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PROJECT FINAL REPORT

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Project acronym: NANOII

Project title: ***Nano****scopically-guided induction and expansion of regulatory hematopoietic cells to treat auto****i****mmune and* ***i****nflammatory processes*

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Name of the scientific representative of the project's co-ordinator, Title and Organisation:
Prof. Dr. Joachim P. Spatz, Max-Planck-Institute, Stuttgart

Tel: +49 711 689-3611

Fax: +49 711 689-3612

E-mail: spatz@is.mpg.de

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

4.1 Final publishable summary report

Executive Summary

NanoII
Nanoscopically-guided induction and expansion of regulatory hematopoietic cells to treat autoimmune and inflammatory processes

NanoII has been a colaborative project within the EU 7th framework program for research on Nanoscopically-guided induction and expansion of regulatory hematopoietic cells to treat autoimmune and inflammatory processes.

This project has been developing novel approaches for directing the differentiaton, proliferation and tissue tropism of specific hematopoietic lineages, using micro-and nanofabricated cell chips. We are using advanced nanofabricated surfaces functionalized with specific biomolecules, and microfluidic cell chips to specify and expand regulatory immune cell for treating of important inflammatory and autoimmune disorders in an organ and antigen-specific manner. A new chip system creates ex vivo microenvironments mimicking in vivo cell-cell-interactions and molecular signals involved in differentiation and proliferation of hematopoietic cells. Key element of the project is the development of a novel high-throughput microscopy system for the identification of optimal conditions.

The educated cells are employed in in-vivo experiments in new mouse models. Our methodology can be further adapted for human cell populations. New perspectives for clinical diagnosis and therapy for important diseases has been achieved.

Summary description of the project context and objectives

The overall goal in this project is the development of a system for the diagnosis and therapy of diabetes and inflammatory diseases through harnessing novel nano-technology based cell chips to direct the differentiation, proliferation and tissue-tropism of specific hematopoietic lineages and to produce large numbers of regulatory cells to be used therapeutically to limit inflammatory and autoimmune processes developed.

Diseases we will treat with the regulatory cells are Type-1 Diabetes Mellitus **(T1DM),** for which therapy will attempt to delay the progression of disease and to facilitate islet transplantation, and Inflammatory Bowel Disease **(IBD).** Both conditions are widespread and highly-debilitating inflammatory diseases for which cellular therapy holds great promise.

In a primary screen, we will use an array of nano-patterned cell chips to systematically identify optimal inductive surfaces. Cells attached to these chips will be examined by high throughput microscopy for robust proliferation, appropriate regulatory phenotype and desirable cytokine profile. Quantitative pre-screening is intended to speed up drug development by reducing the number of animal and human tests needed to identify the best cell populations. Based on this screen, the optimal bio-active surfaces for each cell type will be defined and chips will be scaled up for cell culture. Ex-vivo conditioned and expanded cells will be tested for effectiveness in murine disease models and the methodology will be further adapted for human cell populations and applied to the development of clinical devices to be used in therapy.

Business Strategy: Together with the European companies *Miltenyi Biotec GmbH*, *CellMade, IDEA Bio-Medical Ltd.,* and GenID/*AID GmbH* we will explore business strategies for the transition of our fundamental research developments into an appropriate business market. The inclusion of relevant companies in the **multi-disciplinary research consortium assures the identification of** opportunities for the intended **product developments**. *Miltenyi Biotec GmbH,* GenID/*AID GmbH* and CellMade will undertake the production and commercialization of the conceptually new Nano-pattern chips. The company GenID/*AID GmbH* will explore the capabilities of the developed chip technology for diagnostic applications and will guide the development into a fully integrated routine system including an automated reader. *IDEA Bio-Medical* will develop and commercialize the HTS live cell optical microscope. The biochip/HTS system will find wide application in the life sciences.

Objectives

- Engineering Nanopatterned, hematopoiesis-directed cell chip
- Development of high-resolution, high throughput microscopy for examination of cell chips
- Developing on-chip screens for the effects of bioactive molecules on proliferation, differentiation and function of hematopoietic cells
- Nanopatterned, hematopoiesis-directed, microfluidic cell chip
- Engineering of 2-dimensional cell chip for reprogramming of regulatory T cells
- Engineering of 2-dimensional cell chip for directing myeloid differentiation

- Engineering of 2-dimensional cell chip for generating central memory veto CD8 T cells
- Scaling up the cell stimulation process – from cell chips to “lineage selective cell reactors”
- Preclinical assessment of Type-1 diabetes mellitus (T1DM) therapy using the various *in vitro* generated cell types
- Preclinical assessment of Inflammatory Bowel disease (IBD) therapy using the various *in vitro* generated cell types
- Optimizing the cell-chip system for the selective expansion of human regulatory cell populations, and setting up protocols for phase I clinical trials
- Integrative and Translational Approaches to Industrial Market
- Educational programmes
- Coordination and Project Management
- IDEG – IPR, Dissemination, Ethics and Gender

Description of main S & T results/foregrounds

1. Engineering Nanopatterned, hematopoiesis-directed cell chip

Summary

We designed, developed and validated an original microfluidic chip capable of generating both spatially and temporally varying concentration gradients.
Functional anti-CD3 nano-arrays varying in interparticle distance from 35 nm to 150 nm have been successfully produced and employed for studying human CD4+ T cell response.
We developed techniques to combine microfluidic networks with different substrates.
We designed, developed and validated two novel microfluidic chips based on closed microchambers for controlled cell cultures.
We have developed a fluidic bioreactor that reproduces the physiological Wall Shear Stress (WSS) values that are commonly measured in human veins and arteries.
A diagnostic microarray platform was developed which enables the multiplex detection of antigens/antibodies on an array (up to 15x15 probes) on the bottom of the well of a 96 well microtiterplate.
A Kit was implemented at verification status and was planned for production as IVD.
Nanostructured substrates were prepared by decorating polymer brushes with gold nanoparticles. We have investigated the (time-dependent) functionality of the PEG passivation layer used in the BioChemical chips in combination with monocytes, lymphocytes and platelets.
We have been constructing a flow through nanoporous membranse sensing element for the in situ detection of signaling molecules secreted from cells.
We developed a novel method based on spatiotemporal image correlation spectroscopy (STICS) to measure velocity fields in microfluidic devices. We used this novel method to study the fluid dynamics of the devices developed for the aims of NANOII.
We characterized the fluid dynamics of the microfluidic cell bioreactor. We showed optimal cell spatial patterning in the microchamber and applied chemical gradients for pharmacokinetics experiments.
We evaluated the combined effect of mechanical stresses and topography on endothelial cell adhesion.
In conclusion we were able to successfully integrate the biochip in the WiScanTM instrument. First tests of the suitability of this automated optical microscope for high-throughput screens of chips offering a systematic variation of biofunctionalized surfaces are provided. Our future plans are to use this biochip and to investigate in detail the mechanosensing responses of normal and cancerous cells derived from different tissues.

Description

For a detailed investigation of the cellular mechanosensing responses an array of bio-substrates with different elastic and nanostructured properties (*"biochip")* was developed. We used sterile *48-well cell culture plates* (Greiner Bio-One GmbH, Germany) in order to create a two-dimensional parameter matrix consisting of hydrogels with different parameters (ΔL; EY) in each well. EY denotes the elastic modulus of the supporting substrate and ΔL is the spacing between anchor points for ligand immobilization on the surface of the substrate. Prior gluing and biofunctionalization, all hydrogels substrates were sterilized by UV irradiation. The hydrogels were glued to the bottom of the wells using the ethyl-2-cyanoacrylate tissue adhesive (EPIGLU, Meyer-Haake, Germany).

To optimize the measurement procedure, the biochip was integrated in an automated optical high-resolution microscope for high-throughput screening (WiScanTM instrument, IDEA Bio-Medical, Israel). This WiScanTM instrument has been developed by beneficiary No 13 in WP2 (compare deliverable D2.1). The system is equipped with live cell environmental control and sophisticated data analysis software (WiSoftTM software, IDEA Bio-Medical, Israel). We used the This WiScanTM system instrument in order to characterize the cellular behavior in the *biochip* during different time periods. Each well was scanned using 10x phase contrast objective (Olympus, Japan) and the images were analyzed by WiSoftTM to count the number of cells in each well and to measure their average spreading area as schematically illustrated

and described in Figure 2. Image analysis included: preprocessing steps (e.g. illumination correction, denoising, and background subtraction) as well as object segmentation. To characterize the focal adhesion formation on different hydrogel substrates we used a 60x/0.95 objective (Olympus, Japan) and FITC readout. A unique combination of the three main features of an automated microscopy such as image quality (high content), acquisition speed (high-throughput) and versatile experimental design helped us to overcome many difficulties of the biochip system in terms of image acquisition. In order to overcome such problems as changes of the hydrogel thickness from well to well, in addition to possible thickness gradients within each well, the WiScanTM was programmed to use its extremely fast, laser based auto-focused system to characterize the thickness of the hydrogel in each well and then to scan automatically not only in x and y direction, but also in z direction searching for the image with the best sharpness.

2. Development of high-resolution, high throughput microscopy for examination of cell chips

Summary

IDEA Bio-Medical has developed the WiScan system 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. The microscope is fully automatic and acquiers high-resolution images at a precise focus plane using fast auto focusing procedure.

The development of an automatic liquid handling system capable of providing four individual liquid streams at flow rates ranging from 0.5 nanoliter/second to 30 microliter/second has been achieved.

We developed the software CIV for automated cell migration tracking and large-scale data analysis.

Multi-parameter algorithms to detect and follow the differentiation state of T_{reg} cells generated ex vivo by either traditional methods, or by bio-functionalized nanopatterned surfaces / cell chips based innovative methodologies have been developed.

Description

IDEA Bio-Medical developed the WiScan®Argus system to combine fast acquisition and storage, accurate stage positioning, live cell conditions, a large set of command functions for versatile applications, flexible sample formats, an automatic sample loader and a user-friendly interface. The microscope is fully automatic and acquires high-resolution images at a precise focus plane using a fast auto focusing procedure. Two WiScan machines are currently installed at NanoII participant's laboratories – Prof. Geiger at WIS and Prof. Spatz at MP.

The complimentary image analysis software, WiSoft®, was released and tested for a large variety of applications, including applications for the analysis of hematopoietic cells features.

Technical features of the WiScan®Argus system:

- High-resolution imaging of sub-cellular organelles (mag. up to 100x)
- Ultra high sensitivity water cooled EMCCD camera capable of capturing dim samples.
- Ultrafast, high accuracy Laser AutoFocus
- Automated operation: TeraBytes of image data acquisition capacity
- Versatile use of all multiwell plate formats and cell chips
- Optimized screen path for fast acquisition of multiple images per well
- Multicolor using 7 color led illumination source, or 10 filters using mercury lamp and a white led for transmission images.
- 2D or 3D acquisition, including a routine for image-based selection of a focus plane.
- Live cell environmental cage with temperature and CO2 controllers
- Powerful visualization and analysis tools
- Quantitative analysis and statistics of multiple image features

The WiScan® system (Figure 1) includes tools for easily setting up experiments using a wizard, which guides the user through the setup, and offers a large variety of functions that enable the flexible design of a variety of user-defined experiments.

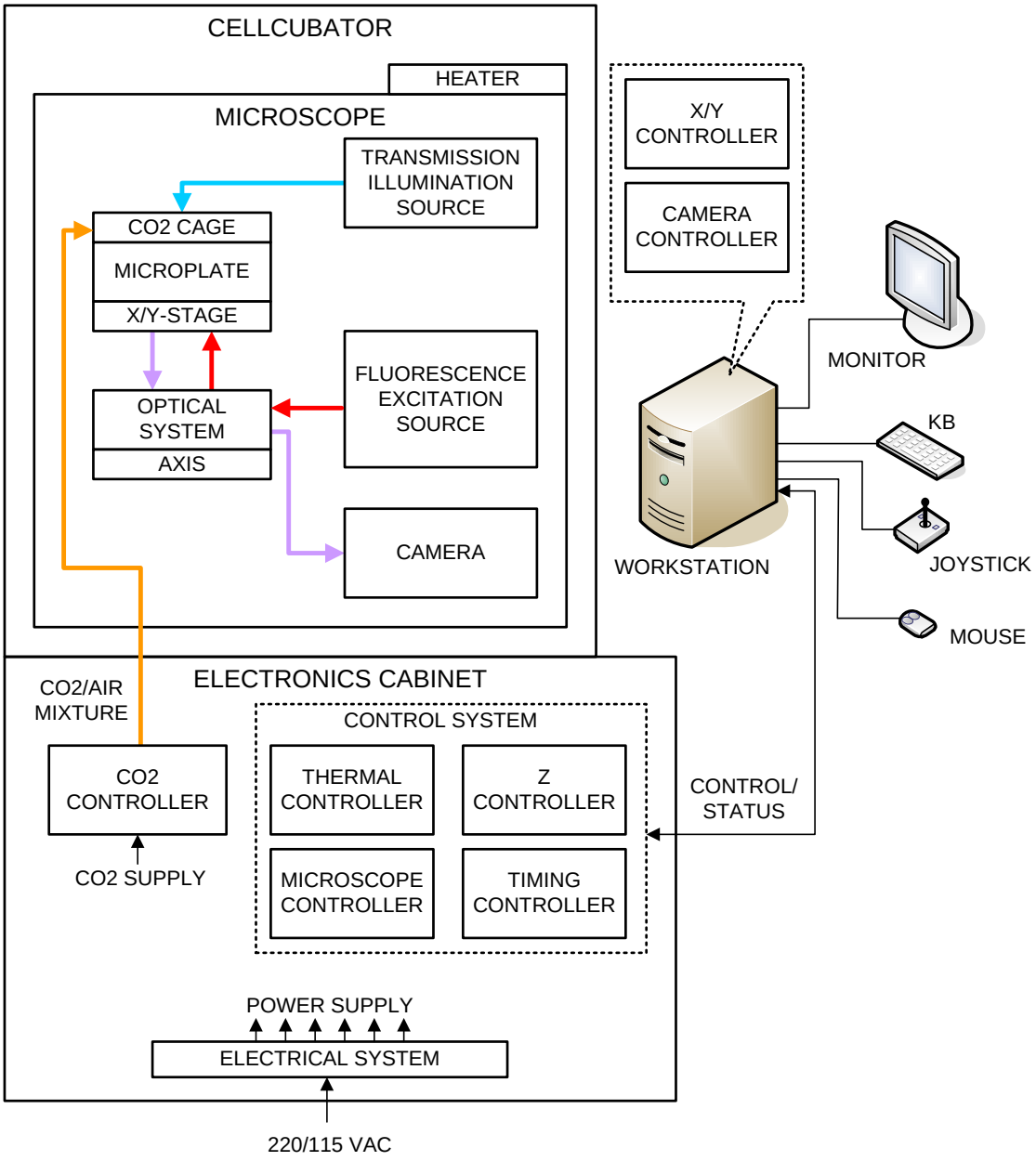


Fig. 1a. WiScan® Argus system block diagram description

Fig. 1b. WiScan® Argus system

WiScan® Argus - features and special adaptations

Adaptation for robotic sample loading:
Automatic sample loading is available using an automatic loader, which was integrated into the WiScan® Argus system (Figure 2). The loader locates the sample at a very high positioning accuracy and also includes automatic covering of the plate with the CO_2 supply cover.

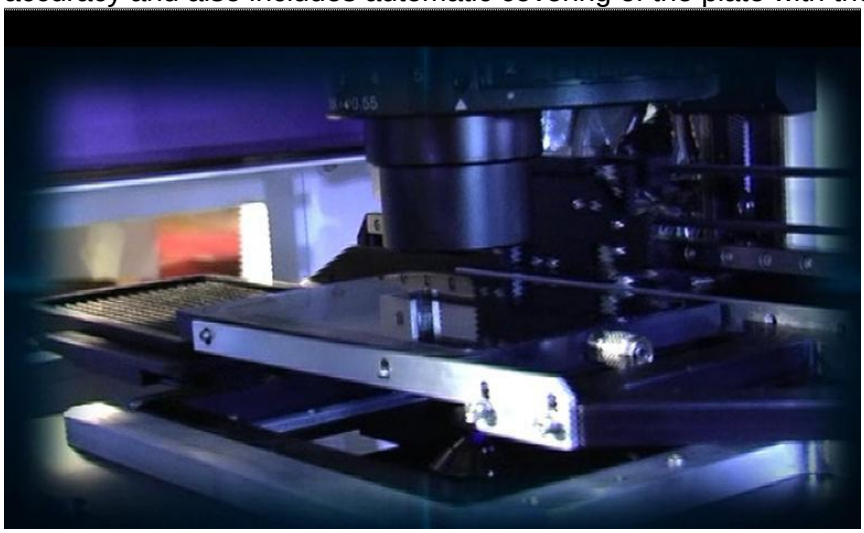


Fig. 2. Automatic sample loader (a view from inside the system)

Adaptation for samples with variable intensity (Exposure map):
A unique feature that's available with the WiScan® Argus system is the option to use an exposure map to define different exposure values for a sample that contains cell clones, which present a variety of fluorescence levels. The exposure map can be defined by the user according to the specific sample that is used for the experiment.

Adaptation to microfluidic:
Wiscan® Argus has a large space at the stage near the plate holder for adding microfluidic devices. This space is included within the heated cage, which therefore keeps the microfluidic supply reservoir at the temperature of the sample.

Adaptation for spotted array and biochip:
Wiscan® Argus allows scanning a flexible sample format. To allow this flexibility, the system offers a set of adaptors for slide or a plate and a special GUI feature that allows the user to define the

plate format. Specifically for biochip, this flexibility allow the user to define a set of points as wells or as fields in a well. Every format of spotted array is accepted.

Image analysis:
WiSoft®, the analysis software, was developed based on a set of algorithms that were developed at the Weizmann Institute of Science by Prof. Zvi Kam and Yuvalal Liron. These algorithms were further optimized at IDEA Bio-Medical for high speed performance. WiSoft® is a modular script-based analysis software, comprised of a large set of analysis functions that can be used to tailor large variety of modules for specific applications. WiSoft® also includes a set of ready-made modules for common applications that are easy to use with a user-friendly application setup wizard. In addition WiSoft® includes tools that support custom development of new functions and algorithms by the user. WiSoft® includes also tools for interactive parameter calibration. It has a large set of evaluation tools to statistically analyze images and to perform plate-based evaluation of the results.
WiSoft® has many visualization tools to allow the visualization of images, movies, montages, graphs and analyzed data in a plate format mode (Figure 3).

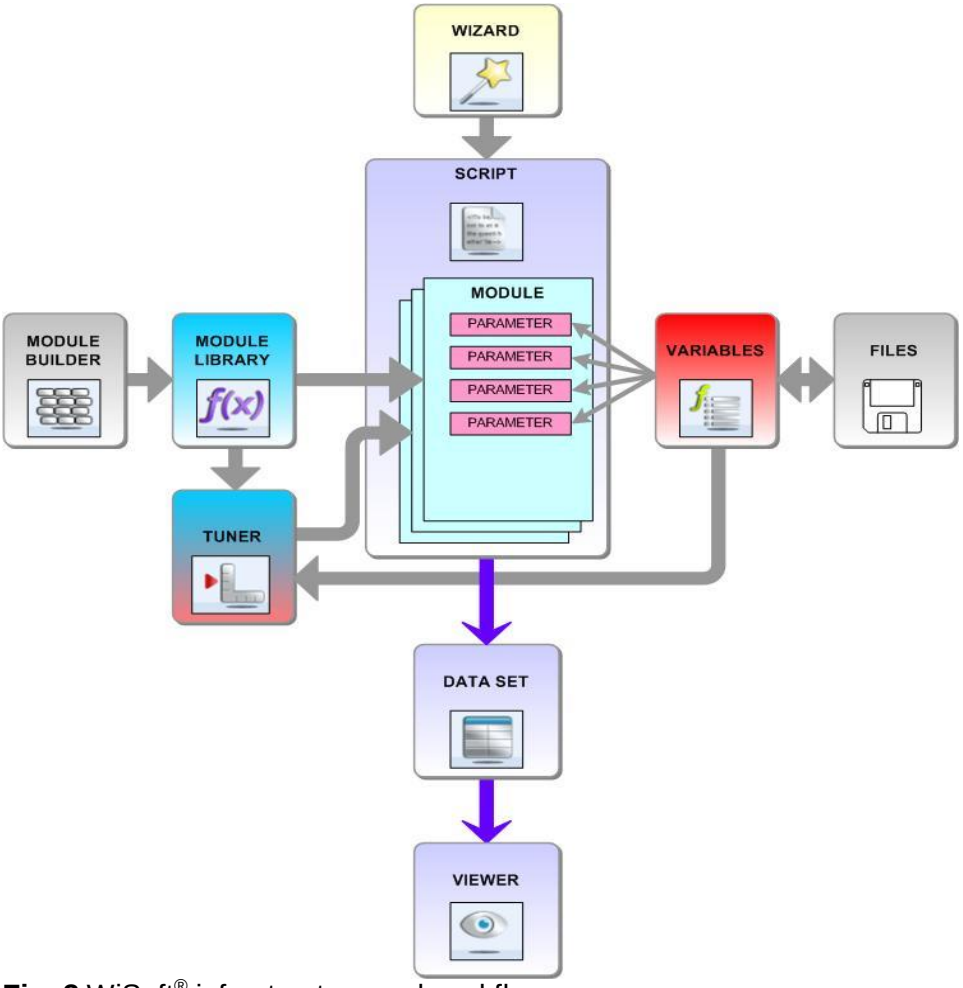


Fig. 3 WiSoft® infrastructure and workflow

3. **Developing on-chip screening for the effects of bioactive molecules on proliferation, differentiation and function of hematopoietic cell lines**

   Summary

   We established 6 colour flow cytometry, that allows us to identify the T cells state of activation, specificity (tetramer-staining), their type of immunologic memory and function (cytokines cell toxins) on single cell level.
   Quantitative cell assays require the preparation of an adhesive substratum consisting extra-cellular matrix peptides immobilize onto a solid support. Nanopattern substrates allow to investigate questions in cellular adhesion down to the nanoscale

level. The homogeneous ligand distribution, controlled distance between ligands, and effective passivation against non-specific adhesion of nanopatter susbtrates allows to study ligand-receptors interaction. The working ”lab on chip” platform, consist of a commercial mask (flexiPERM) with 12 culture wells; on top of a 1x3 inches glass slide, with the passivated PEG 2000 layer and nano-gold dots. Immobility of the substrate is further achieved with an aluminum chamber. The dimensions of the wells resemble a 96 well plate.

Description

Adhesion cell area and elongation was calculated from phase contrast images acquired with the transmitted light microscope. The same 8 pictures used for cell counting were analyzed for manual segmentation. ROI Manager on Image J was the plugin software used to individually segment each cell adhere to the substrate in a field of 10x magnification. The set measurement to analyze the polygons correlates to 0.65 micrometer/ pixel in a 10x picture. Figure 2 illustrate the procedure.
For quantification the polygon fit to an ellipse. The cell area output is report in square micrometers. And the major axis and minor axis in micrometers. The elongation is report without units as it the ratio between major/minor axis. The data is export to a XML file.
Biological image data collection was performed with the microscope system DeltaVision RT, in combination with the softWoRx image analysis software. The inverted epifluorescence microscope allows precise control of stage motion and is used for quantitative microscopy. Retrovirally infected C2 cells self express Pax-YFP to localize punctate focal adhesion structures at the interphase between cells and the ECM components. The chosen filter for detecting paxillin focal adhesion expressing YFP (Yellow Fluorescent Protein) was the FITC filter. The filter is called FITC because its used for detecting Fluoresce in Iso Thio Cyanate with excitation wavelength 490 nm, and emission wavelength =526 nm. Digital images were collected in 40x oil immersion objective + Aux magnification. All pictures were made at the same time exposure, as close as possible to the saturation point, and at the same percentage of light transmission. Standarized conditions set in relation to the RGD control.

The paxilin-YFP focal adhesion analysis was performed using with a mathematical algorithm under MatLab (acknowledgement to Dr. Alex deBeer). Raw cell images often contain diffuse background, which is especially prominent in thick regions of the cell like the nucleus. Subtraction of the average image intensity flattens this background (high pass filtered) and facilitates interactive determination of a uniform threshold level that is below the staining intensity for all matrix adhesion patches. Image segments corresponding to individual matrix adhesion (patches) are then define by the algorithm and approximate to ellipses as shown in figure 3. Such ellipses are then count, and measured (elongation ratio and area). The smallest paxillin structure correspond to 20 sq pixels, which equals an area of 0.5 sq um. The conversion in the delta vision system equipment is 6.25 pixels to 1 um. Parameters were optimized for 52nm RGD control, and used to all the screened data, aiming to get a lower systematic error. However, when plotting the results high amount of artifacts were found in the 70nm and 110nm data (objects such as vesicles close to the nucleous and bright cell-cell borders were identified by the algorithm). Because such artifacts did not correspond to the qualitative observations, where no visual focals are identified at 70nm and 110nm, with the except of few ones on P22.
New parameters were re-adjusted to 70nm and 110nm, allowing a proper signal to noisy/systematic error.

4. Nanopatterned, hematopoiesis-directed, microfluidic cell chip

Summary

PDMS microfluidic chips were coupled to nanopatterned substrates fabricated onto cyclic olefin copolymer (COC) foils. The generation of chemical gradients onto COC nanogratings was demonstrated.

We developed a system that enables us to do LSPR sensing in microfluidic channel with very small measurement areas.
We tested a microfluidics device for performing “ELISA on chip”. We demonstrated measuring levels of the T cell secreted cytokine IL-2, with a sensitivity comparable to conventional ELISA, and a much higher dynamic range.
A new microfluidic chip was designed, fabricated and tested in order to enable high-throughput applications and ameliorate biocompatibility and chemical gradient control.
We have developed two different microfluidic platforms that are capable of forming gradients of biological active compounds in solution.
Development of microfluidics devices that address and solve key challenges in long-term culture and monitoring of primary T cells.

Description

To date we have designed, developed and validated a PDMS device composed of three sets of five fluidic channels that can be housed in a standard tissue culture Petri dish. Thus, in the typical experimental setup, the cells can be first seeded and preconditioned and then exposed to the biochemical gradients produced by the device. The full details of the device operation and architecture are reported in two recent publications[1-2] (Sahai et al.). Altogether our microfluidic device allows for the generation of on-demand gradients of soluble molecules and is optimized for the use in long-term, live cell microscopy set-ups.

The microfluidic device was tested in a biologically relevant system by analyzing cell migration of mouse neutrophils in response to a chemotactic gradient. Two channels within a set of five were filled with either PBS/fluorescein or PBS/fluorescein/IL-8 solutions. As expected, after less than 5 min operation at 0.5 Hz, these two channels formed a stable overlapping gradient (Figure 1). Time-lapse experiments were then carried out to track neutrophil motion in response to the biochemical cue. Migration of cells was automatically tracked and exhibited a clear bias toward the channel delivering IL-8. The corresponding murine neutrophil migration speeds showed a typical distribution (up to 20 □m/min) as reported in the literature. Delivery of PBS/fluorescein without IL-8 from both channels resulted in strongly reduced and random migration. These results demonstrate that stable gradients of chemo-attractants can be created enabling complex migration experiments.

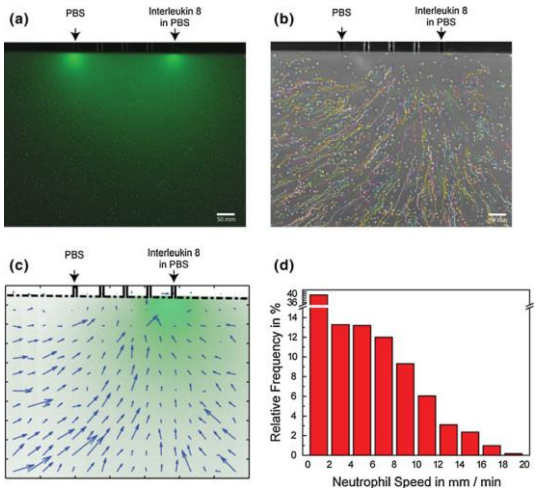


Figure 1: Mouse neutrophil migration in a microfluidic induced IL-8 gradient. **a** The fluorescence image is showing the two channels delivering PBS and Fluorescein with and without IL-8. After 2–3 min, a stable gradient of delivered fluids is achieved. **b** DIC image of automatically tracked neutrophils with migration path. The scale bar is 50 □m. **c** The average direction and speed of the neutrophils are quantified and represented by a vector field with IL-8 gradient as transparent background. **d** Distribution of neutrophil speeds

We used nanopatterned substrates fabricated onto COC (cyclic olephine copolymer) foils to demonstrate the generation of chemical gradients on COC gratings[3-4]. For this a bonding protocol between COC and PDMS was developed and optimized. In particular several tests were carried out to obtain reliable bonding between the two materials composing the biochips: COC as cell culturing substrate and PDMS for the microfluidic network.
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 (Figure 2). The microfluidic chip was aligned and mounted in close proximity to the patterned area present on the COC substrate (Figure 2a). A

fluorescein solution was then delivered through the fluidic channel (Figure 2b) to generate chemical gradients coupled to the underlying topography (Figure 2c).

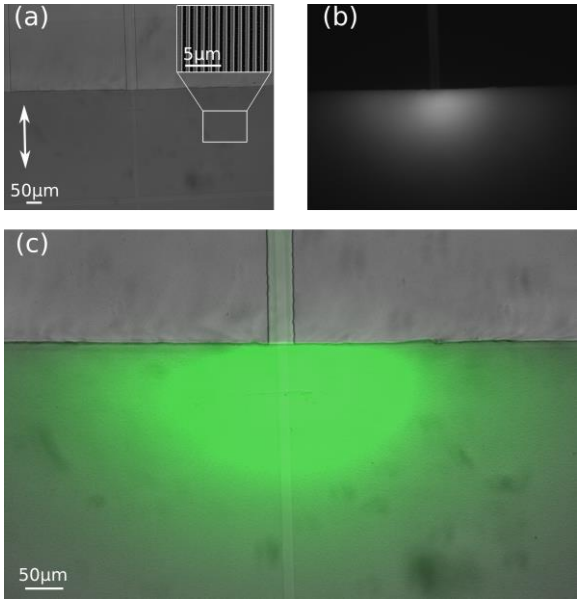


Figure 2: a Complete biochip composed by a microfludic network and a nanostructured substrate. The inset reports a scanning electron microscope image of the nanotopograpy. **b** Fluorescence image of a chemical concentration profile obtained by activating the microfluidic chip. The field of view is the same of (a). **c** Superimposition of (a) and (b).

We developed a protocol to couple a microfluidic chip (Figure 1) to a glass slide structured with nano-dots arrays by means of plasma treatment. Both the surfaces were exposed to oxygen plasma (Table 1), brought in conformal contact at room temperature and left bonding overnight. In order to test the functionality of the coupling, the chip was connected to a pressure line and the channels were filled with water. Complete filling was obtained with a pressure of 2 psi. The pressure was then increased up to 5 psi to verify the absence of leakages from the channels. For this experiment, a chip with 30µm wide and 8µm high channels was used. A total of 15 channels were embedded on a single chip.

The partners have developed a number of fluidic approaches to condition target cells. These approaches include the design and implementation of novel bioreactors, the development of new materials providing controlled physical stimulation to cells, and the purification of soluble and immobilized mitogens for the expansion of hematopoietic stem cells. Additionally, an automated system for live imaging of cell activities, including differentiation and polarization has been developed and tested.

Altogether the fluidic devices, the engineered substrates, the purified soluble molecules, and the multiplex imaging system are now ready and fully functioning. Each component has been made available for the other partners of the consortium in order to optimize the culturing conditions of the target cells. On the same line, the newly developed systems can be applied to perform on-chip ELISA, revealing the secretion of specific cytokines. The two objectives will next converge, revealing what culturing conditions (flow, gradient of soluble or immobilized molecules, physic-chemical properties of the substrate) can induce the growth and differentiation of specific hematopoietic cells and thus the secretion of characteristic sets of signaling molecules.
In WP4, cell loading and viability tests were performed by using HeLa cells (human cell line derived from cervical cancer). HeLa cells were obtained from American Type Culture Collection and cultured in DMEM supplemented with 2mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin and 10% heat inactivated fetal bovine serum (FBS). HeLa cells were expanded in Petri dishes and maintained at 37°C in a saturating humidity atmosphere containing 95% air and 5% CO_2. Cells concentration was proven to be a crucial parameter for having a good cell loading efficiency into the micro chamber. Following some preliminary tests, a cell suspension with $3X10^5$ cells/ml resulted optimal our experiments. Before to be seeded in the chip, cells were harvested by trypsinization and counted in a Coulter cell counter. 450 □l of cell suspension were added in one reservoir (A3) and three hours were waited for complete

gravity driven cell loading. The process is finally stopped by adding fresh culture medium in reservoirs A1 and A2 (see WP1).

Cell attachment, morphology and growth were monitored for 72 hours by bright-field microscopy. Images were acquired every 12 hours. Cell viability was assessed qualitatively by analyzing cell morphological characteristics (e.g. spreading area, motility, blebbing, etc) and quantitatively by measuring the intracellular esterase activity (Calcein AM assay, C3100— Molecular probes, Eugene, USA) as marker of vitality. Cell doubling time was also measured to evaluate cell metabolism. Cells seeded on standard plastic tissue culture dishes were used as control.

Our data clearly show that micro chambers can maintain cells fully viable for at least 72 hours, showing a percentage of living cells indistinguishable from that measured on the control. Proliferation was also not affected by the microenvironment. After an initial, sample dependent, delay time probably related to non-optimal adhesion and loading, the growth rate reached a steady value similar to that of the control. Assuming a constant growth rate, the doubling time (Td) was calculated according to (Vittorio, Quaranta et al. 2011), obtaining Td = 28±3 hrs, a value in line with what reported in the literature (Chen 1988)
the effect of wall shear stress (WSS) on individual cells or cell monolayers can be analyzed as a function of the basal substrate. Flat surfaces are compared to topographically rich substrates, generated by means of nanoimprint lithography, mimicking the features of a basal matrix. These experiments reveal a complex regulation of cell motility yielding effective differentiation and migration only when the correct interplay between WSS and substrate topography is ensured.
The systems was tested and validated using endothelial monolayers grown on flat or topographically modified substrates and exposed to controlled levels of WSS values ranging from 0.35 to 6 Pa within a custom designed parallel plate flow chamber. This allowed us to characterize the combined effect of substrate topography and WSS on endothelial cell polarization, adhesion, proliferation and spreading. Differentiated endothelia are characterized by the monolayer density and polarization. In order to evaluate the differentiation level of our endothelia we characterized the average cell density and cell area in confluent, growth-arrested monolayers on flat substrates or gratings with or without exposure to WSS. Altogether, the results demonstrate that the effects of substrate topography and WSS are similar and result from the enhanced spreading of individual ECs in the monolayers.

In principle the expression and purfication technologies are suitable for transfer into GMP-compliant process for later clinical application and some of the most important proteins are already developed as GMP products including: Cytokines FGF-2, Flt3-Ligand, GM-CSF, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-15, IL-21, and TNF-α, SCF (coming soon). Additionally, the list is completed by the following antibodies: anti CD3, anti CD28, anti CD137
For the expansion of hematopetic stem cells we have developed the Notch ligand Dll4 immobilised on macroscopic beads which can be used for efficient in vitro stimulation of the Notch pathway. Dll4 can also be provided functionalised (biotin, SH-groups) for conjugation to nanosurfaces.
Based on a recently developed total-internal reflection fluorescence (TIRF) microscopy assay capable of detecting unlabeled target molecules with single molecule sensitivity. we have demonstrated fM limit of detections (LODs) for single stranded DNA and virus particles
These results are reported in two recent publications (Gunnarsson, Jonsson et al. 2008) and (Bally, Gunnarsson et al. 2011). This LOD competes very favorable with alternative label-free detection methods compatible with on-chip readout, such as SPR or LSPR (see Chalmers WP4 report of May 31, 2011). In brief, the presence of a suspended (unlabeled) target molecule is detected by its hybridization to *both* a surface-immobilized probe (complementary single strand) and a probe-modified fluorescently labeled lipid vesicle, within the evanescent field of TIRF illumination. Here, the fluorescently labeled lipid vesicle act as a signal enhancement elements, which can be easily detected on an individual basis, each of which thus being a reporter of a single unlabeled target. With the aim to explore if this method would be suitable for ultrasensitive on-chip detection of IL-2, we have developed a protocol for

antibody coupling to lipid vesicles, which act as signal enhancement elements. IgG as well as single-chain antibody (Ab) fragments have been successfully coupled, as shown for the detection of the Alzheimer biomarker B-amyloid (Figure 3.1).

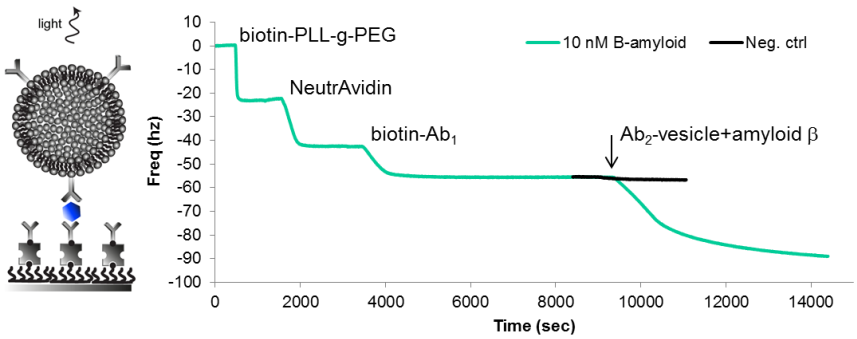


Figure 3.1. On-chip detection of the Alzheimer biomarker B-amyloid.

In order to convert this generic concept from ensemble readout (here quartz crystal micobalance) with nM sensitivity, as shown in the figure above, towards single molecule read-out and sub pM sensitivity for on-chip detection of IL-2, efforts will be devoted to (i) separation of unreacted Abs from Ab-vesicles and (ii) further suppression of non-specific binding, which is partly attributed to the chemical modification of the vesicles used for Ab coupling.

5. Engineering of 2-dimensional cell chip for reprogramming of regulatory T cells

Summary

Software modules to analyze cell features were developed and tested.
We isolated Tregs from standard leukapheresis products by magnetic cell-separation under GMP conditions, using double negative selection (anti-CD8 and anti-CD19) followed by positive selection (anti-CD25).
We have demonstrated in a clinically relevant system that it is possible to generate high numbers of antigen-specific Treg for cellular therapy.
Hardware and software tools have been developed that are required for the visualization, scanning and analyzing BioChip samples.
Use of microwell arrays for analysis and modeling of regulatory T cell differentiation.
We have built and characterized PDMS based MWAs chips, and developed a methodology for long term culture and monitoring of primary T cells within these devices. These devices offer unique advantages over existing technologies for long term monitoring of T cell activation and differentiation with a single cell resolution. We applied these devices to study and characterize the differentiation of mouse CD4+ T cells into regulatory T cells, and also to investigate suppression mechanisms of Tregs.

Description

Interactions of biomolecules with cell surface receptors play an important role in the fields of molecular biology, biochemistry, medicine and diagnostics. Within the last 15 years microarrays have become an important tool for life science researcher in the fields of genomics, proteomics, diagnostics and drug discovery. The aim of our research is the development of a biochip based on a nanostructured surface (fig. 1). This analytical tool can then be used to study interactions between different ligands and cell surface receptors or to study interactions of proteins with other proteins immobilized on the surface.

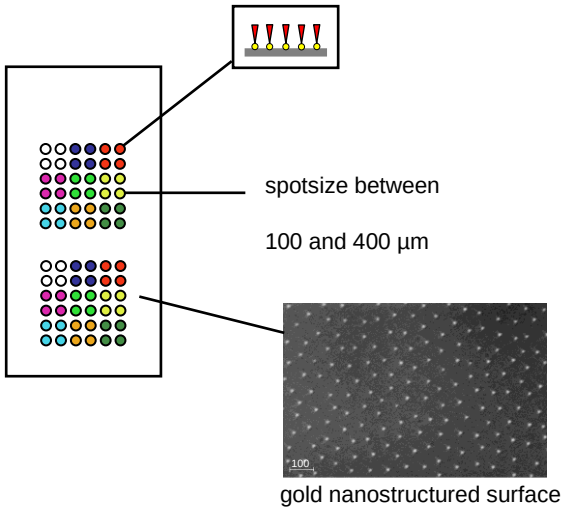


Fig. 1: Idea of the biochip.

As a first step towards this goal we established the biochip (Fig. 2). A glass cover slip is decorated with a gold nanostructure via block copolymer micelle nanolithography. The nanostructure consists of a hexagonal pattern of gold nanoparticles with an interparticle distance between 30 and 200 nm. The separation distance between the dots can be controlled by the molecular weight of the diblock copolymers. Polyethylene glycol is known for its protein resistant character and it is used in our approach to prevent unspecific binding of biomolecules and cells to the glass cover slip. Biofunctionalization of the surface with different biomolecules is achieved with a micro spotter. To prevent unspecific binding of biomolecules to the gold nanoparticles in between the array spots an alkane(PEG)thiole is immobilized on the non functionalized particles. Finally the microarray is washed and only the bioactive molecules which are covalently bound to the gold nanoparticles remain on the surface. For the establishment of all fabrication parameters fluorescent labeled peptides are used.
In conclusion we were able to show that it is possible to develop a biochip on a gold nanostructured surface which is now ready for biological applications.
We aimed to design a platform that can allow long term culture of primary T cells, driving their differentiation into regulatory T cells (Tregs), and monitoring their differentiation state. In addition, we wanted to study the ability of such Tregs to suppress activity of effector T cells, and understand underlying suppression mechanisms.
However, there were several obstacles which we had to overcome to achieve these goals. First, primary naïve T-cells are non-adherent and become highly motile upon activation through their antigen receptor. Second, Treg differentiation is a relatively slow process which takes 3-4 days. As a result, long-term dynamic monitoring of individual cells during the course of activation and differentiation is challenging as cells rapidly escape out of the microscope field of view. We solve these problems using a chip of microwells array (MWA) which is made of a thin layer of PDMS that contains the MWA and is placed inside a glass-bottom 96 well plate.
A potential application of the MWA is the expansion of specific Treg clones with required antigen specificity. For that purpose, it is beneficial to start with microwells containing single cells, and expanding them into clones that can later be isolated for further off-chip characterization or expansion. Our loading scheme results in a random distribution of cells and activation beads in the MWA. Since we are interested in following single cells, we calibrated the average numbers of cells and beads in the loading medium that result in required numbers of cells and beads in the µwells. Additionally, we wanted to verify if the loading scheme is not affected by cell adhesion and clustering, which may reduce the number of µwells containing a single cell.
Establishment of long term culture and live imaging of primary T cells in µwell-arrays allowed us to launch different experimental settings for high throughput single cell measurements that are not possible using standard biochemical techniques. We focus on dynamical changes in gene expression that occur during T cell activation and differentiation into Tregs. We followed

gene expression either using fluorescent proteins as reporters for gene expression, or using continuous staining of cell surface molecules inside the MWA.
Fig. 4a demonstrates dynamic monitoring of gene expression during differentiation of T cells into becoming Tregs. Primary naïve T cells were extracted from a transgenic mouse which expresses GFP fused to the transcription factor Foxp3, which is a key regulator of Treg differentiation. Cells were seeded in the MWA and were induced into Treg differentiation by adding the cytokines TGFβ and IL2 to the growth medium. Expression of Foxp3 in this µwell, indicative of Treg differentiation, starts around 30h after activation, close to the time of first cell division. The total GFP fluorescence in the µwell is plotted in Fig. 4b, showing a steady increase from 30h to 72h (end of experiment).

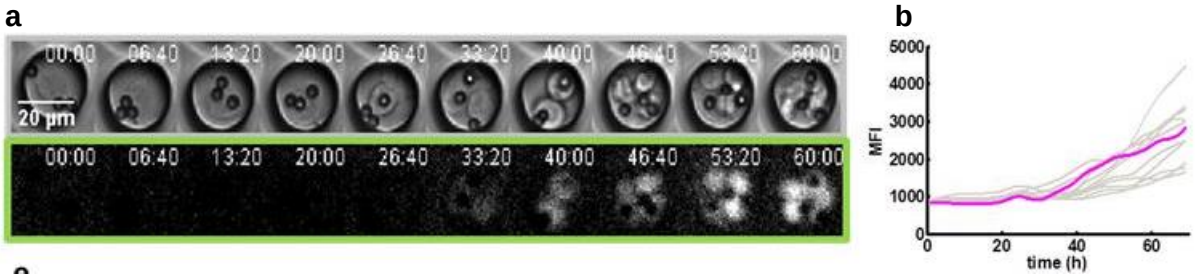


Fig. 4: Dynamics of Treg differentiation followed in a MWA. a) Measurements of Foxp3 up-regulation during differentiation of induced regulatory T cells. Naïve T cells from a transgenic mouse expressing Foxp3-GFP fusion were seeded into a MWA and cultured in the presence of activation beads. Images of one µwell that initially contains one T cell and three beads are shown (Top: bright field; Bottom: GFP fluorescence). b) MFI of the µwell depicted in a, during the course of the experiment (magenta) and of 10 additional µwells from the same experiment.

Monitoring regulatory activity of Treg cells within the device
We further used the device to study mechanisms of suppression by which Tregs suppress proliferation and survival of effector T cells. Compared with existing technologies, our device offers a unique opportunity to decouple contact-dependent and contact-independent suppression mechanisms. Our preliminary results indicate the existence of both types of suppression, and identify contact-independent suppression to be effective only at small intercellular distances of up to about 100µm. We plan to use these unique devices to further study the mechanisms by which contact independent suppression is mediated, by blocking specific cytokines or supplying them at saturating levels.

6. Engineering of 2-dimensional cell chip for directing myeloid differentiation

Summary
Human hematopoietic stem cells were isolated from umbilical cord blood and characterised.
To obtain reference cells for the differentiation of myeloid cells into macrophages and dendritic cells, these cells were derived from human blood monocytes.
The functionalization strategy of the nanopattern surfaces with different choices of relevant peptides and antibodies have been designed and experimentally tested. The success in surface functionality has been tested by quantitiatve QCMD and fluorescent optical microscopy technologies. A nanochip that allows the efficient directed differentiation of monocytes into alternatively activated macrophages or immature dendritic cells has been defined.

Description
We have designed and fabricated a nanochip prototype for the investigation of the activation and adhesion of immune cells, mostly focusing on T cells. The chip is based on micro- and nano-patterning techniques for fine control over ligand density and geometry presented to the cells, together with site-specific immobilization of ligands which preserves their biological activity. Homogenous gold nano-patterns with tunable particle size (3-10 nm) and interparticle spacing (30-200 nm) are produced by block copolymer micellar nanolithography (BCMN). The surface of the nanochip was structured by photolithography, achieving arrays of spots with a diameter of several micrometers. Arrays of spots with large diameters (100-300 µm) were

produced by micro-spotting the proteins of interest. To prevent the non-specific protein adsorption and ensure selective cell interaction with the ligands presented on the gold nanoparticles, the glass area between nanoparticles was passivated by covalent attachment of protein-repellant polyethylene glycol (PEG). As shown in Fig. 1, the Au nanoparticles in between the ligand spots were additionally passivated with an alkane PEG-thiol.

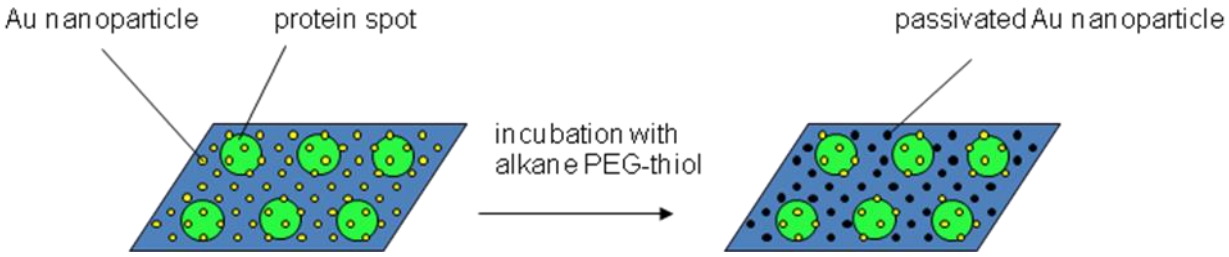


Fig. 1: Passivation of the Au nanoparticles in between the ligand spots during the fabrication of the nanochip.

In order to immobilize T cell relevant ligands in a site-directed manner, gold nanoparticles were functionalized with a thiol-NTA linker and $NiCl_2$, thus allowing oriented binding of single His-tagged proteins. By using His_6-Protein G/ His_6-Protein A, we were able to further attach different activating antibodies and Fc-tagged proteins such as anti-CD3 and Fc-ICAM-1 (see Fig. 2).

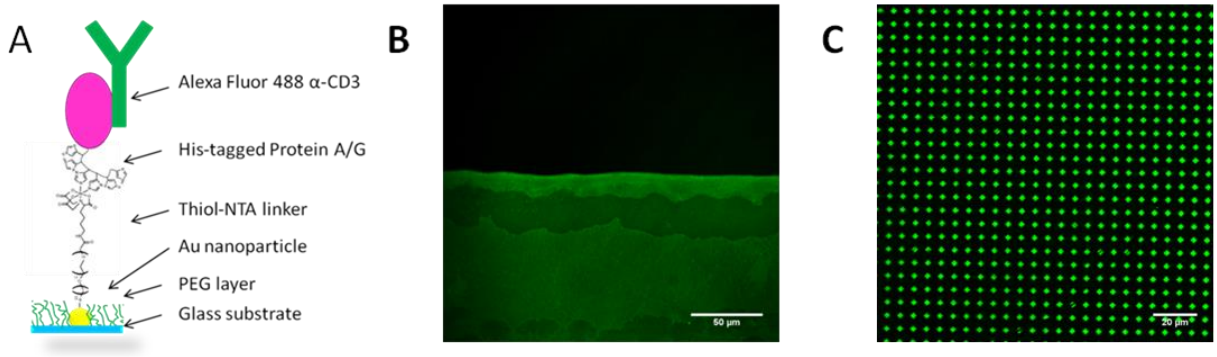


Fig. 2: Anti-CD3 functionalization via thiol-NTA/Ni(II)/His6-ProtG is selectively confined to nanostructured area. (A) Functionalization scheme. (B) Homogenous nano-pattern (interparticle distance 40 nm) with a clear borderline between nanostructured and passivated area of the substrate (anti-human CD3). (C) Micro-spots of nanostructures (spot diameter ~3 µm, interparticle distance 55 nm) surrounded by passivation field (anti-mouse CD3). Passivation of the Au nanoparticles in between the ligand spots during the fabrication of the nanochip.

The successive binding of each component anchoring anti-CD3 to the nano-particles was confirmed on homogenous gold surface by means of QCM-D. Fluorescent microscopy has proven the selective binding of labeled antibody to the nanostructured area, but not on passivated glass (Fig. 2). Jurkat cells (clone E6.1) grown for 1h on anti-CD3 nanochips showed greater extent of activation than on randomly coated anti-CD3 plastic well plates, judging by higher IL-2 production on-chip (Fig.3).

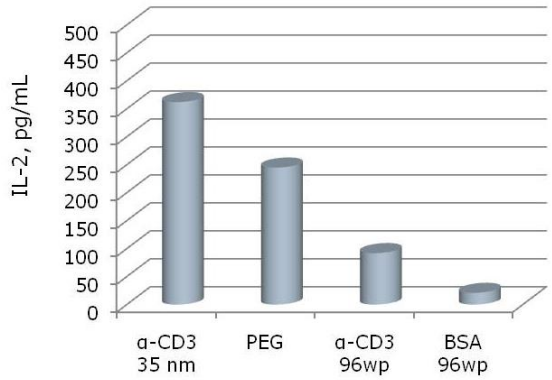


Fig. 3: Homogenous anti-CD3 nano-patterns are bioactive and stimulate T cells to a greater extent than plastic well bottoms randomly coated with anti-CD3 (IL-2 production by Jurkat E6.1 cells grown for 1h on various substrates).

Flexible coupling chemistry allows us to attach with only slight modifications various monocyte relevant adhesion and survival factors, such as anti-CD11b, anti-CX_3CR1 or Fc-CX_3CL1. All of the mentioned molecules are micro-spotted in a format suitable for high-throughput screening microscopy that has already been adapted for the WiScan system, as shown with the fluorescently labeled BSA prototype.
In conclusion we were able to fabricate a prototype nanochip that will allow the efficient directed differentiation of monocytes into alternatively activated (M2-polarized) macrophages (M2-MF) or immature dendritic cells (iDC).

7. Engineering of 2-dimensional cell chip for generating central memory veto CD8 T cells

Summary
Development of protocols for large scale expansion of cytokines and cell culture medium.
Anti-3rd party veto CD8 T cells with central memory phenotype ('Tcm') can support engraftment of T cell depleted bone marrow (TDBM) allografts under reduced intensity conditioning.
Anti 3rd party 'Tcm' support engraftment of TDBM allografts in sub-lethally irradiated mice.
'Tcm' mediated chimerism specifically protects donor skin grafts from rejection.
Anti 3rd party 'Tcm' can induce tolerance to TDBM allografts under an irradiation-free regimen.
In–vivo imaging reveals unique migration and expansion of anti-donor T cells in the presence of donor 'Tcm'

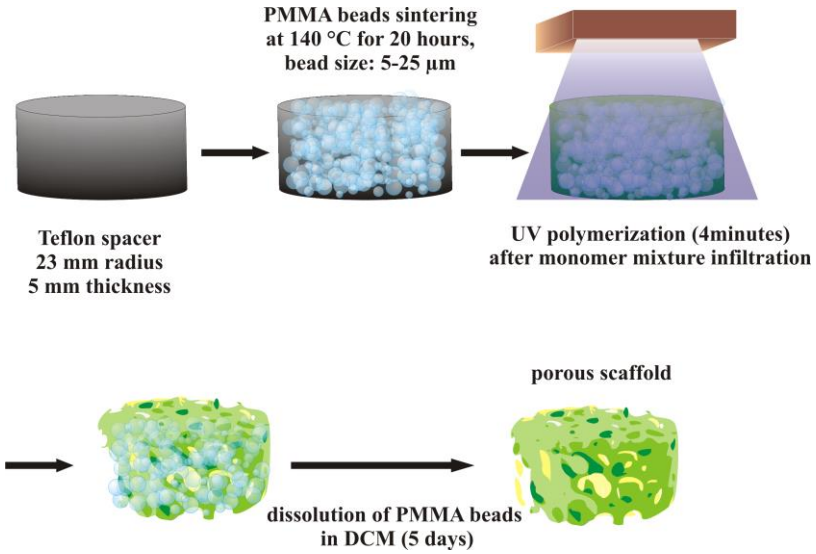
Description
CD8+ T cells were isolated from human blood to provide a protocol for the isolation of these cells. Furthermore, Miltenyi has established isolation procedures for naive T cells, which should be used in GMP compliant processes for the in vitro generation and/or modification of TCM suppressor cells.
We have optimized the nanochip with special emphasize on its application for the generation of central memory veto CTLs. Particularly, we have tested several candidate chip coatings such as poly-L-lysine, PLL-g-PEG, and silane-PEG. In the first two cases the surface is modified in a non-covalent way, while in the third case, covalent bonds between the surface and the polymer are formed. The nanochip prototypes served as substrates for cell cultures for 5-6 days. It turned out that covalently bound PEG is most suited to prevent unspecific cell adhesion and activation.
The marked capacity of the 'Tcm' to tolerate anti-donor T cells demonstrated above in lethally irradiated recipients, indicates that 'Tcm' could potentially enhance engraftment of TDBMT under reduced intensity conditioning (RIC), a difficult challenge due to the robust host immunity remaining after this kind of conditioning.
To that end, BALB/c mice were sub-lethally irradiated [5.5Gy total body irradiation (TBI)] on day -1 and on the next day received $20x10^6$ 'megadose' of nude-C57BL/6 BM, with or without $5x10^6$ allogeneic C57BL/6 'Tcm'. All 6 mice in the control group that received only irradiation, without BMT, survived for more than 6 months following the irradiation, demonstrating the non-

myeloablative nature of the conditioning. While all 13 recipients of BM alone rejected the graft by day 30 post transplant, 9/9 of the mice that received allogeneic 'Tcm', in addition to BM, displayed stable full multi-lineage donor chimerism that persisted for more than 6 months post BMT. As in lethally irradiated mice, enhancement of engraftment by allogeneic 'Tcm' in these sub-lethally mice was not associated with any appreciable GVHD. Thus, the weight and overall appearance of mice receiving allogeneic 'Tcm' were the same as that of control mice which were radio-protected with a transplant of Nude-BM alone.
To verify that the observed enhancement of engraftment is not mediated by residual alloreactive T cells, we also tested 'Tcm' derived from (CB6)F1 mice for their ability to enhance engraftment in this RIC model. The results obtained demonstrate that donor BM engraftment was slightly reduced compared to engraftment in the allogeneic setting. Thus, 6/7 of the mice that received F1 'Tcm', in addition to BM, exhibited only mixed chimerism (81±11%) in contrast to the full chimerism exhibited when using allogeneic 'Tcm' (Fig 1). Nevertheless, this mixed chimerism, induced by F1 'Tcm', was stable and persisted for more than 6 months. The chimeras displayed full donor chimerism in both B cell and myeloid cell compartments and mixed chimerism in their T cell compartment (54±9%). The observed enhanced engraftment exhibited when using fully allogeneic 'Tcm', compared to F1 'Tcm', probably results from residual alloreactive T cells remaining in the allogeneic 'Tcm' preparation, the number of which is below the threshold for GVHD induction.

8. Scaling up the cell stimulation process – from cell chips to "lineage selective cell reactors"

Summary
Development and extensively validation GMP-grade protocols for the isolation of highly purified human CD25+ FOXP3+ Treg cells for efficient expansion using the nano-patterned surfaces (therapeutic surfaces) and cell chips.
Miltenyi has developed and further optimized the clinical large scale separation and expansion of regulatory T cells including the development of large scale and GMP compatible expression strategies for cytokines and antibodies.
A current rapid expansion protocol for naïve Treg (e.g. the optimal cytokine cocktail) has been incorporated into the fabrication of large scale growth vessels for massive ex vivo expansion of Tregs, to be used for *in vivo* preclinical testing in mice has been established.
Miltenyi has developed and further optimized the clinical large-scale separation and expansion of regulatory T cells (see also WP5) including the development of large scale and GMP compatible production strategies for cytokines and antibodies (see also WP4). Miltenyi has further worked on testing various membranes, providing gas exchange for Treg cell culture to improve large scale Treg growth in bioreactors.
Scaffolds of poly(caprolactone) (PCL), poly(ethylene glycol) (PEG), 2-methylene-1,3-dioxepane (MDO) and 2-(hydroxyethyl)methacrylate (HEMA) have been prepared by EPFL. The scaffolds are microporous hydrogels that are biodegradable and nonfouling. They were coated with peptides at various concentrations and decorated with gold nanoparticles to be further used in cell culture experiments.

Description
Biodegradable, micro-porous, polymer scaffolds have been constructed, and their mechanical properties and biodegradation have been investigated.
In a first attempt, the scaffold was constructed by the stacking of multiple films of a microporous biodegradable polymerof polylactide-co-caprolactone (PLC) in the ratio of 2:1. The porosity of the films was achieved via the breath figure technique. Due to some issues with the stacking of the films, three dimension scaffolds were prepared by a sphere-templated process (Scheme 1).



Scheme 1: Schematic representation of the different steps of the fabrication of the porous scaffold by the sphere-templated process.

The mechanical properties of the scaffolds were tested with Minimat machine with 20N load cell. The tensile strength range of the scaffolds is 0.33±0.07 MPa.

The degradability was evaluated in PBS at room temperature and the results showed that the scaffold had good biodegradable properties (Figure 1).

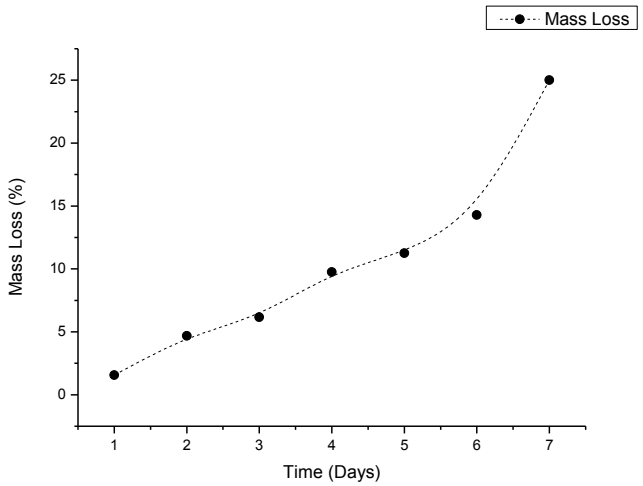
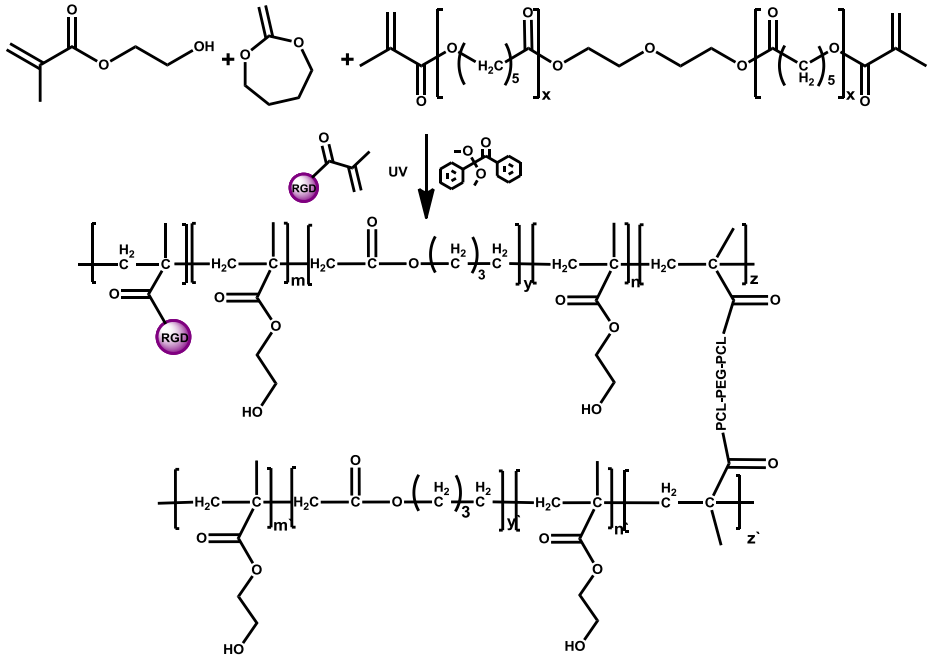


Figure 1: Graph of the mass loss during the degradability experiment.

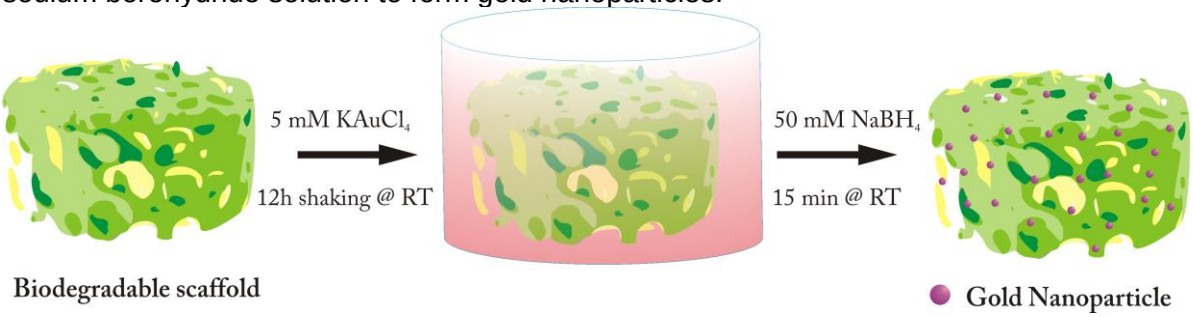
To obtain cell adhesion on the non-fouling polymer scaffold, RGD peptides were integrated as described in Scheme 2.

The presence of RGD peptides was confirmed by XPS analysis No nitrogen peak was visible on the spectra recorded for a non-functionalized scaffold. When RGD peptides were integrated in the scaffold, a peak was observed with its intensity proportional to the amount of peptides present in the scaffold.



Scheme 2: Synthesis of the RGD-functionalized scaffold

For the nanostructuration, the scaffolds were decorated with gold nanoparticles through the reduction of gold(III) ions by sodium borohydride (Scheme 3). In a first step, the scaffolds were immersed in a solution of potassium gold chloride. Afterwards the gold ions were reduced with a sodium borohydride solution to form gold nanoparticles.



Scheme 3: Schematic representation of the fabrication of the gold nanoparticles decorated scaffold.

9. Preclinical assessment of Type-1 diabetes mellitus (T1DM) therapy using the various *in vitro* generated cell types

Summary

A novel transgenic model that allowed us to determine the interelation of Ly6C+ and Ly6C- blood monocytes and their respective circulation half lives has been established.

Description

Monocytes are recruited to sites of inflammation and could hence serve as vehicles to deliver regulatory activities. In an attempt to establish a genetic manipulation protocol for monocytes we aimed to manipulate the cells by lentiviral infection. In a series of pilot experiments we isolated murine monocytes from the bone marrow, transduced them with a lentivirus harboring a GFP reporter gene and cultured the cells in vitro. As seen in Fig. 1 Ly6C+ monocytes were efficiently transduced, and survival of the cells could be improved by addition of Csf-1/ M-CSF to the short time culture.

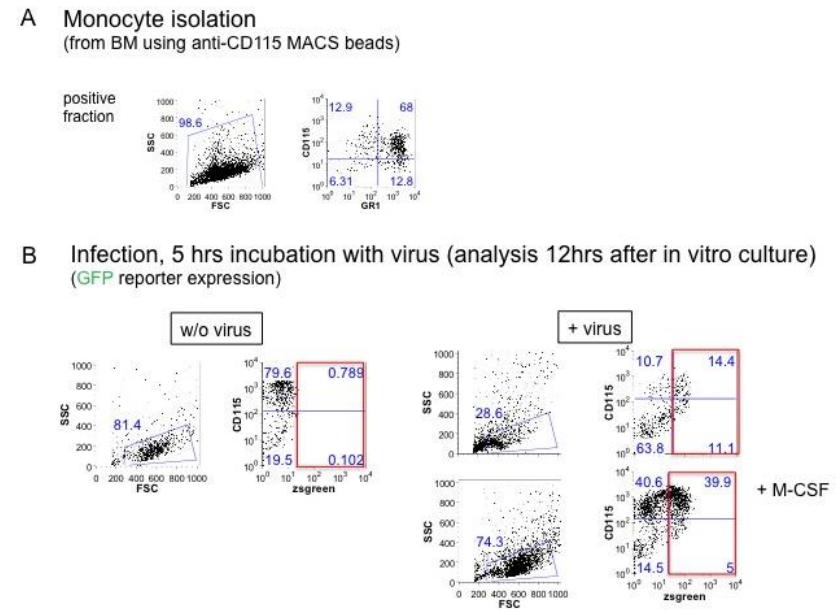


Figure 1. Lentiviral transduction of primary murine monocytes. (A) Monocyte isolate from bone marrow using MACS bead purification. Note that majority of cells are CD115+ and Gr1 (Ly6G/C)+. (B) FACS analysis of monocytes after exposure to lentivirus and 12 hour culture in presence of absence of M-CSF. (note appearance of GFP+ population in transduced samples and that M-CSF addition improves the frequency of transduced cells.

Next we addressed the potential of the transduced cells to give rise to monocyte-descendents in an in vivo context. Here we resorted to an experimental system we had previously established and that is based on the conditional ablation of endogeneous macrophages and their replacement by grafted monocytes. As seen in Fig. 2, while untransduced, GFP- monocytes readily reconstituted the host macrophage compartment, no GFP+ cells could be detected.

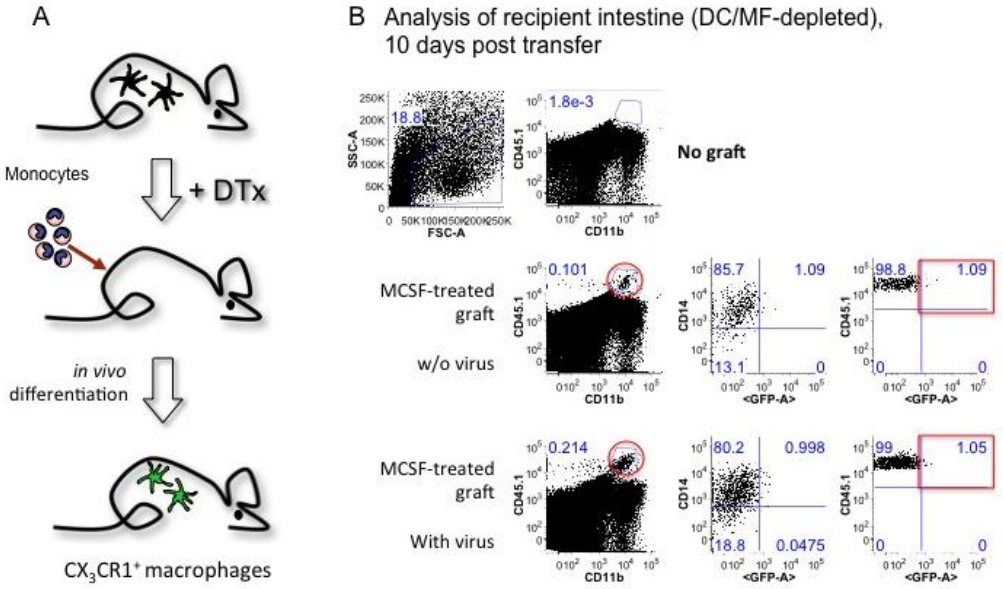


Figure 2. Assessment of in vivo differentiation potential of transduced monocytes. (A) Schematic of transfer model (for details see Varol et al, Immunity 2009) (B) FACS analysis of intestinal macrophage compartment of DC/MF-depleted animals that received transdued of untranduced monocyte graft. Note population of graft derived macrophages, identified according to CD45.1 marker, but absence of GFP+ macrophages.

Collectively, these data strongly suggest that although monocytes can be efficiently transduced, the lentitiviral transduction interferes with their potential to give rise to

macrophages in vivo. Alternative strategies such as the delivery of DNA or RNA via nano-particles should be tested, given that monocytes are phagocytic cells.

10. Preclinical assessment of Inflammatory Bowel disease (IBD) therapy using the various *in vitro* generated cell types

Summary

We have established an experimental pre-clinical model the exT_{regs} by their capacity to prevent inflammatory bowel disease (IBD) / colitis in a pre-clinical mouse model of x-GVHD.
We compared monocyte descendants in healthy gut to monocyte descendants in gut tissue subjected to an acute inflammatory challenge.
We focused on studying the role of CD103 DCs in the inflamed gut. We demonstrated that CD103 DCs are critical for taking up inflammatory bacteria from the lumen of the small intestine and initiating inflammation.

- elucidation of the cells and molecular mechanisms responsible for uptake of inflammatory bacteria from the gut for presentation to T cells

- establishment of therapeutic protocol to manage inflammatory Ag-dependent colitis in animals based on:
a. the depletion of cd1103 DCs
b. blockade of chemokinesis
c. TLR signaling

Description

Intestinal macrophages derive from Ly6C+ blood monocytes that differentiate in the healthy colon into non-inflammatory cells that are critical for the maintenance of gut homeostasis. In a study that is about to be published in Immunity and in which EU7 support is acknowledged, we show that in inflamed tissue monocyte differentiation into resident quiescent macrophages is blocked. Rather the cells give under this condition rise to effector monocytes that respond to bacterial products the encounter in the gut connective tissue and are triggered to secrete proinflammatory cytokines (IL-6, IL-23). Ablation of these cells improves gut inflammation, highlighting these cells as potential future targets for IBD therapy. Our results suggest that once in virto systems for the manipulation of monocytes are in place they should aim to impair the differentiation of Ly6C+ monocytes into effector monocytes and boost their acquisiotion of the non-inflammatory phenotype.
Our studied focused on the definition of monocyte fates in the healthy and inflamed colon to identify targets for the planned in vitro manipulation of monocytes and its use for cell therapy.
We focused on studying the role of CD103 DCs in the inflamed gut. In a study that is was accepted upon minor revisions to Immunity and in which NanoII support is acknowledged, we demonstrated that CD103 DCs are critical for taking up inflammatory bacteria from the lumen of the small intestine and initiating inflammation. We have also demonstrated that they interact with T cells in the mesenteric lymph nodes which are known to be colitogenic. Several treatments were shown to be effective in preventing bacterial sampling, including blockade of chemokinesis, TLR signaling, and depletion of CD103 DCs. These steps point to promising approaches in treating IBD.

11. Optimizing the cell-chip system for the selective expansion of human regulatory cell populations, and setting up protocols for phase I clinical trials

Summary

We focused on developing and testing various rapid expansion protocols designed to generate large numbers of functional T_{regs}, derived from PBMCs of blood donors, to be used for a phase I clinical study.
Miltenyi has developed GMP separation and expansion tools (GMP CD3/CD28 functionalized micro-particles, GMP cell culture media, GMP cytokines) for human CD25+Foxp3+ regulatory T cells. Furthermore Miltenyi has developed GMP tools for direct isolation of antigen-reactive Treg.

We managed to establish a robust, sensitive, fully functional human immune system in mice, suitable for monitoring the functional stability and nature of in vitro generated/induced and adoptively transfered immune effector cells.
The newly developed 3D-light-sheet-fluorescence microscopy technique LSFM set-up allows the analysis of cultured primary immune cell populations such as polyclonal and Ag-specific Treg cells, Mreg cells or central memory veto CD8+ T cells in nanofabricated cell scaffolds to scan cell-interactions in 3D at high-speed and 4-colors simultaneously in a high-throughput manner.
Besides Treg and other T cells, our group has established and evaluated a protocol for the GMP grade isolation and expansion of human Natural Killer (NK) cells.

Description

We have focused on developing and testing various rapid expansion protocol designed to generate large number of functional T_{regs}, derived from PBMCs of blood donors, to be used for a phase I clinical study. For this goal we have been developing and validating standard operating procedures (SOPs) to be used in compliance with good manufacturing practice (cGMP), as follows: (i) SOPs to obtain very pure population of viable naïve $CD45RA^+$ T_{regs}; (ii) SOPs for the ex vivo numeric expansion of naïve T_{regs} (>500-fold expansion); (iii) SOPs for analyzing the functional phenotype of the *ex vivo* generated T_{reg} cell product (exT_{regs}); and (iv) protocols to determine the *in vivo* function of the exT_{regs} by their capacity to prevent inflammatory colitis in a pre-clinical mouse model of x-GVHD.
The unique capabilities and insights from our current rapid expansion protocol for human naïve T_{regs} together with insights developed by the other partners in the various WPs are all currently being incorporated, by the relevant partners (material scientists), into the fabrication the nano-patterned "smart" bio-functional surfaces and cell chip prototype for optimal and reproducible propagation of human exT_{regs}. Such exTregs are to be used initially for *in vivo* preclinical testing in our recently established mouse model. Once finalized the 'optimal' cell-chip prototype and relevant SOPs will be made available to all the clinical participants. In addition, we are initiating discussions with the other partners involved in WP11 for designing a multicenter open label dose-escalation phase I clinical study of the exT_{reg} cell product.
We proved that the anti-CD3 mAbs (aCD3) nano-patterned smart surfaces (therapeutic surfaces) can induce improved activation and proliferation of human $CD4^+$ T cells derived from PBMCs of healthy blood donors compared to "traditional" plastic surfaces coated with anti-CD3 mAbs. On these nano-patterned arrays the aCD3 mAbs was immobilized on gold nanoparticles (AuNPs) in an orientated, bioactive manner and its surface density was finely tuned by varying the inter particle distance. Nanoarrays were fabricated by block copolymer micellar nanolithography (BCML), a self-assembly technique enabling relatively fast nano-patterning of large surface areas that presents an advantage for large-scale clinical applications. Immobilization of aCD3 through nano-patterning had two effects: cell activation was significantly higher on these surfaces than on aCD3-coated simple plastic surfaces and moreover the aCD3 nanoarrays allowed unprecedented fine-tuning of T cell responses. A part of these novel and important findings was very recently published in the high impact journal Nano Letters (Matic et al. *Nano Lett* 2013 Nov;13:5090-7. doi: 10.1021/nl4022623. Epub 2013 Oct 17).
We have developed and tested various rapid expansion protocols (REP) designed to generate large number of functional $CD4^+$ T_{reg} cells, derived from PBMCs of healthy blood donors. For this goal we have developed and validated standard operating procedures (SOPs) to obtain very pure population of viable naïve $CD45RA^+$ T_{regs}, SOPs for the *ex vivo* numeric expansion of naïve T_{regs} (>500-fold expansion), and SOPs for analyzing the functional phenotype of the *ex vivo* generated T_{reg} (exT_{reg}) cellular product.
We have also determined that this exT_{reg} cellular immunotherapy product can prevent harmful T cell mediated immune responses in a pre-clinical animal model. For this aim we set up an experimental model of engrafting human peripheral blood derived T_{con} cells into immune deficient NOD/*Scid*/Gamma^{null} (NSG) mice to mediate "lethal" acute x-GVHD that is associated with multi-organ inflammation with severe colitis. We found, as predicted, that NSG recipients of PBMC-derived T_{con} alone succumbedto severe colitis, whereas contrastingly the NSG mice

that were adoptively co-transfered with exT_{reg} survived until the end of the experiment with only minimal weight loss and no colitis signs.
These multiple pre-clinical data generated in the *NanoII* project strongly supports our working hypothesis that cellular therapy with *ex vivo* expanded post-REP human T_{reg} cells is a practical approach to prevent autoimmunity including Type 1 Diabetes Mellitus (T1DM) and colitis. Thus we aim to proceed in the near future and conduct a phase I study to test the side effects and best dose of blood-derived polyclonal nTreg in subjects (patients) recently diagnosed (<6 months) with T1DM. This study might also allow us to estimate whether this cellular immunotherapy treatment might work in patients to prevent the loss of insulin secretory capacity and the development of life-long T1DM. As stated in the proposal this trial is designed as an open label dose-escalation phase I study. The primary endpoints are dose-limiting toxicity of the exT_{reg} -product and the C-peptide response to a mixed meal at 12 months after study entry. We aim to enroll a total of 30 subjects at the various clinical study sites.Study subjects (Age 8 – 30 with new onset T1DM of 4 - 6 months; stimulated C-peptide levels >= 0.5 pmol/ml; mentally stable; and able to comply with the procedures of the study) will receive I.V. escalating doses of blood-derived exT_{reg} cells on day 0 until the maximum tolerated dose (MTD) is obtained [but not to exceed 1×10^7 CD4+ exT_{reg} cells per kg of body weight]. Patients will then receive a second dose of exT_{reg} cells, at their respective MTD, on day +14.

12. Integrative and Translational Approaches to Industrial Market

Summary

During the various project meetings the potential commercialisation issues and strategies of the industrial partners have been presented to the academic partners.
Furthermore a detailled „Business and Exploitation Plan" has been made based on market analysis and the scientific developments planned in the consortium.
During the various project meetings the potential commercialisation issues and strategies of the industrial partners have been presented to the academic partners. Vice versa, new developments of the academic partners have been scanned by the industrial partners for their commercialisation potential.
We generated induced pluripotent stem cells (iPSCs) from renal epithelial cells present in urine (HEPTECs). Patent situation and market situation have been screened as well and an integration free reprogramming technology was licenced in by Evercyte.
IDEA Bio-Medical interact with scientists in universities and pharmaceutical companies and introduce adaptations to its technology based on their input and requirements.

Description

The main topic is the identification and support of commercialization opportunities of scientific developments within the project, with regard to the development of tools for the use of regulatory T cells in clinical therapies of autoimmunity and inflammatory diseases. The focus of this report are the activities concerning feasibility studies to assess commercialization potential of developments achieved in NanoII as well as an evaluation of potential strategies for market introduction. This was mainly accomplished by industrial partners and coordinated by Miltenyi Biotec together with the project coordinators. During the various project meetings the potential commercialization issues and strategies of the industrial partners have been presented to the academic partners. Vice versa new developments of the academic partners have been scanned by the industrial partners for their commercialization potential. Based on this defined interactions have been started which are summarized below. These activities were supported by the developed monitoring tool, which was used for continuous monitoring throughout the project to identify important developments early-on and provide support to optimize the translational aspects. Furthermore a detailed „Business and Exploitation Plan" has been made based on market analysis and the scientific developments planned in the consortium.

13. Educational programs

Summary

Promoting professional training and development for students and young researcher and to establish a network for further career development in this interdisciplinary area within the European Commission.

Since the initiation of this multi-partner project we have been continuously promoting the professional development Ph.D. students and young researchers and encouraging them to establish a network with their peers, for further career development in the interdisciplinary topics related to ***NanoII.***

Regular workshops of NanoII have been held up.
Here scientists in the fields of modern biology, biophysics, material science, clinical research and industry have been discussing relevant topics of NanoII.
These workshops have been giving to the participants the unique opportunity of sharing expertise in these important fields of research. Especially the interaction between nanotechnology, biophysics, cell biology, theoretical and clinical immunology and relevant industrial partners promote the scientific and industrial system of Europe, resulting in an expansion of the scientific and technological frontier. A strong emphasis has been laid on the training of the young PhD and Postdoc scientists. Therefore a significant contribution to the improvement for the human resource potential has been made. The workshops coincided with the project meetings, so that the presence of the senior-scientists from diverse fields has been giving the opportunity to the young scientists to have fruitful discussions. The participation of industry in the workshops has been shown to the young researchers a necessity of close cooperation between scientific institutions and industry to achieve an efficient technology transfer.
Special training programs have been opened at children's university hospital of Tübingen, the Max-Planck-Institute, the Weizmann-Institute, the Sheba-Medical-Center and the University clinic of Würzburg.
In the frame of the NanoII workshops excellent opportunities and training has been given to the young researchers to present and discuss scientific topics and results.

NanoII PhD/Postdoc Workshops

- Kick off meeting 12.-13. Januar 2010, Stuttgart
- NanoII Meeting 06.-08. June 2010, Rehovot (**Participation of 21 Young researchers**)
- PhD/Postdoc Workshop, Ringberg Castle, Kreuth, Germany (**Participation of 28 Young researchers**)
- NanoII Meeting 05.-07. April 2011, MPI Stuttgart (**Participation of 14 Young researchers**)
- Industrial Workshop 09.05.2012, Stuttgart
- Principal Investigator Meeting 13.09.-14.09.2012, Stuttgart (**Participation of 12 Young researchers**)
- Final Meeting 17.10.-18.10.2013, MPI Stuttgart (**Participation of 16 Young researchers**)

15. IDEG – IPR, Dissemination Ethics and Gender

Summary

The coordinator and the consortium regularly:
(i) identify product development opportunities; (ii) Implement a periodic project-concomitant discussion and brainstorming sessions to explore commercialization opportunities; (iii) oversee IPR protection, (iv) supervise dissemination of **Nano*II*** project results, and (V) monitor Ethical and Gender issues.
We have been working actively on ethical and gender issues.
The progress of the project and discussion of potential IP right protection issues has been accomplished during the bi-annual project meetings.
We have established regular meeting (every 2 months) with our industrial partners for early identification of potential development opportunities.
The Scientific and Project Management Board of ***NanoII*** has been working continuously on

1. Identification of product development opportunities
2. Stimulation of discussions for commercialization opportunities
3. Check of planned publications under consideration of IPR/commercialisation aspects

4. Monitoring of fulfilment relevant ethical and gender related issues.
5. Broad dissemination of NanoII results.

We have established a monitoring tool for potential IP generated during the NanoII project. A discussion session between all industrial partners within NANOII has been organized on 09-05-2012 in Stuttgart to discuss IPR and exploitation strategies.

I. gender aspects

All participating institutions have long-established promotional activities for encouraging women into research careers. The following tables prove let a significant percentage of female researchers were participating in NanoII and performing scientific work. It has been a goal of the project management board that a maximum percentage of female researchers (PhD, candidates, Postdoc associates and group leaders) will be responsible in NanoII.
The gender directive – council directive 2004/113/IC of 14th December 2004 implementing the principal of equal treatment between man and woman in the access to supply of good have been considered in NanoII.
In NanoII female researchers were not only not discriminated but were actively promotive.

female researchers in NanoII

	last name	first name	construction
1	Appel	Silke	University of Bergen
2	Bacher	Petra	Miltenyi Biotec GmbH
3	Cherkes	Ira	Tel Aviv Sourasky Medical Center
4	Desseuax	Solenne	Ecole Polytechnique Federale de Lausanne EPFL
5	Farache	Julia	Weizmann Institute of Science, Rehovot
6	Gold	Julie	Chalmers University, Gothenburg
7	Lee-Thedieck	Cornelia	Max-Planck-Institut für Metallforschung, Stuttgart
8	Lichtenstein	Alexandra	Weizmann Institute of Science, Rehovot
9	Lieber	Shimrit	Weizmann Institute of Science, Rehovot
10	Matic	Jovana	MPI, Stuttgart
11	Mills	Kristen	Max-Planck-Institut für Metallforschung, Stuttgart
12	Mundinger	Tabea	Max-Planck-Institut für Metallforschung, Stuttgart
13	Nagar	Meital	The Sheba Cancer Research Center, Israel
14	Ottmüller	Katja	Universitätsklinikum Würzburg
15	Paran	Yael	IDEA Bio-Medical

16	Polonsky	Michal	Weizmann Institute of Science, Rehovot
17	Preyer	Rosemarie	Autoimmun Diagnostika GmbH
18	Schilbach-Stückle	Karin	Universität Tübingen
19	Mockel-Tenbrinck	Nadine	Miltenyi Biotec GmbH
20	Gamradt	Stefanie	Miltenyi Biotec GmbH
21	Schön	Yvonne	MPI, Stuttgart
22	Shahal	Dr. Tamar	Weizmann Institute of Science, Rehovot
23	Tonazzini	Ilaria	SNS, Pisa
24	Vernitsky	Helly	The Sheba Cancer Research Center, Israel
25	Wandner	Ann-Kathrin	MPI, Stuttgart
26	Yahalom	Yfat	Weizmann Institute of Science, Rehovot

All together a high percentage of female researchers has been realized in NanoII.

II. Ethical issues

The project members have been keeping in mind to comply with the ethical principles contained in the current version of the declaration of Helsinki.
Approvals of the local ethics committees have been obtained where necessary:
Where study subjects have been necessary (e.g. Sheba Medical Center), signed, written informed consent was obtained from all study subjects-compliance with the EU-directives (e.g. 95/46 E.C) on the protection of human data, strictly upheld.
The ethical regulations in the convention for the protection of human rights and dignits of the human being with regard to the application of Biology and medicine convention on human rights and biomedicine.
(Oviedo 4.4.1997) incl the additional protocol to the convention on human rights and biomedicine concerning biomedical research
(Strasbourg 25.1.2005) have been kept by the investigators.
A very important point is the fact that a lot of effort is made by the participating research institution to minimize pain, discomfort and fear of children.

The compliance with the ethics review requirements have been achieved

Potential impact and main dissemination activities and exploitation results

Cellular therapy is generally considered an investigational medicinal product (IMP) of higher risk as compared to other IMPs. Thus, a **first-in-man clinical trial** of an IMP such as cellular therapy with exT_{reg} cells induced by "smart" nano-patterned surfaces (an IMP that specifically targets the immune system) are regulated differently and undergo a painstaking assessment process. The term 'higher risk' means that the assessment of the IMP by the relevant regulatory authorities is done with greater care and by multiple experts. The high risk to patients associated with transition from pre-clinical studies of *ex vivo* generated Treg cells to the very first-in-man clinical trial thus require very rigorous and comprehensive pre-clinical studies to support approval of the clinical trial by the regulatory authorities. For those reasons, they are usually done late in the development of the cellular IMP.

Thus before proceeding to obtain regulatory approval for the planned phase I clinical trial in T1DM patients, we consulted with a few independent physicians highly experienced in first-in-man phase I clinical studies. The general conclusion was that our cellular product is not "mature" enough to obtain regulatory approval. For example at this stage of development our aCD3 nano arrays are not manufactured in accordance with EU GMP (good manufacturing practice) requirements and obviously these "therapeutic surfaces" had not undergone all the analyses, tests or checks necessary to confirm its quality in accordance with the necessary Clinical Trial Authorization guidelines. To address this issue and to ensure that **Nano*II*** brings major benefit to European industry, the **Exploitation board** of the consortium is exploring with our commercial partners the option to commercialize our nano patterning based technology to generate CD4+ exT_{reg} cells. *Miltenyi Biotec GmbH,* one of the world-leader in hematopoietic stem cell therapies is predicted to play a central role in the commercialization of the CD4+ exT_{reg} technology that was developed in the ***NanoII*** project. Obviously, developing such radically new technologies, as developed in ***NanoII***, to the level of a clinical grade cell therapy product is an extensive process that requires a large investment of capital and resources. Moreover, Standard Operating Procedures (SOPs), Batch Record and Drug Master File necessary for cell-based medicinal product will be defined and prepared for submission to relevant regulatory authorities.

Upon translating the technology developed in the ***NanoII*** project into a standardized cellular, GMP-compatible, IMP, the sponsors (***NanoII*** consortium and future commercial partners) will initiate a phase I trial of exT_{reg} in new onset T1DM patients as detailed above. Currently most of the **first-in-man** phase 1 trials in the EU are conducted by CROs. Thus the sponsors may transfer any or all of their trial-related duties and functions to a CRO with a proven track record in cell therapy IMPs. The sponsors will ensure that setting up a rigorous clinical trial data monitoring system such as all potential side effects will be registered and promptly reported to the local regulatory authorities. Electronic Data Capture (EDC) solutions are widely adopted by pharmaceutical companies and CROs as they expedite the time to market for IMP. Thus, all trial data and results will be collected in a standardized data collection electronic form that will be shared by all the groups of the consortium. For this purpose the CRO will install an EDC system for the collection of all the relevant clinical data in electronic format in all the trial sites. The EDC will allow accurate streamline data collection and safety monitoring by the sponsors.
Data management will comply with the following guidelines:
Data management system should include data entry, storage and retrieval, verification, and correction options. Data managers should have computer systems that are secure and allow only authorized access to the data; contain an internal audit trail so that all changes to the data are documented and that entered data is not deleted; backup database; the database setup and verification checks have been tested with "dummy" data before any real trial data are entered; enter the data twice, or once with 100% check of data; keep records of all queries and their resolution; and have a formal procedure for locking and unlocking the database.

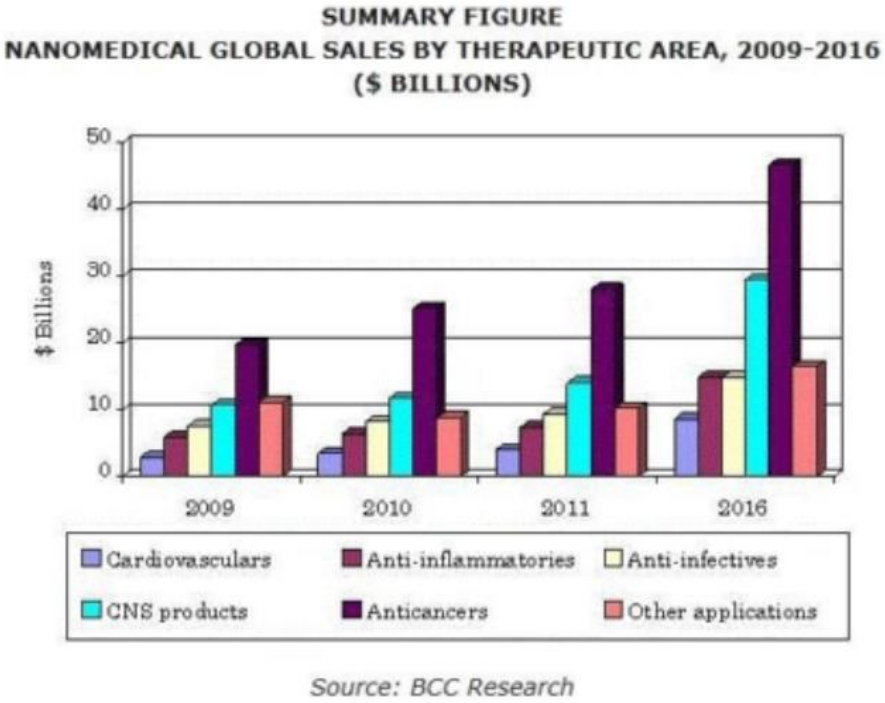
Commercial impact

The possible volume of the cell array market can't be estimated exactly as the technology is still immature and only few products are on the market. It is too young to enable the definition

of a comprehensive business model and strategy. Many perceive it as being in its infant phase. However, conclusions can be drawn from related fields of cellular therapy that is more established in clinical settings. However, the estimations given below should be considered critically. They indicate that there is an enormous economic potential but, as the evident differences in the numbers show, the real market is very difficult to predict because there are still few cellular products and the development was more slowly than initially expected. Consequently, we will start our considerations with 1. the global nano-medicine market, focusing thereafter 2. on biochips taking into account their 3. market dynamics. The therapeutic concept (4.) will lead to the envisaged products and their sales expectation (5.). The report will close with 6. a concept for market entry.

1. Nano-Medicine Market: US

Nanomedicine is already an established market. Unlike some other potential applications of nanotechnology, which are still largely experimental, nanomedicine has already produced a number of significant products in which the nano dimension has made a significant contribution to product effectiveness. The global nanomedicine market reached $63.8 billion in 2010 and $72.8 billion in 2011. The market is expected to grow to $130.9 billion by 2016 at a compound annual growth rate (CAGR) of 12.5% between years 2011 and 2016. The anticancer products market reached $25.2 billion in 2010 and $28.0 billion in 2011. (Fig. 1; Source: New market research report on Nanotechnology in Medical Applications)



Source: BCC Research

Figure1: Turnover by nano-medicine products

2. Global BioChip Market

A biochip is defined as a collection of miniaturized test sites (microarrays) arranged on a solid platform that allows multiple tests to be performed at the same time with increased efficiency and higher speed.

Concentrating the analysis from the entire nano-medicine market to biochips its market share was valued at $2.6 billion in 2010 and is expected to reach $5.6 billion by 2015; growing at a CAGR of 16.7% (Fig. 2). This growth is attributed to increasing applications in cancer diagnostics and expression profiling, the boom in personalized medicine, and government funding. (Source: Global Biochip Markets: Microarrays and Lab-on-a-Chip, BCC research report, 2011)

The global biochips market is segmented into DNA microarrays, protein microarrays (expressional, functional, and reverse), lab-on-chip (LOC), and other arrays that include cell

array and tissue array (frozen array and formalin fixed paraffin embedded array). DNA microarrays represent the largest segment of this market and will continue to be the largest contributor during the study period (2010-2015). However, this segment will be closely followed by lab-on-chip (LOC) in the coming years due to its wide applications and increased adoption by various biotechnology, pharmaceutical companies, and research laboratories. The third largest segment – protein microarrays will be the fastest growing segment; forecasted to grow at a CAGR of 19.9% during the study period due to advances in the fields of genomics and proteomics and improvements in the field of recombinant proteins. Cell arrays, however, are still regarded as a niche market following the rules of the other types of arrays.

Marktsegment	Weltmarktvolumen (Abschätzung für das angegebene Jahr)		CAGR	Quelle
Biochips (DNA, Protein-Analyse, Wirkstoff-forschung)	2,6	6	18 %	BCC 2010 [36]
DNA-Chips	1,3	2,7	15 %	
Mikrofluidische Chiplabore	0,8	2,1	21 %	
Protein-Chips	0,3	0,8	20 %	
Neuentwicklungen Biochips (Glykomik, Gewebeanalysen)	131	265	15 %	

Figure 2: Global value of single biochip products, source: nano.DE-Report, BMBF 2011

The biochip market can be divided into tools, diagnostics and sequencing. Figure 3 indicates the strongest growth for diagnostics until 2016 (Source: Global Biochip Markets: Microarrays and Lab-on-a-Chip, BCC research report, 2011).

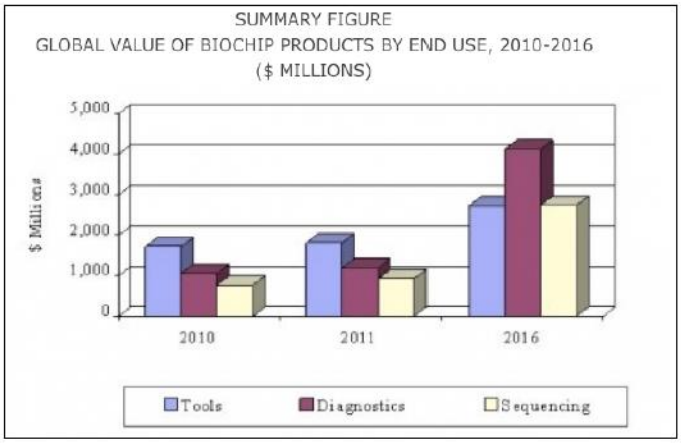


Figure 3: Prognosis global value of biochip products, source: BCC research report, 2011

3. Global BioChip Market Dynamics

The global biochip market is primarily driven by growth in personalized medicine, government funding, technical advancements, and cancer diagnosis. Cancer ranks second after cardiovascular diseases in terms of the number of deaths per year globally and several biochip initiatives are also directed towards fast cancer detection and therapy.
Cross border collaboration across most of the biotech companies has spurred the growth of the biochip market in the Asian subcontinent. Private equity firms and venture capitalists are investing more in countries such as India, China, and Singapore for new opportunities. The government in these countries has planned substantial budget allocations for developing the biotechnology sector. The major biochip players are moving east to take advantage of the growth opportunities. Singapore is the most preferred locations to set up manufacturing facilities for biotechnology and pharmaceutical companies considering its technical

knowledge, resources, and infrastructure. Leading players in the biochip market such as Affymetrix Inc., Fluidigm, and Illumina had set up their manufacturing facilities in Singapore to take advantage of the growing demand and gain competitive edge with large number of regional players.

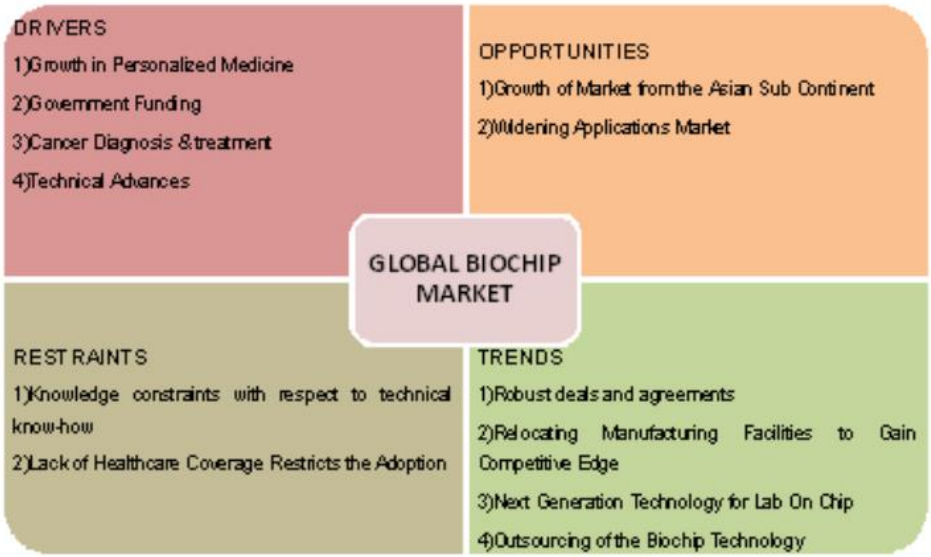


Figure 4: Market drivers
Source: Expert Interviews, MarketsandMarkets Analysis, 2011

4. Therapeutic Concept

The economic potential of regulatory T cells is immense as they can serve several markets: (1) Tregs can suppress GvHD in allogeneic stem cell transplantation settings as envisaged in this trail. (2) They may hamper graft rejection in solid organ transplantation and (3) they can suppress autoimmunity. The market of transplantation is considerable; including the immunosuppressants used in autoimmune diseases it had sales of approximately US$2.7 billion in 2001 with an annual increase of >10%. The market for cellular therapy products in allogeneic transplantation is smaller resulting from the lower patient numbers. We estimate its size as 100-200 million € in 2007 based on the number of expected allogeneic transplantations in the US and Europe (20.000). Treg related products may contribute up to 5% to this market.

Some therapeutic options of cell arrays are outlined in figure 5. They can be used to activate, expand or genetically modify cells that may be derived either from donors or the patient. Thereafter, a large scale expansion step might be necessary before the cells are administered to the patient. Products that will be developed for those kinds of cellular nanomedicine might be next to the cell arrays especially media, cytokines, vectors and automated medical systems performing all the processes leading to formulation of cellular products.

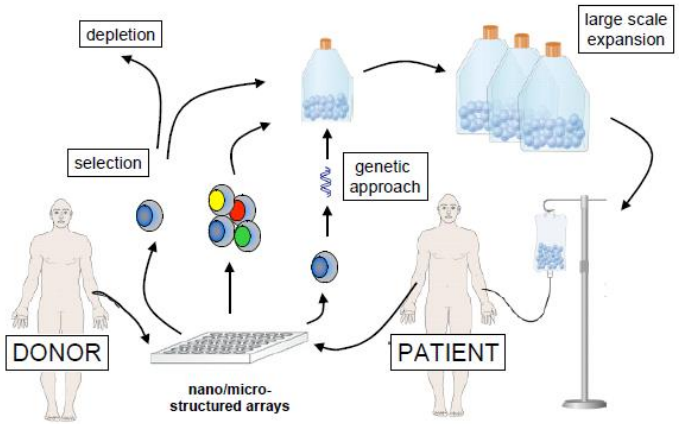


Figure 5: Therapeutic options

5. Products and Sales Expectations

Our business model is based on the provision of complete solutions for the customers: We do not only develop technologies but also provide customized reagents. Hence, most of our sales is generated by consumables. Here, in this context, we will provide research and later on GMP grade disposables and reagents for enrichment, cultivation, amplification and transfection of T cells.

The medical concept is not limited to T cells but can be adapted to cultivation and genetic modification of other immune cells like dendritic cells or B-cells or stem cells. Taking those applications into account, the annual market volume of the reagents is estimated to be two to three times higher than the instrument sales (estimation based on similar systems): 20 Mio € four years after market entry (Fig. 6).

Marketable products will be:

1. Cell Culture Systems including Cell Arrays or BioChips will be developed as disposables (plastic ware) (market volume 5 to 10 Mio € p.a.).

2. (GMP grade) reagents (consumables): Additionally, we will develop innovative reagents, cytokines and media for the isolation, amplification and transfection of cells (market volume 10 to 20 Mio € p.a.).

3. Devices/instruments: An innovative instrument will be developed that combines cell separation, cultivation on biochips, cell expansion and/or transfection in a closed system for the automated generation of GMP grade cellular products (market volume 5 to 10 Mio € p.a.).

Figure 6: Sales Expectation

Potential cost reduction for health care system: For the health care providers there will be a substantial cost reduction since cellular treatments intend to cure tumor and other chronically ill patients. Conventional treatment methods provoke in average costs of more than 100.000 € per year for patients with advanced disease.

5. Market Entry

The time to market for BioChips can be compared to the development of drugs as illustrated in figure 7. It typically takes 10 to 16 years including phase I to III trials in order to gain approval by national and international regulatory authorities.

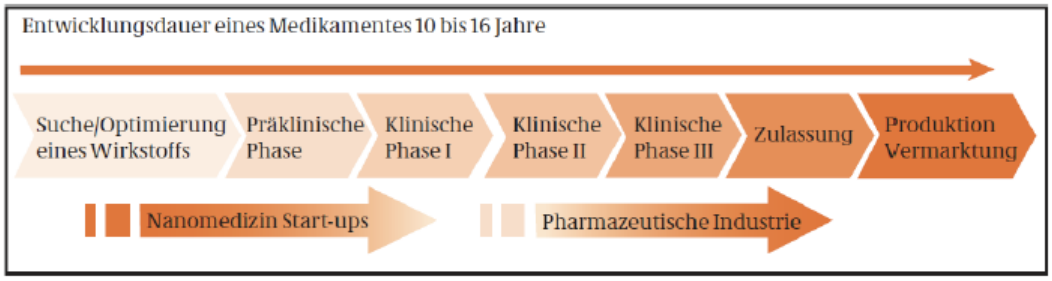


Figure 7: Time to market

A more detailed analysis is presented in Figure 8. After the end of the project in 2013 the

preclinical development is initiated leading to the envisaged clinical trials. Sales, however, will be generated by market introduction of research reagents in 2015 accompanied by corresponding marketing activities that already prepare the customers for the clinical products and applications.
Miltenyi Biotec has a longstanding history in this market. Miltenyi's overall goal is to transfer cellular therapies from research into clinics as a standard method that is reimbursed by the health insurances. Hence, the company started to support the development of clinical protocols for cell isolation already in 2000 and supported more than 50 phase I and II trials since then and is presently starting phase III trials for selected cellular therapy concepts. Additionally, more than 40.000 clinical cell transplantations with the CliniMACS instrument have been performed up to now.

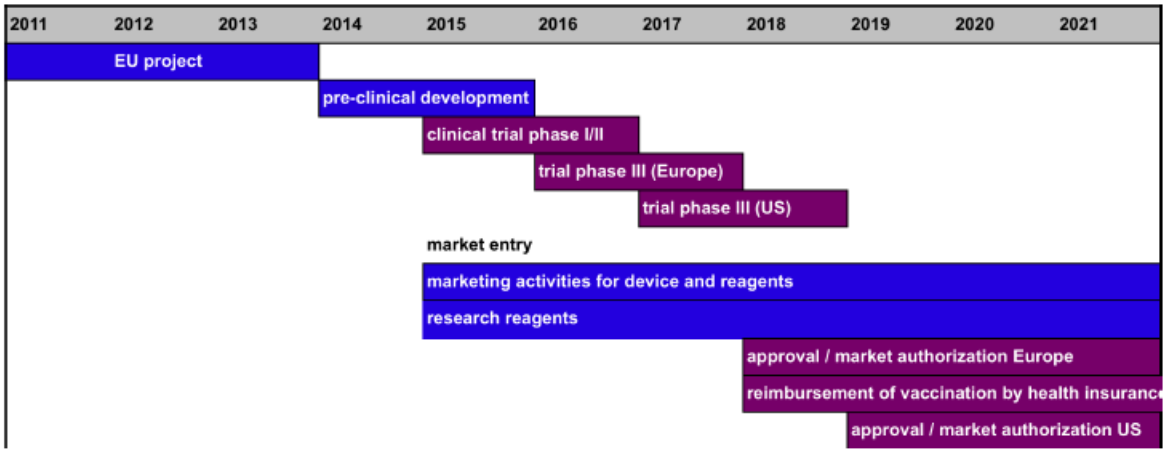


Figure 8: Market Entry

Dissemination impact

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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.
Missirlis, D.; Spatz, J. P., Combined effects of PEG hydrogel elasticity and cell-adhesive coating on fibroblast adhesion and persistent migration *Biomacromolecules* **2013**, accepted.

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.

A lot of publications are in preparation

Conference presentations

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

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

WORKSHOP on NANOMEDICINE AND NANOBIOSYSTEMS (WoMeN), SEPTEMBER 6-8, 2012 Lecce (Italy) (Marco Cecchini, Oral Presentation)

Marco Cecchini, “Neuronal Mechanotransduction: contact guidance and microfluidic devices” Lesson at the PhD school of the Trento University (Trento, ITALY);
Marco Cecchini “Interaction of neuronal cells with nanotopographies” Lesson at the PhD school of Turin University (Turin, ITALY).

“T-cell activation and differentiation at high temporal resolution: Emergent temporal order”, 1st Weizmann-Singapore Conference, Rehovot, Israel, October 2012.

"A micro-well array reveals contact independent suppression of effector CD4 T-cells by regulatory T-cells at short intercellular distances", 14th International Conference on Lymphocyte Activation and Immune Regulation, Newport Beach, California, USA, February 2012.

"A micro-well array reveals contact independent suppression of effector CD4 T-cells by regulatory T-cells at short intercellular distances", World Immune Regulation 5th meeting, Davos, Switzerland, March 2011.

"A micro-well array reveals contact independent suppression of effector CD4 T-cells by regulatory T-cells ", 6th FISEB (Ilanit) Congress, Eilat, Israel, February 2011.

Conferences: Nobel stem cell Meeting in Stockholm, Sweden , June , 2012.

Raisa Gorbacheva Memorial Meeting, St Petersburg , September , 2012.

‘Non-interacting surfaces - microenvironment development for monocytes and lymphocytes’ (Sauter et al., at the Keystone Symposium with the title ‘Understanding Dendritic Cell Biology to Advance Disease Therapies’, March 2013)

Exhibitions

The exhibitions were:

1. High content analysis SF, USA
2. Lab automation SF, USA
3. Medica, Germany
4. Israel life imaging forum, Israel
5. Biotechnica, Hannover 2013

In exhibitions - IDEA Bio-Medical presents its systems and demonstrated their capabilities.

Address of project public website and relevant contact details

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

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

Prof. Dr. Joachim P. Spatz

Tel: *+49 711 689-3611*

Fax: *+49 711 689-3612*

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	Fine Tuning and Efficient T Cell Activation with Stimulatory aCD3 Nanoarrays	Matic J	*Nano Lett.*	monthly	ACS		2013, Nov.	13 (11) 5090-7		
2	Antigen-Reactive T Cell Enrichment for Direct, High-Resolution Analysis of the Human Naive and Memory Th Cell Repertoire.	Bacher P	*J. Immunol.*				2013	190 (8), 3967-76		
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	Ly6C^{hi} monocytes give in the inflamed colon rise to pro-inflammatory effector cells and migratory APCs	Zigmond E.	*Immunity*				December 2012	37:1076-90		

6	Fate Maping reveals Origins and Dynamics of Monocytes and Tissue Macrophages	Yona	*Immunity*				2013	online		Yes
7	Murine anti-3rd-party central-memory CD8+ T-cells promote hematopoietic-chimerism under mild conditioning: lymph-node sequestration and deletion of anti-donor T-cells	Ophir	*Blood*				Dec. 5 2012	online		Yes
8	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
9	Effective polyethylene glycol passivation for the inhibition of surface interactions of peripheral blood mononuclear cells and platelets	Sauter	*Biointerphases*				2013	doi:10.1186/1559-4106-8-14		
10	Antigen-specific expansion of human regulatory T cells as a major tolerance mechanism against mucosal fungi	Bacher P	*Mucosal Immunol.*				2013	in press		
11	Characterization of a novel cell penetrating peptide derived from human	Eva Harreither						in review		
12	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		

13	"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		
14	Unveiling LOX-1 receptor interplay with nanotopography: mechanotransduction and atherosclerosis onset	C Di Rienzo	*Scientific Reports 3*					Article number: 1141 doi:10.1038/srep01141		
15	Imaging intracellular viscosity by a new molecular rotor suitable for phasor analysis of fluorescence lifetime	Antonella Battisti	*Anal. Bioanal. Chem.*				2013	405:6223-6233		
16	On cell separation with topographically engineerined surfaces	D. Franco	*Biointerphases*				2013	doi:10.1186/1559-4106-8-34		
17	Interaction of leech neurons with topographical gratings: comparison with rodent and human neuronal lines and primary cells	Tonazzini I.	*Interface Focus*				accepted for publication			
18	*CD103^{+} DCs are recruited to the intestinal epithelium to sample luminal bacteria*	Julia Farache	*Immunity*				2013	38,3; 581-595		
19	The constitutive phosphorylation of VE-cadherin in vivo in veins is a priming factor necessary but not sufficient for induction of vascular permeability	Orsenigo, F.	*Nature Communications*				2012	3 1208		
20	Creating cell microenvironments in vitro	Elin	*Chalmers University of*				2012			

		Bernson	*Technology*							
21	Development of a microfluidic platform to study cell migration along gradients	Patric Wallin	*Biomicrofluidics*				2012	6, 024131		
22	Monitoring the dynamics of primary T cell activation and differentiation using long term live cell imaging in microwell arrays	I. Zaretsky	*Lab Chip*				2012	12, 5007		
23	Dynamic response diversity of NFAT isoforms in individual living cells	N. Yissachar	*Mol. Cell*				2013	49:322-30		
24	Dynamic single-cell measurements of gene expression in primary lymphocytes: challenges, tools and prospects	M. Polonsky	*Brief Funct Genomics*				2013	12, 99		
25	Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation	Farache J	*Immunity*				2013	21, 38 (3) 581-95		
26	Microstructured platforms to study nanotube-mediated long-distance cell-to-cell connections	Abel, M.	*Biointerphases*				2011	6(1), 22-31		
27	Impact of Local versus Global Ligand Density on Cellular Adhesion	Deeg, J.	*Nano Letters*				2011	11, 1469-1476		
28	Direct assessment of living cell mechanical responses during deformation inside microchannel restrictions	Walter, N.	*Biointerphases*				2011	6 (3), 117-125		
29	The significance of integrin ligand nanopatterning on lipid raft clustering in hematopoietic stem cells	Altrock, E.	*Biomaterials*				2012	33,3107-3118		

30	Circular, Nanostructured and Biofunctionalized Hydrogel Microchannels for Dynamic Cell Adhesion Studies	Kruss, S.	*Lab on a Chip*				2012	12 (18), 3285-3289		
31	Regulation of integrin adhesions by varying the density of substrate-bound epidermal growth factor	Shahal, T.	*Biointerphases*				2012	7,1-11		
32	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		
33	T cell activation is determined by the number of presented antigens	Deeg, J.	*Nano Letters*				2013	13 (11), 5619-5626		
34	The role of integrin-linked kinase in the molecular architecture of focal adhesions	Elad, N.	*Journal of Cell Science*				2013	126,4099-4107		
35	Marker-free phenotyping of tumor cells by fractal analysis of RICM images	Klein, K.	*Nano Letters*				2013	13 (11), 5474-5479		
36	Cell membrane topology analysis by RICM enables marker-free adhesion strength quantification	Klein, K.	*Biointerphases*				2013	8,28		
37	Adhesion Maturation of Neutrophils on Nanoscopically Presented Platelet Glycoprotein Ibα	Kruss, S.	*ACS Nano*				2013	7 (11), 9984-9996		
38	Combined effects of PEG hydrogel elasticity and cell-adhesive coating on fibroblast adhesion and persistent migration	Missirlis, D.	*Biomacromolecules*				2013, accepted			
39	Interface Immobilization Chemistry of cRGD-based Peptides Regulates Integrin Mediated Cell Adhesion	Pallarola, D.	*Advanced Functional Materials*				2013, accepted			
40	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		

41	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		
42	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		
43	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		

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 | Austria	Journal of Tissue Engineering and Regenerative		world

						Medicine, 6 s. 350-350, 2012		
9	WORKSHOP on NANOMEDICINE AND NANOBIOSYSTEMS (WoMeN)	Marco Cecchini	Interaction of neuronal cells with nanotopographies	SEPTEMBER 6-8, 2012	Lecce (Italy)			
10	Lesson at the PhD school of the Trento University	Marco Cecchini	Neuronal Mechanotransduction: contact guidance and microfluidic devices		Turin, ITALY			
11	Lesson at the PhD school of the Trento University	Marco Cecchini	Interaction of neuronal cells with nanotopographies		Turin, ITALY			
12	1st Weizmann-Singapore Conference	Yair Reisner	T-cell activation and differentiation at high temporal resolution: Emergent temporal order	October 2012	Rehovot, Israel			
13	14th International Conference on Lymphocyte Activation and Immune Regulation	Yair Reisner	A micro-well array reveals contact independent suppression of effector CD4 T-cells by regulatory T-cells at short intercellular distances	February 2012	Newport Beach, California, USA			world
14	World Immune Regulation 5th meeting	Yair Reisner	A micro-well array reveals contact independent suppression of effector CD4 T-cells by regulatory T-cells at short intercellular distances	March 2011	Davos, Switzerland			world
15	6th FISEB (Ilanit) Congress	Yair Reisner	A micro-well array reveals contact independent suppression of effector CD4 T-cells by regulatory T-cells at short intercellular distances	February 2011	Eilat, Israel			
16	Nobel stem cell Meeting	Yair Reisner		June , 2012	Stockholm, Sweden			
17	Raisa Gorbacheva Memorial Meeting	Yair Reisner		September , 2012	St Petersburg			
18	Symposium with the title 'Understanding Dendritic Cell Biology to Advance Disease Therapies'	Sauter	'Non-interacting surfaces - microenvironment development for monocytes and lymphocytes	March 2013	Keystone, Colorado, USA			world

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		Patent application in preparation	CD3/CD28 Treg TransACT Reagent	Miltenyi
Patent	Yes		Patent application filed US20110097313A1, EP2306191A1, DE102009040716B4	Ag-specific Treg isolation method	Miltenyi
Patent	Yes		Patent application filed EP11164382.1, EP12165890.0, US 13457878	Sensitive detection of rare antigen-specific Treg	Miltenyi

ADDITIONAL TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND confidential	
Description of Exploitable Foreground	**Explain of the Exploitable foreground**
biochip	The biochip is integrated in an automatic optical high resolution microscope for high throughput screening. It is planned to use this biochip to investigate the mechanic sensing responses of different cell types.
microfluidic chips	A novel technology to reproducibly generate gradients of soluble molecules on nanostructure arrays. These integrated chips can be use to directly manipulate cellular activities.
specialist bioreactor	An instrument to study cell differentiation and migration in a physiological flow environment.
large protocols for large scale protein purfication	Miltenyi has worked on the large scale purification of recombinant proteins (cytokines and antibodies which can be imobilized on nano surface and or used for later Treg manipulation
a PDMS based micro assay chip	This is a system for long term culture in monitoring of primary T cells. The devices offer unique advantages for long term monitoring of T cell activation and differentiation with a single cell resolution.
rapid expansion protocols	With these protocols it is possible to generate large numbers of functional $CD4^+$ Treg cells derived from PBMs
standard operation protocols	These protocols are for the ex vivo numeric expansion of naive Treg
nano arrays	Development stimulatory anti-CD3 nano arrays for controlled activation of T cells
adaptation of Wiscan systems	unique CO2 cover, LED fluorescence, illumination, laser based autofocus, automatic played loader
new method of integration free generation of IPSC	The ability To generate and culture IPSCs and IPSC derived cell types as for example macrophages highly reproducible platform and a continue supply of cellular material
humanized mouse model	the model is important for the in vivo tracking of Treg

4.3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	**229289**
Title of Project:	***Nano**scopically-guided induction and expansion of regulatory hematopoietic cells to treat auto**i**mmune and **i**nflammatory processes*
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'*	***Yes***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	Yes
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	Yes
• Did the project involve Human genetic material?	Yes
• Did the project involve Human biological samples?	Yes
• Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
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?	No
• Were those animals cloned farm animals?	No
• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	

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

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	0	15
Experienced researchers (i.e. PhD holders)	8	31
PhD Students	23	11
Other		

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**23**
Of which, indicate the number of men:	11

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)?

No X Yes- please specify open day MPI

○ 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]: 2.10, 2.5, 1.6, 2.6, 3.1, 3.2

X Associated discipline[1]: 1.4 ○ 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)*	○ ○	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?	**25**
To how many of these is open access[2] provided?	**3**
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	**22**
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).*		**3**
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	X	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:* ***92***

[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	X	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)		