SIXTH FRAMEWORK PROGRAMME

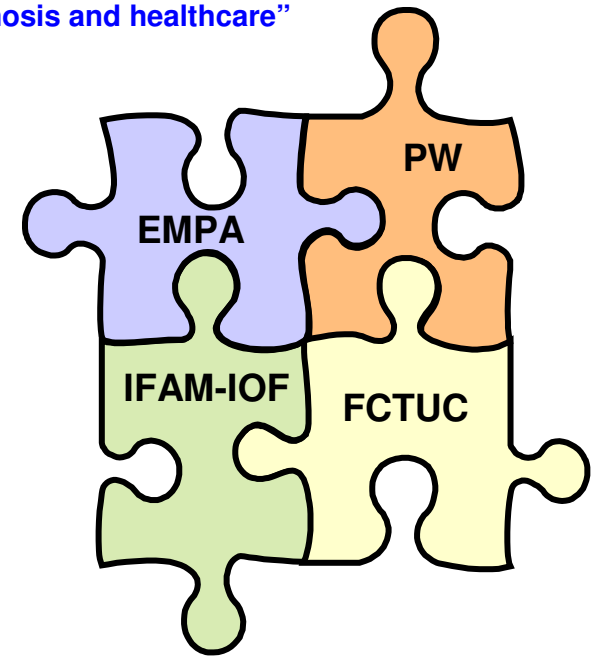
PRIORITY 2&3 – IST& NMP

IST-NMP-2 Bio-sensors for Diagnosis and Healthcare

SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT

Publishable Final Technical Report of the Project CellForce

"CellForce: Development of a single cell based biosensor for subcellular on-line monitoring of cell performance for diagnosis and healthcare"



Contract no.: **NMP4-ct-2005-016626**

Period covered: 1. November 2005- 30 June 2009

Date of preparation: 23.2 2010

Kick-off date of project: 29 November 2005

Duration: 30.June 2009

Coordinator
Arie Bruinink
Empa Materials Science & Technology
arie.bruinink@empa.ch
fax +41-71 274 7788
tel +41-71 274 7695

Content

The Consortium

Table 1. *List of the consortium participants. All participants entered the project at kick-off and left the project after the 44 month period duration of the project*

Partic. Role*	**Participant name**	**Short name**	**Country**
Coordination Cellular Biology	Empa St. Gallen	EMPA	Switzerland
CR Simulation & software development	Fac. Mat. Sci. Eng., Warsaw Univ. of Technol.	PW	Poland
Chemical surface modification of polymers	Dept Eng. Quimica, Uni Coimbra	FCTUC	Portugal
Injection moulding of pillar samples	Fraunhofer Gesellschaft, Bremen	IFAM	Germany
Optical System	Fraunhofer Gesellschaft, Jena	IOF	Germany

1 Summary

The main objective was to develop a very robust and simple device biosensor usable in an industrial and university environment that enables online monitoring of cell forces transduced to the substratum. The substratum consists of an array of pillars which degree of bending relate directly to the forces of the cell contacting these. The premise of the Cellforce sensor was that all parts can be produced solely by industrial processes and exact knowledge on cell attachment to the substratum and functionality at each time point.

During the project much knowledge was gained in each scientific field addressed within the 44 month of its duration. Areas such as the microstructuring of polymer material and the development of on optical measuring device were performed and pushed to their limits. Furthermore, topics such as the development of double gene constructs reporting for the functional state of cells or its shape and attachment as well as of new thermoplastic polymer materials with reduced Young's modulus were addressed within the Cellforce project. A short description of the success story of the project results is listed below:

- Fraunhofer IFAM pushed the injection moulding technology to its limits. For the first time Elastollan pillar mats consisting of hexagonal ordered arrays of 490 000 pillars having a height of 25 µm, a diameter of 5 µm and an intra pillar distance of 5 µm were manufactured in series. A specially adapted mould as well as the injection moulding process for this difficult task was developed within this project.
- Surface coatings that are cell repellent or cell adhesive on a plane surface were found and tested by FCTUC and EMPA.
- A Cellforce optical device was developed by Fraunhofer IOF, including a cell culture flow chamber for the Cellforce pillar array and the electronic equipment. Furthermore Sylgard material was applied to manufactured higher pillars mats with smaller diameters. Masks and Sylgard pillar mats were developed and produced at Fraunhofer IOF.
- The control software for the optical system was developed by PW enabling to monitor cells in the flow chamber. PW also developed the software (mathematical algorithms) to measure the traction forces of the bend pillar structure.
- EMPA: Double gene constructs were prepared enabling to mark and visualise transfected cells (cytoplasm or nucleus) and a gene entity to report for the activity of a

specific gene. Furthermore, a FRET system was developed by EMPA to investigate the interaction between talin and vinculin being the base process and premises for force transduction to the substratum.

- Theoretical models for in vitro toxicodynamics were developed and data regarding CNT toxicity were also gemerated by EMPA
- FCTUC developed new thermoplastic materials with a reduced young's modulus compared to the commercially available Elastollan material.

A large number of experiments were performed applying the different pillar mats during cell cultivation experiments. Unfortunately, it was found that the previously assumed forces of cells are not in the area of 30 nN but in the range below 5 nN. Therefore, no commercially available thermoplastic materials were found having a Young´s modulus lower than the processed Elastollan material. Manually processed Sylgard pillars that measurably bend by these forces tended to glue together and not to bend back to the original position if forces are going to zero. Thus, as a result the stability of the pillars cannot be ensured, in terms of elasticity (bending back to the original position) or collapsing or gluing together of the pillars. The latter stability is not only crucial for industrial production and use, but also for small scale research use. This indicates that the goal of an industrial processed cell-force sensor cannot be reached even if the project period would be extended for a second or a third time.

Nevertheless, due to all the obtained knowledge within the Cellforce project valuable results have been obtained to improve sensory equipment in the field of cell cultivation and detection. New micro structured substrates can be processed in series; the optical detection system can be applied enabling the cultivation of cells within the flow chamber of the Cellforce optical device, etc. Additionally, the cell adhesion of the corresponding proteins e.g. talin and vinculin on surfaces are better understood due to the Cellforce project and will act as important result within other collaborations.

2 Goal of the project

The CellForce project aimed to develop a biosensor. Latter is based on the on-line monitoring of traction forces that each cell transduces to the substratum surface through it's with the cytoskeleton connected focal adhesion points (FA). FA represent the connecting points between the cell and the material surface. The observation of cellular forces may yield facts about cellular physiology, as well as knowledge about the effects of any substances applied to the cells. The approach to visualize these cellular forces by means of a Cell Force Sensor is sketched in Fig. 1.

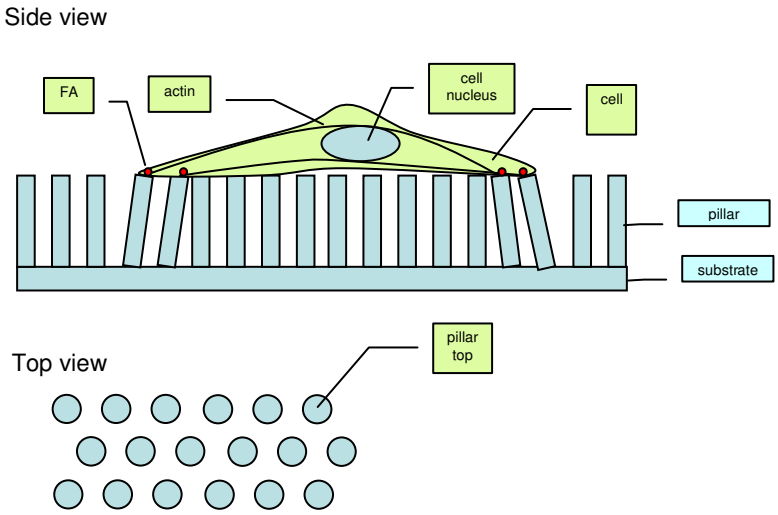


Fig. 1. *Schematic drawing of the sensing principle followed during the project.*

The premises of the CellForce sensor was that by using a soft polymer, shaped to yield a dense array of approximately 5 µm diameter pillars, the action of a force should become apparent by the tilt or deflection of individual pillars. This pillar tilt has to be measured optically. Additional fluorescence markers to label different cellular organelles should be observed in parallel. The aim was to establish a long term (hours…days) measurement system to observe cellular motions and associated forces. It's out of the scope of this report to describe all the different work packages to be combined to this very multi disciplinary system. The project was organised into 5 technical/biological work packages. WP1 focuses on microstructuring, WP2 on optical detection, WP3 on surface coating, WP4 on cell experiments and WP5 on data analysis and simulation. The WP's were designed in a way that each single partner was responsible for one ("his/her") WP.

3 Project Review Criteria and final status

Evaluation issues as mentioned in revised Annex-I and final status

- **WP1:** Materials were selected on which the project was based (Elastollan 1180A50 and Sylgard 184 as reference). The design was updated according obtained knowledge. Bending of the pillars with the mouldable materials used was however not seen. Concerning the material there is no thermoplastic material available on the market having a young's modulus in the range of Sylgard or below. Therefore, it is technically not possible to obtain micro injection moulded pillar carpets with lower bending forces than the Elastollan materials we processed within the Cellforce project. By that the key premise of the project cannot be fulfilled by physical and material reasons.

- **WP2:** The optical system was designed and also the set up. Initial testing revealed a few drawbacks that have been tackled. Corresponding changes were introduced into the system. During the project, the device was transferred to WP5 for software development and to WP 4 to be used experimentally. Lots of materials have been screened for fluorescence properties in order to support material selection.

- **WP3:** The coating procedure on the final 2-D surface was defined. The technology developed for the 2D surfaces is not 1 to 1 applicable for the pillar mats and had to newly developed specifically for the pillared surfaces. Within the time frame of the project it was not possible to obtain surfaces with pillars characterised by cell adhesive surfaces at the pillar top while the rest of the pillar was completely cell repellent.

- **WP4:** Biocompatibility tests of the bulk materials are finished for the obtained materials. Cell reactions on pillar structures were tested. Gene construct data are present including the methodology to monitor cell function on-line using fluorescence based methods other than the envisioned biosensor. Theoretical models for in vitro toxicity were developed. Effects of CNT on cells are measured.
  Cell forces could be detected only in some cases using Sylgard184. As concluded from bending of the reference Sylgard pillars cell forces are much lower as expected. In addition, it was seen using this material that forces of cells probably depends on the rigidity of the substratum and is different for each cell. Pillars of pillar mats able to react to cell forces collapsed.

- **WP5:** Finite element analysis of the pillars of the final set-up was done. Engineering software to recognize a fluorescent cell on top of the pillar is prepared. Activities to translate pillar bending into traction forces and to correlate these traction forces with each other were made.

3.1 Milestones reached as defined by each partner

For all partners the cell force project was a complete success looking to the obtained knowledge and new technologies that were developed. The goals were driven to the biological and physical limits. They can be summarized as follows:

IFAM

Within this project it was possible to develop the series production (micro injection moulding) of a microstructured surfaces having 490 000 pillars with a diameter of 5 µm, a centre to centre distance of 10 µm and a heights of 25 µm. After demoulding all 490 000 pillars were parallel to each other.

These excellent results were never obtained before, not in other institutes or industry. Fraunhofer IFAM also tried to manufacture 4 µm pillars with a height of 20 µm and a distance of 8 µm. The replication of these small geometries is below the limits of the micro-injection moulding process

Nevertheless, we already got a lot of feedback from the community that it is impressive to manufacture such a high number of pillars in series. The obtained results will be important for other application areas such as lab on chip systems or implant surfaces, where microstructures in the lower micron range are significant concerning cell adhesion or detection of biological molecules.

IOF

All goals that were set in Annex-I were completely achieved.

First, a set-up using full LED based illumination system has been developed. This enables to sequentially take two different fluorescence images as well as a standard transmission mode image avoiding any moving parts in the system. Therefore, the system is well prepared to be applied in quantitative, long-term experiments.

Second, Sylgard 184 pillar maths with different pillar diameters and heights have been moulded and delivered to support EMPA experimental work. During the project, the limits of the moulding process have been pushed above the limits of pillar stability (see section 4). Therefore, we conclude, that using technology enables to prepare pillars that bend by their intrinsic gravity.

FCTUC

The project was certainly for FCTUC very ambitious. However, during the project FCTUC got good and important results: Polyurethanes cell repellents and polyurethanes with good adhesives properties. The results were published recently in high impact journals highlighting the quality of the work. However, the main problem was to coat only the top surface. It was not possible in the project to get the pillars with the right mechanical properties and the right distance between them. Unfortunately, we could not use Sylgard 184 that would have been more easy to handle. However, we got very important objectives. We can now better define the chemical composition of the substrate as result of different chemical modifications. This may for other projects important cues and materials.

PW

All goals that were set in Annex-I were completely achieved with the restriction of describing effects of living cell behaviour under different conditions.

EMPA

Of all delivered and own prepared materials, including pillar-structured mats, the biocompatibility and cell spreading behaviour could be assessed. Statements regarding cell behaviour and CellForce set-up were made. New ways to vitally label cell components and the interaction between them using the double gene construct technology (transfection) in combination with FRET could be successfully developed. The quality of the work is highlighted by two prices that were obtained, i.e. for the best oral presentations of Anne Born in a national (SSB) and international (ESB) conference.

3.2 Concluding remarks regarding the progress made during the project

In the present project materials had to be selected that need to meet requirements of different disciplines: optics, biocompatibility and cell adhesion properties, moulding and reproductions properties, elasticity/stiffness characteristics, durability. For this we had to push the limits of a variety of methodologies. The overall search of bulk and coating material is based on an assumption regarding the forces that cell are able to transmit to the surfaces.. Our results suggest that the forces are much smaller as expected. Part of the published data are based regarding cell forces are based on dried fixed cells without taking the drying process induced shrinkage into account. This means that the design of the pillar has to be accordingly adapted: smaller diameter of the pillars or increase of the length of the pillars, less stiffer materials. This has consequences for coating procedure and the analysis software. In addition, based on recent reports of others it must be assumed that cells are able to adapt their forces depending on the stiffness of the substratum making it with current available materials and techniques impossible to define and produce optimal pillar characteristics for a biosensor for industrial applications. After submission of the project proposal the evaluators of the project recognized the present project as a highly risky project but with very large impact if the CellForce biosensor could be made. Each of the workpackages reached their personal goals at least to a large extend. A consortium network was built in this time and besides this project partners are connected now to each other within the frame of other projects. Although the final goal, the CellForce biosensor, could only partly be achieved, because of physical, biological and technical limitations the present project must be seen as successful.

3.3 Summary per Workpackage/Partner

3.3.1 WP 1. Microstructuring

Name of the Partner:	Fraunhofer IFAM
Name of the responsible Person:	Dr. Natalie Salk
Address:	Wiener Str. 12 D-28359 Bremen
Phone:	+49-421-2246-196
e-mail address:	Salk@ifam.fraunhofer.de

Tasks

Task nr	Short description	Reached?	Remarks
1.1	Selection of suitable materials	yes	With the knowledge at beginning of project
1.2	Determination of sensor design	yes	With the knowledge at beginning of project
1.3	Production of microstructured pattern	yes	With the knowledge at beginning of project
1.4	Characterisation of parts	yes	
1.5	Improvement of processing parameters	yes	
1.6	Production of surfaces for cell culture tests	yes	With the knowledge at beginning of project, no pillar bending could be realized

Deliverables

Deliverable nr	Short description	Reached?	Remarks
D 1.1	Bulk material samples for coating procedures	yes	Different bulk material samples were send DEQ
D 1.2	Material and design selection	yes	delayed
D 1.3	Microstructured patterned test surfaces	yes	Delayed due to long mould delivery time
D 1.4	Microstructured surfaces for cell tests	yes	With the knowledge at beginning of project, no pillar bending could be realized

Milestones

Milestone nr	Short description	Reached?	Remarks
M 1.1	Material is selected	yes	With the knowledge at beginning of project
M 1.2	Surface is defined and characterised	yes	With the knowledge at beginning of project
M 1.3	Production procedure is defined	yes	

Summary

The objective of Fraunhofer IFAM within the CellForce project was the development of a series production process for preparation of the microstructured CellForce-Sensor. Based on the knowledge at the beginning of the project adequate materials concerning their biological, optical and mechanical behaviour were selected. The processing and microstructuring abilities of the material were tested on a laboratory injection moulding machine. For the preparation of the CellForce-Sensor by injection moulding Elastollan 1180A and Elastollan 685A were determined.

All partners together defined a hexagonal pillar structure as the suitable design for the CellForce-Sensor. Therefore, an injection moulding tool with 490000 holes of 25 µm depth and with a diameter of 5 µm was developed and acquired. For manufacturing of the Sensors a variotherm injection moulding process was developed and optimized achieving a series production of well defined microstructured patterns.

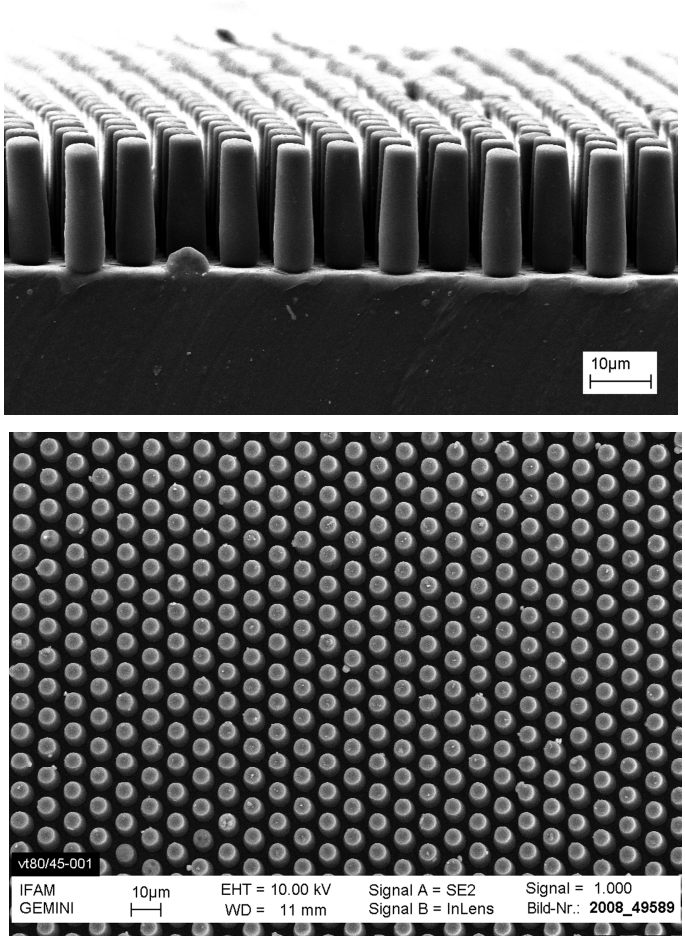


Fig. 2. *Pillar mat made of Elastollan 1180 A. Each pillar have a height of 25 µm and width of 5 µm (Top: Side view; bottom: top view).*

3.3.2 WP 2. Optronics

Name of the Partner:	IOF Jena
Name of the responsible Person:	Dr. Norbert Danz
Address:	Albert-Einstein-Str. 7 07745 Jena Germany
Phone:	+49 (0) 3641 – 807750
e-mail address:	Norbert.danz@iof.fraunhofer.de

Tasks

No	*Short description*	*Reached?*	*Remarks*
2.1	Material Selection	Yes	Material fluorescence analysis
2.2	Design optical system	Yes	Basis for task 2.3
2.3	Set-up optical system	Yes	Transferred to EMPA
2.4	Support system use	Yes	System realignment & ongoing discussions

Deliverables

No.	*Short description*	*Reached?*	*Remarks*
D 2.1	Design of optical part	Yes	
D 2.2	Optical set-up	Yes	Transferred to Warsaw and EMPA St. Gallen, finally

Milestones

No.	*Short description*	*Reached?*	*Remarks*
M 2.1	Optical design freeze	Yes	
M 2.2	Set-up finished	Yes	Slightly delayed due to overall delays during material selection; therefore cost neutral extension of the project

General Summary

Within the CellForce project, the IOF Jena developed the optical / technical part of the measurement system. Therefore, an optical system with LED illumination has been developed to enable the sequential exposure of two different fluorescence images as well as a transmission image. Thus, cellular organelle might be labelled by markers like fluorescent proteins, while the transmission image is intended to enable the analysis of pillar substrate surface (as transducer for cellular forces).

In order to select appropriate sensor material for the pillar surface, different polymer samples had been tested for intrinsic fluorescence, both with and without surface treatment. Additionally, different pillar samples had been prepared from the silicone Sylgard 184.

All software, associated with the control of the set-up and the analysis of images, has been developed by partners (Warsaw University of Technology).

3.3.3 WP 3. Coating

Name of the Partner:	FCTUC, Department of Chemical Eng., Univ. of Coimbra
Name of the responsible Person:	M. H. Gil
Address:	Polo II Pinhal de Marrocos, 3030-290 Coimbra
Phone:	351 239798700
e-mail address:	hgil@eq.uc.pt

Tasks

Task nr	Short description	Reached?	Remarks
Task 3.1	2-D coating: Cell repellent and cell adhesive coating definition.	Yes	
Task 3.2	3-D coating	No	It was not possible to modify only the top of the pillars. With all the techniques tested, a part of the pillar sides was probably also modified.
Task 3.3	Coating surfaces for cell culture tests	Yes	

Deliverables

Deliverable nr	Short description	Reached?	Remarks
D 3.1	Production of coated plane surfaces for cell culture tests	Yes	
D 3.2	The technology (& production) for cell repellent pillar side and adhesive pillar top coating	No	It was not possible to modify only the top of the pillars. With all the techniques tested, a part of the pillar sides was probably also modified
D3.3	Coated sensor surfaces for cell culture tests	Yes	

Milestones

Milestone nr	Short description	Reached?	Remarks
M3.1	Expected results are the production of new materials for the pillars and the development of a suitable coating procedure to improve cells adhesion at the top but repellent on the side.	No	It was not possible to modify only the top of the pillars. With all the techniques tested, a part of the pillar sides was probably also modified

Summary

A polyurethane elastomer was chosen, due to its physical and chemical properties. Elastollan 1180A50 was surface modified with several components in order to optimise cell repellent and cell adhesive surface coatings on plane bulk materials.
Also, the development of a coating technique resulting in a pillar structure which is cell repellent on the side and cell adhesive on the top of each pillar.

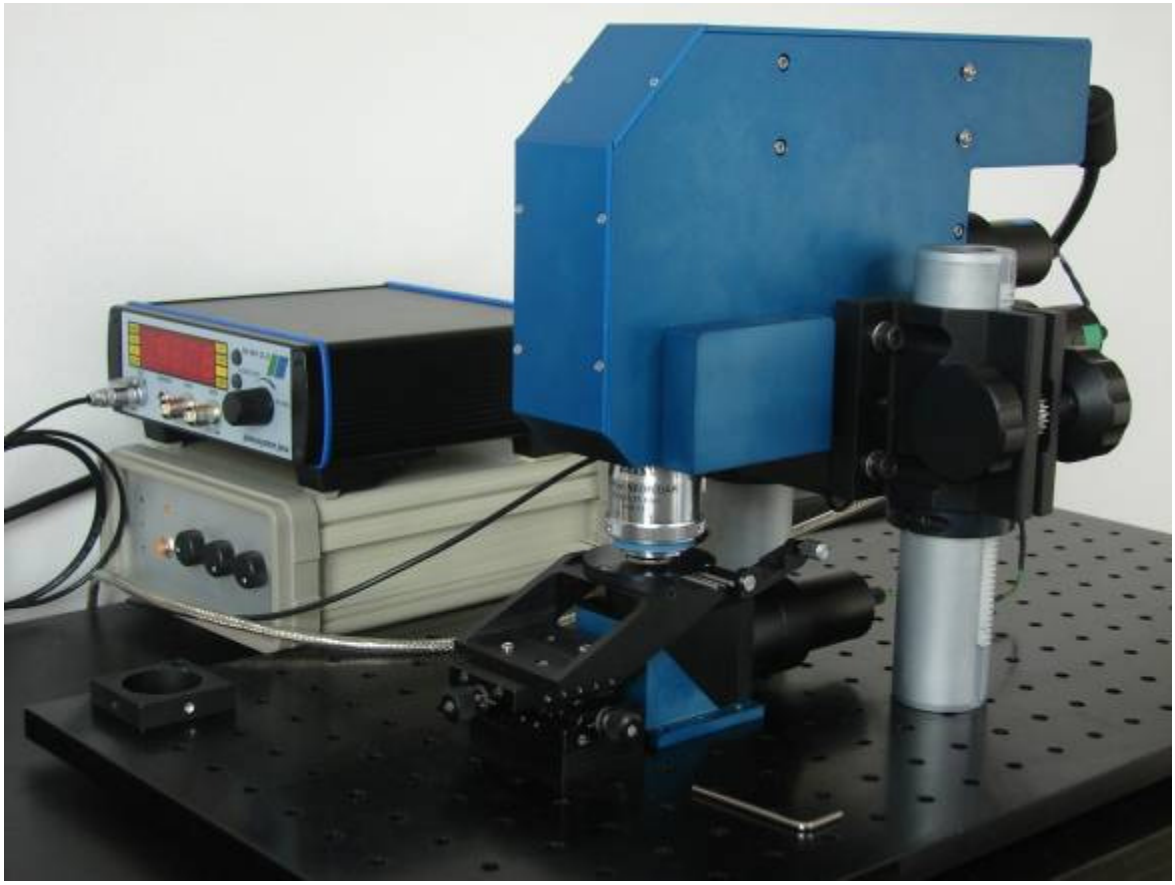


***Fig. 3**. CellForce sensor.*

3.3.4 WP 4. Cell Experiments

Name of the Partner:	Empa
Name of the responsible Person:	Dr. Arie Bruinink together with Dr. Jean-Piere Kaiser and Anne Born
Address:	Lerchenfeldstr. 5, CH-9014 St. Gallen
Phone:	+41 71 274 76 95
e-mail address:	arie.bruinink@empa.ch

Tasks

Task nr	Short description	Reached?	Remarks
T4.1	Biocompatibility	yes	
T4.2	Cell attachment and spreading on plane surfaces	yes	
T4.3	On-line monitoring attachment, cell migration on pillars	yes	
T4.4	Gene reporter constructs development	yes	
T4.5-8	Behaviour of cells on pillared surface with or without toxic compounds	partly	No pillar mat was available with which we could monitor cell forces.

Deliverables

Del. nr	Short description	Reached?	Remarks
D4.1	Correct bulk materials	yes	
D4.2	Correct coating materials	yes	
D4.3	mechanical pillar property	no	No pillar mat was provided with pillars to which cells only attached at pillar top
D4.4	OK for correct pillar coating	no	No pillar mat was provided with pillars to which cells only attached at pillar top
D4.5	Construct technology	yes	
D4.6-9	Algorithm forces & functional state	no	This deliverable is dependent on D4.4

Milestones

Milestone nr	Short description	Reached?	Remarks
M4.1	Choice bulk material set	yes	
M4.2	Coating procedure set (2D)	yes	
M4.3	Surface structure set	yes	
M4.4	Coating procedure set (3D)	no	No pillar mat was provided with pillars to which cells only attached at pillar top
M4.5	Definition constructs, cell isolation procedure set	yes	
M4.6	Algorithms set	no	This milestone is dependent on M4.4
M4.7	Biosensor potency defined	yes	

Summary

The aim of our workpackage in this project was to investigate and to monitor cell attachment and spreading on different coated pillared structured surfaces. Since cell spreading and cell movement is an active process slight effects on cell function may directly be reflected by the size of the focal adhesion points and the contractile forces submitted to the underlying surface. The contractile forces of a single focal adhesion point may directly be dependent on the functional state the cell. The extent and change in contractile force is assumed to be influenced at a very early phase of test compound treatment and long before other indices of cell state like mitochondrial activity is changing.

The idea was to measure the contractile force and thereby to monitor the influence of toxic compounds such as heavy metals, inorganic and organic nanoparticles, drugs and other organic compounds on cell behaviour. In addition it was proposed to obtain early indications of cell differentiation; for example differentiation of human bone marrow cells to osteoblasts, by measuring differences of the contractile forces during the differentiations process.

During the first phase of the project a material with optimal properties regarding biocompatibility that also is optimal regarding optics, biocompatibility and cell adhesion properties, moulding and reproductions properties, elasticity/stiffness characteristics, durability.

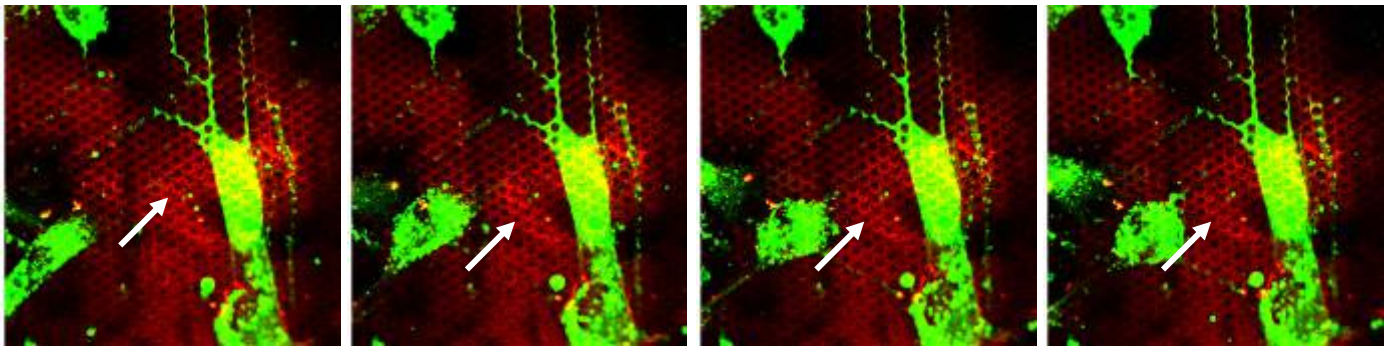


Fig. 4. *Migration of human bone cells (HBC) on Fibronection / Fibrinogen Alexa Fluor 647 conjugated coated pillar-structured Sylgard 184 (PDMS). DiI stained HBC were seeded on the Fibronectin/Fibrinogen Alexa Fluor 647 conjugated pillar structured Sylgard 184 (PDMS) surfaces with pillar diameter: 5 µm, distance between the pillars: 1 µm and height: 25 µm. The migration of the cells was monitored for 2 hours. Pictures were taken every 30 minutes for 2 hours. Please note the left bottom with cells migrating to the picture centre (arrow).*

3.3.5 WP 5. Data analysis and simulation

Name of the Partner:	Faculty of Materials Science and Engineering, Warsaw University of Technology
Name of the responsible Person:	Wojciech Swieszkowski
Address:	Woloska 141, 02-507 Warsaw, Poland
Phone:	+48226608792
e-mail address:	wojciech.swieszkowski@materials.pl

Tasks

Task nr	Short description	Reached?	Remarks
T5.1	Numerical simulation of the pillar. Development of mechanical finite element models (FEM) of a pillar. FEM simulations of cell – pillar interaction. Selection of a proper material for the pillar.	yes	
T5.2	Data analysis. Data acquisition and processing from the Cell Force sensor. Control software for optical system built by IOF Jena.	yes	
T5.3	Calculation of the traction forces and the cell position and shape..	yes	
T5.4	Visualization of cells.	yes	
T5.5	Simulation software. The additional software for biological algorithms. The simulation of a cell behavior, i.e. contraction, movements and traction force.	partially	A basic, simple model of contraction forces was implemented. The unsuccessful measurements of cell forces freeze the development of the simulation.

Deliverables

Deliverable nr	Short description	Reached?	Remarks
D5.1	The material properties for the pillars (Task 5.1)	yes	
D5.2	Software to record and analyse forces	yes	
D5.3	The software product to simulate cell behaviour	partially	The only basic, simple model of contraction forces was implemented. The unsuccessful measurements of cell forces freeze the development of the simulation.

Milestones

Milestone nr	Short description	Reached?	Remarks
M5.1	The decision is made which material might be used for the pillars (together with Task 1 of WP1).	Yes	
M5.2	The traction forces, positions and shapes of the cells are monitored during the time of experiments	Yes	
M5.3	The observation of cell behavior will help to define a algorithm describing the mechanisms of biological processes connected with traction forces and changes of the cell shape and position (together with Task 5 of WP 4).	No	The lack of the cell forces observation did not allow constructing new hypotheses about relationship between cell dynamics and biological processes.
M5.4	The simulation software may be a first step in developing of complex computer system aiding diagnosis and patient specific treatment of various diseases.	No	The lack of the cell forces observation makes it impossible to propose new algorithms of diseases detection.

Summary

The work package 5 main objectives are: a mechanical modelling of a cell – pillar interaction, the simulation software and the software for an image registration, analyzing and cell forces visualization. All tasks were carried out.

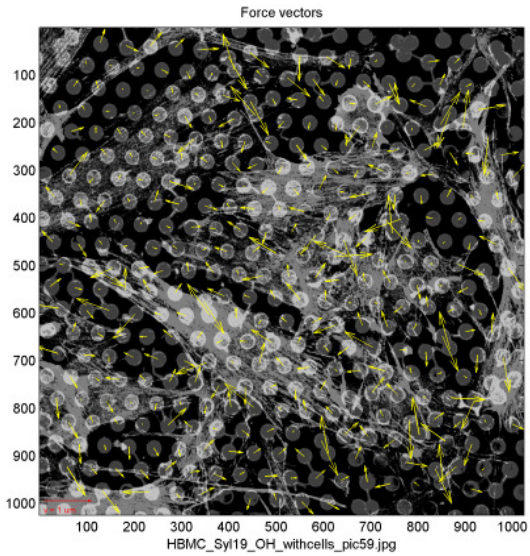


Fig. 5. *Sylgard pillar matrix with HBMC. Identified vectors of contracting forces.*

3.3.6 WP 6. Management

Name of the Partner:	Empa
Name of the responsible Person:	Dr. Arie Bruinink
Address:	Lerchenfeldstr. 5, CH-9014 St. Gallen
Phone:	+41-71-2747695
e-mail address:	arie.bruinink@empa.ch

Summary

Meetings were held after 0, 6, 12, 17, 21, 24, 31, 35, 41 and 44 month. A progress report was delivered each half year to the EU commission and every 3rd month to the partners of the project.

4 General thoughts and SWOT analysis

Fig. 6. *Correlation between pillar material, pillar length (um) and force (N) to bend the pillars by 1 µm*.*

*: Formula used: $E=(4/3)*(1/\pi)*(L^3/r^4)*F*(1/d)$; $F=(3/4)*\pi*(r^4/L^3)*E*d$

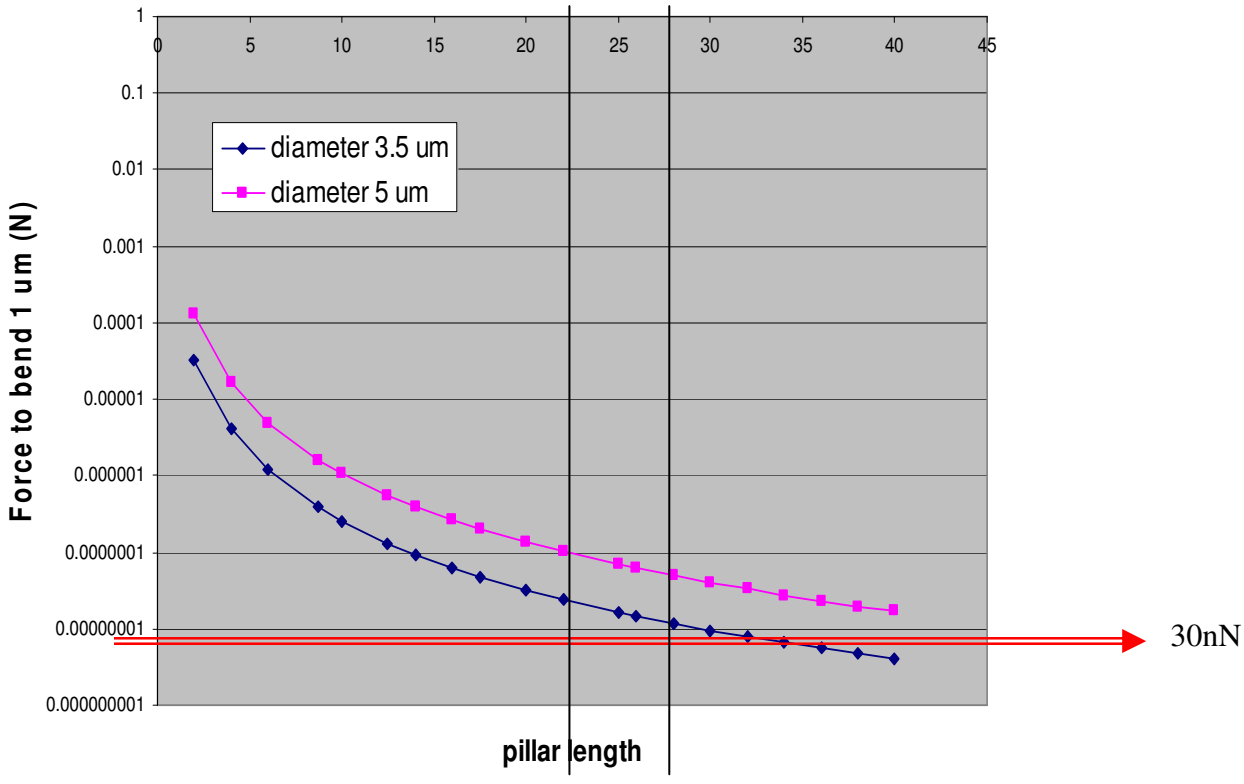


Fig. 7. *Correlation between pillar material, pillar length(µm) and force(N) to bend the pillars by 1 µm using Elastollan 1180A and two different pillar diameter*

Pillar-like structures are used by various teams to measure forces (Table 1 and 2). The forces that we expect to measure are around 30 nN with maximal values around 80 nN. Based on this formula and the relationship shown in figure 1 we can calculate the following:

- -Sylgard 184 (e-module =2MPa) pillar and 5 µm diameter. Force needed for 1µm bending: Pillar height 10um :184 nN, 15 µm : 54.5 nN and 25 µm : 11.7 nN

- -Elastollan 1180A (11.7MPa) 5 µm diameter. Force needed for 1µm bending: Pillar height 25µm: 68.9 nN, pillar height 35 µm: 25.1 nN

These values we have to keep in mind by our further optimisations of the sensor. So far we did not see any bending with Sylgard pillars with diameter of 5 µm and height of 17 µm. It must be noted that for the moulding the expect ratio L/r is important. By increasing this value the difficulties to remove the pillars out of the form also increases. However by slightly decreasing the pillar diameter with the same expect ratio (for instance 5 or 10) we are able to decrease the force needed to bend the pillars (Fig. 3)

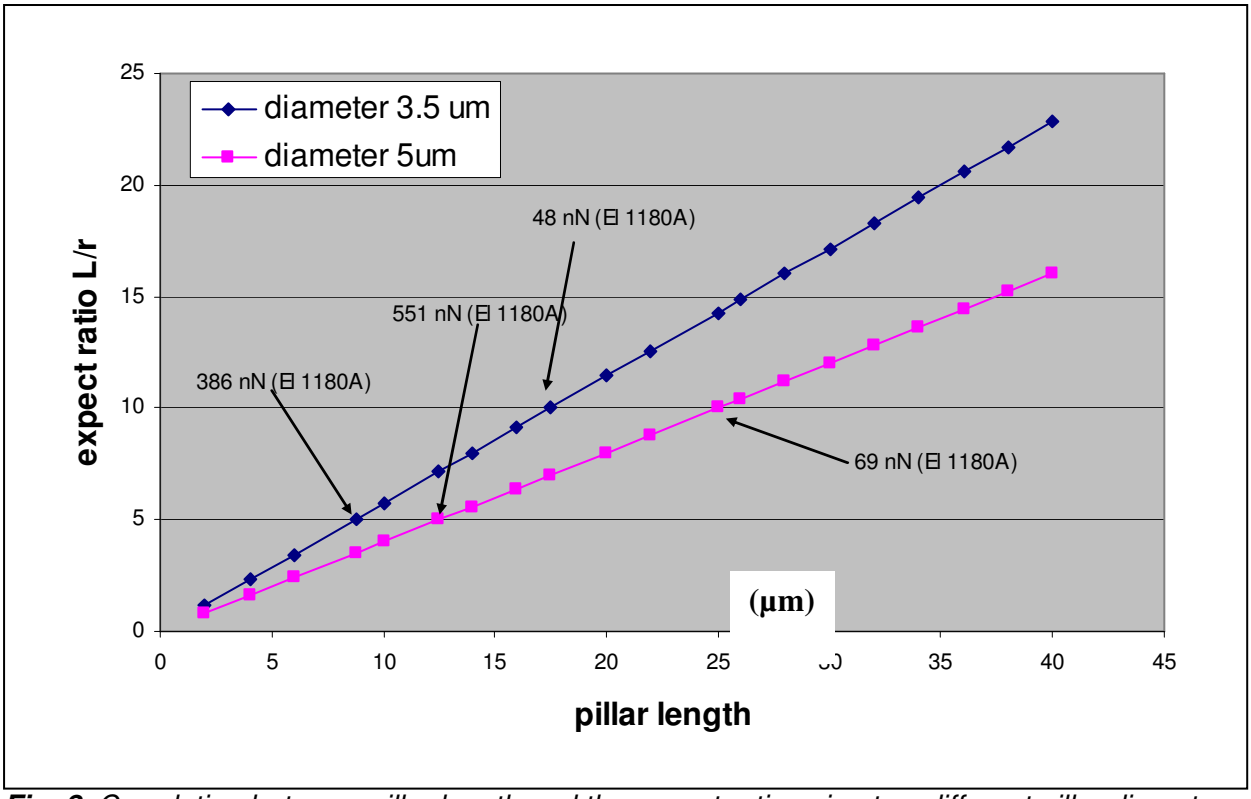


***Fig. 8**. Correlation between pillar length and the expect ratio using two different pillar diameters.*

The latest data of the EMPA showed that pillars with the dimensions 5 height and 1 um diameter could be bend by some cells. Production of pillar mats which could be visualized by the CellForce sensor using Sylgard should thus lie on the blue curve of the graph showing below. It may, however, be that more mature and larger focal adhesion points are formed on pillar with increased diameter transducing more force to the pillars. A marginal range seems to exist between optimal pillar sizes enabling to measure cell forces and pillar characteristics that result in pillar collapse or gluing together. Several experiments were made to find the optimal range but the optimum could not been found, probably partly because of the reaction of the cells to the stiffness and the variation between the cells.

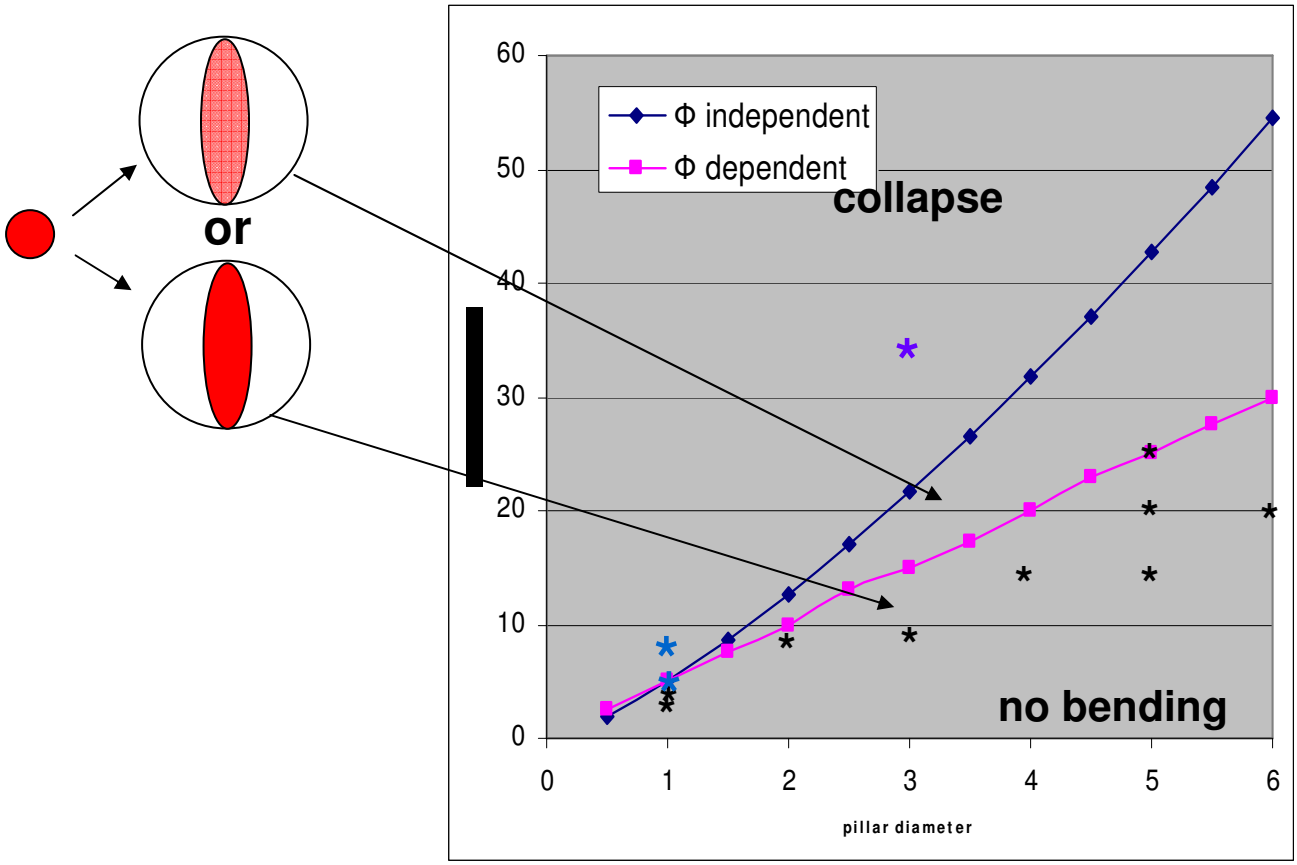


Fig. 8. *Theoretical curves based on recent data obtained in WP4 showing that that Sylgard 184 pillars with the dimensions 5 height and 1 um diameter could be bend by cells. The blue curve assumes that with increasing pillar diameter the same cell force is transmitted to each pillar even if more space for adhesion is present. The pink curve assumes that the force transduced to each pillar is directly related to the pillar diameter. Stars represent experiments using Sylgard pillar mats with different pillar diameter and length. In the black star experiments no bending was found, in the blue one bending was seen and in the experiment with pillar dimensions designated by the violet star the pillars glued together and collapsed.*

4 SWOT analysis of the project

4.1 Strength

- By pushing the limits of all methodologies and techniques to their limits. Much knowledge was gained on each field. Our network is established.
- Various contributions were made as publications, and conference presentations (See chapter 5). Several papers are currently in preparation.

4.2 Weakness

Materials

-For moulding only certain materials are possible. The stiffness and biocompatibility are additional limiting factors.

-It is not possible to go below certain pillar sizes (height, diameter) and interpillar distances. Here we pushed the moulding limits. Weak pillars collapse, stiff pillars cannot be bend.

-Numerous coatings were made. Biocompatibility is here a limiting factor. The difficult part was to solely coat the pillar top without also coating the sides and without making the pillars to collapse.

-Behaviour on plane and structured surfaces was not the same.

Cells

-Cells adapt probably their forces according the stiffness of the substratum and not all cells react the same.

-Cells go in between the pillars if they have a chance to adhere.

Team composition

-An overlap in competences would have been helpful to improve support of each other of this highly complex project.

4.3 Opportunities

-Optical Cellforce set-up was prepared and software to use it was made

-Micrometer pillars of 25 um length with 5 um diameter could be reproducibly made using Elastollan. This optimised technology can be used for the production of other micro(structured)parts.

-Methods to modify the surfaces with possibilities to control the cell adhesion to polyurethanes based materials. So, this material can be used due to its bulk properties and its surface can be modified either to be cell adhesive or cell repellent, without affecting the bulk properties. The base material used (Elastollan 1180A50) may be used in other applications beside the CellForce sensor.
-A set of new gene double constructs were made and a set-up of Fret and Flim analysis was developed to be used also in other projects.

4.4 Threats. Information for other scientist that want to work in this field

-Cells adapt probably their forces according the stiffness of the substratum
-Cells go in between the pillars if they are able to adhere even if less as on the top of the pillars
-The base material used (Elastollan 1180A50) may be used due to its bulk properties and its surface can be modified either to be cell adhesive or cell repellent, without affecting the bulk properties.
-If ever tried in future it becomes extremely challenging to coat pillars below a certain stiffness as postprocess since they collapse or glue together due to the forces applied to the pillars by this treatment. By that a preprocessing technique to obtain cell repellent pillars with solely on top a cell-adhesive surface would probably be optimal. So far, no such process is currently available.
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5 Final Plan for Using and Dissiminating the Knowledge

Within the frame of this project many data were obtained in all involved disciplines. They were or soon will be published (see 5.1 Strength). The obtained knowledge will not be used for the development of a product because of the above mentioned physical and biological limitations. It is not envisioned that a spin-off will be created, a patent will be made or knowledge out of this project will be otherwise commercially used. However, in each lab the knowledge was created that will help to create new projects and by that indirectly may lead to patents and products. However, at this stage no prognosis can be made in this regard.

5.1 Publishable results

Papers

-Alves, P., Coelho, J.F.J., Haack, J., Rota, A., Bruinink, A. and Gil, M.H. (2009). "Surface modification and characterization of thermoplastic polyurethane". Eur. Polym. J., 45: 1412-1419

-Müller, M., Wolf, M., Rösslein, M.; *MUSE:* Computational aspects of a GUM supplement 1 implementation*;* Metrologia, 2008, 45, 586-594

-Alves, P., Kaiser, J.-P., Haack, J., Salk, N., Bruinink, A. and Gil, M.H. (2009) Surface modification of thermoplastic polyurethane in order to enhance reactivity and avoid cell adhesion. *Colloid and Polymer Science 287*, 1469-1474

-Alves, P., Kaiser, J.-P., Haack, J., Salk, N., Bruinink, A. and Gil, M.H. "Thermoplastic polyurethane surface modification by grafting to avoid cell adhesion". – Ready to be submitted

-Bruinink, A., Hasler, S., Manser, P. (2009) In vitro effects of SWCNT: Role of treatment duration. *Physica Status Solidi B* 246, 2423-2427

- Born, A.K., Rottmar, M., Lischer, S., Pleskova, M., Bruinink, A. and Maniura-Weber, K. Correlating cell architecture with osteogenesis: first steps towards live single cell monitoring. *Eur Cell Mater* 287, 1469-1474

-Rota, A., Haack, J. "Spritzguss bringt Materialvielfalt und Fromfreiheit", SMM Schweizer Maschinenmarkt 2007, 14/15, 68-71

- Salk, N., Haack, J., Kaiser, J.-P., Bruinink, A., „Mikrospritzguss von Biosensoren zur Messung von Zellkräften", Tagungsband 14. Heiligenstädter Kolloquium, September 2008, ISBN 978-3-00-025695-0

-Salk, N. , HaackJ., (2009) Herausforderungen an den Mikrospritzguss von biologischen Sensoren“, Kunststoffe und Kunststoffe international 2/2009, Medizintechnikausgabe

Book chapters

Bruinink, A. (2008) In vitro toxicokinetics and dynamics: modelling and interpretation of toxicity data. In: Preclinical Development Handbook (ed. S. C. Gad), John Wiley & Sons, Inc. New York, pp 509-550.

Müller, M., Wolf, M., Rösslein, M. Measurement uncertainty calculation using *MUSE*. In:AMCTM VIII Advanced Mathematical and Computational Tools in Metrology). In Mathematics For Applied Sciences Vol. 78 World Scientific (2009)

Conference presentations/proceedings (oral-poster)

Alves, P., Coelho, J.F.J., Kaiser, J.-P, Spohn P., Haack, J., Rota, A., Bruinink, A. and Gil, M.H. (2007) Modification of polyurethane based materials for biomedical applications. FBPS07, Ghent, Belgium (Poster)

Born, A.K., Bruinink, A., Maniura, K. (2007) Live-cell monitoring of fluorescent cytoskeleton and adhesion proteins for the development of a biosensor. USGEB, Basel, Switzerland (Poster)

Born, A.K., Rottmar, M., Maniura, K., Bruinink, A. (2007) Correlating cell architecture with osteogenesis: First Steps towards live-cell monitoring. ECM VIII, Bone Tissue Engineering, Davos, Switzerland (Poster)

Born, A.K., Rottmar, M., Maniura-Weber, K., Bruinink, A., Krug, H. (2007) Live-cell monitoring tools for cell-surface interaction investigations. CFN Summer School on Nano-Biology, Bad Herrenalb, Germany (Poster)

Born, A.K., Rottmar, M., Pleskova, M., Maniura, K., Bruinink, A. (2007) Live-cell monitoring tools for cell-surface interaction investigations. Biosurf VII, Functional Interfaces for Directing Biological Response, Zürich, Switzerland (Poster)

-Born, A.K., Bruinink, A., Maniura, K. (2007) Analysis of focal adhesion protein interactions using fluorescence resonance energy transfer. American Society for Cell Biology, 47th Annual Meeting, Washington, DC, USA (Poster)

-Alves, P., Kaiser, J.-P., Bruinink, A. and Gil, M.H. (2008) Thermoplastic polyurethane surface modification in order to obtain carboxyl groups into the membrane surface. ANM, Aveiro, Portugal (Poster)

-Alves P., Kaiser, J.-P, Haack, J., Rota, A., Bruinink, A. and Gil, M.H. (2008) Surface modification of thermoplastic polyurethane in order to enhance reactivity. Chempor 2008, Braga, Portugal (Poster)

-Born, A.K., Bruinink, A., Maniura, K. (2008) Analysis of focal adhesion protein interactions using fluorescence resonance energy transfer. USGEB, Biology Meets Engineering: New Landscapes in Life Sciences, EPFL Lausanne, Switzerland (Poster)

-Born, A.K., Bruinink, A., Maniura, K. (2008) Cellular mechanosensing: Analysis of molecular interactions between fluorescently tagged focal adhesion proteins talin and vinculin. 14th Swiss Conference on Biomaterials, Basel, Switzerland (oral)(**Awarded** as best young scientist presentation)

-Born, A.K., Bruinink, A., Maniura, K. (2008) Analysis of focal adhesion protein interaction using fluorescence resonance energy transfer. 8th World Biomaterials Congress, Amsterdam, Netherlands (poster)

-Born, A.K., Bruinink, A., Maniura, K. (2008) Analysis of focal adhesion protein interaction using fluorescence resonance energy transfer. GRC Biointerface Science, Aussois, France (Poster)

-Born, A.K., Bruinink, A., Maniura, K. (2008) Analysis of focal adhesion protein interaction using FRET. Joining Forces: Sensing and Manipulating in Live Cells, ETH Zürich, Switzerland (Poster)

-Alves, P., Kaiser, J.-P., Bruinink, A. and Gil, M.H. (2009) Surface modification of a thermoplastic polyurethane (TPU) to reduce cell adhesion. Euromat2009, Glasgow, UK (Poster)

-Alves, P., Bruinink, A. and Gil, M.H. (2009) Surface modification of thermoplastic polyurethane in order to enhance reactivity. POLYMCON'09, Calicut, India (oral)

-Born, A.K., Bruinink, A., Maniura, K. (2009) Analysis of molecular interactions between focal adhesion proteins talin and vinculin using FRET. 32nd Annual Meeting of the German Societies for Cell Biology, Konstanz, Germany (Poster)

-Born, A.K., Bruinink, A., Maniura, K. (2009) Analysis of molecular interactions between focal adhesion proteins talin and vinculin using FRET. ESF-EMBO Symposium Biological Surfaces & Interfaces, Sant Feliu de Guixols, Spain (oral)

-Born, A.K., Bruinink, A., Maniura, K. (2009) Analysis of molecular interactions between focal adhesion proteins talin and vinculin using FRET. 22nd European Conference on Biomaterials, Lausanne, Switzerland (oral)(**Awarded** as best young scientist presentation)

-Wolf, M., Müller, M., Rösslein, M., Gander, W. (2008) Measurement uncertainty calculation using MUSE *A software project to support the measurement community with a freely available implementation of GUM supplement 1"*; Conference Proceeding: Conference on advanced mathematical and computational tools in metrology and testing June 23-25, ENS Cachan, France

-Wolf, M., Müller, M., Rösslein, M., Gander, W. (2008) Messunsicherheit - Softwaregestützte Modellierung und Simulation komplexer Messvorgänge / Measurement Uncertainty - Software based modelling and simulation using complex measurement procedures" Conference Proceeding: VDI Tagung - Messunsicherheit praxisgerecht bestimmen 12 & 13 November 2008

-Müller, M., Wolf, M., Rösslein, M. (2008) Measurement uncertainty calculation using *MUSE*; Conference Proceeding: AMCTM 2008. June 23-25, ENS Cachan, France; Conference Proceeding (2008)

-Müller, M., Wolf, M., Rösslein, M., Gander, W. (2007) Limits of the uncertainty propagation: Examples and solutions using the Monte Carlo Method; 13. Internationaler Metrologie Kongress, Lille France 18 - 21 June 2007 Conference Proceeding

-Plaggenborg, T. (2007) Mikro-Spritzgießen in der Medizintechnik", Compamed Frühjahrsforum June, Dortmund, Germany (oral)

-Salk, N., Haack, J., Kaiser, J.-P., Bruinink, A., Rota, A., (2008) Biological sensor for cell monitoring via micro injection moulding technology", Conference Proceedings: EUSPEN Conference 2008, May, Zurich, Switzerland (Poster)

-Hein, S., (2009) Micro manufacturing in the field of Biomaterials-Technology. Hanover Fair 2009, Micro Technology Forum, April, Hanover, Germany (oral)

-Haack, J., Hein, S., Salk, N., (2009) Functionalized micro products made of adapted materials", accepted as Conference Proceeding: 4M/ICOMM 2009 Conference, 23. – 25. September, Karlsruhe, Germany (Poster)

-Glisovic, A., Salk, N. (2009) Mikrofertigung von Biomaterialien", 5. Jahrestagung des Arbeitskreises Mikrosysteme für die Biotechnologie und Lifesciences e.V., München, Juni 2009 (oral)

Other output

Training courses

-FCTUC: One month in the Department of Organic chemistry of the Ghent University, to work with a plasma device.

-FCTUC: One-day seminar about XPS (X-ray photoelectron spectroscopy) and AES (Auger Electron Spectroscopy) in CEMUP (Centro de materiais da universidade do Porto), in Porto.