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PROJECT FINAL REPORT

Grant Agreement number: 229294

Project acronym: NANOCARD

Project title: Nanopatterned scaffolds for active myo**card**ial implants

Funding Scheme:

Period covered: from month 1 to month 48

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4.1 Final publishable summary report

Executive Summary

NanoCARD
Nanopatterned scaffolds for active myocardial implants

The overall goal of this project was to create nanopatterned scaffolds for myocardium implants.
Cell therapy and tissue engineering are opening novel therapeutic perspectives for myocardial repair. The introduction of new myocytes into diseased areas of the heart can improve the hearts mechanical properties. The therapeutic utilities significantly hampered by the paucity of cell sources for human cardiomyocytes and by the high degree of donor cell death following cell grafting.
Therefore the fundamental different concept of NanoCARD aims to provide a biomaterial which is based on high throughput screens for identifying specific and necessary signals of extracellular matrix. These signals are activating specific cell programs such as a cell adhesion and the induction of differentiation of stem or progenitor cells to functional myocytes. The implant materials present smart nanopatterned interfaces to cells. These surfaces present chosen biomolecules in nanoscopically defined spatial patterns on biologically inert, biodegradable substrates.
A key point has been the identification of parameters required to drive cardiac tissue formation.
The scientific bases of NanoCARD is the fact, that the activity and fate of all living cells is exquisitly regulated by the cells and environments and particularly by the adhesive interaction with neighbouring cells and with the extracellular matrix. Therefore the design and use of nanostructure and specifically biofunctionalized surfaces with desirable molecular properties offers unique opportunities for programming cell responses and functions.
This biomimetic implant technology concept is based on the interaction between living cells and synthetic, nanostructured interfaces.
The economic potential is remarkable because myocardial infarction is an important disease worldwide.

Summary description of the project context and objectives

Since many previous attempts to engineer tissue scaffolds were based on trial and error approaches, NanoCARD aims to create a *conceptually new biomimetic, nanoscopically designed implant.* The implant's highly tailored material properties will encourage the selective adhesion of stem and progenitor cells and direct their differentiation, enabling replacement myocardial tissue to be generated *ex vivo*.
The development of conceptually new implants is based on five pillars: (1) High-Throughput Screens for identifying specific extracellular matrix material parameters as signals for directing stem or progenitor cells towards specific cardiac tissue generation *in vitro*; (2) Implant synthesis by integration of the set of material parameters identified by HTS into a cell support: so-called "*therapeutic surface*". The implant will be based on a microporous mulitlayered stack of "*therapeutic surface*"; (3) *in vitro* recruitment of cells and cardiac tissue formation; (4) *in vivo* (animal) testing of implants; (5) development of commercialization strategies for the new implant concept for cardiac tissue generation together with the companies *Qiagen*, *Idea Bio Medical*, *CellMade*, *Evercyte and Cell System.*
The *in vitro* HTS will reduce the number of animal experiments, which is in close agreement with the ethical aims of EU guidelines.
The biochip/HTS system will find wide application in the life sciences.

Objectives

Objective 1: Development of nanostructured, biofunctionalized and biodegradable cell supports which have the capacity to vary cell ligand type, ligand spacing, and substrate stiffness for exploring and manipulating cellular interactions with non-biological surfaces

Objective 2: High-Throughput Screens (HTS) for identifying implant material parameter: Exploration of the interaction mechanisms between cells and biomaterial by automated live cell optical microscopy

Objective 3: Characterization of the physicochemical properties of extracellular matrices in vivo and in vitro; exploring how the interaction mechanisms between selected cell types and matrix impact cell fate.

Objective 4: Translation of the set of identified cell response-specific material parameters to implants for *in vivo* applications

Objective 5: Transition from basic scientific understanding to the industrial market

These objectives are achieved via workpackages covering the following areas:

- Synthesis of Artificial Cell Environments on the Molecular Level by Self-Organization Principles
- Development of the BioChip
- Development of the automate optical microscope for application of High-Throughput Screens
- High-throughput screening of endothelial cells (EC) and cardiomyocytes (CM)responses to nanoengineered cell environments, Systems Biology Analysis
- How cell-made extracellular matrix co-regulates protein expression profiles and cell fate
- Synthesis of the implant

- Directed stem cell differentiation into endothelial cells (EC) and cardiomyocytes (CM) by the implant
- *In vivo* assessment of therapeutic potential of the implant
- Integrative and Translational Approaches to Industrial Market
- Co-ordination and project management
- Dissemination, IPR & Knowledge Exchange - DIKE

Description of main S & T results/foregrounds

1.Synthesis of Artificial Cell Environments on the Molecular Level by Self-Organization Principles

Summary

Biodegradable polymer scaffolds have been constructed, and their mechanical properties and biochemical potential have been investigated. Furthermore, the surface of the scaffold has been modified with a polymer brush that will enable their future patterning with cell adhesive ligands. In order to examine the interaction between the synthesized scaffold and the heart a rat in-vivo model was used. Poly(lactide-co-caprolactone) (PLCL) porous and non-porous scaffolds were implanted in healthy rat hearts.

In addition, PLCL-porous scaffolds were implanted in rats after the induction of myocardial infarction.

Evaluating the biocompatibility of the polymer substrates we monitored the behavior of human bone marrow stem cells (hBMCs) in terms of cell adhesion, spreading, actin organization and focal adhesion formation using a confocal microscope. Our analysis of focal adhesion formation and actin organization on tested substrates reveals that the number of pores strongly affects cellular adhesion.

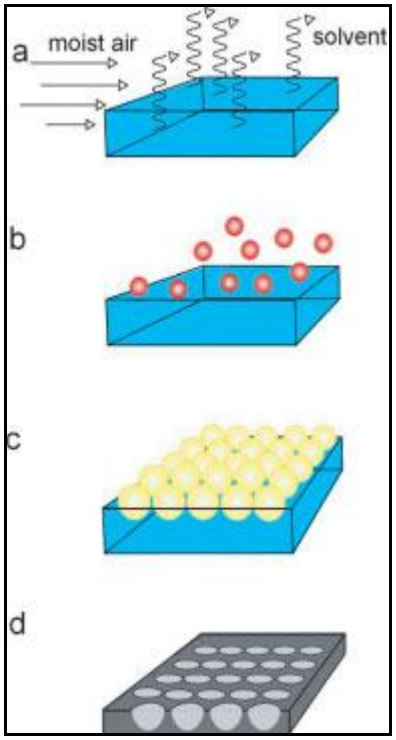
We demonstrate distinct nanotopography-induced cell phenotypes, characterized by different morphology, LOX-1 diffusivity and oligomerization state.

A model combines microfluidic flow, mass transport of the chemotractant and binding of the molecules on a model cell surface in a single model.

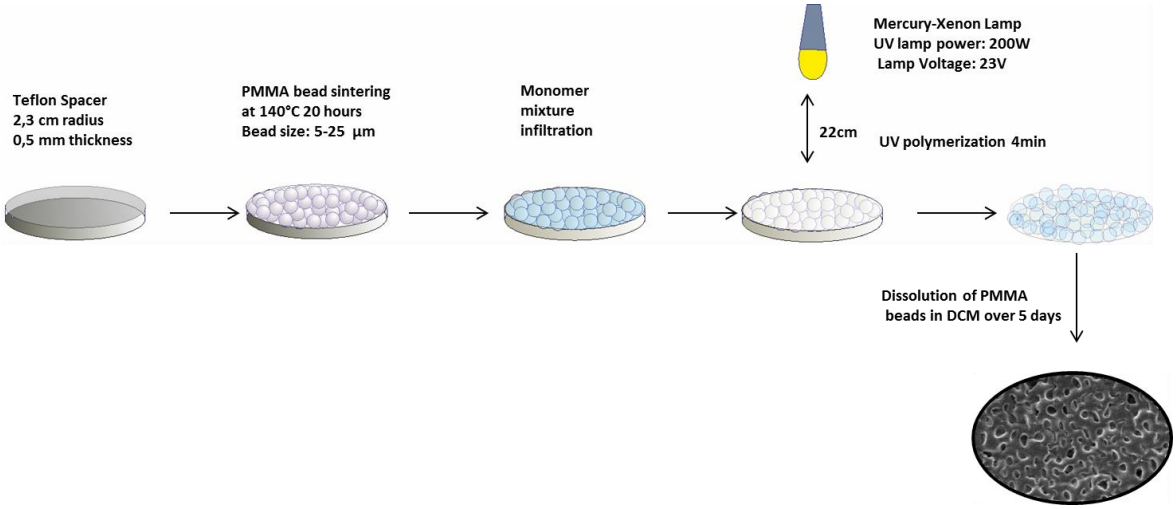
Description

Micro-porous films

The mechanism of the breath figure technique to create well-ordered porous films from polymers in an organic solvent has been well reported in literature.



The breath figure technique allows great control over the formation of the porous material, due to the fact that there is a number of conditions which can be varied that will have an effect of the process, such as, air flow speed, humidity content (%), organic solvent choice, polymer molecular weight and polymer solution concentration. The first scaffolds were obtained by stacking of the microporous PLCL films that were prepared with the breath figure technique. Due to the some problems with the stacking of the films, 3D scaffold preparation method was changed to the sphere-templated fabrication method (Scheme 1). Sphere-templated fabrication method allows controllable pore size and high interconnectivity between pores.



Scheme 1: Sphere-templated fabrication method for scaffold synthesis.

Biofunctionalization and nanostructuration of the biodegradable, 3D, interconnected-microporous and non-fouling polymer scaffolds were achieved. They are prepared by random copolymerization of 2-methylene-1,3-dioxepane (MDO) and 2-(hydroxyethyl) methacrylate (HEMA) monomers and a PCL-PEG-PCL crosslinker.

Mechanical Studies

Young's modulus measurements of scaffolds were carried out by using Minimat machine with 20N cell load. Figure 1 shows the average stress-strain graph and the average Young's Modulus

of the scaffolds that were obtained with the following monomer mol ratios: HEMA/MDO/PCL-PEG-PCL : 27/5/1. This composition gives the Young's Modulus of the scaffold as 0.33±0.07 MPa, which is close to the value of the Young's Modulus for a heart rat tissue 0.59±0.22 MPa.

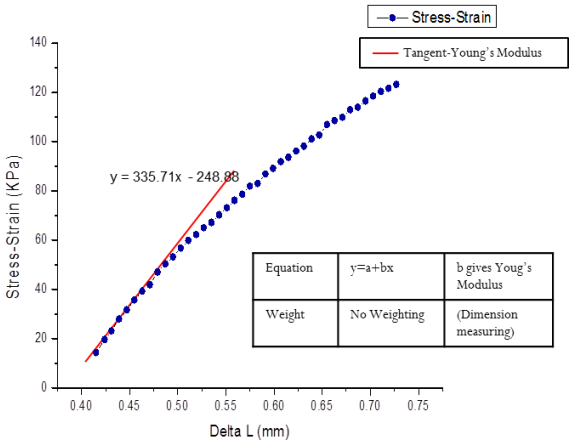
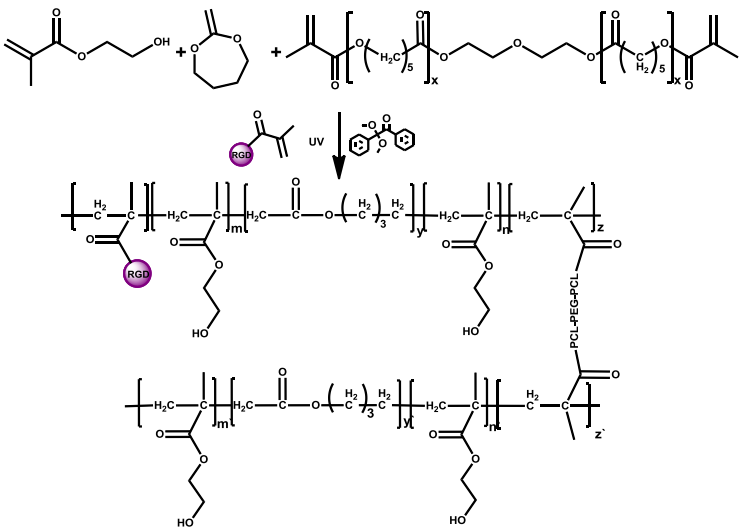


Figure 1: Avarage stress-strain graph for biodegradable scaffolds.

Biofunctionalization

Biofunctionalization of scaffolds with cell adhesive peptides (RGD) was performed by addition of RGD-labeled methacrylate monomer to the monomer mixture before polymerization (Scheme 2). The methacrylated RGD peptide is the only monomer with nitrogen atom. High resolution N1s X-Ray Photoelectron Spectroscopy (XPS) shows an increase of the nitrogen peak while increasing the feeding ratio of the RGD peptide in the monomer composition. It means that the amount of peptide integrated in the scaffold could be tuned with the amount of peptide containing monomer in the feeding ratio.

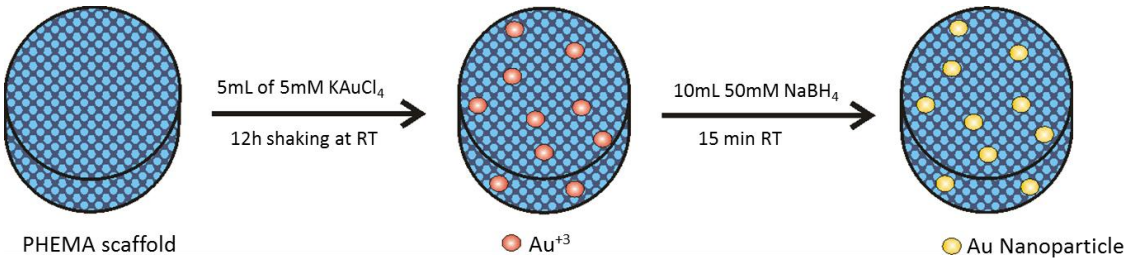


Scheme 2: Scheme for the synthesis of RGD functionalized scaffolds.

Cell Studies

In order to evaluate the cells bio-fouling property of scaffolds cell studies were carried out with different concentration of RGD peptide inserted scaffolds. RGD functionalized scaffolds were incubated with GFP expressing HeLa cells and attachment of cells was examined with confocal microscopy. Cell cytoplasm forms were observed.

The nanostructuration of scaffolds was obtained by reduction of Au^{+3} in sodium borohydride ($NaBH_4$) (Scheme 3). First, scaffolds were immersed into aqueous potassium tetrachloroaurate ($KAuCl_4$) solution and Au^{+3} ions were absorbed in the scaffolds after shaking for 12 hours. After that $NaBH_4$ was added for the reduction of Au^{+3} ions to Au nanoparticles (Scheme 3).



Scheme 3: Schematic representation of Gold nanoparticle decoration of PHEMA scaffolds.

2.Development of the BioChip

Summary

We demonstrated the possibility to implement a microfluidic device on topographically-modified substrates yielding on-demand local 3D gradients of soluble molecules with controllable spatial and temporal profiles.

Protocols for the fabrication of biochemical chips on glass supports and on polymer support have been established. Chips are adapted and used for cell experiments within the project.

Cell tests with such substrates proved the suitability of the chip for cell culture studies.

The design of a general platform allowing for mechanical stimulation of cells by lateral stretching of an elastomer membrane is achieved.

We have developed two different microfluidic platforms that are capable of forming soluble gradients.

We showed that our systems are compatible for cell culture and cells could be maintained up to at least one week.

Description

The goal of high throughput screening (HTPS) remains the fabrication of miniaturized laboratory reactors, called micro-arrays that can work in parallel and be compatible with high sensitivity detection systems to monitor their outputs. Complex analyses can be performed within a few hours with the help of microarrays or “biochips”.

The manufacture of microarrrays and biochips benefit from the use of a great number of nanofabrication tools. Surface molecular modification techniques include: self-assembled mono-layers, surface spin coating with polymers or colloids) to control properties of the array interface at the molecular level (adhesion, hydrophobicity,friction) and as shown in this research, the Diblock Copolymer Micelle Nanolithography (BCML) technique.

In addition, HTPS approaches must fullfill certain criteria in order to be useful in a research diagnostic laboratory. They must be able to perform a large number of assays rapidly and simultaneously in a user friendly manner and be small in format. They must be configured to provide robust and reproducible results that allow standardization and comparison of experiments performed in different laboratories.

Figure 1 illustrates the preliminary experiments.

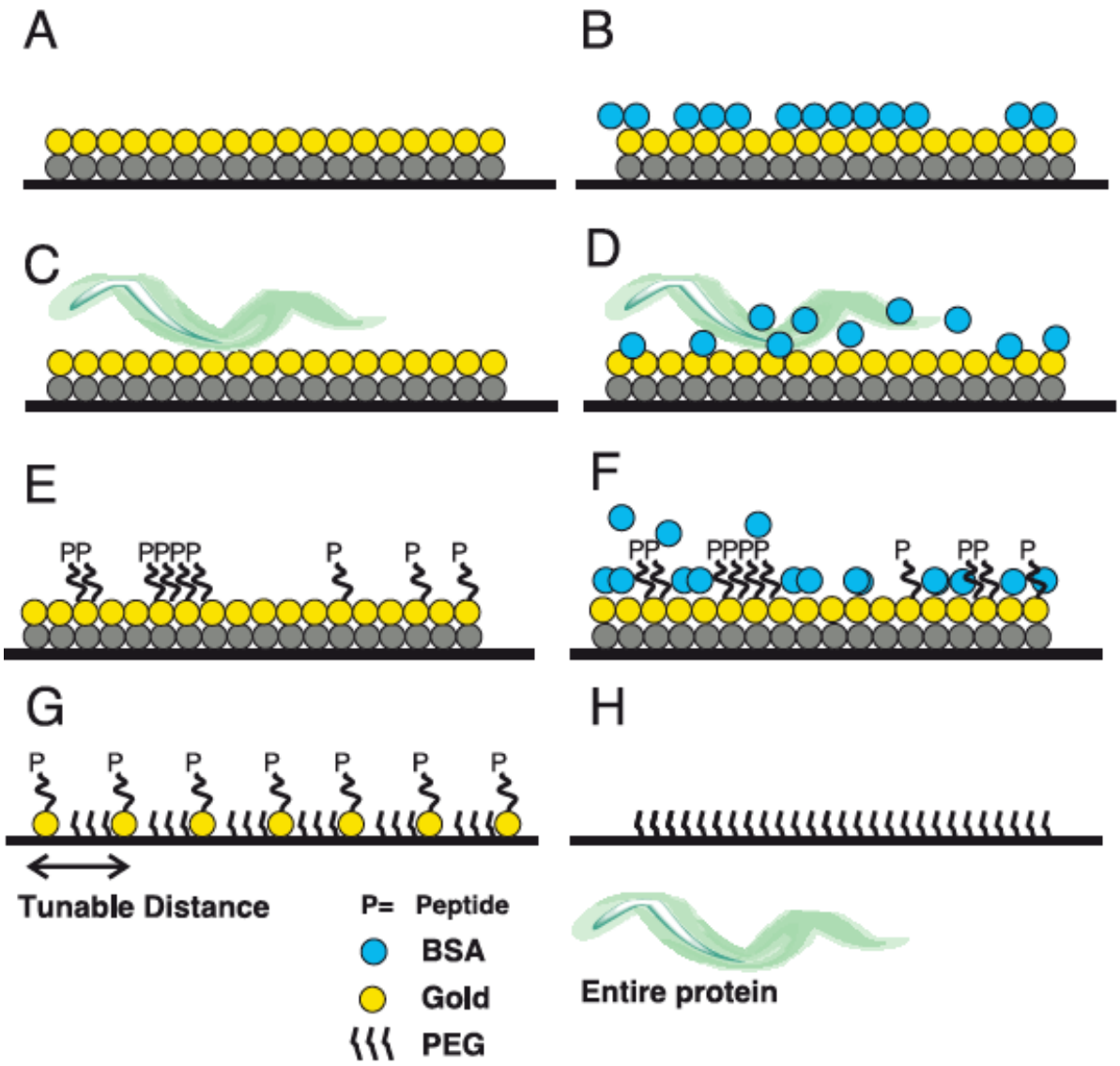


Figure 1: Illustration showing preliminary cell adhesion experiments. (A) Gold (B) Gold + BSA (C) Gold and Fibronectin (D) Gold and Fibronectin + BSA (E) Gold and RGD peptide (F) Gold and RGD peptide + BSA (G) Gold-nanodots with RGD passivated with PEG 2000 (H) Glass surface passivated with PEG 2000

Key technology for the production of biochips is the micellar nanolithography (BCML). This technique uses self-organization principles to arrange micelles on the surface of flat glass substrates and it allows for patterning the surfaces with hexagonal arrangements of gold nanoparticle. The particles have a size of typically 8nm which is comparable to the size of cellular membrane receptors. Within the project the production of these nanoscopically-structured surfaces has been up scaled to meet the requirements of producing large numbers of surfaces for all project-relevant applications.

The method to precisely functionalize the biochip surface with Au-NP has been further extended to be applicable to more complex 3D-like environments. This is an important step to use polymers developed within WP1 and to account for more in-vivo like cell culture conditions.

The design of a general platform allowing for mechanical stimulation of cells by lateral stretching of an elastomer membrane is achieved. Several prototypes following different approaches and designed for specific purposes have been developed and manufactured. Examples are given in Fig. 7. One setup is particularly suited for live cell imaging application and allows for the observation of cells during the experiment by phase, DIC or fluorescent microscopy. Other setups use a six-well format and can apply uniaxial tensile strains to 48 wells with 8 different amplitudes or frequencies. All experimental platforms are suitable to manipulate cells over long time periods (weeks). Test runs with cells under regular culture conditions (T= 37°C, 5% CO2 and high

humidity) were successful and the setups are used for screening cell reaction upon application of various mechanical stimulations (WP4).
A prototype biochip composed by a fluidic network and a textured COC substrate was fabricated and characterized. Complete biochips were realized by bonding a PDMS microfluidic network onto the grating. The microfluidic chip was aligned and mounted in close proximity to the patterned area present on the COC substrate. A fluorescein solution was then delivered through the fluidic channel to generate chemical gradients coupled to the underlying topography.

Production of surfaces for biochemical chips

Key technology for the production of the biochemical chips is the micellar nanolithography (BCML) which is illustrated in Fig. . This technique uses self-organization principles to arrange micelles on the surface of flat glass substrates and it allows for patterning the surfaces with hexagonal arrangements of gold nanoparticle. The particles have a size of typically 8 nm which is comparable to the size of cellular membrane receptors. Within the project the production of these nanoscopically-structured surfaces has been up scaled to meet the requirements of producing large numbers of surfaces for all project-relevant applications.

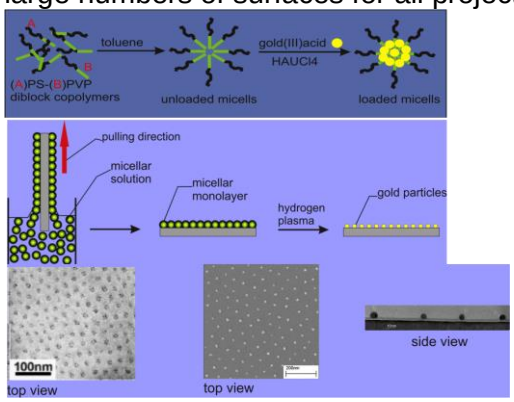


Figure: Schematic representation of the Micellar Nanolithography used for nanopatterning oft he biochip surfaces.

In order to immobilize several different peptides or other biomolecules on a chip a novel protocol was developed. Briefly, surfaces are patterned with small gold nanoparticles (Au-NP) in a hexagonal order by BMCL (BCML) as described above. The inter-particle distance can be adjusted between 30 and 200 nm. To prevent unspecific protein absorption, the surface is covered with a PEG-passivation layer. Several lengths of PEG molecules were tested. For the final chip we use typically PEG 2000 which gets immobilized on the glass (between the Au nanoparticles) via silane chemistry. This procedure provides a stable protein passivation layer on the glass but still allows the chemical modification of the Au nanoparticles with other molecules

Initial cell adhesion studies on the biochemical chip

The chips were used for cell adhesion experiments to proof their suitability for larger scale cell culture studies. In first test, rat embryonic fibroblasts were cultured on the biochips for several days. The biofunctionalization as well as the passivation of the surface between the micro-spots was demonstrated to be stable over that time period. An illustrative example of cells adhering on RGD-functionalized micro-spots is given below. The chips' dimensions, optical and mechanically handling properties are ideally suited for high content screening microscopy within NanoCARD.

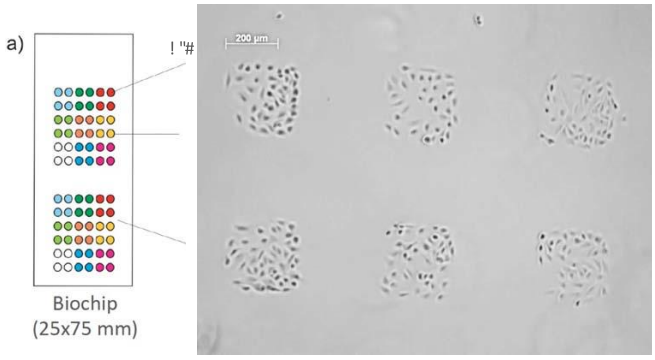


Figure 1: Rat embryonic fibroblasts on biochip surface with micro-spotted peptides (RGD). Cells adhere only on the RGD-modified spots. Within each spot the peptide is specifically bound to the Au-NP (not visible with this magnification).

Biomechanical Chip Design

Biochip Fabrication – general properties

The substrate system used in the *bio chip* was designed in a way that allowed for varying the biophysical and biochemical properties on the chip surface simultaneously.

Distances between the ECM mimicking peptides (biomolecules) range between 30nm and 150 nm. The size of anchor points itself is chosen not exceed a limit of about 10 nm to assure single receptor-ligand interaction. The mechanical properties of substrates were chosen to be with 3kPa and 90 kPa with additional very stiff support of 4 MPa, serving as a control substrates for the in vitro tests.

Cell tests with biomechanical chip

Initial functional test of the chips were performed with various cell lines such as normal human dermal fibroblasts (NHDF) and CCL-121, poorly differentiated fibrosarcoma cells from the acetabulum. Both cell types were trypsinized and seeded on the biofunctionalized substrates surfaces in appropriate media containing 10% fetal bovine serum (FBS). The experiments were performed after desired cell-substrate interaction time under cell-culture conditions (5 % CO_2, 37^o C). To optimize the measurement procedure and characterization of the cellular behavior on the biomechanical chip during different time periods, a high throughput screening (WiScan^{TM} instrument, Idea-Bio, Israel), equipped with live cell environmental control and analysis (WiSoft^{TM} software, Idea-Bio, Israel).

3.Development of the automate optical microscope for application of High-Throughput Screens

Summary

The WiScan family of systems to include fast acquisition and storage, accurate stage positioning, live cell conditions, large set of command functions for versatile applications, flexible sample formats, automatic sample loader and user friendly interface has been developed. The microscope is fully automatic and acquiers high-resolution images at a precise focus plane using fast auto focusing procedure.

Tools for image processing and data analysis were added to WiSoft the image analysis software. Many biological applications were studied using the WiSoft including – GFP spot detection, cell cycle evaluation, translocation of fluorescnce protein, variation in mitochondria and ER, studies of fluorescently labeled Celegans, focal adhesion morphologicl changes, tube formation and more. We have improved usability and stability of the software and the system has been tested extensively.

We have also been working on some simple image analysis tools and ImageJ scripts to automate processes like cell counting and analysis.

Description

Adaptation for robotic sample loading:

The WiScan® Argus system was designed to enable robotic loading and unloading of biological samples. Thus, cells can be cultured at an automatic liquid handling system and then transfered to the WiScan® Argus screening microscope automaticallly using a robotic arm.

Biochip readout:

The researchers prepared a spotted array of adhesion sites on a standard glass slide and seeded cells on the array. After fixation the cells were labeled for nuclei and for actin fibers. The biochip unique structure was defined at the WiScan® system as a spotted array, and the array was scanned using 20x/0.75 objective and two wavelengths for DAPI and FITC readout. The images were analyzed to count the number of cells in each spot and to measure the average actin fiber length (Figure 3).

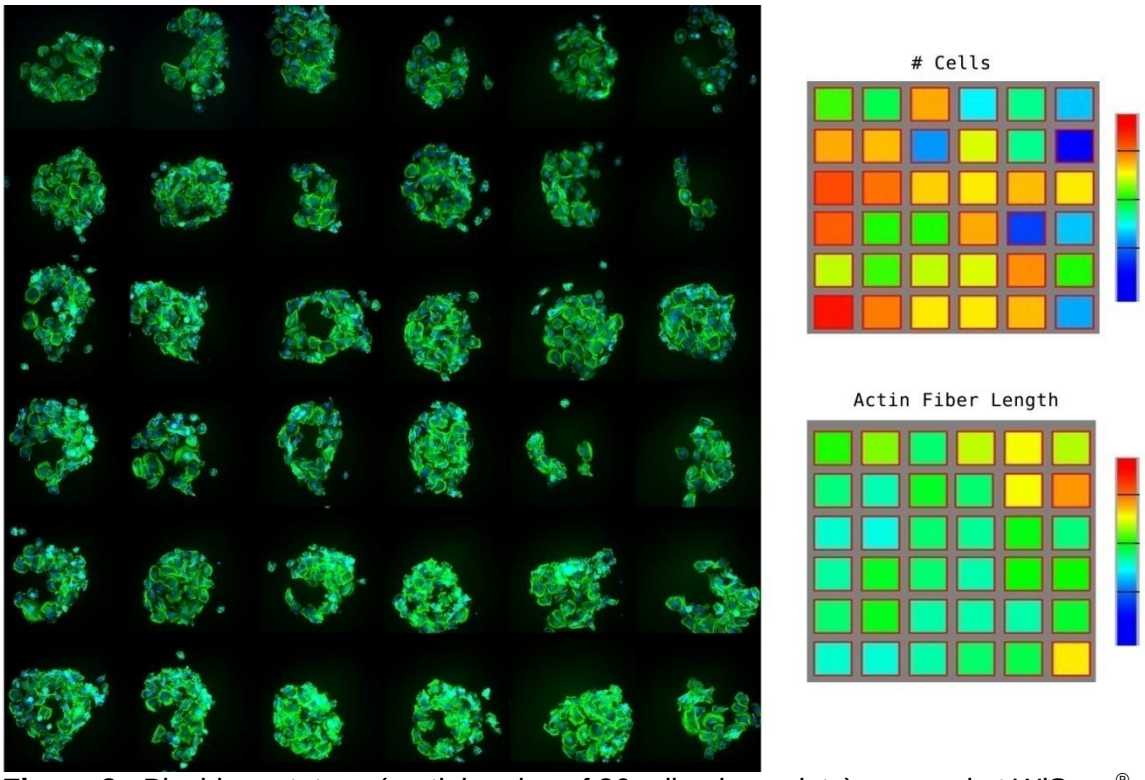


Figure 3 - Biochip prototype (partial region of 36 adhesion points) scanned at WiScan® using 20x magnification. Cells are labeled with DAPI for nuclei and with phalloidin-FITC for actin. The number of cells per adhesion point and the actin fiber length was calculated using WiSoft® and these two parameters are presented for each well in color scale.

4.High-throughput screening of endothelial cells (EC) and cardiomyocytes (CM)responses to nanoengineered cell environments, Systems Biology Analysis

Summary

We tested the adhesion of rat mesenchymal stem cells to LACL and LAGA membranes. In addition, we tested the adhesion of inflammatory cells (rat splenocytes) to the synthesized surfaces.

Studies were performed on cell responses of human mesechymal stem cells (hMSC). The cells were cultured on nanostructured RGD-functionalized Biochip surfaces with different developed compliances. hMSC stem cell morphology and structure of adhesion sites and cytoskeleton depends on both parameters: ligand spacing and substrate stiffness.

All the produced surfaces were used for the induction of EC and/ or CM development, proliferation.

We have established an experimental system to test the effect of substrate rigidity on the proliferation, differentiation, and the commitment of rat cardiomyocytes.

We found that modulating surface rigidity effects rat cardiomyocyte proliferation.

Protocols for determination of cell specific markers of endothelial cells were developed using primary human endothelial cells (HUVEC and HAoEC). In addition, in order to establish

reproducible and stadradizable cell models, HUVECs were immortalized using human telomerase and characterized in detail.

Description

We have selected to work, at this stage with cells of 2 origins, namely chick embryos and newborn rats. Detailed procedures for the cultivation of these two cell types were developed, optimizing the condition for reaching full differentiation into functional heart cells, namely – well organized sarcomeric organization, and beating. To study both features, we have specifically fluorescently labeled the cells, at different time points after plating, for a variety of sarcomeric proteins (e.g. actin, □-actinin, myosin (cardiac)), and examined the cells for beating, using live cell microscopy.

The cells, from the same origins, were extensively examined, following labeling for multiple cytoskeletal components, enabling the visualization and quantification of multiple cellular parameters including sarcomere length, nuclear organization, sarcomere registration, cell shape and polarization, matrix adhesion structure, composition, orientation and connection to the contractile apparatus. In addition, the cells were examined by transmission electron microscopy for the organization of their contractile machinery.

Towards the development of synthetic tissue scaffolds for the regeneration of the heart muscle after myocardial infarction, we have set the experimental system, including the choice of cells for the study (primary cardiac myocytes (PCM) from chicken or rat origin, prepared as above) and characterization of the cellular response to variations in the properties of synthetic substrates.

Our preliminary finding showed selective adhesion of cardiac **fibroblasts** on the high-density signal hydrogel surface and higher population of **cardiocycytes** on the lower density signaling surfaces. These finding are based on the labeling of the cells to several sarcomeric and adhesion-associated cytoskeletal proteins, including actin and alpha actinin enabling the assessment of Z-band formation across the cardiomyocytes. In order to evaluate whether these results are differentiating outcome, or of selective adhesion, we are repeating these experiment with both the soft hydrogel surfaces and on the rigid glass surface.

Highlights of the PDMS elastomers results -

Soft PDMS substrates facilitate cardiomyocytes proliferation. We found that modulating surface rigidity effects rat cardiomyocyte proliferation. Specifically, softer PDMS substrates of 20kPa and 5kPa elevate neonatal rat cardiomyocyte proliferation by over 50%. We would perform additional experiments to further characterize the proliferating cardiomyocytes and to assess the mechanism that leads to this phenomenon.

Substrate rigidity effects sarcomeric organization of cardiomyocytes. We compared 1 day old rat cardiomyocytes cultured on either relatively rigid (2MPa), or relatively soft (5kPa) surfaces. Substrate rigidity effects the sarcomeric architecture of newborn rat cardiomyocytes, as evident by the sarcomeric markers MHC, myomesin, and Troponin T. Cardiomyocytes on the 2MPa substrates are more polarized, exhibit well organized sarcomeric structures and directionality. On the soft 5kPa substrate cardiomyocytes are radial and exhibit disorganized sarcomeric structures.

5.How cell-made extracellular matrix co-regulates protein expression profiles and cell fate

Summary

Different ECM and VEGF concentrations were investigated on their effect on tube formation of HAoEC in co-culture with HAoSMC.

Characterization of the biophysical properties of extracellular matrix that cells assemble and remodel on nanopatterned surfaces with varying ligand density and substrate rigidity.

Studies of cell assembled ECM on the biochips.

Preliminary studies whether hMSCs sense the topographical differences in the surfaces.

Fabrication of cell-derived ECM scaffolds on NanoCARD chips.

We found that the optimal conditions for vessel-network formation is low fibrin concentration (7.5 mg/ml-final concentration) and high cell density (0.3X10^6 – huvec + 0.06X10^6- fibroblasts).

Our results show that mechanical forces induce differentiation of the cells into the mesoderm direction.

Different ECM and VEGF concentrations were investigated on their effect on tube formation. Adding VEGF to the used ECM had only limited effects on tube formation in the three models.
Stem cell engineering and conditioning: Since none of the available stem cell sources can guarantee that none of the injected stem cells might differentiate into cancer cells, we started to ask how stem cells could perhaps be conditioned to minimize tumorogenesis (C. Moshfegh & V.Vogel, **patent filed** on Oct 3, 2013).

Description
In comparison to flat surfaces, HFFs on NanoCard surfaces formed much less ECM. Also, the ECM exhibits a broader range of FRET ratios within a given matrix and even within individual fibrils, indicating a broad range of matrix strains. Although a variety of FN conformations co-exist in cell culture, our preliminary experiments show that the mean matrix tension alters with the underlying nano-topographyat a ridge width of 2 and 1.75 µm, significantly less matrix was assembled when compared to the smaller ridge width, 1.5µm and 1.25µm respectively. The trends of the mean FRET ratios are different as shown in the Figure below. The FRET ratios were highest at intermediate ridge width.

6.Synthesis of the implant
Summary
We have implanted the preliminary cardiac scaffold (LACL porous scaffold) in healthy rat hearts. Cardiac performance was measured using high frequency echocardiography in order to determine whether the implant interferes with normal cardiac function (n=4). Also, implanted hearts were evaluated by histology to detect signs of tissue inflammation or any other structural abnormality.
Description
Seeding EC and CM on implant material in vitro and investigating cardiac tissue formation.
MPI performed experiments with endothelial cells (EC) and smooth muscle cells (SMC) on biochips with different ligand spacing and peptide functionalization. Experiments demonstrate that cell adhesion of both cell types depends on the nano-scaled ligand spacing (40 vs. 90 nm) but not on the selection of one of the two different adhesive peptides (RGD and REDV).
Co culture of GFP or RFP-huvec cells and fibroblasts were grown on PLLA\PLGA in vitro for 10 days, during this time the scaffolds were monitored using confocal for the formation of vessel-like networks.
Spontaneous contraction and vessel like structure formation were observed between days 4-6.

7.Directed stem cell differentiation into endothelial cells (EC) and cardiomyocytes (CM) by the implant
Summary
We have explored endothelial-fibroblast interactions in 2D and 3D constructs and the influence of scaffold mechanical properties on formation of vessel-like structures. Moreover, we have examined the influence of scaffold patterning and topography on cell attachment, proliferation and organization.
Seeding stem cell derived cells (cardiomyocytes and endothelial progenitors and common progenitors) on biofunctionalized surfaces designed to induce specific attachment and differentiation and analysis. Histological characterization of the engineered tissue and biocompatibility tests. In addition, biosafe generation of iPSCs was tested by using recombinant Yamanaka proteins as media additives. Surprisingly, Oct4 was found to contain a functional peptide transduction domain allowing direct cellular uptake.
We used mechanical forces in purpose to differentiate embryonic stem cells (ESC) into the mesoderm germ line, as a precursor cells for both endothelial cells and cardiomyocytes.
We seeded endothelial cells and mouse ESC in defined patterns (using PDMS stamps) in purpose to mimic cardiac tissue.

Description
Effect of different RGD spacing on endothelial cells proliferation and survival

We have used three different RGD spacing (i.e. 32, 58 and 102 nm spacing between two neighboring RGD molecules) to investigate the influence of RGD spacing on EC attachment and organization.
The spacing between the RGD molecules plays a key role in cell attachment and organization.

Effect of different RGD spacing on fibroblasts proliferation and survival

When fetal fibroblasts were seeded on PEG gels, we observed some morphological changes between the different surfaces. The fibroblasts are more elongated and able to create cell-to-cell interactions with the increase in RGD spacing.
Fibroblasts seeded on higher density and low spacing (i.e. 33 nm) cover glasses proliferated more than fibroblasts seeded on lower density and high spacing (i.e. 74 nm).
We can see that in the of 33 nm spacing, we have approximately 50% of proliferating cells, while in the 74 nm spacing, we have only 10% proliferating cells.

Effect of different RGD spacing on endothelial-fibroblasts organization

When EC and fibroblasts were seeded together on RGD cover glasses no significant difference was observed between low (i.e. 74 nm spacing) and high (i.e. 33 nm spacing) density RGD.

Non Canonical Wnt Pathway:

In order to evaluate the effect of Wnt signaling through the non canonical Wnt pathway we evaluated the effect of Wnt-11 and Wnt 5a conditioned media on hESC cardiac differentiation. Wnt 11 conditioned media applied at equal volumes with culturing media during the initial 8 days of differentiation resulted in a 2-fold increase in the percentage of contracting areas.

We aimed at evaluating whether non canonical Wnt signaling effected cardiac differentiation during early hESC differentiation (day 1-4) suggesting a potential role mesoderm induction or late hESC differentiation (days 5-8) suggesting influence on commitment to the cardiac lineage. While early exposure to Wnt5a conditioned media resulted in significant upregulation of cardiac markers late exposure to Wnt5a conditioned media did not result in upregulation of cardiac markers. Activation of the non-canonical WNT pathway is known to induce cardiac specification through protein kinase C (PKC) and Jun N-terminal Kinase (JNK) activation in the xenopus laveis model. Selective JNK inhibition did not significantly affected the proportion of the contracting areas significantly ($p=0.70$, $n=150$). However, PKC inhibition by bisindolylmalmeimide I reduced significantly the proportion of contracting areas by 67%.

Canonical WNT Signaling

To evaluate the effect of activation of canonical Wnt signaling on differentiating hESC, we applied Wnt3A conditioned media during the first 8 days of differentiation.

In conclusion, we have showed that: 1) Non-canonical Wnt signaling promotes human embryonic stem cell differentiation into the cardiac lineage through both Wnt11 and Wnt5a. 2) The time frame in which non-canonical Wnt signaling exert its cardiogenic effect is between days 1-4 of hESC differentiation. 3) Among the suggested signaling pathways through which non canonical Wnt is known to mediate its effect, activation of PKC but not JNK results in enhancement of cardiogenesis in hESC. 4) Activation of canonical Wnt signaling during the suspension period inhibits hESC differentiation to the cardiac lineage.
In order to examine mESCs differentiation under the different mechanical conditions real time PCR was used for examining representative genes of the three germ layers. We started by examining the genes expression after two days of oscillations
One can notice that under static stretch significant change couldn't be observed in the three germ layers vs. control. However, by using oscillatory stretching significant change in oscillations vs. control was observed both in bry, pax6 and nestin, representative genes of the mesoderm and ectoderm layers.

Establishment of an efficient hiPSCs cardiomyocyte differentiation system. Since the initial demonstration that beating cardiomyocytes can be generated from both hESC and hiPSCs using the spontaneous, but relatively inefficient, serum-dependent embryoid-body (EB) differentiation system, several important improvements were made. In this part of the project we evaluated a number of strategies in an attempt to establish a well-defined, scalable, serum-free, directed

hiPSCs cardiomyocyte differentiation system. These different suggested methods were inspired from lessons learned from embryology and are based on sequential manipulation of the BMP, Activin/nodal, and Wnt pathways. Comparing the different strategies we noted that in our hands the most effective, reproducible, and cost-effective differentiation system was a modification of the method suggested by Lian et al. (PNAS 2012).
This method is based on the use of small molecules to manipulate a single signaling-pathway (the canonical Wnt pathway). This method is based on initial activation of the Wnt pathway by CHIR-99021, a GSK3 inhibitor, to facilitate mesoendoderm formation followed by Wnt inhibition by IWP-2 or IWP-4 to induce cardio-mesoderm formation. Optimization of this strategy in a monolayer approach resulted in a highly-efficient cardiomyocyte differentiating system.

Induction of anisotropic cardiomyocyte alignment by nano-patterened films.

Ordered alignment of cardiomyocyte cells, similar to the anisotropic in-vivo cardiomyocyte alignment, is one of the major challenges in myocardial tissue engineering. Anisotropic alignment may allow achieving tissues with better force forming capabilities and lower risk for arrhythmogenicity. To this aim we collaborated with Marco Cechinni's group (Pisa, Italy) and seeded nano-patterned scaffolds containing grooves with a variety of geometries. Scaffolds were generated from COC and PDMS, the unpatterened borders of the scaffolds were used as controls. Both neonatal rat ventricular cardiomyocytes and hiPSCs derived cardiomyocytes were seeded on the scaffolds. Scaffolds were covered prior to seeding with fibronectin to improve cell attachment and were followed microscopically for two weeks. Additionally, time-lapse imaging was generated to allow real-time follow up of the alignment kinetics. Seeding of both neonatal rat ventricular cardiomyocytes(NRVC) and human induced pluripotent cardiomyocytes (hiPSC-CMs) demonstrated that the cells can attach to the surface. Most of the cells survived following seeding and the ordered alignment process was started within hours following seeding. Following 24 hours, most cell in all groups (COC/NRVCMs, COC/hIPSCs, PDMS/NRVCMs, PDMS/hiPSCs) were generally aligned to the direction of the nano-patterned grooves.

8.In vivo assessment of therapeutic potential of the implant

Summary
We have selected a preliminary list of 7 candidate ligands known to affect the migration of adult stem cells, and tested the migration of rat mesenchymal stem cells towards these ligands.
We tested the ability of the same candidate ligands to induce migration of inflammatory cells.
We tested the safety, as well as the therapeutic potential of PLC scaffolds implanted to rat heart after the induction of myocardial infarction. PLC scaffolds were implanted either in single layer or in 4 stacked layers.
There was no indication of cardiac or systemic toxicity of the implanted scaffolds.
However, no positive effect was seen on cardiac function.

Description
We have previously implanted tissue constructs seeded with human embryonic stem cells derived cardiomyocytes (hESC-CM), endothelial cells (HUVECs) and mouse embryonic fibroblasts (mEFs) onto rat myocardium. The cells were seeded in PLLA/PLGA scaffolds and grown for two weeks *in-vitro* pre-implantation and then for an additional 3 weeks *in-vivo*. We have shown that the engineered constructs beat synchronously and form endothelial vessel networks (1,2). We have demonstrated vascularization of the grafts and organization of functional blood vessels within the scaffold. We also have showed that hESC-CM seeded with HUVECs and mEFs are able to contract synchronously and express cardiac markers (1,2).
Following the development of spontaneously synchronized beating areas, the engineered muscle constructs were engrafted to the left ventricular surface of immunosupressed rats. Two weeks after transplantation, histological analysis revealed the presence of vascularized cardiac tissue.
Following these experiments we then proceeded to infarct rat model. We have seeded PLLA/PLGA scaffolds with HUVECs, human foreskin fibroblasts (hff) and hESC-CM. One week later we induced infarct in rats. The infarct was confirmed by Ultra-Sound Electrocardiography (U.S-EKG). One week after infarct induction, we tried to implant our scaffolds onto the infracted area; unfortunately the scaffolds didn't attach to the infracted area. Ongoing studies are trying to establish a suitable protocol for implanting the scaffold onto the infracted area and to test biofunctionalized surfaces.

We have implanted tissue constructs containing skeletal myoblasts, EC and human neonatal dermal fibroblasts (HNDF) around the femoral artery of mice to enhance angiogenesis in the engineered muscle tissue. The cells were seeded in PLLA/PLGA scaffolds and grown for 10 days *in-vitro* pre-implantation and then an additional 4-12 days *in-vivo*. We have shown that the engineered constructs have blood perfusion and promoted angiogenesis.

To explore the ability to establish a 3D supportive environment for generation of a vascularized engineered tissue which can induce angiogenesis in the implantation arae we used PLLA (50%)/PLGA (50%) biodegradable scaffolds. We evaluated 3 cell culture combinations: (1) scaffolds seeded with myoblasts alone; (2) a co- culture of neonat fibroblasts and EC and (3) a tri-culture of myoblasts, fibroblasts and EC.
We tested the safety, as well as the therapeutic potential of PLC scaffolds implanted to rat heart after the induction of myocardial infarction. PLC scaffolds were implanted either in single layer or in 4 stacked layers.
There was no indication of cardiac or systemic toxicity of the implanted scaffolds. In addition, implantation of 4 stacked scaffolds resulted in attenuation of the end-diastolic diameters which can indicate improved cardiac remodeling. Also, implantation of 4 stacked scaffolds resulted in reduction of left ventricular fibrosis, as indicated by reduced infarct size.
However, no positive effect was seen on cardiac function.

9.Integrative and Translational Approaches to Industrial Market
A monitor has been sent to all NANOCard groups (see below) in which they could indicate achievements that they thought are possibly interesting for further development by industry. These techniques will be evaluated together with the industrial partners within the project for suitability for commercial use in the future. None of the partners indicated techniques or other achievements possibly transferable to industrial partners or ready for commercialization.

Partner No.: Partner institution name and address	**Scientist responsible:**	**Contact:** Tel.: Fax: E-Mail:

	1	2	3	4	5	6
No.	**Title of Invention/development**	**Short description of the invention/development**	**Development status**	**IP-right status**	**Commercialisation status/activities & next steps**	**Support requested/additional comments**
1						
2						
3						

1: Short but meaningful title describing the technical nature of the invention/development
2: Briefly describe the (technical) nature/basis of the invention or development and indicate the commercialisation perspective, e.g. potential products, application field, etc...
3: Which status does the invention/development have: early development, technical proof of principle, product development phase, ready for release to market, ...
4: Which manuscripts (in prep, in press), publications, patent applications or patents do exist, please give correct citation and or patent (application) number. Who owns the patent?
5: Which measures for commercialisation have been taken already: contact to commercial enterprise, contact to investors, company founded,... and what are the next steps to accelerate commercialisation.
6: Which support from NanoII partners (especially SME, which one?) would accelerate commercialisation of your development, e.g. help with IP rights, contact to commercial enterprises, general input regarding product development & commercialisation, input regarding business development, ...

If you have additional comments regarding commercialisation of your development/invention? Please add them also here.

11.Dissemination, IPR & Knowledge Exchange - DIKE

I. Dissemination

1. **After the periodic project commitment discussions at the consortium meetings to explore commercialization opportunities, the dissemination in high impact scientific journals has been launched.**
2. **NanoCARD has been producing a lot of high impact scientific publications.**

Publications

Franco et al. 'Accelerated endothelial wound healing on microstructured substrates under flow' Biomaterials. 2013

Vittorio et al. 'Endothelial differentiation of mesenchymal stromal cells: when traditional biology meets mechanotransduction' Integrative Biology. 2012

Wieringa et al. 'Nanotopography induced contact guidance of the F11 cell line during neuronal differentiation: a neuronal model cell line for tissue scaffold development'. Nanotechnology. 2012

Meucci et al. Biocompatible noisy nanotopographies with specific directionality for controlled anisotropic cell cultures. Soft Matter, (2012).

Sahai et al. 'Microfluidic chip for spatially and temporally controlled biochemical gradient generation in standard cell-culture Petri dishes. Microfluidics and Nanofluidics. 2011

Licentiate Thesis, Patric Wallin "Creating cell microenvironments in vitro", Chalmers University of Technology, Sweden, 2012

Master Thesis, Elin Bernson "Development of a microfluidic platform to study cell migration along gradients", Chalmers University of Technology, Sweden, 2012

Patric Wallin, Carl Zandén, Björn Carlberg, Johan Liu and Julie Gold "Integration of Electro Spun Nanofibers in Microfluidic Gradient Channels"
Biomicrofluidics 6, 024131 (2012)

Emmert et al, Intramyocardial transplantation and tracking of human mesenchymal stem cells in a novel intra-uterine pre-immune fetal sheep myocardial infarction model: a proof of concept study, PloS One, 2013;8(3):e57759.

Emmert et al, Transcatheter based electromechanical mapping guided intramyocardial transplantation and in vivo tracking of human stem cell based three dimensional microtissues in the porcine heart. Biomaterials 34(10): 2428-2441.

C. Moshfegh & V.Vogel, Reprogramming of pluripotent stem cells for improved control of their differentiation pathways, European Patent Application, filed on Oct 3, 2013.

Patric Wallin*, Elin Bernson*, Vera Hirschfeld-Warneken, Francesco Mazzotta, Davide Franco, Aldo Ferrari, Joachim Spatz, Julie Gold, "Endothelial cell migration dependence on cell-adhesion ligand spacing", in manuscript.

"Interaction of SH-SY5Y Cells with Nanogratings During Neuronal Differentiation: Comparison with Primary Neurons", I Tonazzini, A Cecchini, Y Elgersma, M Cecchini, Advanced healthcare materials, 2013, DOI: 10.1002/adhm.201300216

"Easy Monitoring of Velocity Fields in Microfluidic Devices Using Spatiotemporal Image Correlation Spectroscopy", Marco Travagliati, Salvatore Girardo, Dario Pisignano, Fabio Beltram, Marco Cecchini, Anal. Chem., 2013, 85 (17), pp 8080–8084

"Cell Guidance on Nanogratings: A Computational Model of the Interplay between PC12 Growth Cones and Nanostructures", PN Sergi, IM Roccasalvo, I Tonazzini, M Cecchini, S Micera, PlosONE 2013, DOI:10.1371/journal.pone.0070304

"Neuronal differentiation on anisotropic substrates and the influence of nanotopographical noise on neurite contact guidance", I Tonazzini, S Meucci, P Faraci, F Beltram, M Cecchini, Volume 34, Issue 25, August 2013, Pages 6027–6036

"Unveiling LOX-1 receptor interplay with nanotopography: mechanotransduction and atherosclerosis onset", C Di Rienzo, E Jacchetti, F Cardarelli, R Bizzarri, F Beltram, M Cecchini, Scientific Reports 3, Article number: 1141 doi:10.1038/srep01141

"Imaging intracellular viscosity by a new molecular rotor suitable for phasor analysis of fluorescence lifetime" Antonella Battisti, Silvio Panettieri, Gerardo Abbandonato, Emanuela Jacchetti, Francesco Cardarelli, Giovanni Signore, Fabio Beltram, Ranieri Bizzarri, Anal Bioanal Chem (2013) 405:6223–6233

D. Franco, F. Milde, M. Klingauf, F. Orsenigo, E. Dejana, D. Poulikakos. M. Cecchini, P. Koumoutsakos, A. Ferrari*, and V. Kurtcuoglu. Accelerated endothelial wound healing on microstructured substrates under flow. Biomaterials 34, 1488-1497 (2013).

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A lot of publications are in preparation

3. **Several international conference contributions have been made (see below).**

<u>Conference presentations</u>

Dr. Emanuela Jacchetti (SNS) participated to the Workshop "NANOTECHNOLOGIES FOR HEALTH CARE", where she presented her results about the interaction between cells and nanogratings, obtained within the NANOCARD project.

Patric Wallin, Elin Bernson and Julie Gold “Simulation of Chemotractant Gradients in Microfluidic Channels to Study Cell Migration Mechanism in silicon”
Conference Paper, Comsol User meeting Milano (2012)

Scandinavian Society for Biomaterials Annual meeting 2011 “Preparation of peptide-functionalized Au nanodot and lipid bilayer surfaces for studying cell focal adhesion formation”

Lab-on-a-chip world congress 2011 “Patterned electrospun microfibers integrated in a microfluidic system to study cells in complex microenvironments”

Scandinavian Society for Biomaterials Annual meeting 2012 “Patterned electrospun microfibers integrated in a microfluidic system to study cells in complex microenvironments”

TERMIS world congress 2012 “Microlfuidic gradient systems to generate defined cell microenvironments and cellular fate processes” Journal of Tissue Engineering and Regenerative Medicine, 6 s. 350-350, 2012
T3Net meeting in Manchester, UK

Patric Wallin, Elin Bernson, Julie Gold, “Microfluidic gradient systems to generate defined cell microenvironments and study cellular fate processes”, Materials for Tomorrow/Materials for Health Oct 1-2, 2013, Chalmers University of Technology, Sweden, and

BRC Biomaterials Research Centre Annual Meeting, “Biomaterials associated infection, inflammation and immune function”, Nov 18, 2013, Göteborg University, Sweden

Neuroscience 2013, Society for Neuroscience’s annual meeting, November 8-13, San Diego (CA) - USA. Mechanotransduction of hippocampal neurons: role of ubiquitin ligase E3a (Ube3a) in neurite contact guidance. *Tonazzini I, Van Woerden GM, Meucci S, Elgersma Y, Beltram F[1] and Cecchini M*. Poster presentation.

Neuroscience 2013, Society for Neuroscience’s annual meeting, November 8-13, San Diego (CA) – USA. Interaction of neuronal cells with nanotopographies and the impact of nanotopographical noise during neurite path finding. M. CECCHINI, I. TONAZZINI, S. MEUCCI, F. BELTRAM. Poster Presentation

E-MRS 2013 Spring Meeting, European Materials Research Society meeting, May 27-31, Strasbourg - FRANCE.
Anisotropic nanostructured substrates and neuronal cells: neurite contact guidance in patho-physiological models. *Tonazzini I, Meucci S, Van Woerden GM, Elgersma Y, Beltram F and Cecchini M.* Oral presentation

E-MRS 2013 Spring Meeting, European Materials Research Society meeting, May 27-31, Strasbourg - FRANCE.
“Shaping the biological identity of implant materials: A topographical approach” *Ferrari Aldo*, Invited oral Presentation

4. **Several exhibitions the products have been shown (see below).**

The exhibitions were:

1. High content analysis SF, USA
2. Lab automation SF, USA
3. Medica, Germany
4. Israel life imaging forum, Israel

II. Intellectual property rights

Project participants are working closely with suitable consultants to safeguard their intellectual property rights and to ensure the best technology transfer from the project. Therefore the participation of Qiagen and other industrial partners at the meetings have been very helpful, especially to maximalist control over the project outcome. To guarantee the IPR protection the coordinator is discussing with experts before publishing any results the possibility of the protection of the results. After this check of the manuscript by the

coordinator whether a protectable result has been achieved or not, the results were published in high impact scientific journals.
The coordinator has been organizing an industrial workshop to achieve the best possible exploitation of results.

III. Knowledge exchange

a. Public website of the project has been established. All informations related to the scientific goals and achievements of the project have been put into the wiki. http://www.is.mpg.de/NanoCARD

b. An eternal project web page has been created for the exchange of protocols, methods, results and specific organizational questions as well as making the whole details of NanoCARD available for the different participants.

c. Every scientific group has been participating at the NanoCARD meetings. The different groups have been giving talks, so that the very lively knowledge exchange has been guaranteed. Different labs offered training courses to use the different microscopical material and cellular systems.

d. An important instrument for the knowledge exchange has been the PhD- and Postdoc Workshops as well as the principal investigator meetings**.**

NanoCARD Meetings/PhD Workshops

- Kick off meeting 12.-13. Januar 2010, Stuttgart
- NanoCARD Meeting 06.-08. June 2010, Rehovot
- Brussels Meeting 31.01.2011 with the European Commission
- NanoCARD Meeting 05.-07. April 2011, MPI Stuttgart
- Midterm review in Brussels 30.06.-01.07.2011with the European Commission
- Principal Investigator Meeting 13.09.-14.09.2012, Stuttgart
- Final Meeting 17.10.-18.10.2013, MPI Stuttgart

e. Regular meetings with the industrial partners for early identification of potential protection development opportunities for the European industry have been taken place.

Potential impact and main dissemination activities and exploitation results

I. Scientific and technological impact and actual NanoCARD context

Heart failure affects over 14 million people worldwide and is a leading cause of death. Since cardio-myocytes have very little regenerative capacity, therapies are limited at present. The introduction of exogenous stem-cell-derived cardico-myocytes holds promises but there are up to date several challenges remaining: That includes the delivery, integration, rejection and cellular maturation. Reprogramming adult fibroblasts into induced pluripotent stem cells (iPSCs) that are similar to embryonic stem cells addresses some issues, but others, including efficient directed differentiation into CMs and effective delivery, remains a challenge. Novel surface technologies to supporting heart muscle cell growth as proposed in NanoCARD may offer new and unique tools.

There were several reviews published in the last two years giving an up-to-date overview of tissue engineering approaches for cardiac repair. Several of the publications follow similar strategies as proposed in the NanoCARD project. An overview of such strategies is given in (1). A general understanding of several publications is that the engineering of heart tissue requires an mulit-parametric approach by providing an environment to the cells which considers, next to the chemistry, the micro and nanostructure of the scaffolds, the mechanics and also active mechanical and/or electrical stimulations ((2), (3)).

1. Cell sources for heart muscle regeneration

There are several key publications demonstrating the potential use of different cell types for repairing infarcted heart tissue. The reprogramming of adult cells into pluripotent cells or directly into alternative adult cell types promises new pathways for regenerative medicine in this area. Idea et al. reported previously that cardiac fibroblasts, which represent 50% of the cells in the mammalian heart, can be directly reprogrammed to adult cardiomyocyte-like cells in vitro by the addition of Gata4, Mef2c and Tbx5 (GMT) ((4)). Quian used a similar approach to reprogram in vivo (mouse) resident nonmyocytes of the murine heart can into cardiomyocyte-like cells ((5)). This *in vivo* delivery of GMT decreased infarct size and modestly attenuated cardiac dysfunction up to 3 months after coronary ligation. Delivery of the pro-angiogenic and fibroblast-activating peptide, thymosin b4, along with GMT, resulted in further improvements in scar area and cardiac function. These findings demonstrate that cardiac fibroblasts can be reprogrammed into cardiomyocyte-like cells in their native environment for potential regenerative purposes. A recent review list stem cell sources for regenerative treatment of ischemic heart muscle tissue and typical markers of these cells (6). In addition, there is an overview about clinical trials and stem cell types used with these. Most of the human clinical trials performed to date used blood- and bone marrow-derived stem cells that are defined by different characteristics. According to Brenner et al., two encouraging trials utilizing cardiac stem cells from heart biopsies have been launched and are still recruiting; first results are expected soon ((6)). Most of these trials involved, however, only the administration of bone marrow-derived stem cells after myocardial infarction and did not use scaffold materials or specifically designed surfaces for cell differentiation or expansion. As such they followed a different approach compared to the NanoCARD strategy.

The reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs) opens new possibility in regenerative medicine. A large pool of fibroblasts exists in the postnatal heart and Ieda et al. reported recently that a combination of three developmental transcription factors (Gata4, Mef2c, and Tbx5) quickly and effectively reprogrammed post-natal cardiac or dermal fibroblasts into differentiated cardiomyocyte-like cells ((7)). These directly induced cardio-myocytes expressed cardiac-specific markers and showed spontaneous contractions in vitro. Fibroblasts transplanted into mouse hearts one day after transduction of the three factors also differentiated into cardiomyocyte-like cells in vivo.

Chip technologies, Materials, Scaffolds and chip for heart tissue regeneration

Independent of the cell source major challenges of heart muscle repair remain to be the cell survival, cell fate determination and engraftment as well as vascularization after transplantation. Several strategies of tissue-engineering combining scaffolds and different cell types have been developed and were partially adapted for specific application to enhance stem cell function. Different approaches and scaffold techniques for cardiac cell therapy are described by Karam et al. in a recent review ((9)). Several advancements in the field of biomaterials for heart muscle scaffolds are discussed. Examples are given for 2D studies and 3D supports for the cells also mimicking the structural architecture of the heart. In some studies hydrogels of different types were used to control the biophysical and biochemical microenvironments of transplanted cells. Interestingly, stacks of cells-seeded polymeric sheets, as similarly proposed in NanoCARD, were already suggested in 2002 by Shimizu et al. ((10)).

Several studies of biomaterial scaffolds for heart muscle regeneration or tissue engineering, cell types and stimulation signals are documented in a recent review ((1)).
Several approached have been proposed to use micro- or nanofabrications techniques to modify surfaces of scaffold polymers in order to direct stem or progenitor cells to cardio-myocytes.

For example, polymeric scaffold surfaces have been modified by specific recognition sites for cells using technologies like the Molecular Imprinting ((11)). The authors of this publication claim that this technique is extremely suitable to produce intelligent matrices usable in the field of heart tissue engineering characterized by their particular capacity for molecular recognition of extracellular proteins (collagen, fibronectin, laminin, vitronectin, etc.) that favor cellular adhesion or cell functions. It is proposed that the polymeric matrices will show a capacity for increasing the adhesion and growth characteristics of the cells on the scaffolds thanks to the presence of nanosites that are complementary and selective towards specific peptide sequences presented by ECM proteins and with which specific integrins interact.
Other approaches followed the strategy to incorporate resilience-imparting protein found in all elastic human tissues into hydrogels to form a highly elastic scaffold (12). These hydrogels, with photocrosslinked methacryl-tropoelastin, facilitated the attachment, adhesion, alignment, function, and intercellular communication of cardiomyocytes by providing an elastic mechanical support mimicking mechanical in vivo properties.

Considering that MI cannot be used directly on biodegradable polymers, which are a fundamental requisite for tissue engineered scaffolds, biostable polymers in the form of nanoparticles will be realized. They will be used in small quantities to modify degradable materials, so that these will retain their ability to serve as temporary scaffolds.

There are several publications reporting on the impact of surface modification of culture systems on stem cell differentiation or expansion. Focus is often on nanotopography of polymer support but also on the functionalization of the surface with peptides. To the later approach there is one publication reporting a similar technique for nanopatterning by loaded block-copolymers as commonly used in the NanoCARD consortium. The group demonstrates, next to variation in adhesion of mesenchymal stem cells (MSC), that MSC osteogenesis is reduced on surfaces with increased lateral RGD spacing while adipogenic differentiation is increased ((13)).
Many more publication report on the behavior of various stem cells controlled or directed by topographic surface features. Zouani et al., for example, demonstrated that the depth (on a nanometric scale) of micro-patterned surface structures allowed increased adhesion of human mesenchymal stem cells (hMSCs) with specific differentiation into osteoblasts in the absence of osteogenic medium (14). There have been also reports on the screening of stem cell responses to surface featured by a chip approach.
Generally, the approach of using chip technologies and high content screenings to explore the complex interaction of stem cells with cues from their environment is well accepted and widely followed(16-18).

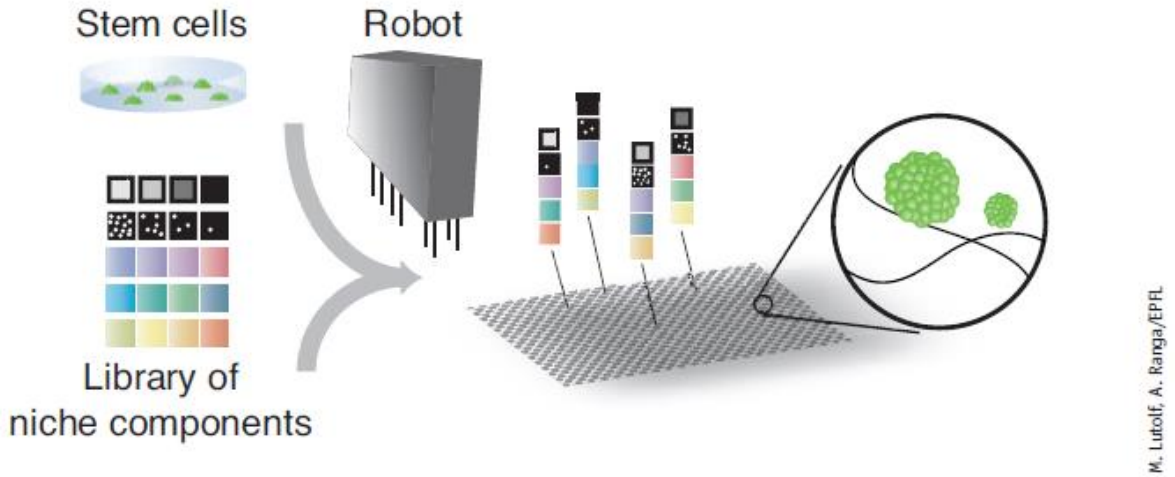

Figure 11: Schematic representation of high throughput approaches in stem cell applications. Hydrogel engineering combined with robotics can generate several hundreds to a thousand printed artificial niche candidates in one experiment (from (16)).

Devices for mechanical cell manipulations in regenerative medicine

Mechanical forces are ubiquitous in our body and greatly affect the development and functional homeostasis of tissues. In particular, in vivo mechanical stimuli within muscles and blood vessel walls may play an important role in differentiation of stem cells to various phenotypes. Previous work has shown that mechanical strain has an effect on proliferation and differentiation on many cell types also on MSC, including up-regulation of various SMC contractile markers (8–10), and the cells aligned perpendicularly to the axis of strain (11). Cyclic stretching has been used for improved tissue engineering on heart muscles for more than one decade. Zimmermann at al. reported, for example, first results by using simple mechanical devices (19,20). There are also several reports on stretching devices within the last 3 years (21,22), some of them trying to mimic specific *in vivo* conditions for tissue engineering (23). Parallel to the development of a stretching device for live cell microscopy within NanoCARD designs for similar devices have been reported (24-26).

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Dissemination impact

Dissemination

NanoCARD has been producing a lot of high impact scientific publications , conference contributions and exhibition participations

1. **Publications**

Franco et al. ‘Accelerated endothelial wound healing on microstructured substrates under flow’ Biomaterials. 2013

Vittorio et al. ‘Endothelial differentiation of mesenchymal stromal cells: when traditional biology meets mechanotransduction’ Integrative Biology. 2012

Wieringa et al. ‘Nanotopography induced contact guidance of the F11 cell line during neuronal differentiation: a neuronal model cell line for tissue scaffold development’. Nanotechnology. 2012

Meucci et al. Biocompatible noisy nanotopographies with specific directionality for controlled anisotropic cell cultures. Soft Matter, (2012).

Sahai et al. ‘Microfluidic chip for spatially and temporally controlled biochemical gradient generation in standard cell-culture Petri dishes. Microfluidics and Nanofluidics. 2011

Licentiate Thesis, Patric Wallin “Creating cell microenvironments in vitro”, Chalmers University of Technology, Sweden, 2012

Master Thesis, Elin Bernson “Development of a microfluidic platform to study cell migration along gradients”, Chalmers University of Technology, Sweden, 2012

Patric Wallin, Carl Zandén, Björn Carlberg, Johan Liu and Julie Gold “Integration of Electro Spun Nanofibers in Microfluidic Gradient Channels”
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Emmert et al, Intramyocardial transplantation and tracking of human mesenchymal stem cells in a novel intra-uterine pre-immune fetal sheep myocardial infarction model: a proof of concept study, PloS One, 2013;8(3):e57759.

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C. Moshfegh & V.Vogel, Reprogramming of pluripotent stem cells for improved control of their differentiation pathways, European Patent Application, filed on Oct 3, 2013.

Patric Wallin*, Elin Bernson*, Vera Hirschfeld-Warneken, Francesco Mazzotta, Davide Franco, Aldo Ferrari, Joachim Spatz, Julie Gold, “Endothelial cell migration dependence on cell-adhesion ligand spacing”, in manuscript.

"Interaction of SH-SY5Y Cells with Nanogratings During Neuronal Differentiation: Comparison with Primary Neurons", I Tonazzini, A Cecchini, Y Elgersma, M Cecchini, Advanced healthcare materials, 2013, DOI: 10.1002/adhm.201300216

"Easy Monitoring of Velocity Fields in Microfluidic Devices Using Spatiotemporal Image Correlation Spectroscopy", Marco Travagliati, Salvatore Girardo, Dario Pisignano, Fabio Beltram, Marco Cecchini, Anal. Chem., 2013, 85 (17), pp 8080–8084

"Cell Guidance on Nanogratings: A Computational Model of the Interplay between PC12 Growth Cones and Nanostructures", PN Sergi, IM Roccasalvo, I Tonazzini, M Cecchini, S Micera, PlosONE 2013, DOI:10.1371/journal.pone.0070304

"Neuronal differentiation on anisotropic substrates and the influence of nanotopographical noise on neurite contact guidance", I Tonazzini, S Meucci, P Faraci, F Beltram, M Cecchini, Volume 34, Issue 25, August 2013, Pages 6027–6036

"Unveiling LOX-1 receptor interplay with nanotopography: mechanotransduction and atherosclerosis onset", C Di Rienzo, E Jacchetti, F Cardarelli, R Bizzarri, F Beltram, M Cecchini, Scientific Reports 3, Article number: 1141 doi:10.1038/srep01141

"Imaging intracellular viscosity by a new molecular rotor suitable for phasor analysis of fluorescence lifetime" Antonella Battisti, Silvio Panettieri, Gerardo Abbandonato, Emanuela Jacchetti, Francesco Cardarelli, Giovanni Signore, Fabio Beltram, Ranieri Bizzarri, Anal Bioanal Chem (2013) 405:6223–6233

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Kruss, S.; Erpenbeck, L.; Schön, M.; Spatz, J. P., Circular, Nanostructured and Biofunctionalized Hydrogel Microchannels for Dynamic Cell Adhesion Studies. *Lab on a Chip* **2012,** *12* (18), 3285-3289.

Rolli, C. G.; Nakayama, H.; Yamaguchi, K.; Spatz, J. P.; Kemkemer, R.; Nakanishi, J., Switchable adhesive substrates: Revealing geometry dependence in collective cell behavior. *Biomaterials* **2012,** *33* (8), 2409-2418.

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Kruss, S.; Erpenbeck, L.; Amschler, K.; Mundinger, T. A.; Böhm, H.; Helms, H.-J.; Friede, T.; Andrews, R.; Schön, M.; Spatz, J. P., Adhesion Maturation of Neutrophils on Nanoscopically Presented Platelet Glycoprotein Ibα. *ACS Nano* **2013,** *7* (11), 9984-9996.

Pallarola, D.; Bochen, A.; Böhm, H.; Rechenmacher, F.; Spatz, J. P.; Kessler, H., Interface Immobilization Chemistry of cRGD-based Peptides Regulates Integrin Mediated Cell Adhesion. *Advanced Functional Materials* **2013**, accepted.

Platzman, I.; Janiesch, J.-W.; Spatz, J. P., Synthesis of Nanostructured and Biofunctionalized Water-in-Oil Droplets as Tools for Homing T Cells. *Journal of the American Chemical Society* **2013,** *135*, 3339-3342.

Platzman, I.; Muth, C. A.; Lee-Thedieck, C.; Pallarola, D.; Atanasova, R.; Louban, I.; Altrock, E.; Spatz, J. P., Surface properties of nanostructured bio-active interfaces: impacts of surface stiffness and topography on cell-surface interactions. *RSC Advances* **2013,** *3* (32), 13293-13303.

Rahmouni, S.; Lindner, A.; Rechenmacher, F.; Neubauer, S.; Sobahi, T. R. A.; Kessler, H.; Cavalcanti-Adam, E. A.; Spatz, J. P., Hydrogel micropillars with integrin selective peptidomimetic functionalized nanopatterned tops: a new tool for the measurement of cell traction forces transmitted through αvβ3 or α5β1 integrins. *Advanced Materials* **2013,** *25*, 5869-5874.

Rechenmacher, F.; Neubauer, S.; Mas-Moruno, C.; Dorfner, P. M.; Polleux, J.; Guasch, J.; Conings, B.; Boyen, H.-G.; Bochen, A.; Sobahi, T. R.; Burgkart, R.; Spatz, J. P.; Fässler, R.; Kessler, H., A Molecular Toolkit for the Functionalization of Titanium-Based Biomaterials That Selectively Control Integrin-Mediated Cell Adhesion. *Chemistry – A European Journal* **2013,** *19* (28), 9218–9223.

Harreither E., Rydberg H.A., Åmand H., Jadhav V., Fliedl L., Benda C., Esteban M.A., Pei D., Borth N., Grillari-Voglauer R., Hommerding O., Edenhofer F., Nordén B., Grillari J.* Characterization of a novel cell penetrating peptide derived from human Oct4, Cell Regeneration, in press

A lot of publications are in preparation

2. Conference presentations

Dr. Emanuela Jacchetti (SNS) participated to the Workshop "NANOTECHNOLOGIES FOR HEALTH CARE", where she presented her results about the interaction between cells and nanogratings, obtained within the NANOCARD project.

Patric Wallin, Elin Bernson and Julie Gold “Simulation of Chemotractant Gradients in Microfluidic Channels to Study Cell Migration Mechanism in silicon”
Conference Paper, Comsol User meeting Milano (2012)

Scandinavian Society for Biomaterials Annual meeting 2011 “Preparation of peptide-functionalized Au nanodot and lipid bilayer surfaces for studying cell focal adhesion formation”

Lab-on-a-chip world congress 2011 “Patterned electrospun microfibers integrated in a microfluidic system to study cells in complex microenvironments”

Scandinavian Society for Biomaterials Annual meeting 2012 “Patterned electrospun microfibers integrated in a microfluidic system to study cells in complex microenvironments”

TERMIS world congress 2012 “Microlfuidic gradient systems to generate defined cell microenvironments and cellular fate processes” Journal of Tissue Engineering and Regenerative Medicine, 6 s. 350-350, 2012
T3Net meeting in Manchester, UK

Patric Wallin, Elin Bernson, Julie Gold, “Microfluidic gradient systems to generate defined cell microenvironments and study cellular fate processes”, Materials for Tomorrow/Materials for Health Oct 1-2, 2013, Chalmers University of Technology, Sweden, and

BRC Biomaterials Research Centre Annual Meeting, “Biomaterials associated infection, inflammation and immune function”, Nov 18, 2013, Göteborg University, Sweden

Neuroscience 2013, Society for Neuroscience’s annual meeting, November 8-13, San Diego (CA) - USA. Mechanotransduction of hippocampal neurons: role of ubiquitin ligase E3a (Ube3a) in neurite contact guidance. *Tonazzini I, Van Woerden GM, Meucci S, Elgersma Y, Beltram F[1] and Cecchini M*. Poster presentation.

Neuroscience 2013, Society for Neuroscience's annual meeting, November 8-13, San Diego (CA) – USA. Interaction of neuronal cells with nanotopographies and the impact of nanotopographical noise during neurite path finding. M. CECCHINI, I. TONAZZINI, S. MEUCCI, F. BELTRAM. Poster Presentation

E-MRS 2013 Spring Meeting, European Materials Research Society meeting, May 27-31, Strasbourg - FRANCE.
Anisotropic nanostructured substrates and neuronal cells: neurite contact guidance in patho-physiological models. *Tonazzini I, Meucci S, Van Woerden GM, Elgersma Y, Beltram F and Cecchini M.* Oral presentation

E-MRS 2013 Spring Meeting, European Materials Research Society meeting, May 27-31, Strasbourg - FRANCE.
"Shaping the biological identity of implant materials: A topographical approach" *Ferrari Aldo*, Invited oral Presentation

3. Several exhibitions the products have been shown

The exhibitions were:

- High content analysis SF, USA
- Lab automation SF, USA
- Medica, Germany
- Israel life imaging forum, Israel

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Address of project public website and relevant contact details

Project website address: *http://www.mf.mpg.de/NanoCARD/*

Name, title and organisation of the scientific representative of the project's coordinator:

Prof. Dr. Joachim P. Spatz

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E-mail: *spatz@is.mpg.de*

4.2 Use and dissemination of foreground

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available)	Is/Will open access provided to this publication?
1	Endothelial differentiation of mesenchymal stromal cells: when traditional biology meets mechanotransduction	Vittorio	*Integrative Biology*				2012			
2	Nanotopography induced contact guidance of the F11 cell line during neuronal differentiation: a neuronal model cell line for tissue scaffold development	Wieringa	*Nanotechnology*				2012			
3	Easy Monitoring of Velocity Fields in Microfluidic Devices Using Spatiotemporal Image Correlation Spectroscopy	Marco Travagliati	*Anal. Chem.*				2013	85 (17), 8080–8084		
4	Accelerated endothelial wound healing on microstructured substrates under flow.	D. Franco	*Biomaterials*				2013	34, 1488-1497		
5	Biocompatible noisy nanotopographies with specific directionality for controlled anisotropic cell cultures	Meucci	*Soft Matter*				2012			

6	Microfluidic chip for spatially and temporally controlled biochemical gradient generation in standard cell-culture Petri dishes	Sahai	*Microfluidics and Nanofluidics*				2011			
7	Guidance on Nanogratings: A Computational Model of the Interplay between PC12 Growth Cones and Nanostructures	PN Sergi	*journal.pone*				2013	DOI:10.1371, 70304		Yes
8	Interaction of SH-SY5Y Cells with Nanogratings During Neuronal Differentiation: Comparison with Primary Neurons	I Tonazzini	*Advanced healthcare materials*				2013	DOI: 10.1002/adhm .201300216		
9	"Neuronal differentiation on anisotropic substrates and the influence of nanotopographical noise on neurite contact guidance	I Tonazzini		Volume 34, Issue 25			Jul 05	6027–6036		
10	Unveiling LOX-1 receptor interplay with nanotopography: mechanotransduction and atherosclerosis onset	C Di Rienzo	*Scientific Reports 3*					Article number: 1141 doi:10.1038/srep01141		
11	Imaging intracellular viscosity by a new molecular rotor suitable for phasor analysis of fluorescence lifetime	Antonella Battisti	*Anal. Bioanal. Chem.*				2013	405:6223-6233		
12	On cell separation with topographically engineerined surfaces	D. Franco	*Biointerphases*				2013	doi:10.1186/1559-4106-8-34		

13	Interaction of leech neurons with topographical gratings: comparison with rodent and human neuronal lines and primary cells	Tonazzini I.	*Interface Focus*				accepted for publication			
14	Integration of Electro Spun Nanofibers in Microfluidic Gradient Channels	Patric Wallin	*Biomicrofluidics*				2012	6,024131		
15	Intramyocardial transplantation and tracking of human mesenchymal stem cells in a novel intra-uterine pre-immune fetal sheep myocardial infarction model: a proof of concept study	Emmert	*PloS One*				2013	8(3):e57759		
16	Transcatheter based electromechanical mapping guided intramyocardial transplantation and in vivo tracking of human stem cell based three dimensional microtissues in the porcine heart	Emmert	*Biomaterials*					34(10):2428-2441		
17	Reprogramming of pluripotent stem cells for improved control of their differentiation pathways	C. Moshfegh & V. Vogel	*European Patent Application*				2013	Filed on Oct 3		
18	Endothelial cell migration dependence on cell-adhesion ligand spacing	Patric Wallin					In manuscript			
19	Microstructured platforms to study nanotube-mediated long-distance cell-to-cell connections	Abel, M.	*Biointerphases*				2011	6(1), 22-31		
20	Impact of Local versus Global Ligand Density on Cellular Adhesion	Deeg, J.	*Nano Letters*				2011	11, 1469-1476		

21	Circular, Nanostructured and Biofunctionalized Hydrogel Microchannels for Dynamic Cell Adhesion Studies	Kruss, S.	*Lab on a Chip*				2012	12 (18), 3285-3289		
22	Regulation of integrin adhesions by varying the density of substrate-bound epidermal growth factor	Shahal, T.	*Biointerphases*				2012	7,1-11		
23	Biselectivity of isoDGR peptides for fibronectin binding integrin subtypes α5β1 and αvβ6: conformational control through flanking amino acids	Bochen, A.	*Journal of Medicinal Chemistry*				2013	56,1509-1519		
24	Gold-nanoparticle-decorated glass microspheres	Gadomska, K. M.	*Particle & Particle Systems Characterization*				2013	30 (11),940-944		
25	Fabrication of multi-parametric platforms based on nanocone arrays for determination of cellular response	Purwaningsih, L.	*Beilstein Journal of Nanotechnology*				2011	2,545-551		
26	Elastic moduli of living epithelial pancreatic cancer cells and their skeletonized keratin intermediate filament network	Walter, N.	*Biointerphases*				2011	6 (2),79-85		
27	Marker-free phenotyping of tumor cells by fractal analysis of RICM images	Klein, K.	*Nano Letters*				2013	13 (11), 5474-5479		
28	Cell membrane topology analysis by RICM enables marker-free adhesion strength quantification	Klein, K.	*Biointerphases*				2013	8,28		
29	Adhesion Maturation of Neutrophils on Nanoscopically Presented Platelet Glycoprotein Ibα	Kruss, S.	*ACS Nano*				2013	7 (11), 9984-9996		
30	Switchable adhesive substrates: Revealing geometry dependence in collective cell behavior	Rolli, C. G.	*Biomaterials*				2012	33(8),2409-2418		
31	Interface Immobilization Chemistry of cRGD-based Peptides Regulates Integrin Mediated	Pallarola,	*Advanced Functional*				2013, accepted			

	Cell Adhesion	D.	*Materials*							
32	Synthesis of Nanostructured and Biofunctionalized Water-in-Oil Droplets as Tools for Homing T Cells	Platzman, I.	*Journal of the American Chemical Society*				2013	135,3339-3342		
33	Surface properties of nanostructured bio-active interfaces: impacts of surface stiffness and topography on cell-surface interactions	Platzman, I.	*RSC Advances*				2013	3 (32), 13293-13303		
34	Hydrogel micropillars with integrin selective peptidomimetic functionalized nanopatterned tops: a new tool for the measurement of cell traction forces transmitted through αvβ3 or α5β1 integrins	Rahmouni, S.	*Advanced Materials*				2013	25,5869-5874		
35	A Molecular Toolkit for the Functionalization of Titanium-Based Biomaterials That Selectively Control Integrin-Mediated Cell Adhesion	Rechenmacher, F.	*Chemistry – A European Journal*				2013	19 (28), 9218-9223		
36	Characterization of a novel cell penetrating peptide derived from human	Harreither, E.	*Cell Regeneration*				2014			

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
1	Neuroscience 2013, Society for Neuroscience's annual meeting	*Tonazzini I*	Mechanotransduction of hippocampal neurons: role of ubiquitin ligase E3a (Ube3a) in neurite contact guidance	November 8-13	San Diego (CA) - USA			world
2	Neuroscience 2013, Society for Neuroscience's annual meeting	M. CECCHINI	Interaction of neuronal cells with nanotopographies and the impact of nanotopographical noise during neurite path finding	November 8-13	San Diego (CA) - USA			world
3	E-MRS 2013 Spring Meeting, European Materials Research Society meeting	*Tonazzini I*	Anisotropic nanostructured substrates and neuronal cells: neurite contact guidance in patho-physiological models	May 27-31	Strasbourg - FRANCE			european
4	E-MRS 2013 Spring Meeting, European Materials Research Society meeting	*Ferrari Aldo*	Shaping the biological identity of implant materials: A topographical approach	May 27-31	Strasbourg - FRANCE			european
5	Scandinavian Society for Biomaterials Annual meeting 2011	Julie Gold	Preparation of peptide-functionalized Au nanodot and lipid bilayer surfaces for studying cell focal adhesion formation	May 4-6, 2011	Bohuslän on the Swedish west coast			european
6	Lab-on-a-chip world congress 2011	Julie Gold	Patterned electrospun microfibers integrated in a microfluidic system to study cells in complex microenvironments	25-26 September 2012	San Diego (CA) - USA			world
7	Scandinavian Society for Biomaterials Annual meeting 2012	Julie Gold	Patterned electrospun microfibers integrated in a microfluidic system to study cells in complex microenvironments	May 8-9,2012	Uppsala, Sweden			european
8	TERMIS world congress 2012	Julie Gold	Microlfuidic gradient systems to generate defined cell microenvironments and cellular fate processes	September 5-8	Hofburg Congress Centre | Vienna |	Journal of Tissue Engineering and		world

					Austria	Regenerative Medicine, 6 s. 350-350, 2012		
9	NANOTECHNOLOGIES FOR HEALTH CARE	Emanuela Jacchetti	interaction between cells and nanogratings	May 25th-26th, 2012	Trento, Italy			european
10	Comsol User meeting	Patric Wallin, Elin Bernson and Julie Gold	Simulation of Chemotractant Gradients in Microfluidic Channels to Study Cell Migration Mechanism in silicon	2012	Milano			european
11	Materials for Tomorrow/Materials for Health	Patric Wallin, Elin Bernson and Julie Gold	Microfluidic gradient systems to generate defined cell microenvironments and study cellular fate processes	Oct 1-2, 2013	Chalmers University of Technology, Sweden			european
12	BRC Biomaterials Research Centre Annual Meeting	Patric Wallin, Elin Bernson and Julie Gold	Biomaterials associated infection, inflammation and immune function	Nov 18, 2013	Göteborg University, Sweden			european

Section B (Confidential or public: confidential information to be marked clearly)
Part B1

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	Yes	2013 Filed on Oct 3	*European Patent Application* 13004776.4	Reprogramming of pluripotent stem cells for improved control of their differentiation pathways	C. Moshfegh & V. Vogel

ADDITIONAL TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND confidential	
Description of Exploitable Foreground	**Explain of the Exploitable foreground**
New microfluidic chip	To enable high throughput applications and ameliorate biocompatibility and chemical gradient control
Data analysis	Implementation of software tools for image processing and data analysis is high throughput experiments. This includes fast data acquisition and storage as well as a robotic handling system.
Extracellular matrix scaffolds and biochips	Nanogratings can be exploited to produce an aligned a cellular ECM scaffolds
iPSCs generation protocol	Development of a mRNA based reprogramming protocol for the generation of iPSCs that can be differentiated into cardiomyocytes.
cardiomyocytes differentiation system	We developed an efficient hiPSCs cardiomyocyte differentiation system
Novel myocardial infarction model	We developed a novel myocardial infarction model to track human mesenchymal stem cells after direct intra-myocardial transplantation
Development of Cell Image Velocimetry (CIV)	Provision of a methodology and software for cell layer segmentation and flow detection inside migrating cell layers

4.3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	**229294**
Title of Project:	***Nano**patterned scaffolds for active myo**cardi**al implants*
Name and Title of Coordinator:	*Prof. Dr. Joachim P. Spatz*

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	Yes
• Did the project involve Human biological samples?	Yes
• Did the project involve Human data collection?	
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	Yes
• Did the project on human Embryonic Stem Cells involve cells in culture?	Yes
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	Yes
• Were those animals transgenic small laboratory animals?	Yes
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	

RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	No
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	1
Work package leaders	2	5
Experienced researchers (i.e. PhD holders)	6	21
PhD Students	13	10
Other	1	2

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**32**
Of which, indicate the number of men:	8

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?	○ X	Yes No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
X	Set targets to achieve a gender balance in the workforce	○	○	X	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

X Yes- please specify MPI-OPEN-DAY

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

X Yes- please specify public website

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

X Main discipline[1]: 1.4,1.6,2.5,2.6,2.10

X Associated discipline[1]: 1.1,3.4,3.1,3.2 ○ Associated discipline[1]:

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*	○ X	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[1] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**40**
To how many of these is open access[2] provided?	**1**
How many of these are published in open access journals?	**1**
How many of these are published in open repositories?	
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available X no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[3]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**1**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**1**
	Registered design	
	Other	
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

X	Increase in employment, or	X	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:* ***128***

[2] Open Access is defined as free of charge access for anyone via Internet.
[3] For instance: classification for security project.

Difficult to estimate / not possible to quantify	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

X Yes ○ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes X No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

X	Press Release	❑	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
❑	Brochures /posters / flyers	X	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	X	English
❑	Other language(s)		