bone repair with materials/biomolecular therapeutics and used these materials as a quantitative, designed and controllable platform for probing hypotheses regarding the fundamentals of osteodifferentiation, osteogenesis, and bone repair, with an emphasis on the role of angiogenesis in these processes. Our outcomes were both translational (to induce bone repair) and fundamental (to develop material and molecular tools for understanding osteodifferentiation, osteogenesis and bone repair more deeply).

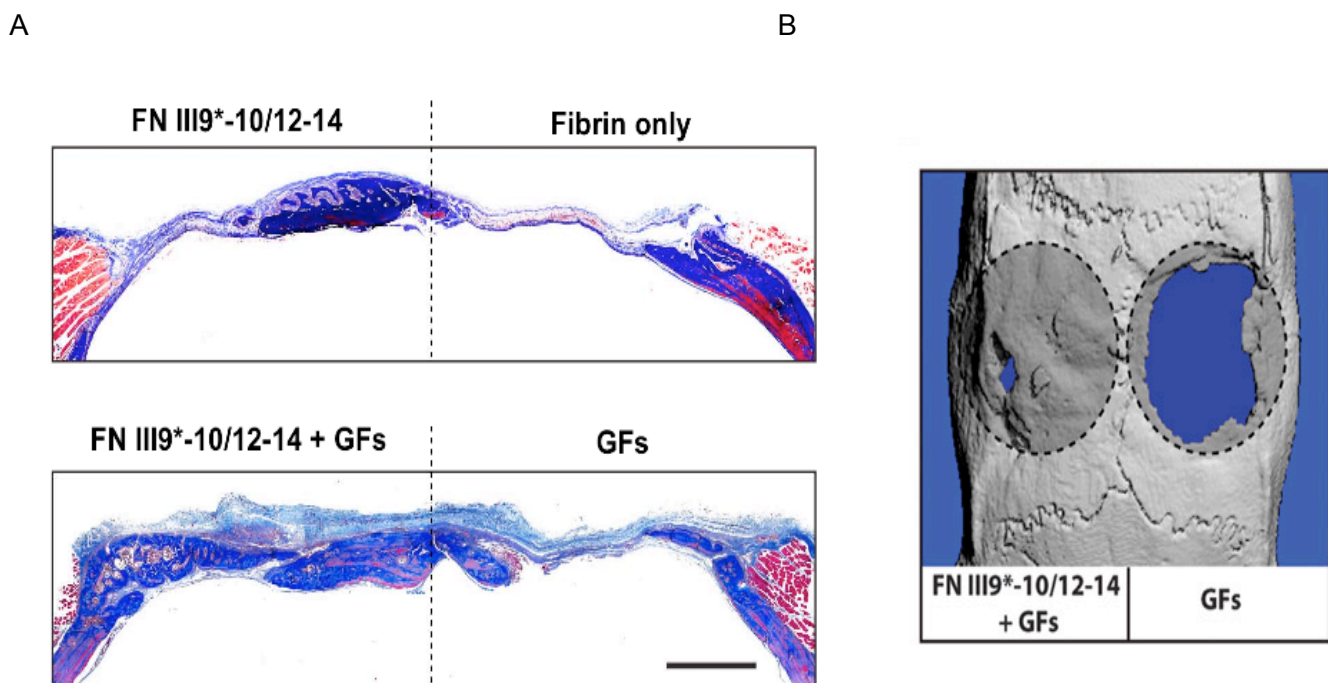
Our underlying hypotheses were focused on functionalised materials: the predominant therapy for bone repair has been to use very high concentrations of BMP-2, equivalent to the amount present in ~1000 humans which, while also being very expensive, indicated that there were clear inadequacies in the localized release with an optimal time course. The problem of high dose BMP-2 received increased media attention during the latter

stages of the project as increased rates of cancer had been reported in those patients receiving the morphogen; unsurprising considering that BMP-2 is a member of the Transforming Growth Factor family of morphogens.

### *i) Fibrin*

The multi- domain fibronectin fragment (FN III9-10/12-14) simultaneously binds integrins and growth factors which were bound the matrix via the FN fragment and evaluated for their retention in fibrin gels and their ability to promote bone repair. Preclinical development indicates the potential for the use of the FN fragment (FN III9-10/12-14) to allow growth factors, in this case BMP-2 and PDGF-BB to be bound to the healing matrices and that the addition of the FN fragment increases bone tissue deposition in calvarial defects through the recruitment of bone progenitor cells (Figure 27).

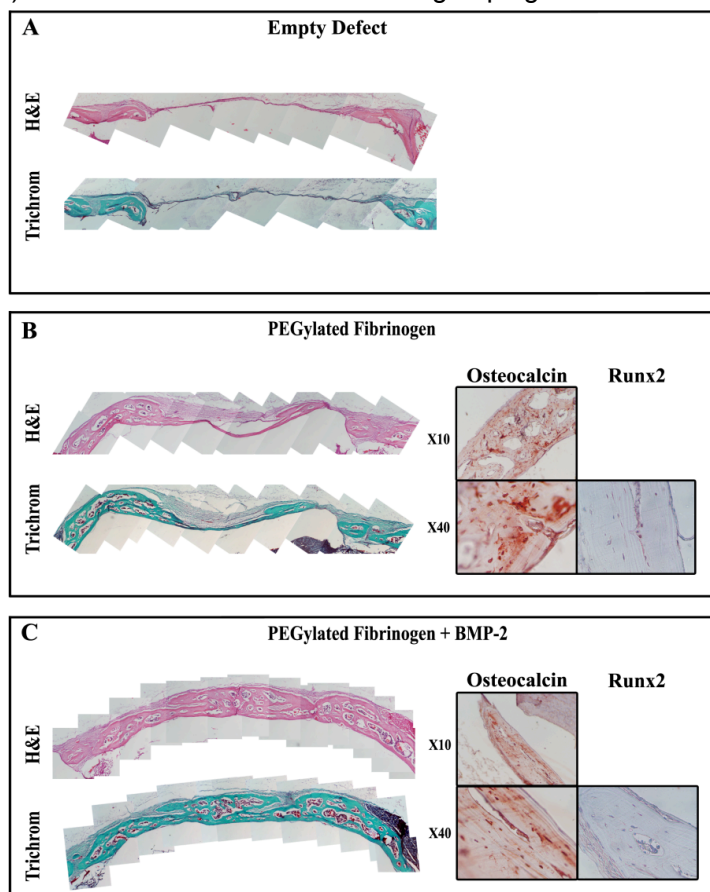
**Figure 27. Bone healing in the rat calvarial model is enhanced by delivering BMP-2 and PDGF-BB within the FN III9\*-10/12-14 microenvironment.** A) Four groups were tested: fibrin only, fibrin functionalized with FN III9\*-10/12-14, fibrin containing GFs only, and fibrin functionalized with FN III9\*-10/12-14 and containing GFs. Four weeks after treatment, bone regeneration was analyzed by histology (Masson's trichrome stain for collagen and bone tissue appearing in blue). Representative histological sections at the center of the defect are shown. Scale bar, 2 mm. (B) Representative skull reconstitution is shown for FN III9\*-10/12-14–functionalized matrix with GFs and for the fibrin matrix with GFs. The defect area is shaded.



### *ii) Fibrinogen polymer*

The use of the fibrinogen polymer for supporting bone regeneration was very successful. The hydrogel is easy to use as it is photo-polymerized in situ and appears to degrade almost completely in 8 weeks. The controlled release of matrix bound BMP-2 generates superior bone formation as compared to the material only in both a subcutaneous ectopic bone formation, in which bone marrow with hematopoietic and mesenchymal stem cells was generated, and in cranial defect repair (Figure 28).

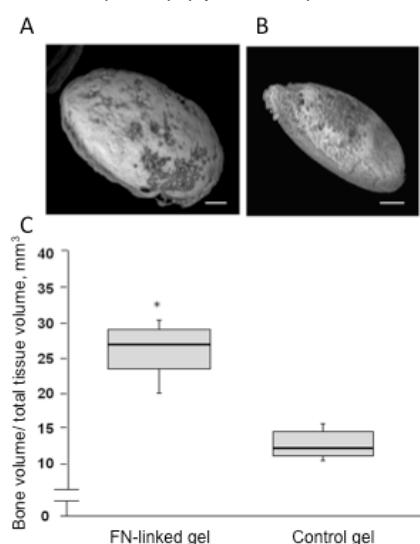
**Figure 28. Histological examination of cranial transplants of PEG-Fibrinogen plugs following implantation in vivo.** A) Representative images of empty defects stained with H&E and Trichrome, B) defects treated with PEG-Fibrinogen plugs and evaluated by H&E, Trichrome, Osteocalcin and Runx2 expression, and C) defects treated with PEG-Fibrinogen plugs with matrix bound BMP-2.



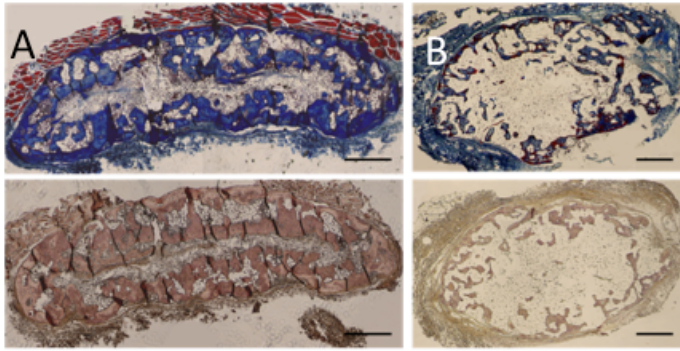
### iii) Hyaluronic acid

Hyaluronic acid (HA) hydrogels were functionalized to allow for the attachment of a fibronectin (FN) fragment and loaded with BMP-2. The formed hydrogels were injected subcutaneously into rats to measure bone formation. After 8 weeks we had shown that linking of the cell-adhesive fibronectin fragment resulted in more homogenously distributed bone tissue (such bone tissue should be less fragile thus excluding the risk of further fracture), which may be due to infiltration of the surrounding pluripotent cells into the corresponding hydrogel material (Figure 29 and 30).

**Figure 29. Ectopic bone harvested after 8 weeks of HAI implantation.** Representative images of (A) FN-linked HA and (B) control HA when BMP-2 was delivered at concentrations 20  $\mu\text{g/mL}$  and the volume of each hydrogel implant was 200  $\mu\text{L}$ . (Scale bars, 1 mm). (C) Micro-CT analysis and quantification of ectopic bone volume ( $\text{mm}^3$ ) (\* $p < 0.01$ ).



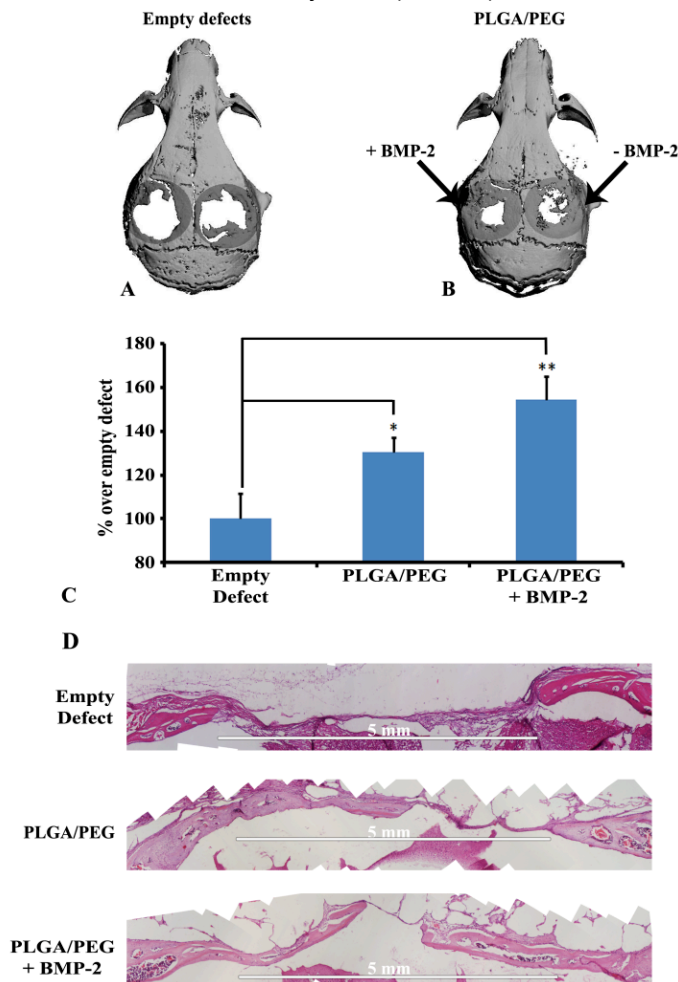
**Figure 30. Cross sections of ectopic bone resulting from hydrogel implantations (n=6).** (A) FN-linked hydrogels compared to (B) Control hydrogels. Representative cross sections were stained with Masson's Trichrome (upper images) and H&E (bottom images) (Scale bars, 100 $\mu$ m).



#### iv) Porous scaffolds

The PEG-PLGA-PEG porous scaffolds are very conducive for bone regeneration; they are temperature-sensitive, highly porous, have strong mechanical properties, are biocompatible and degradable. The use of BMP-2 with porous biomaterials were tested for establishing their ability to support bone formation. The scaffolds effectively released BMP-2 in assays, are cell adherent and induced osteogenic differentiation and proliferation. Regeneration of bone in preclinical models is achieved when they were implanted in cranial defect models (Figure 31). The porous biomaterials evaluated in combination with BMP-2 are advantageous for bone healing, with the absence of BMP-2 also inducing significant bone repair, indicating that very low doses of BMP-2 could be used.

**Figure 31. Evaluation of bone healing in a cranial defect model.** Images of A) empty defects, or B) PEG-PLGA-PEG scaffolds (left) with BMP-2 or (right) without BMP-2. C) Quantification of the percent closure of the cranial defect, as compared to an empty defect. D) Histological H&E sections (top) empty defects, (middle) PEG-PLGA-PEG scaffold only, and (bottom) PEG-PLGA-PEG scaffolds + BMP-2.



## 7 - Cardiac tissue repair therapy development

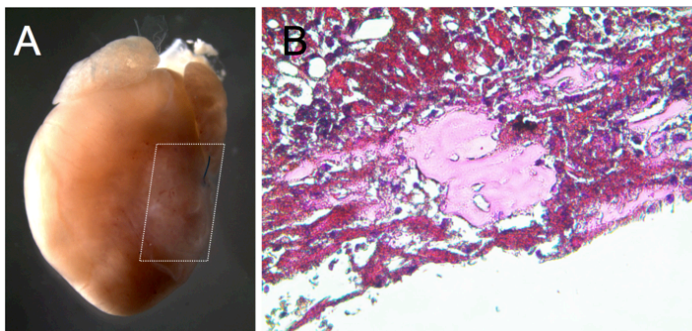
Chronic heart failure (CHF) post- myocardial infarction (AMI or MI) is the consequence of a deficit in functional myocardial contractile cells (myocytes), which are not replaced by any of the clinical therapies presently in use. Cardiac regenerative approaches to fill this necessity have been proposed. These regenerative therapies have been mainly based on different versions of autologous cell therapy. Several of them have been clinically tested but most have been proven to be only marginally effective, if at all. Unfortunately, even if their efficacy were to be significantly improved, none of these protocols (including the most promising use of autologous cardiac stem cells) could solve the severe public health problem, have a measurable impact in the everyday clinical setting, or affect the natural course of the disease. Given the high demand in time, human and economic resources, these techniques can only benefit a very small fraction of the candidate patients. Even with improved clinical efficacy, autologous therapies based on extracting cardiac stem cells would fail to satisfy the criteria of affordability, be readily available to treat the acute phase of the disease, and would remain inaccessible for treatment in the majority of clinical centers.

In order to develop effective cardiac therapies and significant improvements in the treatment of injured myocardium, focus was placed on the integration between novel tissue engineering biomaterials and the development of induced pluripotent stem cells (iPS) technology. Injectable biomaterials were considered the best approach to act as cell carriers in the ischemic area, and also prevent associated cardiac remodelling, which would improve cardiac mechanical properties. By utilising a cell-reprogramming technology this would permit us to obtain autologous differentiated cells, such as cardiomyocytes (CMs), directly from somatic tissue which together would address the practical barriers and socioeconomic need.

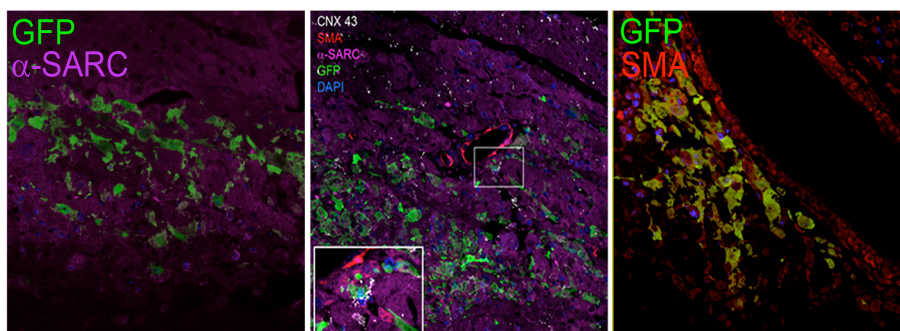
Induced pluripotent stem (iPS) cells were obtained after neonatal CMs reprogramming which were comparable to murine ES cells in the expression of cardiac associated transcription factors. Microarray analysis of global gene expression in iPS cells derived from the cardiac compartments identified upregulation of genes directly involved in cardiogenesis. Preliminary screening revealed that Fibrinogen Polymers (FP) proved to be the support for the CM-derived iPS, which tested in myocardial infarct models to assess efficacy.

This demonstrated that CM-derived iPS cells survive, integrate, and differentiate in the host myocardium, significantly improving cardiac function, as compared to control samples injected without iPS cells (Figures 32 and 33)

**Figure 32. FP injected in the adult mouse heart.** (A) heart tissue 1 month after Acute Myocardial Infarction (B) Representative samples evaluated 7 days after infarction histologically by hematoxylin and eosin staining.

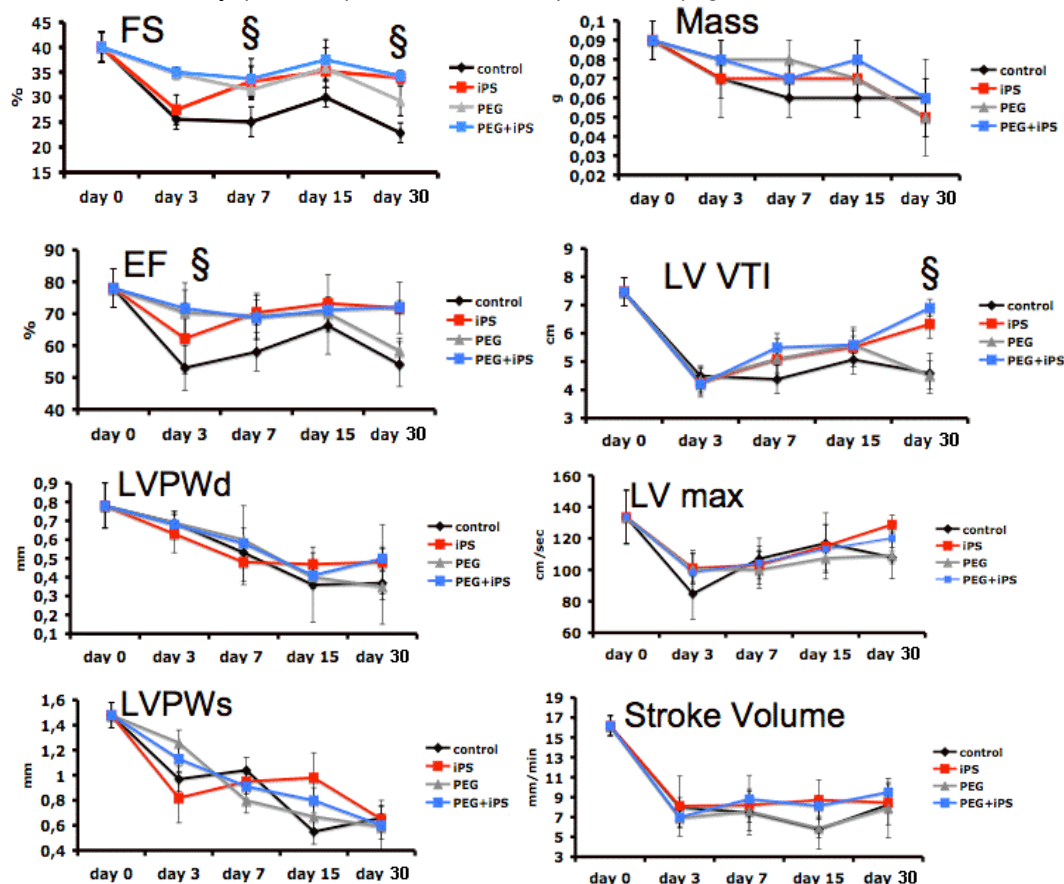


**Figure 33. Representative immuno-fluorescence images of sections of myocardium 3 days after myocardial infarction and concomitant transplantation of GFP-expressing mouse CM-derived iPS cells (n=3) supported by FP.** Functional integration of CM-derived iPS cells in the host myocardium is demonstrated by the expression of connexin 43 (white) among GFP-positive (green) cells and  $\alpha$ -sarcomeric (SARC)-positive cells (magenta).



Functionality (Echo-Doppler analysis) of the combined iPS-FP transplant demonstrated increased cardiac output and improvement of systolic and diastolic functions after myocardial infarction (Figure 34). A significant amelioration in the anatomical parameters of the iPS and iPS+biomaterial treated groups compared with phosphate-buffered saline-treated control group was observed with a significant improvement in hemodynamic parameters as soon as 1 week after myocardial infarction.

**Figure 34. Analysis of myocardium transplants by Echo-doppler analysis.** Echo-doppler parameters at 3, 7, 15, and 30 days after myocardial infarction in mice engrafted with (red lines) CM-derived iPS cells; (grey lines) biomaterial only; (blue line) biomaterial + CM-derived iPS cells; or (black lines) the PBS-treated control group. (Left panels) Measurements made of Fractional Shortening (FS), percent Ejection Fraction (EF), left ventricular free wall thickness in diastole (LVPW); (Right panels) cardiac mass, integral velocity/time (LV VTI), maximum velocity (LV max); stroke volume (stroke vol); §,  $P < 0.05$ .



This confirmed that iPS cell methodology integrated with FP produced a tissue engineering approach which was efficacious and could ameliorate cardiac function when introduced into an infarcted myocardium. The engineered cells had generated bigger structures compared to the control which essentially also created a harmonious cell orientation, probably caused by the movement of the tibialis muscle. This aspect should not be underestimated in view of clinical application. The coordinated orientation of the cells in artificial cardiac tissue allows a synchronous contraction dictated by electrical cardiac stimuli.

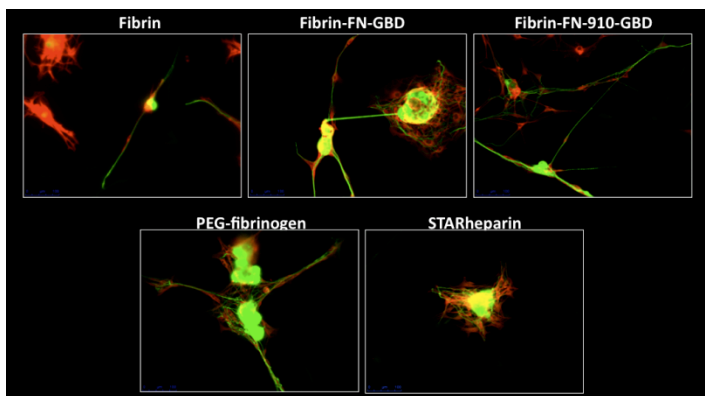
The potential to use these CM-derived iPS cells is substantial because they can grow, survive and maintain their undifferentiated state without feeder layers in vitro. This is a particularly useful for fast and reliable personalized medicine approaches for acquired or congenital cardiac disorders, as these cells can be generated from the host and reimplanted as a syngenic graft with a readily available material to generate fully functional cardiac tissue and repair the damage. We are presently translating this work to larger animals to confirm this data prior to moving the studies into humans.

## 8 - Nervous tissue repair therapy development

Development of regenerative approaches to neural disease focused on traumatic injury to the nervous tissue itself, predominantly manifested as spinal cord injuries in humans, which is where we placed our focus. Recent human clinical work has demonstrated that correct application of stimuli at the site of injury, within hours of the injury occurring, matched with long term rehabilitation can alleviate some of long term outcomes of the injury. The hypothesis is that the initial correct treatment prior to tissue rehabilitation has to be tri-partite. There must be an anti-inflammatory/anti-fibrotic component, as the damaged tissue is rapidly infiltrated by scar tissue which stays and 'blocks' the rejoining of the fibres; there must be an angiogenic component to rapidly restore a functional blood supply to the tissue to permit it to repair and there must be some level of instruction to the undamaged neural tissue to enable them to rejoin with the broken neural networks.

Compared to the four other sectors of application within Angioscaff, which have all received extensive focus in the regenerative/repair field, in the neural field, this focus of research is very much in its infancy as concepts and approaches to treating such injuries have had to be reconfigured which meant that conceptually developing therapies would start from a blank canvas.

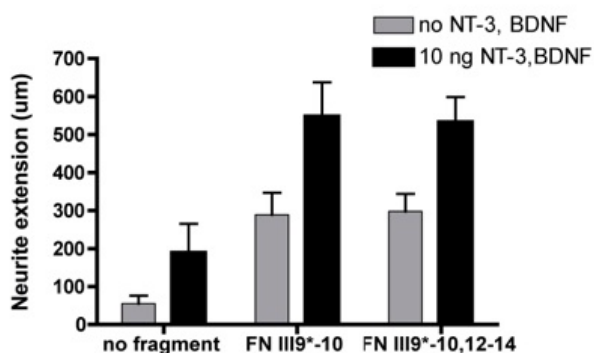
Initial effort was focused on identifying the best 'soft' biomaterials for the neural tissue itself; adult Dorsal Root Ganglia (DRG) explants were grown on starPEG-heparin gel and different fibrin gels. DRG neurites had an increased length of 50% in both PEGylated fibrin gel and FN-GBD and FN-910-GBD gel when compared with negative control. With the use of heparin gel, the length of DRG neurites increased by an additional 20% (Figure 35).



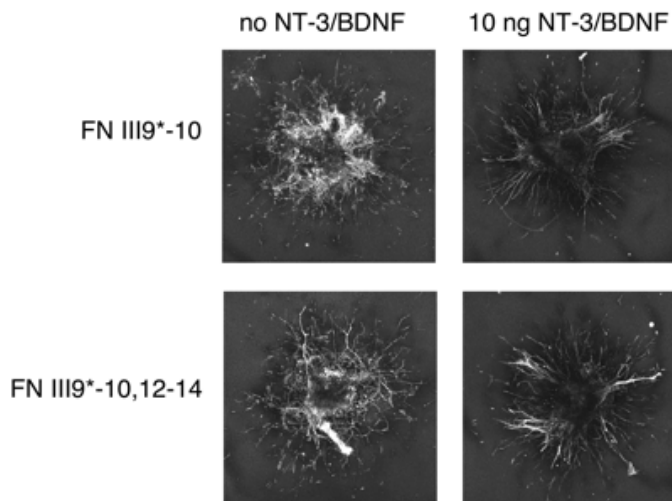
**Figure 35. DRG neurite length was measured in different gels. Red staining = s100; Green staining: Betall tubulin.**

PEG arrays were used to test different FN9-10,12-14 batches and to demonstrate that high doses of fibronectin fragments showed superior activity, for high capture of the growth factors (10 ng/mL BDNF or NT3) to be tested (Figure 36 and 37). These were extended to include the fibrin like hydrogels to generate TG-PEG, to colocalise the fibronectin fragments and the growth factors which permitted the neurites to grow much more radially oriented, compared to hydrogels without neurotrophic factors, where a much more random extension was observed.

**Figure 36. Quantification of neurite extension inside TG-PEG gels.** Gels were modified with FN III9\*-10, FN III9\*-10,12-14, or no fragment. Media with (black bars) 10 ng/mL BDNF and NT3 or without (grey bars) were added to the samples.

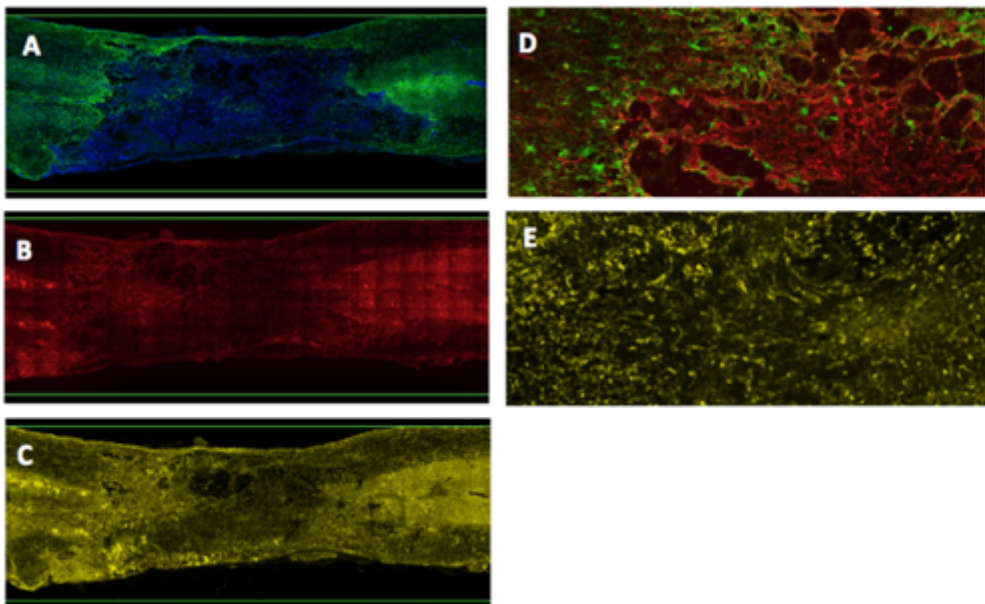


**Figure 37. Representative images of neurite extension inside TG-PEG gels.** Gels were modified with FN III9\*-10, FN III9\*-10,12-14 with or without 10 ng/mL BDNF and NGF.



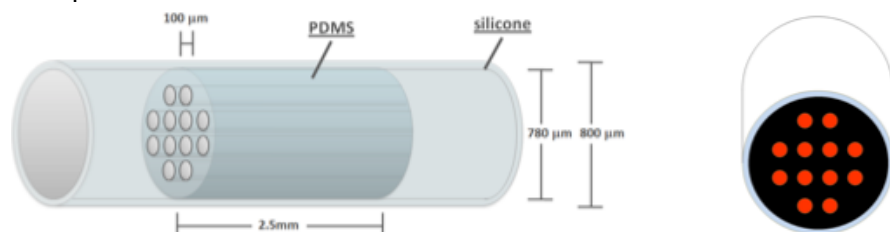
An in vivo spinal cord contusion study was performed to analyze cavity formation, the amount of reactive astrocytes, scar tissue formation, and neurite extension. Following pressure injury, growth factor loaded TG-PEG was applied. Histology of the spinal cords revealed that the addition of FN fragments to the gel improves spinal cord recovery (Figure 38).

**Figure 38. 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).



Based on these results and single channel implantation experiments, fibrin and FN-910-GBD fibrin was assessed in promoting axonal growth in microchannel using dorsal root injury model to determine if the implanted fibrin-related materials are supporting regeneration in a harsh regenerating condition. A schematic of the microchannel conduit design is shown in Figure 39; These conduits were implanted into the severed dorsal root of adults rat, which also had the growth factor loaded biomaterials present.

**Figure 39. A schematic diagram of the microchannels conduit used for implantation (logtitudinal and cross section).** Twelve 100  $\mu\text{m}$  (in diameter) microchannels were casted in a 2.5 mm long polydimethylsiloxane (PDMS) tube. The microchannel unit was then inserted into a 800  $\mu\text{m}$  silicone cuff which is used for nerve stump attachment.

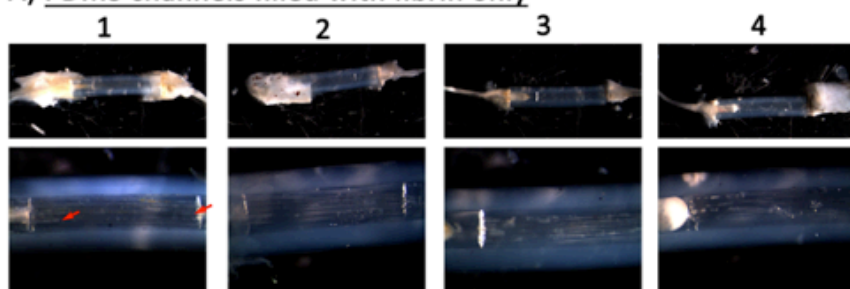


The microchannel conduits recovered from the injured animals five weeks after implantation indicated that 4 rats demonstrated some degrees of axonal regeneration down the channels (Figure 40, red arrows). Among these 4 rats, one was implanted with TG-PEG alone, one was with FN910-GBD fibrin and two were with FN910-GBD fibrin plus NT3. In the channels which demonstrated regeneration (9 in total), 7 of them grew more than half way down the channels. The rest of the 8 rats did not show any regeneration.

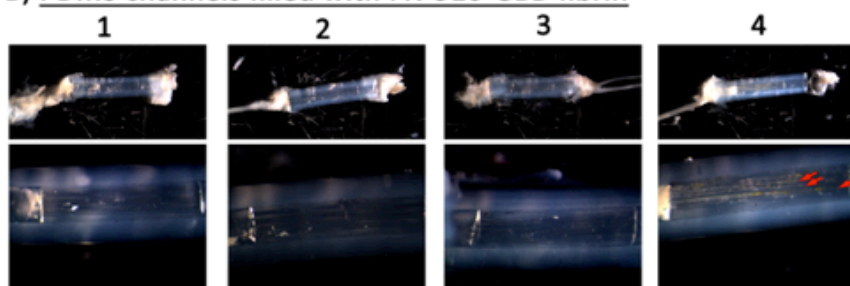
One of the possible reasons for this high variability in regeneration could be due to the design of the microchannel conduits. The microchannel conduits were made up of material that is highly anti-adhesive and hydrophobic. Unless the cut nerve ends were put juxtaposed to the channels where they would meet the fibrin (the red area in Figure 40), the regenerating nerve ends retracted and stopped regeneration when they interacted with the anti-adhesive PDMS surface (the dark area in Figure 40). Nonetheless, the long regeneration distance demonstrated by the 4 rats suggests that the fibrin-based materials are supporting regeneration even in harsh condition.

**Figure 40. Increased axonal regeneration of the dorsal root in some TG-PEG filled microchannels.** Here shows the microchannel conduits recovered from the several dorsal root models five weeks after implantation. The channels were filled with A) fibrin only, B) FN-910-GBD fibrin and C) FN-910-GBD fibrin with NT3. Regenerated axons (indicated with red arrows) were found in A1, B4, C1 and C2.

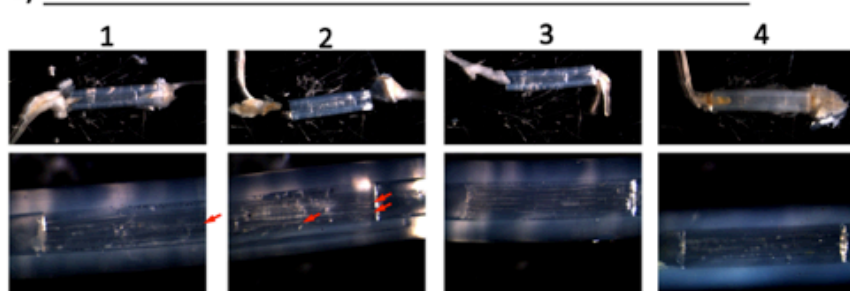
**A) PDMS channels filled with fibrin only**



**B) PDMS channels filled with FN-910-GBD fibrin**



**C) PDMS channels filled with FN-910-GBD fibrin and NT3**



Fibrin with and without FN-9\*10-GBD strongly supported the growth of DRGs in vitro, as compared to other biomaterials. These fibrin gels also appeared promising in vivo for allowing increased axon growth in a microchannel prototype in sciatic nerve injury model and also dorsal root injury model used for enhancing nervous system regeneration. In general, these highly crosslinked fibrin materials are suitable for multichannel fabrication for use in implantable channels for nerve regeneration.

We have demonstrated the potential for nerve growth to be correctly stimulated in a clinically comparable disease model, and potentially identified the best biomaterial/morphogen combinations to permit that growth to happen; however significant work is required to continue this study and demonstrate a feasible regenerative therapy using this approach. Once proven in addition to treating patients with spinal cord injury there is also the possibility to apply the same innovations within the neuroprosthetics and robotics arenas which attempt to link conscious decision making, information technology and the biological process of correctly reactivating damaged neural tissue.

## **9 - Skeletal muscle repair therapy development**

There are no effective therapies to treat traumatic or genetic skeletal muscle diseases: once the tissue is gone it is impossible to replace. We chose to leverage clinical advances in treating the genetic disease, Muscular dystrophy and the associated models which, like the human disease manifest as progressive muscle wasting, to develop effective muscle treatments. There are, several different therapeutic options that are currently under clinical investigation, including cell transplantation using adult muscle progenitors. These progenitor cells could be transplanted into large animal dystrophic muscle to partially repair the damaged myofibres and clinical trials utilising cell therapies are presently underway. Generating functionally restorative therapies for rare congenital disorders represents the more difficult scenario for restoring muscle, which if performed will permit the rapid development of approaches to restore tissue functionality in the plethora of diseases and traumatic tissue events which damage muscle tissue and prevent the continuation of a long and healthy life.

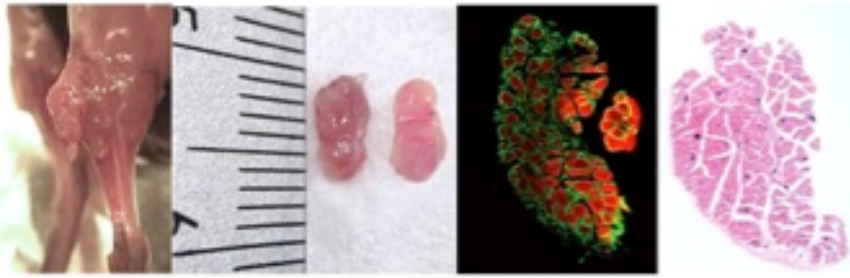
A number of adult-derived progenitor cells have been isolated, characterized and used for this purpose. While these attempts at promoting skeletal muscle regeneration represent a major step forward in finding novel treatments for MD, they have also been limited by extremely poor cell survival and lack of integration of grafted cells with the native skeletal muscle. These limitations motivated our approach to engineer the developed biomaterials to be instructive in promoting skeletal muscle differentiation/maturation, that are protective to the grafted cells (from an environment characterized by chronic inflammation which is exacerbated by transplantation), that are bioresorbable to facilitate controlled graft integration, and that are injectable to be consistent with the surgical approach for cellular therapeutics in humans. While this approach is limited to intra-muscular injections in localized forms of muscular dystrophies or localized muscles in forms affecting most of the musculature, it promises a different level of efficacy in comparison with systemic delivery.

Of the 6 different biomaterials developed, the Fibrinogen Polymers (FP) proved to be the most permissive for muscle cell growth when grown with the clinically tested muscle stem cell, the Mesangioblast (Mab). The FP was able to promote the maturation of well-differentiated myofibers and was deemed the most promising material in *in vitro* assays.

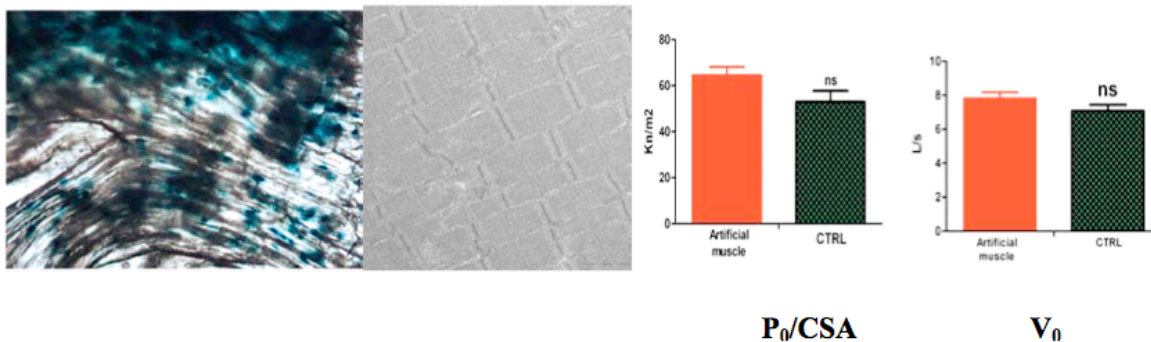
Using laser ablation for confining the construction of microchannels, the muscle fibres became oriented, aligned and cylindrical, comparable to real skeletal muscle fibers. Immunofluorescence and protein analysis revealed the expression of skeletal muscle contraction proteins such as Tropomyosin, Myosin heavy chain, Troponin, and others.

To evaluate the materials in vivo, subcutaneous implants were made in Rag2/gammaChain<sup>-/-</sup> immunocompromised mice of the FP with marked muscle stem cells (Mabs-LacZ). The LacZ permits a staining of the cells in situ, in which positive cells become blue and distinguishable from the native muscle tissue. FP was injected into injured Anterior Tibialis (AT) with the cells, which caused an increased survival of transplanted cells and an overall improvement in cell engraftment as measured by the newly regenerating myofibers formed. FP also enhanced skeletal muscle differentiation of engrafted cells with well-defined skeletal muscle organization and differentiation and permitted the growth of a completely new muscle (Figs. 41 and 42).

**Figure 41. Artificial muscle developed within 8 weeks over the host TA (n>2).** Comparable size, morphology and protein expression with host TA were seen.

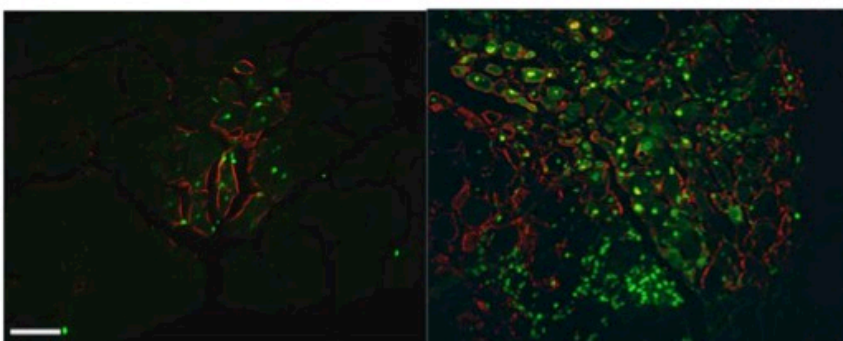


**Figure 42. X-Gal staining of freshly dissected muscle bundle (n=3).** Staining shows Mabs-LacZ artificial muscle origin and reveals mature sarcomere myofibril organization, specific force ( $P_0/CSA$ ) and maximum shortening velocity ( $V_0$ ) was similar to the host TA indicating muscle functionality. The host is indicated in green, while the FP+cell combination is indicated in orange.



In the context of treating potential rare diseases, Mabs and FP were combined, injected and polymerized *in vivo* into alpha Sarcoglycan null mouse (aSG-KO) TA. This mouse represents an *in vivo* model of muscular dystrophy sharing some of the pathophysiological characteristics of the human condition. PF again induced increased the survival of transplanted cells and led to an overall improvement in cell engraftment as measured by the newly regenerating myofibers formed. FP seemed to enhance skeletal muscle differentiation of engrafted cells and provided recovery from the genetic defect (Fig. 43).

**Figure 43. aSG expression analysis by immunofluorescence on TA section from aSG null mice.** After 5 weeks treatment, Mabs (left) and PF embedded Mabs (right) were evaluated. aSG (red) and LacZ (green) immunofluorescence of aSG expression localized in proximity of LacZ positive engrafted myofibers revealed increased numbers aSG positive fibers due to increased numbers of engrafted LacZ positive Mabs in aSG-KO treated with PF embedded Mabs. Protein assays confirmed the aSG expression in PF embedded Mabs treated muscle.

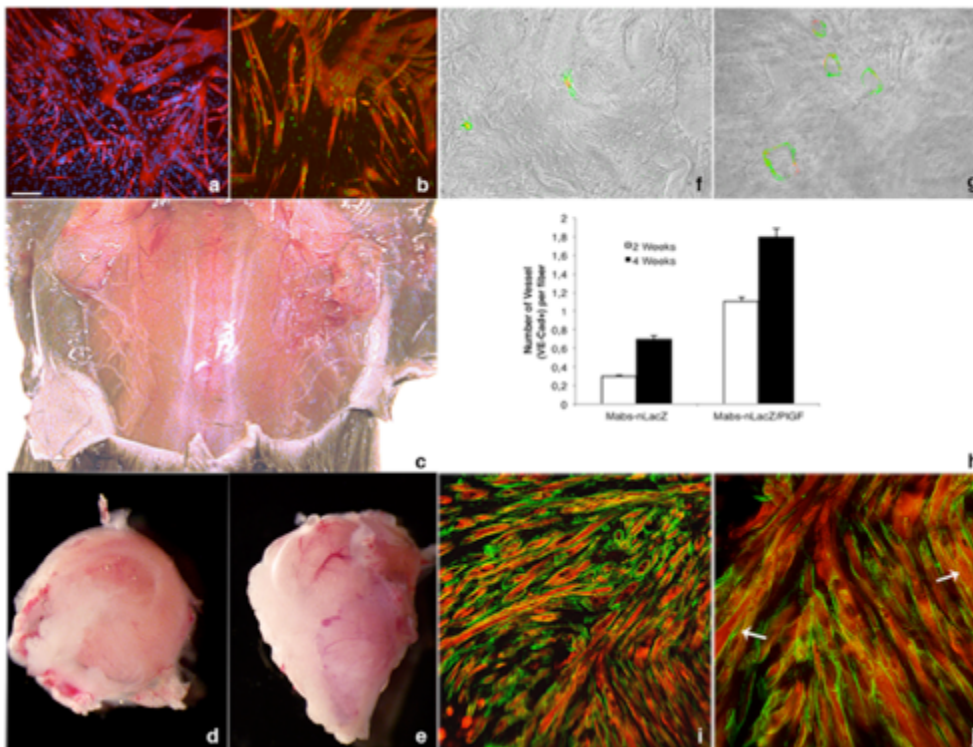


In order to ameliorate implant functionality and increase angiogenesis, we tested the pro-angiogenic factor (VEGF) which was added to the FP material and embedded with Mabs to enhance vessel formation and to

guarantee oxygen and nutrient delivery to the *in vivo* generated artificial tissue. FP, VEGF and Mabs were polymerized together in cylindrical molds and then implanted subcutaneously into immunocompromised Rag2/gammaChain<sup>-/-</sup> mice.

The results using VEGF were not promising as we observed vessel forming muscle edemas, which have previously been demonstrated with VEGF (Gargioli et al., 2008). Thus, we moved to adding Mabs that express the growth factor PIGF into PF matrices. Using this system, the addition of PIGF greatly influenced the ability of the host to recruit blood vessels, thus assuring oxygen and nutrient supply, and therefore muscle functionality (Figure 44).

**Figure 44. PIGF transduction does not influence myogenic differentiation but does promote blood vessel recruitment.** (a, b). Immunofluorescence staining for MyHC (red) and LacZ (green), counterstained with DAPI labelling nuclei (blue) for wild type Mabs (a) and Mabs-nLacZ expressing PIGF (Mabs-nLacZ/PIGF) (b) revealing that the myogenic capability of Mabs is not affected by lentiviral infection and/or by PIGF expression. (c-e). Macroscopic comparison between *in vivo* dorsal subcutaneous Mabs-PF implant loaded with:  $1.5 \times 10^6$  Mabs-nLacZ/PIGF on the right and  $1.5 \times 10^6$  Mabs-nLacZ on the left, revealing increased blood vessel recruitment when PIGF is expressed. (f, g). Immunofluorescence analysis for Smooth Muscle Actin (SMA) (green) and VE-cad (red) on Mabs-PF dorsal subcutaneous implants (4 weeks) sections (g) expressing or (f) not expressing PIGF, showing increased blood vessel number under PIGF influence. (h). Capillary/muscle fiber ratio calculated at different time points (2 and 4 weeks after implantation) by scoring VE-cad-positive vessels highlighting the increasing number of blood vessels in the Mabs-PF implants expressing PIGF. (i, j). Laminin (green) and MyHC (red) immunofluorescence analysis revealing healthy muscle-like tissue formation characterized by time related mature myofiber differentiation as indicated by the cross-striations (arrows) at (j) 4 weeks not yet visible at (i) 2 weeks. Scale bar values: (a, b) 50mm, (c) 300mm, (d, e) 100mm, (f, g) 10mm, (i, j) 20mm. Representative images from n=3.



We have proven that PF embedded Mabs can totally reconstruct a new muscle in the live tissue. When combined with the growth factor PIGF there was an increased vessel recruitment in *de novo* generating artificial tissue compared to unmodified Mabs. These data demonstrate the ability of PIGF to enhance vessel recruitment thereby allowing for the formation of artificial muscle tissue that is healthy and functional, when used in combination with state of the art biomaterials. The future use of this combination has a broad and high impact application; target diseases include, but are not restricted to urinary incontinence, forms of Muscular Dystrophy which affect small muscle tissues, and post traumatic injury, including those following surgery. Both the target cell and the optimised material are clinically validated, and therefore a combination approach, testing the system in the various disease models and in large animals is necessary as the linking steps to translating these innovations to the human scenario.

## **Impact of the Angioscaff partnership**

If published figures are to be believed the market average return on investment on industrial R&D in the life science sector is between minus 2 to minus 7%, which implies that by performing research, companies are in fact destroying their own value. When combined with the well published patent cliff this has resulted in serious questions on identifying where the next cost effective and profitable product is going to come from, being asked. Solutions are not forthcoming and indeed seem increasingly elusive, which has been compounded by the perfect storm of financial recession combined with a socio economic demographic based on an increasing ageing population with tissue degenerative diseases who spend nearly as much time in retirement as they did working, and cost increasingly more as they age. While published data indicate that new drug approvals maybe holding steady or indeed increasing according to some reports, and this does bode well for confidence in the R&D and approval process, there does seem to a worrying increase in the reimbursement agencies refusing to buy the therapies as they are not considered to be cost effective for the patient.

While problematic, this is also a unique opportunity to pull together opposing cultures in the life science sector, match them with industry, and generate real value, which leverages the issues that are facing the industry and turn these into market drivers and enablers. In the context of the ageing demographic, the fact that academic research focuses generally on specific issues related to rare or less common diseases (as those with high commercial value are already extensively addressed), there is real strength and opportunity.

Collaborations, joint ventures, and partnerships between industrial and academic partners, simultaneously focusing both on fundamental and translational research is not a new phenomenon. However in the field of degenerative diseases to be treated by regenerative approaches, to create any significant impact, this is the necessary and ideal scenario; not only do the key molecular and cellular triggers for the disease have to be understood, but also their impact on tissue function defined so that potential underlying regenerative mechanisms can be stimulated; insights of which can be rapidly translated into therapeutic reality. Critically, the outcome should be a limitation of the degenerative process, matched with a restoration of tissue function, as opposed to a palliative treatment.

Amongst many diseases, and across tissues, some of the major obstacles to restoring function are shared (angiogenesis, inflammation, fibrosis, stimulation of endogenous cells to repair or replace the damage with exogenous cells) therefore insights can generate therapies for rare diseases with an impact on more common disorders and vice versa. Extended to the development of the therapies themselves, if each underlying technology can be targeted to the underlying problem in one specific disease, it can then be tailored to enable an existing mono-applicable therapy to become multi-applicable. If extended to the global issue of keeping the ageing population operational and contributing to society, such innovations represent the real impact of regenerative medicine research. Whether this is achieved by using chemical entities to stimulate endogenous cells, transferring biological growth factors or attempting to reconstruct the tissue, the outcome remains highly beneficial and lucrative, and low costs should be achievable if the system can be both rapidly tailored and incorporate existing innovations.

It is obvious that therapies need to be created and validated cheaper and faster, and are de-risked as much as possible prior to clinical translation, nowhere more than in the previously high cost sector of regenerative medicine. To generate a lower cost product which do work for the patients and therefore agencies are ready to reimburse, the product should be designed for known disease targets, or at least well described diseases with the capacity to be easily adapted for new ones; an area where those working in fundamental and translational degenerative disease and regenerative medicine research have the advantage and was manifested in the partnership.

## ***Generating knowledge***

The single largest impact of the Angioscaff partnership, has been the enormous amount of insight and volume of fundamental knowledge produced by the partnership, which cannot be valued but only observed in the contractual reports and number of high quality publications generated (total of 89 by the end of the project). The selection of the partnership was based on the criteria of bringing together some of the strongest and most renowned teams working in degenerative disease and regenerative medicine working in Europe and known as opinion leaders around the world. All of them had made extensive prior investments in their sectors and the resources and expertise they brought to the collaboration. Each of the biomaterial teams had made seminal contributions in the field of design and application of polymers as both experimental tools and human therapeutics.

All of the teams involved in the validation of the novel materials had prior and ongoing clinical trial experience and therefore integrated into the project the constant perspective of real evaluation of approach to be developed

as therapy and whether application, patient compliance and the required primary endpoints for success could be achieved. The preceding twenty five pages illustrating the highlights of the collaboration represents a fraction of the results, both negative and positive, all beneficial, generated by leveraging an extensive previous knowledge in the sector into a focused strategy. The more translationally focused outcomes will be moved towards the clinical setting, negative outcomes from tested hypotheses will be used as guidelines to design ones that will be applicable, while many of the positive results represent early stage concepts which will continued to be developed.

This continued development and partnering will occur due to the collegial perspective of the need to develop cures that was held by all the teams, and that the cross fertilisation within the partnership between teams who knew of but had never worked with each other, has laid the foundation for continued collaborations as insights generated. Critically, these same insights are presently being used to establish collaborations with other teams not from the network who can fill a resource hole e.g. large animal modelling or to leverage the outcomes for other application e.g. delivery of biopharmaceuticals, vaccines and chemical entities for other purposes. This is an essential impact due to the complexity necessary to collaborate with teams who are not necessarily of the same expertise; something that happens frequently in regenerative medicine and as such teams are now far more empowered to enter in more complex collaborations because they now have a much deeper understanding of the actual needs and level of understanding of the partners and eventually generate a measurable value as inventions become therapies.

### ***Innovations in regenerative medicine and value adding Intellectual property***

Creating tangible outcomes with measurable value in early stage research is not possible; the valuation models used by business development which rely on risk adjusted net present value perceives all innovation which has not entered clinical trial to have a value of zero, as the risk of high attrition dilutes out any accurate measurement.

However, each of the partners brought in a significant amount of prior intellectual property which via the work performed as part of the portfolio projects increased their area of application and future potential value, as tabulated in table 4.

**Table 4: Prior IP brought into the project and value added by Angioscaff**

<b>Team leader</b>	<b>Prior IP brought into Angioscaff</b>	<b>Value added by Angioscaff</b>
Jeffrey A. Hubbell	“Drug delivery matrices to enhance wound healing” US20100845354 20100728 “Growth factor modified protein matrix for tissue engineering” JP20100067010 20100323	Engineered into state of the art multi morphogen binders and confirmed as an effective morphogen delivery vehicle with potential to be used for all degenerative diseases.
Jöns Hilborn	“Composition for the formation of gels” US2011008444	Engineering enabled growth factor addition for bone and establishment of a drug delivery system
Josep A. Planell	“Injectable composite material suitable for use as a bone substitute” US2011091554	Engineered into a morphogen binding finely structured material system which was also naturally angiogenic
Dror Seliktar	“Matrix composed of a naturally-occurring protein backbone cross linked by a synthetic polymer and methods of generating and using same” WO20060233855 “compositions and methods for scaffold production” EP EP2150282 (A2)	Following composite changes repurposed into an effective regenerative therapy adjuvant for cell therapy combinations treating cardiac and skeletal muscle disease
Kevin M. Shakesheff	“Porous particles” WO2008041001	Fine tuning engineering for second stage composite generating new intellectual property
Carsten Werner	“Bioactive hydrogel” US2012058943	Engineered to include growth factor binding capacity and validated in soft tissue systems
Ranieri Cancedda	“Biomembrane for tissue regeneration” US2012141559	Engineered to include cells and confirmed in bone repair systems
Sabine A. Eming	“Proteolysis Resistant Active VEGF” US2012258915	Approach used to generate new VEGF mutants which had significantly enhanced angiogenic activity
Thomas Eschenhagen	“Multiloop engineered heart muscle tissue” US2009061410	Technology altered to generate a functional heart muscle tissue combining materials and

		cells which can be used as a screening system
Giulio Cossu	<p>“Method for establishing and expanding multipotent stem cells” WO03095631 ,</p> <p>“Method of treatment for muscular dystrophy WO2007088050 -.</p> <p>“Skeletal muscle periangioblasts and cardiac mesangioblasts, method for isolation and uses thereof” WO2007093412</p>	Cells combined with selected bioactive materials can significantly augment the transplant potential of muscle stem cells in congenital disorders and can also grow a brand new muscle for traumatic injury.

### ***Generating new IP***

The partners also filed 4 new patent applications, based on the prerequisites for ensuring that the innovations were ‘non obvious’ as compared to the prior patents owned, which were as follows:

W Holnthoner, H Redl “One step method for casting hydrogels for tissue engineering”; application submitted in August 2012.

K Shakesheff “Injectable compositions comprising polymeric particles and hydrogel” WO2012028881

J Planell "Nanostructured material, process for its preparation and uses thereof" application number PCT/IB2011/052112

J Hilborn, D Ossipov, O Varghese “ Hyaluronic Acid (HA)- based delivery systems” Application number: WO2010SE50596 20100531: WO2010138074

### ***The patient need and market forces***

The future impact for patients and by necessity economic growth for those companies that develop and sell regenerative medicine products as regenerative medical approaches become first line therapeutics has been enhanced by the outcomes of Angioscaff. The areas of application are broad, as confirmation of application for one disease in one tissue, implies that with modifications the approach can be used for many diseases for that same tissue and by default repurposed to be applicable to other diseases. Below we indicate the sectors, needs and market sizes where the the outcomes of the project will be applicable.

### ***Biopharma and pharma therapeutic delivery***

Innovations in biomaterial development have been the driving force behind advances in sustained-release technology for injectable therapeutics, particularly for biodegradable drug polymers that can be used as depots and implants which could increase patient compliance by decreasing side effects and reducing the amount of therapeutic that has to be administered. Patient compliance to therapy significantly impacts therapeutic efficiency health outcomes and healthcare budgets, with a world wide importance.

Virtually all medicines are highly sensitive to changes in available concentrations of bioactive substance, which affects the efficacy, safety and tolerability of the therapeutic. The route of administration and local presentation of a therapeutic at the site needed therefore has a significant effect on its therapeutic concentration within the body.

“Patient noncompliance with therapy regimens is often thought to be the single greatest threat to successful treatment in chronic conditions. Low rates of adherence to treatment substantially contributes to increased levels of mortality, as well as accelerated disease progression, which in turn results in hospitalization or other costly procedures that ultimately place an economic burden on healthcare budgets” (source Datamonitor “innovations in drug delivery) while the World Health Organisation has stated that lack of compliance to therapeutic treatment “is a worldwide problem of striking magnitude” and that “the consequences of poor adherence to long-term therapies are poor health outcomes and increased health care costs.”

The delivery route and mode of presentation is thus driven by more than simple patient acceptability, as the properties of the therapeutic (such as its solubility, duration of need to elicit an effect and level of metabolism) determine optimal administration routes.

Advanced and affordable therapeutic delivery systems, which can be designed and tailored for a direct personalized medicine with drug specific release profiles represent value maximizing approaches for the generation of “best in class therapeutics”. This design opens new opportunities to develop new therapeutics and the repositioning/reformulation of existing ones, maximizing value for large pharma looking to stay viable. The total therapeutic delivery market is in the region of €82bn.

Optimising drug delivery improve pharmacokinetics and pharmacodynamics by enhancing therapeutic stability, the therapeutic efficacy and half-life in the patient. Advanced targeted delivery system will increase patient

compliance; preserving the pharmacological action; reducing toxicity, and the antigenicity of the agents administered. Importantly, and as demonstrated in Angioscaff, it also reduces the amount of therapeutic that would have to be administered greatly reducing the medical costs that would have to be reimbursed, while simultaneously increasing efficacy.

In the context of therapeutics that did not pass during development, there is the potential to readdress their application through combination with therapeutic delivery systems to extract value from the costs of development. Furthermore for therapeutics that proved to be efficacious and value generating, potential reformulation generates the opportunity to extend patent life, destroy competing generics and maintain market share. This can be performed for the primary targets, and for maximum value extraction by repositioning the drug in other therapeutic areas: the market has numerous examples of this being performed as a value adding strategy (Glivec, Thalidomid, Viagra, Exubera).

Within Angioscaff, two delivery systems were successfully generated; one which linked a selection of structural biomaterials to any selected factor via a promiscuous growth factor binding domain which was demonstrated to be highly effective in a number of tissue systems in eliciting significant tissue repair and a second which could effectively deliver any kind of therapeutic directly into a cell. Both are poised to have play a significant role in what has become a very complex healthcare market.

### *Soft tissue repair*

The application of the successful soft tissue repair developed as part of the project is incredibly broad. Soft tissue regenerative medicine targeting the epithelia and epidermis is designed for patients who have poor therapeutic alternatives, with obvious high social and clinical impact. This includes epidermal, ocular and mucosal diseases which have a large range of clinical manifestation.

For instance, in Italy there are approximately 6,000 patients/year receiving a corneal graft from organ donors. Only a fraction of them would require regenerative medical treatment. Those patients, however, have severe symptoms, loss of vision, poor alternative therapy and can even undergo eye bulb removal. For those patients, tissue restoration means cornea functionality and complete recovery of visual acuity. In the case of total destruction of the corneal surface, a clinical situation that requires regeneration of both corneal and conjunctival epithelia, there are no available therapies. When applied to bilateral ocular surface lesions, the technology developed in this project would determine an extraordinary improvement of the quality of life: patients who are virtually blind could resume a normal life-style. Similarly, patients with large oral lesions often need complex surgical procedures. The proper restoration of such surgical lesions is however hampered by the lack of an epithelial replacement, leading to severe pain and discomfort for the patient. Such patients would enormously benefit from the availability of technologies that restore or replace the epithelia.

In 2009, European sales of skin replacements and substitutes and active wound repair modulators totaled approximately €340 million, with skin replacements and substitutes accounting for 48% of sales and active wound repair modulators accounting for the remaining 52% of sales. Wounds that do not heal within three months are often considered chronic. The vast majority of chronic wounds can be classified into three categories: venous ulcers, diabetic, and pressure ulcers. A small number of wounds that do not fall into these categories may be due to causes such as radiation poisoning or ischemia.

Diabetic ulcers (DUs) are a devastating medical problem and possibly the most difficult soft tissue to demonstrate primary endpoints being reached as part of therapeutic development. There are 26 million diabetics in the US and that number is growing. As a result, 1.5 million DU's are being treated annually in the US alone. In Europe there are 20 million diabetics, and of these, 1.0 to 1.4 million have DUs. The US diabetic foot ulcer market is €2.6 billion and growing. The European market for DU's is also €2.6 billion. The cost of failure to treat DU's is often amputation, a decreased quality of life and over €35,000 in associated medical costs<sup>3</sup>.

Less life threatening disorders such as scar contractures following burns, dermatitis, psoriasis and potentially rarer diseases such as Epidermolysis Bullosa all represent significant patient need and product potential.

Within Angioscaff we used traumatic tissue damage as the model for demonstrating that biofunctionalised materials offers potential and can be developed into an effective therapy, which can be used to treat all of the above clinical needs.

### *Bone repair*

The global orthopedic market is growing at 10% per year and anticipated to reach \$50 billion by 2016. In Europe alone the market is expected to reach €10 billion in 2016. In China specifically, the worlds second largest market, its worth approximately €14 billion per year, with 13% of this being for implantable devices.

**The Frost and Sullivan market report on ‘Biomimetics inspired by Nature’ stated ‘In recent times, the orthopedics and sports injury markets are undergoing a transition as there has been an increase in demand for the adoption of biologically active treatments. This would mean that the industry would shift from the use of traditional, passive, highly invasive metallic devices to more advanced, bioactive and nano biomimetic devices. This trend may be seen from the fact that osteobiologics, in particular bone grafting related products, is one of the fastest growing segments within the orthopedics market’.** The report also indicated that biostimulatory approaches had the highest probability of success and level of attractiveness for the market.

The reason for the sustained global market growth is due to:

- An increase in the ageing population. From 2012 to 2020, this growth rate was expected to reach 3.1 per cent as the baby boomers become 65 years old. The total population, in comparison, is growing at a rate of 0.9 per cent. However, this rate is expected to decrease to 0.8 percent by 2020. The aging population has been very active and hence there has been constant stress to their bodies. Orthopedic surgeries are performed on people of all ages, but many of the degenerative conditions that lead to the need for surgery affect people in middle age or later in life.
- The improving medical infrastructure and consequent rise in diagnosis rates in emerging markets in Asia, Africa, and Latin America also present growth opportunities.
- The number of overweight people worldwide is expected to increase by 44 per cent between 2005 and 2015. As the risk of orthopedic related disorders is more in overweight people, this trend is expected to fuel demand in the global orthopedic medical devices market.

Within the orthopedic sector, approximately 15 companies control 95% of the worlds market in orthopedic medical devices, which includes novel biomaterials, however the market is very fragmented in which each market Tier is controlled by its own market forces based on price differences or quality; nonetheless the sector as a whole is investing in innovation as basic patents of most orthopedic devices and implants have expired, leading to genericisation and commoditisation. Thus ‘continued innovation’ or value additions such as improvement of materials is becoming very competitive and perceived of high value.

While the non medical device sector is also targeting this market (in 2012 there were 5 NCEs in development for fractures, 5 for spinal fusion, 4 for bone repair, 3 for bone regeneration and 1 for osteo induction) the biomaterial sector is considered to be the future. In 2011 the orthopaedic biomaterials market was worth about 14.5% of the global orthopaedics market and sales of biomaterials are expected to grow about 10% annually and could surpass €10 billion in 2016.

Present state of the art products are based upon materials sciences implants made of metals combined with biological substances for better incorporation in the natural bone. In addition, re-absorbable materials in implants that fulfill their function during a period of time and then gradually undergo re-absorption are emerging. Growth factors alone such as BMP2 are also considered as products, however in the context of large bone defects, efficiency is questionable.

Currently, the highest focus sector, and one that was developed in Angioscaff involves materials implanted with growth factors rendering it bioactive that stimulate cellular. When commercialised, these are likely to revolutionise the orthopedic sector, but is very dependent on combination products being approved by the authorities.

#### *Heart repair*

In the Western world alone presently there are over 9 million people suffering of heart failure where the treatment available (with the exception of heart transplant) are only palliative. Yearly, more than a million new patients join this group. Extended to the rest of the world which has witnessed a significant increase in cardiac disorders the future impact of the data generated is large.

The Frost and Sullivan report ‘Advances in cardiovascular therapy’: stated

*“A fundamental driving force in the cardiovascular therapeutic field is the realization that pharmaceuticals can only go so far in the treatment of cardiovascular diseases. With increasing complexity of the genetic makeup and the role of multiple factors in the etiology of cardiovascular diseases, there has been a drive to think beyond the ordinary and consider the use of technologies such as tissue engineering and stem cells in managing cardiovascular diseases and leading to treatment of the disease.”*

The prevention and amelioration of pathological cardiac remodelling as a consequence of acute or chronic ischemic heart, myocardiopathies due to inflammatory, infectious or genetic causes is an immediate

requirement. The average life-span after the first episode of heart failure for these patients is of ~5 years. By 2016 the annual global market for all cardiovascular products is estimated to reach €160bn, with the top ten companies estimated to possess half of that market. Critically, cardiac disease and myopathies are global issues.

Due to its prevalence and its disabling long term sequels, Ischemic Heart Disease (IHD) is a critical challenge for the health care systems of the developed world and increasingly also for developing countries. In each the EU and USA, over 1 million acute myocardial infarctions (AMI) are treated annually. Angioplasty, combined with stent implantation and new pharmacological regimes, when applied promptly, has been successful in re-establishing the perfusion of the ischemic myocardium and has reduced significantly post-AMI early mortality, which now stands at >10%. Despite the remarkable reduction in early mortality, these new therapies do not recover the injured tissue or the cells lost and fail to prevent the subsequent degenerative process of cardiac remodeling which ultimately leads to Chronic Heart Failure (CHF). Paradoxically, the reduction in early mortality due to AMI has aggravated an epidemic of late CHF, which is now suffered by >12 million patients in the EU and the USA, with ~500,000 new patients added every year. Post-MI CHF is a terminal disease with an annual mortality rate of ~18% after the first episode (average life-span ~5 years), for which the only curative treatment is heart transplantation.<sup>1-4</sup> This treatment is available only to a minute fraction of candidate patients due to donor scarcity, cost, and the requirement for long-term immunosuppression<sup>5</sup> with its many deleterious effects. The fact that the worldwide number of annual heart transplants peaked in 1994 at 4,429 cases and has steadily decreased since then to about 3,000 annual cases underscores the need to develop a myocardial regeneration protocol which could restore myocardial function to an unlimited number of patients.

The outcomes of the Cardiac work achieved in Angioscaff, exceeded our expectations. The insinuations of the data indicate that an 'off the shelf' cardiac repair strategy is both feasible and affordable.

### *Muscle repair*

The outcomes of the work performed in combining biofunctionalised scaffolds with cells and the total muscle repair obtained provides a ground breaking approach that could be used to treat the following:

**Muscular dystrophies:** The inability to produce dystrophin and other proteins usually linking the cytoskeleton to the membrane and the ECM in muscle causes several genetic diseases collectively known as muscular dystrophies. Duchenne Muscular Dystrophy (DMD), the most common and one of the most severe forms, is due to mutations that affect the X-linked *DMD* gene and affects 1 in 3,500 newborn boys and there are 800 new cases annually in the EU. The burden for DMD healthcare in Europe is €75,000/annum/patient, amounting to an annual bill of €60 million; an effective treatment will therefore be of significant economic benefit to the EU. The stem cell therapy itself for DMD has been estimated to cost approximately €300,000 euros per patient (G.Cossu, personal communication); this would be extremely cost effective in both contributing to reducing the health burden and generating novel marketable products, which together will give socio-economic benefits to the EU community. The potential cost saving to the health care systems, given that DMD patients can live into their 40's, is over 5.5 million per patient, while the value given to the patient and their families is immeasurable. Being the most common, DMD has been so far the most studied form of MD and thus it may serve as a paradigm for new treatments. However it is unlikely that a topic treatment may be effective in all patients' muscles that are affected in this form. Nevertheless, treating selected target muscles, such as the dorsal muscles in children (to prevent lordosis), intercostal muscles (to enhance ventilation) and the hand muscles in adult patients (to maintain motility) may result in great benefits for the patients and increased quality of life and partial independence. In addition, less common forms, such as Oculo-Pharyngeal (OPMD), Becker dystrophy or distal (DD) muscular dystrophies are restricted to few specific muscles, even though they compromise the autonomous life and in some cases (OPMD) are lethal due to the inability of patients to swallow food and even liquids. These forms represent ideal target for a topic treatment also because cells may be derived from non-affected muscles (e.g. thigh) and thus would not require genetic modification nor immune suppression for the patient as in the case of heterologous cells.

**Urinary Incontinence (UI):** This is an extremely common problem, and has a significant impact on quality of life, the vast majority of those who experience the condition do not undergo treatment, in part due to cost, embarrassment, or fear of risky surgical procedures. There is therefore a very strong demand for less costly, less invasive and more tolerable, discreet, nonsurgical UI therapies. UI is due to both age-related muscle degeneration and to iatrogenic lesions in young women due to ephysectomy during delivery. Trials with myoblasts injections are ongoing but the low engraftment of cells injected in saline solutions lowers efficacy. The global UI therapeutics market was worth approximately €2.5 billion in 2009. In 2001 the market was valued at €1.4 billion and it grew at an approximate CAGR of 7.8% from 2001 to 2009. The global UI therapeutics market is expected to reach €3.4 billion by 2017 growing at a CAGR of 3.5%. Existing therapies simply do not repair the non or dysfunctional tissue.

**Surgical management of malignant lesions of the oro-facial region:** Tumors of the splanchnocranium often require demolitive surgery. Patients survive but with mutilations that severely limit their normal life functions and usually abolish their social life. Plastic reconstruction is major challenge and could be enormously helped by

the possibility of developing in situ, artificial muscles, as the proponents have demonstrated to be possible by combining biomaterials and stem, at least for the small Tibialis anterior of the mouse (Fuoco et al in preparation). The possibility of combining this novel approach with ongoing bone and teeth reconstruction would immensely help this challenging novel therapeutic strategy

**Hysterectomies:** According to the U.S. Dept. of Health & Human Services, 25% of all women (16 million) suffer from fibroid symptoms, leading to 250,000 annual hysterectomies – a highly invasive surgery with many side effects. The problem is so widespread that a third of all U.S. women have undergone hysterectomy by age 60, with fibroids being the most common reason. In 2006, combined sales of products for the treatment of the three most common benign conditions affecting the uterus (Endometriosis, fibroid tumors, and menorrhagia) were totaled approximately \$415.6 million; reaching approximately \$760.3 million in the year 2010. The hysterectomy process removes a significant portion of the tissue, which results in extensive remodelling and debilitation; processes that could be both optimised and decreased in time with a highly effective muscle restoration therapy. In addition, less traumatic and life threatening or altering issues, such as hernia repair which is the most common surgical procedure performed, can also be treated easily with such an approach, reducing scar formation, restoring tissue integrity and decreasing the possibility of repeat herniation

### *Neurological repair*

Neurodegenerative diseases, including stroke, spinal cord injury and Parkinson's disease, which could be treated with tissue repair approaches, are the major causes of chronic disability in European communities with a market size estimated to reach €17 billion by the year 2014. With the increasing number of elderly people, coupled with successful treatment of non-neurological causes of chronic illness, the incidence of neurodegenerative disease is increasing. Approximately 1% of people over 65 years of age are likely to develop a neurodegenerative disorder.

Europeans suffer nearly one million strokes each year, highlighting the need for efficacious therapy and the tremendous market potential for effective stroke therapy. Between 15-30% of ischemic stroke victims are permanently disabled and 20% require prolonged institutional care. As a result, stroke is one of the most common causes of long-term serious disability and represents an economic burden similar in scale to myocardial infarction, the symptoms of which could be treated using vascular repair and neurostimulatory therapies to potentially restore function.

Spinal cord injury is estimated to affect at least 330 000 people (paraplegia and tetraplegia) with over 15 000 new cases reported each year. In two-thirds of cases, road accidents are the cause of injury, with sporting accidents making up another 10%. Most occur at a young age: average age of 19; about 80% of males with spinal cord injuries are aged 18-25 years. The cost of treatment and aftercare for sufferers is phenomenal: the average lifetime costs directly attributable to spinal cord injury for an individual injured at age 25 range from € 0.45 M to € 2.1 M and have to prepare to spend an average of forty years or more in a wheelchair. It is known that stimulating angiogenesis and preventing fibrosis as soon as possible after injury, using locally administered morphogens and factors directly into the site of damage, immediately after the accident (followed by rehabilitation) will significantly increase the possibility of the patients retaining motor function.

Treating Parkinson's Disease (PD) with conventional drugs and newer innovative therapies such as deep brain stimulation and/or the use of Apomorphine or DuoDopa® cost between 8000 and 40,000 Euro a year per patient treated. Indeed in the UK the total cost of PD is estimated to be between 449 million and 3.3. billion pounds annually. Thus any therapy that can reduce disability and dependency on expensive drugs that only offer symptomatic benefit would be a major breakthrough. Regenerative therapies, likely involved cell transplantation with the neuromorphogen/material complex or morphogen/materials alone will not only reduce the costs of care in the short term but also in the long term by altering the natural history of treated disease. and significantly reduce patient morbidity and mortality and be highly cost-effective.

Despite posing the greatest challenges for tissue repair in the Angioscaff project, the long term potential to adapt the developed material/morphogen systems similar to that achieved with the heart (iPS cells) and skeletal muscle provides a long term potential therapeutic, that despite its complexity will actually restore function to the tissue.

### *Sport and activity related tissue damage*

In addition to tissue specific disorders indicated above, other circumstances can induce tissue damage. Among them, occupational injuries and sport-related injuries often cause life-altering conditions. Two examples are cited below:

In high intensity sports, hardly a game or championship goes by without a sprain, strain or break. Just before a championship, it is not ideal for a team to lose one of its star players. Strain injuries, which are common in sport, cause the rupture of large myofibril bundles leading to muscle regeneration and formation of scar tissue and

new myotendinous junctions at the level of the rupture. To avoid the risk of reruptures, early remobilization is required to induce correct growth and orientation of regenerated myofibers. The problem is to improve the healing process. A lot of the work has initially been in bone, but the more exciting area is the soft tissues. These soft tissues that cushion and hold joints together—tendons, ligaments and cartilage—heal slowly, if at all. Part of the problem is that the blood that helps other tissues heal after injury hardly reaches them. One of the challenges of sport medicine is to reach the tissues that are injured. Thus the problem of sports medicine could find solutions in our project. Indeed, the central goal of EndoStem is to identify molecules that can be used either alone or in combination to activate muscle repair through endogenous muscular or/and vascular stem cell mobilisation and activation.

Sport and activity-related tissue damage can impair the patients' life as badly as other degenerative disorders however none of these traumas receives the same profile as the major life debilitating disease however restoring the capabilities of those injured at work or activities performed in their free time has become a major issue, especially because those injuries have a considerable economic impact. For example, based on the latest data available (generated in 2004 by a French health insurance company), occupational injuries in France alone resulted in a net loss of 48 million working days, which, in other words, corresponds to shutting down a 130,000-people company for one year. In addition, over €6 billion were spent by insurance companies to compensate those whose injury resulted into life-altering disabilities.

In the United States, direct costs for occupational injuries are estimated to be over \$50 billion per year while indirect costs such as loss of wages or workplace disruption costs reach \$150 billion (source: *Costs of Occupational Injuries and Illnesses*, University of Michigan Press, 2000). In France, direct medical costs incurred after sport injury reach €200 million euros per year, without considering the resulting absence of work, which corresponds to about 4% of total absenteeism (source: *British Journal of Sport Medicine*, 2008). If matched with effective pain management, the materials generated in Angioscaff could offer rapid tissue repair at a very localised level and with likely more effect; the endogenous cells necessary for repair of the tissue are still local and available, but simply lack the correct stimuli and structure.

#### *Non human-health related markets*

While we have naturally focused the potential developments on human healthcare needs, in the context of pure market development, the outcomes of Angioscaff also have the potential to be developed for both human cosmetic and veterinary sectors, which have stand alone large market values.

(Sources of information: BCIQ, MDDI, Datamonitor, Research and Markets, Forest and Sullivan, Kalorama)

#### **Spin off companies**

Two spin off companies were created from innovations that arose from the work performed: The first, Delivery Limited which was based on the delivery technologies created by the team of Jons Hilborn from Uppsala. The technology itself has a very low cost of production and can be engineered to deliver all types of therapeutics (chemical entities, nucleic acids and proteins). The hyaluronic acid component which forms the core of the technology means that the 'payload' of the delivery vehicle is taken into the cell by receptor mediated cytosol intact. As the pharmaceutical field is looking to repurpose existing therapeutics, readdress earlier stage therapies which were abandoned due to inefficient delivery e.g. toxicity due to high dose necessary, or poor effect due to poor pharmacokinetics, and explore new therapies such as siRNA, the potential of the application is very wide.

The second company (originally named Promimetic Limited), renamed to Echino Limited (after the Echinoidea Class of organisms which have the capacity to regenerate limbs and organs throughout their life span) was created based on the combined work of Professor Dror Seliktar from the Technion and Professor Giulio Cossu from the UCL. The capacity to create *de novo* a completely functional tissue *in situ* is groundbreaking for soft tissue and the potential applications extremely broad based on the flexible tailoring that forms the core part of the innovation; the fact that both the cell component and the biomaterial component have progressed through clinical trials, means that conceptually the product is de-risked. The largest barrier we face with continued development is dependent on a change in regulatory perspective of combination tissue repair products.

Despite the particularly difficult financial market for hi-tech life science companies and that early stage capital for start up companies is sparse, we aim to keep the companies functional and continue the product development using any source of funding possible so that when the funding markets do become more conducive we can rapidly grow these companies.

#### **Linking with the public: general public and scientific community**

The pre-clinical nature of the work being performed decreased the potential to attract stakeholder interest in the work being performed; the prior experience of all the partners involved in stakeholder liaison clearly indicates that interest is only received when clinical testing is publicised. Stakeholders in general have great difficulty in being interested in early stage research unless there is a near short term application, and observing what other

outreach entities perform (Eurostemcell, the Association of Science and Discovery Centres, The European Network of Science Centres and Museums), extensive investment is necessary in the establishment of the outreach activity itself and its continued population with information. This was not designed into the budget of Angioscaff and therefore these activities were limited by these constraints. The added factor was based on human consumption of media and the rationale behind it, which in the context of hi-tech at a conceptual phase of development is linked to controversy which polarises opinion and fosters debate e.g. the use of human Embryonic Stem Cells. The field of work of Angioscaff was not controversial.

In addition to the project website, a total of four press releases were made: one referring to the kick off of the project and three highlighting the advances made during in years 2, 3 and 4 of the project, while to the scientific community 86 oral and poster presentations were given in invited seminars, international meetings and congresses.