5.7 Exploratory projects

5.7.1 Investigations of mussel adhesive proteins for application in biomaterial research and in clinic

5.7.1.1 Background and description

This project was co financed with another SSF-financed program, MASTEC (Marine Science and Technology). The aim of the project was to investigate the cohesive and adhesive properties of byssal proteins from the common blue mussel. Many natural adhesives have an extremely high adhesive strength to solid surfaces. Examples are certain adhesives from blue mussel and certain algae. If the adhesion process and the chemical cross-linking of the adhesives can be controlled, it will open many new application areas in biomaterials. Examples of new application areas may be different forms of tissue adhesives, and different application areas in dentistry.

Figure 5.6: A blue mussle hanging in its byssal threads, firmly attached to the surface above. (H. Elwing)

5.7.1.2 Scientific results

A dominating part of the project was to develop Quartz Crystal Microbalance with dissipation measurements (QCM-D) for the analysis of cross-linking of adsorbed monolayers of mussel adhesive proteins. This work was successful, and we have also used the technique for the investigation of other marine adhesives, for example adhesives form algae. The most prominent finding is that we, in real time, can monitor chemical cross-linking of the marine adhesives. This demonstrates a possibility to control the cross-linking reaction, which is a prerequisite for the development and use of marine adhesives as a biomaterial "glue". One Ph.D. thesis was evolved from the project, namely, Camilla Fant: "Studies on Cross-Linking and Protein-Protein Interactions of Adhesive Proteins from the Blue Mussel" Ph.D. Dissertation Göteborg University 2002. The project ended in 2002.

5.7.2 Supported biomembranes

5.7.2.1 Background and description

Supported phospholipid bilayer (SPB) membranes have received growing interest during the last decade. One reason is their potential or proven value for applications in design of biosensors, coatings of medical implants, drug delivery, drug screening, catalytic interfaces and as biologically inert surfaces (Sackmann, E., Science, 1996. 271: p. 43-48. and Tampé, R., *et al.*, H.C. Hoch, L.W. Jelinsi, and H.G. Craighead, Editors. 1996, Cambridge University Press: Cambridge. p.201-221.) A potentially very interesting application for the future is to use these membranes as supports for controlled stem cell growth and differentiation. A second reason, followed up in teh SSF programme BIOMICS is to huse such layers - or intact vesicles - for biosensing platforms.A third reason for the interest in SPBs is that they are presenting a number of challenges of purely scientific nature, e.g., what are the mechanisms behind the autocatalytic and self-assembling processes involved in SPB formation? Functionalized membranes constitute interesting model systems for cell membranes and may provide a route to understand - or at least study - some aspects of cell-mediated processes such as cell-cell interaction, biological signal transduction and budding. During the past few years, many different types of supported membranes have been produced and characterized. The most promising technique to form bilayers is self-assembly from vesicles in solution.

5.7.2.2 Scientific results

The above questions have been addressed in this project (and in other related projects) over the past six years (see A.5 ref 7.2:7 and references therein) by experimental and mathematical simulation methods. First the formation of bilayers from vesicles in solution was studied. Today a thorough –but not complete – understanding has been achieved of how an initially clean surface is converted to a completely bilayer covered surface (on e.g. a silica surface). The methods employed were QCM-D, SPR and AFM. AFM provides microscopic information (Postdoc. Michael Zäch 100% supported by the programme). The picture that emerged is: Vesicles (25-200 nm) that arrive at the surface are first adsorbed intact. When the coverage reaches a critical value, the vesicles rupture and fuse to bilayer patches. As more vesicles are added, they continue to rupture and fuse until a complete bilayer is covering the surface. This picture is corroborated by Monte Carlo simulations (ref. 7.2:1).

The above mapping was followed by measurements of protein adsorption and cell attachment/growth on these surfaces, showing a high degree of protein and cell resistance (partly within the Biomaterials consortium). Then SPBs were made active by incorporation of functional molecules such as biotin and membrane proteins (mainly within BIOMICS and Chalmers Bioscience initiative). The current activities are focused on two directions (in this programme and in a new FP6 EU STREP project “Nanocues”). The first direction is nanoscale patterning by AFM of surface patterns containing alternating areas of bilayers, intact vesicles and empty surface. Such patterning is very interesting for steered cell growth and sensing. This part of the project continues until the end of 2004 with remaining funding from this project. The second direction is binding of stem cell specific peptides (Dr. J. Gold) to the bilayers and studies of specific cell interaction with such functionalized bilayers (collaboration with Prof. Ernest Arenas, KI). This part will successively merge into the EU STREP project “Nanocues”.

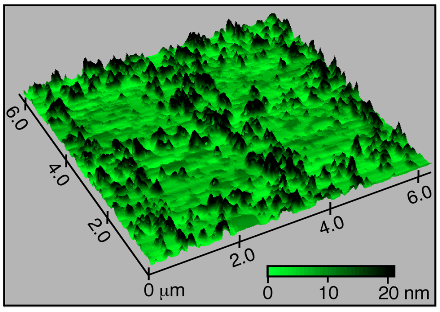

Figure 5.7: Pattern of coherent lipid bilayer patches in a sea of surrounding, unruptured vesicles (black protrusions) on a solid support as produced by scanning probe lithography. The pattern was produced by intentionally causing ruputure of vesicles by scanning at an increased imaging force. (M. Zäch)

5.7.3 Neural stem cell culture model

5.7.3.1 Background and description

The influence of controlled surface chemistry and topography on neural stem cell differentiation *in vitro* is the topic of this project. It is a collaborative extension of the TFPS and STIM projects, with input also coming from projects funded by Chalmers Bioscience Program, VR and the SSF Bioelectronics Programme (O Orwar). One main contribution from the SSF Bioelectronics Programme is the establishment of a new cell culture facility at Chalmers, which finally became functional in June 2003 after a ca. 2.5 year delay. Surfaces developed within the STIM and TFPS projects, together with surfaces containing differentiation "trigger" molecules (from VR/CHA-Bio projects), are studied in a rat neural stem cell culture model in collaboration with Professor Peter Eriksson, Inst. of Clinical Neuroscience, Dept. of Experimental Neuroscience, Sahlgrenska University Hospital, Göteborg. The project is planned to continue until September 2004.

5.7.3.2 Scientific results

Work to date has focussed on the biochemical modification of surfaces and their influence on neural stem cell (i.e. AHP - adult hippocampal progenitor) attachment, proliferation and differentiation. Laminin adsorbed on a layer of polyornithine is the standard culture surface for AHP cells (control). CNTF is a strong inducer of glial cell fate in AHP cells. Fibroblast growth factor, when present in the culture media, will prevent differentiation of AHP cells. All of these proteins, when adsorbed to glass, promote cell attachment, spreading and cell body extensions. Image analysis derived parameters for cell area and shape elucidated differences in cell morphology on different proteins. We are evaluating if cell shape at 24 hrs can be used as an early marker of differentiation. We have obtained 3 surfaces which induce glia, neuron and no differentiation, respectively.

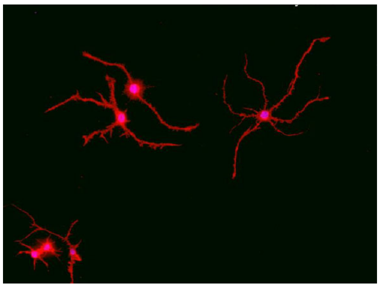

Figure 5.8: Actin-stained AHP cells on a laminin coated surface. (J. Kelly, J. Gold)

Work in progress include studying neural stem cell attachment, proliferation and differentiation on nanostructured biopolymer surfaces (produced in collaboration with the 5th framework EU programme "Nanomed") as well as microstructured pyramid surfaces in PDMS (silicone), which were developed by the TFPS project and used by the STIM project in other cell models.

5.7.4 Bone cell culture model

5.7.4.1 Background and description

This project was a collaboration between Dept. of Handicap research (HKF) at Göteborg University and IFM at Linköping University. The goals of the project were to prepare a bioactive functionalized implant surface that shows: (i) a thinner fibrous tissue formation around implants and (ii) a quick functional integration to tissues, initially to bone. Cell attachment and spreading, the expression of phenotype, the production of mineralized deposits and gene expression (DNA-arrays available via SWEGENE) were used to evaluate the bone cell response.

5.7.4.2 Scientific results

Surface evaluation revealed similar roughness for the turned control and the anodised surface with Mg^{2+} ions incorporated, while the blasted surface demonstrated a rougher surface profile.

LDH (lactatdehydrogenas) values were generally low for all surfaces (within the range of 0.8-1.6 µkat/l) but were slightly increased after LPS stimulation and after 72h. TNF-α was transiently higher day one and after LPS stimulation, especially on the turned control surface. Further, IL-10 levels were generally low irrespective of time. Increased IL-10 amounts after LPS stimulation and after 24h were observed for all surfaces. The total cell numbers decreased on all surfaces from 24h to 72h but there were no major difference between stimulated and non-stimulated wells. Acute monocytic phenotype 27E10 marker dominated on all surfaces while the expression of the chronic RM3/1 marker was almost absent on all surfaces both at 24h and 72h.

Conclusion: The present study indicates a surface topography and chemistry related difference in the acute inflammatory response with a stronger acute inflammatory response to the turned control compared to the blasted and the anodised surface with Mg^{2+} ions incorporated. The project will continue until the end of year 2004.

5.7.5 Lotus-leaf effect

5.7.5.1 Background and description

The main idea of this project is to explore the correlation between surface structure, wettability, and contamination of different material surfaces in an approach inspired by the mechanisms of the so-called Lotus Effect. When a water droplet runs over a superhydrophobic surface, like a Lotus leaf, the droplet takes all the dirt with it from the surface, and the surface seems to be self-cleaning.

More specifically, the lotus leaf project includes:

(i) preparation of micro- and nanopatterned surfaces using different techniques
(ii) their topographical and chemical characterization, and
(iii) pointing out and exploring possible applications

The project is a close collaboration between the Dept. of Applied Physics and the Dept. of Electrical Engineering at Chalmers and GU. This is an example of collaboration between the Biocompatible Materials and the ELIS (also SSF) programmes.

5.7.5.2 Scientific results

The major result up to now, is the preparation of super-hydrophobic polymer (PDMS) surfaces by pulsed laser treatment. Micro- and nanofabrication has been combined to prepare micropatterned silicon samples that are used as templates for the molding of PDMS. The polymer samples are characterized by optical and electron microscopy, interferometry, AFM, contact angle and XPS. Electrical measurements to test the long-term durability are initiated. The typical sample size in this project is many square centimeters and the technique also enables the use of non-flat surfaces. Two papers are in preparation and the project will end in June 2005.

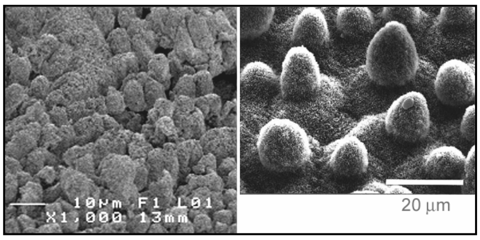

Figure 5.9: Left: SEM image of surface produced within the project. Right: SEM image of the surface of a Lotus leaf. (D. Chakarov, P. Holgerson)

5.7.6 Theoretical modelling and simulations

5.7.6.1 Background and description

Theoretical modelling of such a complex situation as the structure and processes at a biointerface is a great challenge. Due to the complexity and many unknowns, mainly highly phenomenological models are feasible to simulate the real situation. Still, it is highly desirable to develop both such complex models and also much simplified models, in order to provide simulation tools that can organize and incorporate the growing mass of experimental information into descriptive models. The usefulness of such models is both in order to analyze data, and to organize the knowledge into conceptually reasonable and transparent models, and of course, to guide experiments. In the present work we have addressed a number of issues relevant to this program. The methods of choice were Monte Carlo (MC) simulations (MCS), and in some cases mean field approximation (MFA). This whole work was made possible by the long standing and extremely productive collaboration with ProfessorV.P. Zhdanov from the Boreskov Institute in Novosibirsk. He recieved a guest professorship at GU during this project period.

5.7.6.2 Scientific results

The first addressed problem was protein adsorption, including protein unfolding at surfaces. This also included processes in adsorbed 2D protein layers such as nucleation and condensation of 2D islands. A second area was simulations of protein folding –one of the long standing challenges in theoretical molecular biology. Significant progress was made in understanding different moves and the role of residue interactions in these MC models (one licentiate thesis).

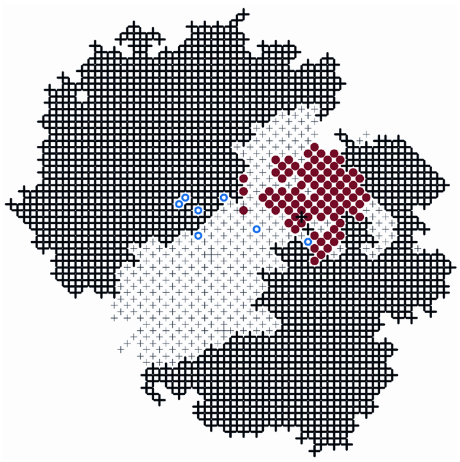

Figure 5.10:. One of the patterns obtained during Monte Carlo simulations of the 2D cellular growth [ref 7.5:23]. The simulations started from a single stem cell. After a while, there were cells of four types: Open, blue circles show stem cells. Filled, red circles and thin and thick plus signs represent cells of the other three types.

Another area that has been treated over several years in a number of publications is vesicle adsorption, and their subsequent rupture and fusion to a bilayer (see section 5.7.2). These simulations led to a phenomenological model which today is the basis for our current picture of bilayer formation, and which is also guiding new experiments to fill in "white spots" in the model.

More recently cellular processes have been addressed. One is glycolytic (metabolic) oscillations in cells. This has in turn led to a collaborative paper with Dr Agneta Richter at Karolinska Institute (KI) to model the Ca^{2+} related oscillations that her group recently reported in Nature.

Finally and more recently a scheme has been developed to mimic, by MC models, stem cell division and differentiation in a 2 D layer with an underlying surface influencing the cell-cell signalling and evolution. Although at a primitive stage we judge this as an extremely important tool for future development in this area and for tissue engineering. It will also be part of the EU Nanocues project and of the collaboration (Dr. J. Gold) with e.g. Prof. Ernest Arenas at KI.

5.8 Projects which were initiated as cooperation with academic groups initially outside the programme

5.8.1 Background

In autumn 2002 it was decided to extend the scope and network of the Biocompatible Materials programme by inviting academic research groups outside the initial programme participants to send in proposals for collaboration projects with research groups in the programme. The call for proposals was issued via the Project Leaders, encouraging colleagues not yet involved in the programme to send in proposals (the latter had to be the applicants).

The proposals were evaluated by the Board at the board meeting in December 2002, and four of them were approved for funding.

In total, 2189 kSEK were allocated, and the projects demonstrate clearly that the focus of science has changed from "conventional" biomaterial aspects towards more fundamental aspects of biocompatibility. At the same time they have generated new contacts, network and "new blood" into the programme.

5.8.2 Cell-force sensor

This project, with significance to biomaterials as well as basic cell biology and even drug screening research, is a collaboration between the Bioimplant Research Group, Dept. for Medicine, Surgery and Orthopaedics at Lund University (Prof. L.M. Bjursten), and the Chemical Physics group at Chalmers/GU (Dr. J. Gold). Before this project started, silicon substrates with vertical micropillars were developed by Sarunas Petronis at Chalmers in the Time and Functionally Programmed Surfaces project as a well-controlled porous surface model for studying cell behaviour at surfaces. Initial experiments in cell culture indicated that cells growing on top of the pillars deflected the micronwide pillars, and presented a new way of measuring the force exerted by cells on the underlying surface. The idea to use this for cell force measurements was the basis for the new project. By knowing the stiffness (or spring constant) of the pillars, measurement of the deflection of the top of the pillar can be converted to the force needed to create the deflection. These are the forces which the cells are exerting on the pillars. In this project, time-lapse microscopy is used to monitor living cells migrating on the pillared surfaces, as well as to follow the real time deflection of the pillars. Image analysis of video sequences allows the plotting of individual force vectors exerted by the individual focal contacts. Since the pillars are on the order of one or a few microns in size, many pillars are located under individual cells. It is therefore possible to map the force vectors occurring around a single cell at any instant in time. Many cell types have been examined and differences in forces exerted by different cell types have been observed. This project has now evolved into a Ph.D. student project, with funding recently obtained from other external funding sources (VR, Chalmers Bioscience Programme).

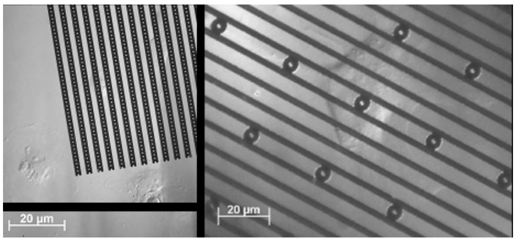

Figure 5.11: SEM images of the cell-force sensor (S. Petronis, N. Tymchenko, L.M. Bjursten, J. Gold)

The project was approved by the Board at the board meeting on December 2, 2002, with a grant of 168 kSEK. Additional funding of 1340 kSEK was approved at the board meeting on June 3, 2003.

5.8.3 Neural stem cell differentiation under electrical stimulation

A collaboration between Chalmers/GU and Rensselaer Polytechnic Institute, Troy, NY, USA has been established through this project. Despite the crucial importance of the nervous system, the field of neural stem cell differentiation is still quite unexplored. The underlying hypothesis for this *in vitro* research project is that electrical stimulation induces asymmetric differentiation in neural stem cells. Cells are to be cultured at Chalmers on conductive polymeric biomaterials formulated at Rensselaer and characterized at Chalmers. The effects of electrical parameters (such as magnitude of the current, duration of exposure etc.) on neural stem cell proliferation and differentiation based on phenotype expression will be investigated using a laboratory setup developed at Rensselaer and reproduced at Chalmers. Johan Gustavsson, scholar from Chalmers, will travel to Rensselaer for 3 months (January - March 2004) to learn to use the experimental setup and the conducting polymeric cell culture substrates. Experiments will be performed upon his return to Chalmers, with an estimated project completion date of Sept 2004.
The project was approved by the Board at the board meeting on December 2, 2002, with a grant of 110 kSEK.

5.8.4 Probing interactions between phospholipids and phosphatidyl choline containing polymers

This project is conducted in collaboration between the Chemical Physics group at Chalmers/GU (Dr. J. Gold) and the Polymer Chemistry group at the Ångström laboratory, Uppsala University (Prof. J. Hilborn). The Uppsala group is working with biodegradable polymers, in this case polyesters (PCL) or polycarbonates (PTMC) with incorporated phosphoryl choline (PC) head groups. Phospholipids with the PC head group together with various proteins constitute a large part of natural cell membranes and therefore this biomimetic polymer holds promises for a novel type of biomaterial. In this project, the PC-containing phospholipid-like polymers are to be investigated in terms of interaction with natural phospholipids. Experiments have for instance included the ability to make PC lipid bilayers on top of the PC polymer but also to study the interaction of phospholipids with the PC containing polymer. The group at Uppsala has developed a novel synthesis to fabricate the polymeric material. Following the synthesis, the material is covalently attached onto the QCM crystal. All interaction experiments are performed using the QCM-D technique at Chalmers/GU. Regarding the coating of the QCM crystals both a spin-coating method and a grafting method have been evaluated, where the grafting method has been the most successful. Addition of sonicated POPC vesicles resulted in adsorption of intact vesicles.

The project was approved by the Board at the board meeting on December 2, 2002, with a grant of 271 kSEK.

5.8.5 Mucin modification of biomaterials: Biopolymer adsorption kinetics and surface arrangement

Mucins are macromolecular proteoglycans that are found in tear fluid, saliva and other lubricating secretions. These molecules are known to adsorb to a variety of surfaces, particularly to hydrophobic surfaces, but the adsorption process is not well studied. Mucin coated surfaces give rise to a significantly reduced adsorption of proteins, bacteria, and cells, so an increased knowledge about the adsorption process is of large interest. In this collaboration project between the Centre for Surface Biotechnology at Uppsala University (Prof. K. Caldwell), Dept. of Chemistry at KTH, and the Dept. of Applied Physics at Chalmers/GU, the adsorption behaviour of mucin from bovine saliva onto hydrophobic model surfaces is studied. The adsorption process is characterized in terms of its various phases, surface arrangements at steady state and competitive adsorption with smaller proteins that are also present in saliva.
The complex starting material (commercial bovine submaxillary mucin) has been fractionated to yield a well-defined fraction with a molecular weight of $2x10^6$ Dalton (by static light scattering). The adsorption behaviour of this fraction on hydrophobic model surfaces (polystyrene) has been studied using QCM-D. Given that ultra-high molecular weight materials of the mucin type are present in a multitude of conformations, reflecting the concentration of the polymer, much attention has been focused on finding conditions with reproducible adsorption behaviour. Such conditions have now been identified, and an aliquot of “standard mucin” has just been forwarded to KTH (Dr. Eva Blomberg, YKI) for adsorption studies by means of AFM and surface force apparatus. It is anticipated that the final result of our joint program will be at hand on June 1, 2004.

The project was approved by the Board at the board meeting on December 2, 2002, with a grant of 300 kSEK.

5.9 Projects in cooperation with industries

5.9.1 Background

In autumn 2002 it was decided to extend the scope and the industry network of the Biocompatible Materials programme by inviting industries so far not engaged in the programme to send in proposals for collaboration projects with research groups in the programme. The proposals were evaluated by the Board at the board meetings in December 2002 and February 2003, and five of them were approved for funding. In total, 2069 kSEK were allocated for the projects, with 1286 kSEK coming from the programme and 783 kSEK from the industries. The projects demonstrate clearly that the knowledge and the facilities associated with the programme to a considerable extent can contribute to R&D activities in established as well as in start-up companies.

5.9.2 ToF-SIMS-analysis of complex surface modifications

The company which submitted the application, Carmeda AB, is a company focussing on blood compatible surfaces. The technology, which is known under the name of CBAS™ (Carmeda Bioactive Surface), is now well-established, and used in a number of bloodrelated products. The technology is based on a unique mechanism to link heparin molecules to a surface, keeping the clotting prohibiting properties of the molecules intact. The heparin molecules can be linked to a large number of substrates like e.g. thermoplastics and metals.

The purpose of the project was to get a deeper understanding of the relation between molecular surface composition and biological function of heparin surfaces, and also to obtain an increased understanding of how secondary ion mass spectroscopy, measured with TOF-SIMS, reflects the molecular composition and structure of complex surfaces. The collaborating research partner was SP in Borås, also a partner in the project "Tribology of Articulating Joints" (see section 5.3).

> Helena Franzén at Carmeda says:
> ”Generally speaking, we believe that an exchange of knowledge, as represented by this project, is of great value for both parties, since we benefit from each others competence and resources. It is also of great value that we together, because of differences in background, can get new ideas how to approach the scientific questions we are working with. Another important issue is that a small company like Carmeda does not have the possibility to in-house have all the competence or all the scientific instruments needed, like e.g. the ToF-SIMS instrument at SP that is used in this project. It is of great value for us to have access to resources in this way. Further, we hope that this cooperation will lead to further exchange of knowledge in the future, to the benefit for both parties."

The project was approved by the Board at the board meeting on February 21 2003, with a grant of 130 kSEK. The contribution from Carmeda was 145 kSEK and from SP 53 kSEK.

5.9.3 QCM-D based characterization of lipid-based sensor templates developed for SPR analysis

The company which submitted the application, Biacore AB, is the world leader in the detection and monitoring of biomolecular binding using surface plasmon resonance (SPR) technology. The company develops, manufactures and markets advanced bioanalytical systems that provide real-time quantitative data on binding interactions between biomolecules. The project was based on using the QCM-D technique, at Dept. of Chemical Physics (Dr. F. Höök), and at Biacore, to study vaarious aspects of the Biacore chips.

> Stefan Löfås, VP, Chief Scientific Officer, says:
> "The Biacore sensor surfaces (or sensor chips) are the core components in the commercial Biacore instrument systems and thus of central importance for the technology. The funding of this project has facilitated the research together with the Department of Applied Physics at Chalmers/GU which had probably not been possible to do otherwise.
> The most significant benefits for Biacore in this collaborative project are as follows:
> - Crucial funding for a bilateral focused project with clear objectives and agreed commitment from both partners.
> - Access to an interesting complementary surface characterisation tool.
> - Access to world class experts (its inventors) for the experiments and interpretations of the data.
> - Extension of the scientific network.
> - Increased knowledge and competence rising among Biacore employees for characterisation methods of importance.
> - Possibility to interact and transfer technology know-how that otherwise had been impossible or a tedious and costly process if done separately.
> - Results of great interest for both partners, which also will be beneficial for Biacore in the future development of the core surface technology."

The project was approved by the Board at the board meeting on February 21 2003, with a grant of 325 kSEK. The contribution from Biacore was 130 kSEK.

5.9.4 Preparation, characterization and immune system activation in blood by new conducting electrode materials in pacemaker applications

The company which submitted the application, St Jude Medical AB, manufactures mechanical and tissue heart valves, pacemakers, implantable cardioverter defibrillators, and electrophysiology catheters.

The purpose with the project is to design a material for electrodes, that do not activate the inflammation system via contact activation by blood. Activation by blood causes fibrous encapsulation of the electrodes, which leads to the need for higher pulse voltage, which in turn leads to the need for more frequent changes of batteries.

The collaborating research partner is Professor Pentti Tengvall at LiU, also a partner in the programme project "Time- and functionally programmed surfaces" (se section 5.6).

Susanne Nilsson, Sr Manager Material Technology at St. Jude Medical AB, says: "Collaborations with Swedish universities and institutes are here very important tools for ensuring a high level on research and development. The access to the expertise and front end research in different areas is a critical tool for a medical device company as basic research funding is limited as the focus for the company is and shall be on applied research.
Projects like the one financed by the SSF, results in an innovative and creative problemsolving, facilitating the development of new functions and applications as well as optimised properties of existing products, as the competence for everybody involved is increased."

The project was approved by the Board at the board meeting on December 2, 2002, with a grant of 140 kSEK. The contribution from St Jude was 140 kSEK.

5.9.5 Soft- and bone tissue reactions on loaded microimplants with different surface modifications

The company which submitted the application, Osspol AB, is in its start-up phase and is not yet on the market with its product, a new dental implant system.

The purpose with the project was to explore the knowledge that was built up in the project "Optimal surface topography for bone anchored implants" (see section 5.2), where different titanium surfaces were investigated in *in vivo* studies on rabbits, and under the restrictions that the studied surfaces should be possible to manufacture in a large-scale production to reasonable cost.

In this project, these investigations were extended to comprise human tissue, using microimplants that were inserted close to regular implants on patients undergoing treatment for edentulism. This is an established technique, but was here modified in the sense that the microimplants had a part penetrating the mucosa, and were loaded via the patients own prostheses. The surfaces studied were based on the knowledge about surface structures built up in the project discussed in section 5.2, and on a recently developed technique to dope the surface oxide with ions expected to enhance the healing process.

Lennart Carlsson, CEO, says: "It is extremely valuable to have direct access to the academic research, especially for smaller companies where the need for expertise and resources often vastly exceeds resources within the company. The best way to gain knowledge for us is to run concrete projects together with academic research groups. This particular project is of considerable importance for us, since it focuses on properties of the implant that are crucial for its performance".

The project was approved by the Board at the board meeting on December 2, 2002, with a grant of 290 kSEK. The contribution from Osspol was 40 kSEK.

5.9.6 Interaction of antithrombin with different types of heparin surfaces with implications for cellular interactions

The company which submitted the application, Corline AB, is a high-tech company at the very fore-front of research, development and manufacturing of biocompatible surfaces for medical devices in contact with blood.

The goal of the project was to explore the mechanisms for antithrombin interaction with immobilised heparin as related to the molecular structure of the immobilised heparin and the implications for cellular reactions and performance in whole blood tests. The collaborating research partner was Professor Rolf Larsson at UU, also a Project Leader for the project “A systematic approach to improve blood compatibility of biomaterials for cardiovascular applications” (see section 5.5).

”The activities within this research project are excellent illustrations of a mutually beneficial collaboration between industry and academia”, says Professor Larsson. ”Industry, in this case Corline, provides interesting and relevant study objects and questions, and academia can use their sophisticated instruments and knowledge as part of an ongoing education of young scientists to provide useful answers published in peer-reviewed scientific reports.”

The project was approved by the Board at the board meeting on February 21, 2003, with a grant of 401 kSEK. The contribution from Corline was 275 kSEK.

6 The "graduates" of the programme

In appendices A6-A9 all relevant data are included regarding the students who participated in the programme.

The students were mainly recruited on a traditional basis via undergraduate courses, master degree theses etc., and one of them were recruited by a broad public advertisement. There were also a number of students, so called “free movers”, who were not initially appointed to a particular group or project, but who instead were given the opportunity to choose freely a topic and a group in materials science for their thesis work. One of them chose to work in the Biocompatible Materials programme. Both SSF and Chalmers were supporting the free mover positions with special grants of 3 MSEK/year, i.e. 12 MSEK, and 6 MSEK/4 years, respectively. All students followed courses developed within the programme “Graduate School in Materials Science”, and the additional courses needed were chosen according to the specific individual needs and graduate programmes.

A unique feature with the graduate training of the programme was that the students did their research work within the framework of interdisciplinary projects. They were therefore not limited to only one specific research group, but were encouraged to understand and acquire knowledge over the traditional academic borders. The pronounced project form adopted for the research work provided an understanding and training for the students to work according to specific plans towards well-defined goals. The availability of the Graduate School in Materials Science constituted a major facilitator for such interdisciplinary work and studies.

In total, nineteen (19) students graduated from the program, fourteen (14) with a PhD exam and five (5) with a Licentiate exam. Of these 19 students, six (6) are women, i.e. 32%. Of the 19 students, six (6) were financed 100% by SSF.
Another four (4) students are expected to get their Ph.D exams in 2004-2005, yielding a total of 18 Ph.Ds.

7 Impact of the programme – on industry and society

Throughout the programme, the issue of its impact on industry and society was of considerable and continuous concern to the programme management and the programme Board. The Board in itself comprised extensive knowledge about the needs and requirements from the industrial society as well as from the health care system, which was of great help to the programme management in getting support and resources for different exposing and contact creating activities.

In principle, the impact of this programme on industry and society is strongly associated with virtually all aspects of health care, ranging from medical device and pharmaceutical companies to clinical treatments and diagnosis of patients. Also, areas like biosensors and tailoring surfaces with specific properties like low friction or fouling resistant surfaces (Lotus leaf effect), are within the scope of this programme. However, the impact of the research of the programme on the health care system will not be “visible” in terms of e.g. products for still a number of years. The lead time from research to products and/or clinical procedures is very long, as a minimum 10 years, but we are convinced that the research represented by this programme will sooner or later be manifested economically in a profound way, and will also be carried further by research in other programmes and projects, like the newly started EU project.

Several of the projects have enjoyed continuous support in terms of money and advice from different industries, reflecting the traditionally good contacts between the academic system and the industrial society (see Table 7.1). Via these contacts, knowledge about the research in the programme has been transferred to parties with an interest to exploit the results.

Table 7.1: Industrial support/ involvement

Project	Industry involved
Optimal surface topography for bone anchored implants	Nobel Biocare
Tribology of articulating joints	AstraTech AB, Scandimed AB, Bone Support AB, Poli Hi Solidur , TA Contrast
Screening of tissue integrated materials	-
A systematic approach to improve blood compatibility of biomaterials for cardiovascular applications.	Jomed International AB, Corline Systems AB
Time and functionally programmed surfaces	Pacesetter AB

The network of informal and formal industry contacts is regarded as an important asset for the future, generated by this programme. The impact of this continuous support has not been evaluated by the programme Management, since it has to be considered as activities that are not initiated and supervised by the programme.

“Industrial road show”
During the period April 2001 – June 2002 a number of industries were visited, with the purpose to expose the two programmes ”Biocompatible Materials” and ”Molecular Engineering in Polymer Science” to the R&D staffs. All the selected industries are active in areas where the research in the programmes represents existing or potential areas of interest, like e.g. biomaterials and DNA research. The overall impression was that there is a profound interest among industries to assimilate the knowledge built up in the programmes, e.g. via research contracts with the academic groups or via regular courses in selected topics like Biomaterials. Read more in A.14.

Within the programme, a number of activities have been performed, with the purpose to inform the industry about the programme in general, and to engage interested industries in collaboration projects. The overall impression was that there is a profound interest among industries to assimilate the knowledge built up in the programme, e.g. via research contracts with the academic groups or via regular courses in selected topics like Biomaterials. The small projects that were run very late in the program, between the academic groups and new industries (still ongoing in some cases) are likely to generate continuous collaborations beyond the end of this programme.

Collaboration projects initiated by industries
To further strengthen the industrial interest in the programme, the Board decided in autumn 2002 to call for proposals for collaboration projects between industries and research groups within the programme. A number of proposals were evaluated by the Board, and as a result five projects were approved. The projects are described in section 5.9, and they clearly demonstrate two important reasons for industrial engagement: (i) The possibility to have temporary access to advanced research instruments with pertinent analytical resources, and (ii) the possibility to have temporary access to front line knowledge, thereby expanding their own technology platform(s) into new business opportunities. Read more in A.14.

Closely linked to industrial collaborations is the issue of Intellectual Property Rights (IPR). For many researchers, patenting of results from their research is a natural ingredient in their professional life. For others, the patenting process is an unknown world with very little attraction to them. This was reflected in the fairly low number of patents produced within the framework of the projects. Several efforts were made, in terms of information and encouragement, to get the researchers to write patents. Nevertheless, very little happened until the Board allocated specific resources for a project called the “Patent project”. This project involved gathering participants from the programme and professional patent lawyers, with the invaluabel participation of the Board member Mats Leijon (with >250 patents) into an “exercise” that consisted of an inventory of patentable ideas followed by writing and filing some of them. (Read more in A14-15.) The patent applications and approved patents that have been produced within the framework of the Biocompatible Materials programme are listed in appendix A.11.

The patent project
During the year 2000, a series of meetings were arranged with a group of researchers from the Biocompatible materials programme and three experienced patent engineers. As a bridge between these two groups of people and as an enthusiastic leader and source of inspiration was Professor Mats Leijon, member of the Board. Several patentable ideas were identified, drafts of patent applications were written and the whole group together discussed and commented the texts. This process resulted in that a number of patent applications were submitted, but also, which was the main purpose, that the group of researchers are now much better educated in the process of writing and filing patents and in patent strategical thinking. From the evaluation of the project (see appendix A.15) it was clear that the researchers involved in the project definitely will consider to patent ideas of commercial interest in the future. Read more in A.14.

The Biocompatible Materials programme was presented at the “2002 års Medicintekniska Konferens”, 8-9 October, at Huddinge University Hospital in Stockholm. The conference is an annual event, arranged by SSF and VINNOVA (The Swedish Agency for Innovation Systems). All major projects were presented by the Project Leaders together with their students. The conference programme is enclosed as appendix A.16 to this report.

8 Impact of the programme – on the academic system

The Biocompatible Materials programme comprised a large number of research groups at different universities. Already from the beginning of the programme, the need for scientific collaboration between different disciplines were identified and manifested through the organization of the research in well-defined projects. Each project was lead by a project leader, with pronounced responsibility for budgeting, planning and follow-up of the work, even in those cases where several departments and several universities/ institutes were involved.
In Table 8.1 below, the organizational project structure is presented for the five main projects and the six exploratory minor projects (sections 5.2-5.7):

Table 8.1: Organizational project structure for the main and the exploratory projects

Project	C1	C2	GU1	GU2	GU3	LiU	UU1	UU2	LU	KI	SP	HH	GS
Optimal surface topography for bone anchored implants	x		PL										
Tribology of articulating joints					x	x			PL		x		
Screening of tissue integrated materials (STIM)	PL			x						x			
A systematic approach to improve blood compatibility of biomaterials for cardiovascular applications							PL	x				x	x
Time and functionally programmed surfaces (TFPS)	PL					x							
Investigations of mussel adhesive proteins for application in biomaterial research and in clinic	x			PL									
Supported biomembranes	PL												
Neural stem cell culture model	PL												
Bone cell culture model; functionalized bone materials *in vivo*			x			PL							
Lotus-leaf effect	PL	x											
Theoretical modelling and simulations	PL									x			

C1: Dept. Applied Physics, Chalmers and Göteborg University
C2: Dept. Electric Power Engineering, Chalmers
GU1: Dept. Prosthetic Dentistry/Dental Technology, GU
GU2: Dept. Cell and Molecular Biology, GU
GU3: Dept. Biomaterials/Handicap research, GU
LiU: IFM Applied Physics, LiU
UU1: Dept. Clinical Immunology and Transfusion Medicine, UU
UU2: Dept. of Physics, UU
LU: Dept. Orthopaedics, LU
KI: Microbiology and Tumour Centre, KI, Stockholm
SP: Dept. Chemistry and Materials Technology, SP
HH: Huddinge Hospital, Dept. transplantation surgery
GS: Gävle Sjukhus

PL: Project Leader
x: Project partner

Table 8.2 below, shows the corresponding table drawn for the projects mentioned in section 5.8 ("Cooperation with academic groups outside the programme"):

Table 8.2: Projects with academic cooperation outside the programme

Project	C1	KTH	M	RPI	UU3	UU4
Cell-force sensor	PL		x			
Neural stem cell differentiation under electrical stimulation	PL			x		
Probing interactions between phospholipids and phosphatidyl choline containing polymers	x					PL
Mucin modification of biomaterials: biopolymer adsorption kinetics and surface arrangement	x	x			PL	

C1: Dept. Applied Physics, Chalmers and Göteborg University
KTH: Dept. Chemistry, Surface Chemistry, Royal Institute of Technology
M: Dept. Experimental Research, MAS
RPI: Dept. Biomedical Engineering, Rensselaer Polytechnic Inst., Troy, NY, USA
UU3: Centre for Surface Biology, UU
UU4: Dept. Polymer Chemistry, UU
PL: Project Leader
x: Project partner

As one can see from the tables above, cooperation between different universities have been quite extensive, and in total eighteen different departments at twelve universities, research institutes and hospitals have been involved in the programme.

The Biocompatible Materials programme was throughout its life time run in close co-operation with the programmes "Molecular Engineering in Polymer Science" and "Graduate School in Materials Science". The programmes had the same Board and the same programme management, and were run according to the same organisational principles. Also the programme "High Performance Outdoor Electrical Insulation (ELIS)" was linked to the Biocompatible Materials programme, but it was organised somewhat differently and acted more independently because of an obviously smaller common denominator in terms of engineering and basic science.

The close organisational connections between these four programmes were strengthened through different collaboration projects and common activities.

- All Project Leaders meetings were organized with participation from all four programmes.
- A joint application for funding of scientific instruments were submitted from the programmes "Biocompatible Materials", "Molecular Engineering in Polymer Science" and ELIS. The amount applied for was 26 MSEK, and the final approved funding was 10 MSEK.
- Special training in writing patents was organized as an interprogramme activity.
- At the "industrial road show" (see section 7), the two programmes "Biocompatible Materials" and "Molecular Engineering in Polymer Science" were presented on an equal basis, taking into account the recipients special needs and interests.
- The project "Lotus-Leaf effect" is a collaboration project between the Biocompatible Materials Programme" and ELIS.

There were also collaborations with other SSF-funded programmes/ centres. Within the project “A systematic approach to improve blood compatibility of biomaterials for cardiovascular applications”, characterization of heparin surfaces were performed at CAMM (Centre for Advanced Molecular Materials) at Uppsala University. Also, one of the exploratory projects, “Mussel adhesive proteins” was a joint effort between Biocompatible Materials and the SSF-funded programme MASTEC.

The main responsible departments in the programme are all part of the international scientific community, and therefore have collaboration with universities abroad as a natural ingredient in their research activities. The following of their colleagues abroad have been explicitly mentioned in the context of the Biocompatible Materials programme (see Table 8.3):

The Wallenberg application
In 1998 an inventory was done in the four SSF funded programmes “Biocompatible Materials”, “Molecular Engineering in Polymer Science”, “Graduate School in Materials Science” and “High Performance Outdoor Electrical Insulation” regarding need for scientific equipment. The inventory resulted in a list of suggested instruments, and an application was sent in to “Knut and Alice Wallenbergs stiftelse”. The amount of money applied for was 26 MSEK, and was approved with 10 MSEK. The instruments are now placed at the different universities and institutions, and have in a profound way strengthened the experimental and analytical resources of Swedish biomaterials research. Read more in A.14.

Table 8.3: International collaboration

International collaborator	C1	GU1	GU3	LiU	UU1	LU	SP
Nagasaki University Japan			x				
Kyoto University, Japan			x				
Rensselaer Polytechnic Institute, New York, USA	x		x				
ICR, London, England						x	
Risoe Research Center, Denmark						x	
Department of Orthopaedics, Oxford, England						x	
Department of Biomechanics, Stanmore, England						x	
Department of Biomechanics, Nijmegen, Holland						x	
Department of Orthopaedics, UCLA, USA						x	
Orthopaedics Laboratory, USC; L A, Calif., USA				x			
Department of Orthopaedics, Rigshospitalet, Oslo, Norway							x
Industrial Materials Institute, National Research Council, Quebec, Canada							
EPFL, Lausanne, Schweiz							x
Leipniz Research Laboratories for Biotechnology and Artificial Organs, Hannover							x
Dept. of Pathology and Laboratory Medicine, Univ. of Pennsylvania					x		
University of Tromsö, Norway					x		
Dept. of Clinical Engineering (John Hunt), Univ. of Liverpool, England	x						
McMaster University (John L Brash), Ontario , Canada				x	x		
Div Pharmacology and Therapeutics, King’s College, London	x						
Université Catholique de Louvain	x						
ETH Zürich, Switzerland	x			x			
Institute of catalysis, Novosibirsk, Russia	x						

C1: Dept. Applied Physics, Chalmers and Göteborg University
GU1: Dept. Prosthetic Dentistry/Dental Technology, GU
GU3: Dept. Biomaterials/Handicap research, GU
LiU: IFM Applied Physics, LiU
UU1: Dept. Clinical Immunology and Transfusion Medicine, UU
LU: Dept. Orthopaedics, LU
SP: Dept. Chemistry and Materials Technology, SP

The Biocompatible Materials programme has played an important role in the development of Swedish biomaterials research, from being focused on traditional macroscopic biocompatibility aspects towards a more fundamental approach, where also the basic mechanisms on a molecular and cellular level are studied. Also, the programme has continuously encouraged and supported collaborations between Swedish universities and acted as a catalyst in the development of cross-scientific research constellations, like e.g. in the STIM project (see section 5.4) where a very important step was taken when cell biology and molecular biology groups were engaged, adding competence and resources that in a profound way strengthened the project.

The Neural stem cell project
In one of the projects, "Neural stem cell differentiation under electrical stimulation", the purpose was explicitly to establish cooperation between Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA, and Department of Applied Physics, Chalmers, in order to capitalize on the recognized and established strengths of the respective institutions and thus give graduate students and postdoctoral fellows opportunities to receive training in advanced biomaterial surface preparation and characterization at Chalmers, as well as cellular bioengineering techniques that have been established and developed at Rensselaer.

It is obvious, that the Biocompatible Materials programme in a profound way has strengthened the Swedish biomaterials research. New collaboration constellations have been established, mainly as a result of the scientific development of the area, but, indeed, also because of a clear catalytic impact from this programme. New approaches of nano- and microfabrication and associated characterization have been developed, and new knowledge platforms are established. The importance of a close contact between academic research and industrial R&D is implicitly realized from the high intensity and frequency of industrial contacts in the programme, following the general trend that emphasizes the competitive importance of very direct impact from academic research on the industrial product development.

9 Lessons from the programme

One important lesson learnt from the programme Biocompatible Materials is that it is advantageous to build the programme on a clear project structure with Project Leaders that are responsible for the scientific outcome, as well as the planning and follow up of the activities in their projects. In this programme, the project structure, together with the broad competence of a Board comprised of representatives from the academic world and the industry, made it possible to adopt a strategy with a flexibility that was necessary in order to cope with the rapid scientific development of the area.

A second lesson is the value and importance of having a committed Board, but which does not have a vested interest in the funding from the programme for projects which they participate in.

A third lesson is the importance of always having sufficient free resources (not locked up) for new initiatives, or for amplification of successful ongoing projects etc.

It is well known, that the scientific development in bioscience has been catalysed by cross-scientific collaborations between research groups, which traditionally have worked independently from each other. The flexibility described above made it possible to encourage and even fund new constellations of researchers, and even to include academic research groups outside the programme and industries in collaboration projects.

However, in contrast to the production of purely scientific results, whose time scale is well known and established, and in some sense "business as usual", it has also been realized that the time scale to get research results implemented in an industrial environment is very long. In general terms, it is not possible to see an economic impact of even very applied research within a time scale typical for a research programme of the present kind. It is therefore more important to try to continue to establish industrial relations and collaborations, and even start-ups that will begin or continue when the programme is finalized. This requires an ongoing encouragement and education of the researchers in issues like patenting and entrepreneurship, and associated financial means. In this programme a considerable effort was put into the "patent project" (see section 7), which indeed resulted in several patents, but where the entrepreneurial and financial consequences were not fully realized when the project started. It should therefore be of some concern to the Foundation how to cope with transfer of knowledge to the Swedish industry, during the delicate period when the knowledge is projected into patent applications, while it is still too early to engage venture capital or industries.

Finally, one important lesson is that a dynamic field like biointerfaces develops so rapidly that it is not possible to predict the development even over the lifetime of a programme like the present one. Therefore high flexibility in content and reallocation of resources and directions is important, even if the *general* goals and deliverables are fixed.

10 Outlook

The understanding of the basic mechanisms, on a cellular and molecular level, that regulates the interaction between living tissue and foreign materials is rapidly increasing, and the Biocompatible Materials programme has in a profound way contributed to that Swedish research in this area has been strengthened and deepened. A platform of knowledge and methods of high importance for the future has been established. This also means that the label "Biomaterials" in many respects has become too narrow - or ill defined. Biocompatibility may be a better name. "Biointerfaces" is in our view an even better label, although also that name can be misinterpreted. It is obvious that the same basic knowledge is valuable in e.g. tissue engineering, biosensors and biochips, medical implants, and even in non-bio areas like environmentally friendly protection coatings.

A bottle neck is still means to measure cell – surface interactions accurately, but this is expected to improve reasonably rapidly with the development of new diagnostic tools. There still exists no reliable translation procedure from *in vitro* to *in vivo* with regard to medical implants, although many pieces have been developed. It is hard to predict the pace of improvement here.

Mathematical modelling and simulations are expected to rapidly be much more important, both at the fundamental level (protein adsorption/folding/unfolding, biomembrane formation and processes etc.) and at the engineering level (e.g. modelling of bioreactors for tissue engineering). Also, the diversification of the biointerfaces research to all the fields mentioned above plus new areas is expected to continue.

The groups and the individual researchers that have been involved in this programme are in general very active in exploiting the knowledge in new projects. Various parts of the programme will be sustained through other funding channels. For example, the biosensing part of the programme in Göteborg has found additional funding within Biomics (SSF) and Chalmers Bioscience initiative and in one subproject within the SSF-programme Photo-nano. There are some EU projects either funded already (STREP NANOCUES coordinated by Prof. B. Kasemo), or in the pipeline for funding (e.g. a Marie Curie scholarship and one Network of Excellence). Some activities are continuing within a project funded by Vinnova's programme BIONANOIT. All these have to a significant or decisive part their roots in the present programme. The situation for the biomaterials research relevant for medical implants and tissue engineering is more uncertain. We have not made an effort to map all the funding resulting from the programme. The above should be seen as examples, not as a full covering picture. Generally speaking several parts of the programme seem to find new funding, while others have a tougher perspective ahead.

The industrial and clinical applications will most certainly also come, although the several years' perspective typical for product and method development in this area will make it difficult to have an obvious traceability back to the original research.

11 Economic report

A summary of the annual economic reports is enclosed in A.17.