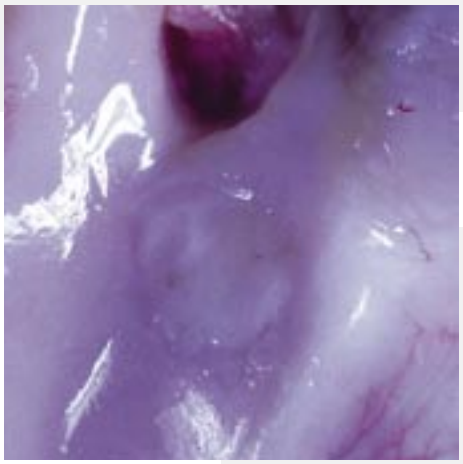
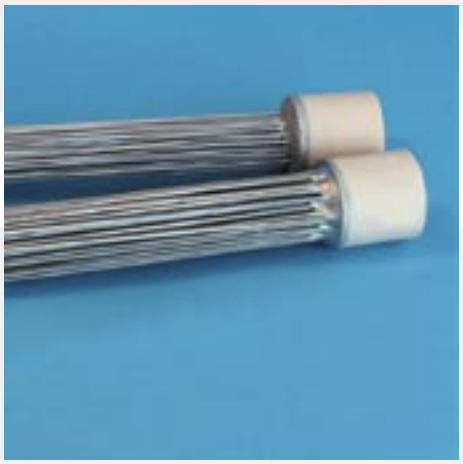
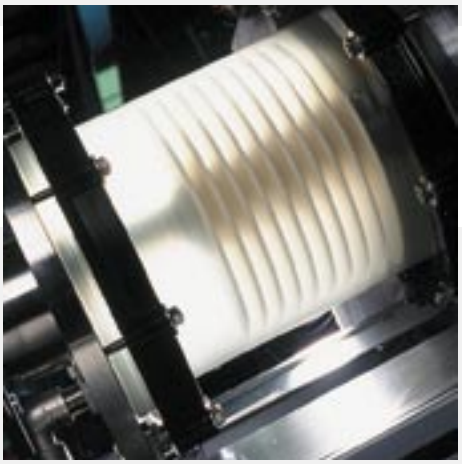
Fraunhofer Institut Grenzflächen- und Bioverfahrenstechnik

Biennial Report 2002 / 2003







Profile

Fraunhofer Institute for Interfacial Engineering and Biotechnology
(Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik IGB)
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Managing Director

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Fraunhofer IGB develops and optimizes processes and products in the fields of

New molecules for pharmaceutics and chemistry:
Therapeutic proteins, target screening, diagnostic tools and assays, microarrays, screening for new enzymes, fermentation process development, downstream processing.

Organoid cell systems:
Three-dimensional organoid test systems, tissue engineering and manufacturing of tissue engineering products in compliance with GMP guidelines, contract manufacturing of cell and gene therapy drugs.

Functional materials and membranes:
Interface engineering of polymers, textiles, non-wovens and membranes, biomaterials and biomimetic interfaces, organic and inorganic membranes and membrane modules.

Bio- and membrane processes for environment and renewable energy:
Reprocessing and conversion of organic waste materials, generation of biogas, waste water purification and water management, bioremediation and biotreatment, production of chemicals and energy by microalgae.

In addition to R&D in contract for our customers, we offer analytical services of reliable and constant quality. In the years 2000, 2001 and 2002, assessors from an internationally recognized accreditation body (Deutsche Akkreditierungsstelle Chemie DACH) have certified that the quality of analytical methods and test procedures at Fraunhofer IGB fulfills international requirements.

For our achievements we can rely on the outstanding know-how of our staff, 147 employees. 90 per cent are technicians and scientists in biology, chemistry, physics and engineering sciences. The interdisciplinary orientation and the integration of the Fraunhofer IGB into excellent research networks secure scientifically founded results for our customers.

We convert the gained research results into new industrial products and procedures. Complete solutions from the test tube to the pilot plant as well as process development and scale-up engineering to industrial dimensions belong to our assets. Among our customers are companies of different industries, municipalities, federation and countries. For our customers benefit, the Fraunhofer IGB has also established and strengthend its international contacts, particularly in Europe.

New technology for health, environment and industry

Biennial Report 2002 / 2003

Fraunhofer Institute for
Interfacial Engineering and Biotechnology IGB

Editorial 2002 / 2003

A research institute dedicated to applied research in Life Sciences and related areas has to cover the full range of the value chain having an acknowledged competence in basic research as well as in product generation appropriate for a direct implementation in industry. That means having the competence and view on technological practicability and on the chances of success in the market. This is for sure one of the special strengths of a Fraunhofer Institute. This is highly acknowledged by our industrial customers and especially by young and small enterprises, which find it quite attractive to use our technological infrastructure and cooperate based on specific competencies and technologies of the Fraunhofer IGB. In addition the know-how in creating and handling intellectual property is to be mentioned. Over all this gives us a position as a first supplier, not only for small and medium technology oriented enterprises but also for global players proven by many examples of customer retention for many years.

There has been a dramatic change in the landscape of the research market during the last two years. Large industrial firms have increasingly emphasized their interest to adopt results of developments close to the market. On the other hand, the biotech start-up scene was shaken in an earthquake-like mode by the negative trends on the stock market. As a consequence, the venture capital market is acting reluctantly in comparison to the enthusiasm three years ago. The shake-out of enterprises turned out as a real backlash and influences the total research landscapes negatively thus also hampering government funding of applied research and of research in alliances between institutes and companies. This development also has an impact on the Fraunhofer IGB, leading to the necessity of rechecking our strategy and its adaptation to the present situation.

For the current year, special emphasis has to be directed onto the Sixth Framework Programme of the European Union, which requires substantial efforts for joining the application rush. Invitations for participation by renowned partners all over Europe may give us confidence for fruitful networking.

We are again proud of our increasing success in environmental biotechnology. The market shows openness for our concepts aiming at a sustainable and economic attractive solution in large-scale dimensions offering a tremendous potential also regarding export possibilities.

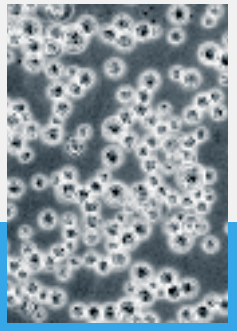
Another highlight are our achievements in the field of nanobiotechnology. The Fraunhofer IGB in-house synergies in biochemistry, molecular biology, interfacial chemistry and engineering make us attractive for joint projects with research institutes and industry. The IGB is seen as a pacemaker in this field.

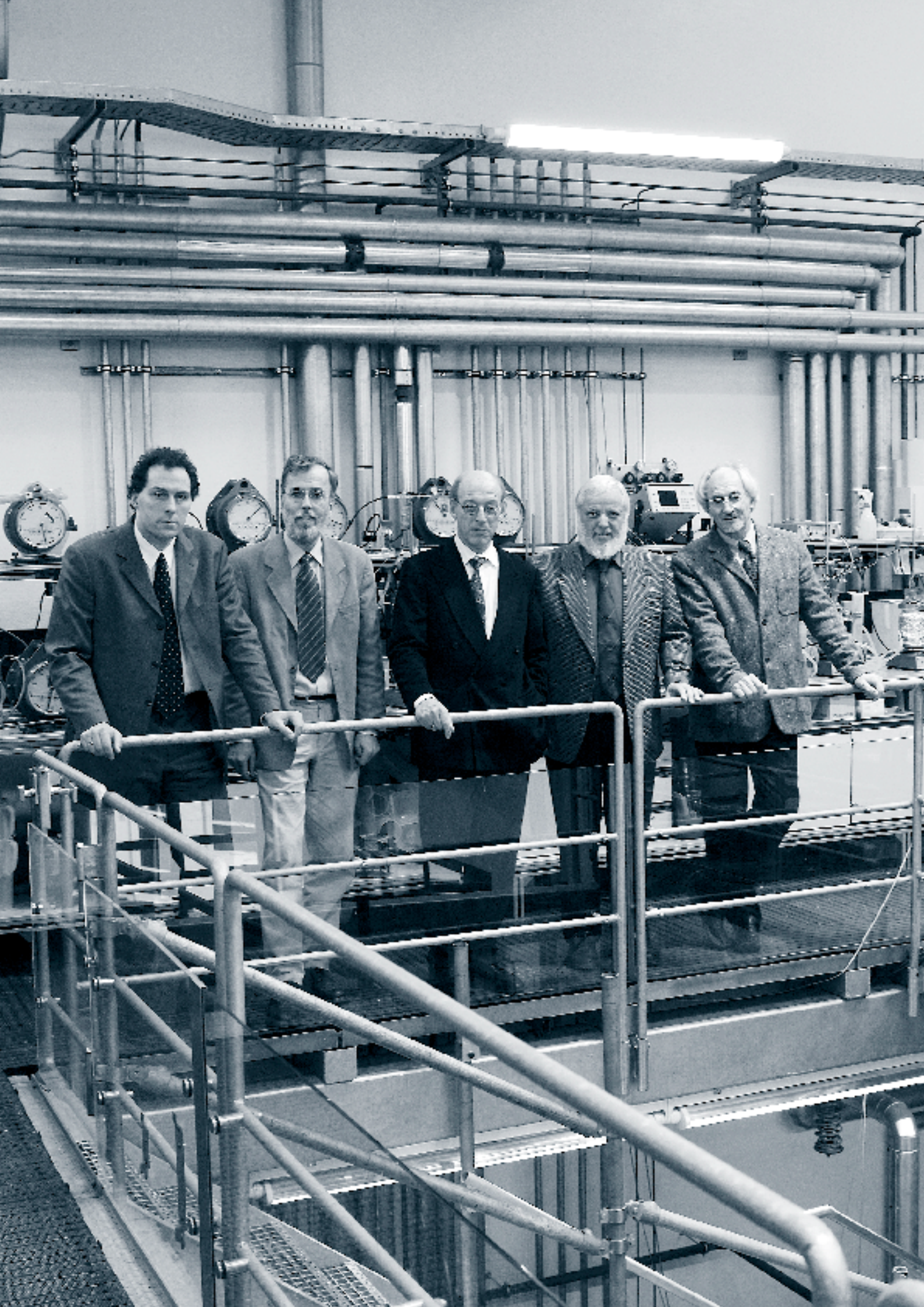
One of our current major topics is the orientation of our in-house expertise to a stronger focus on applied research in cell biology by launching an application for GMP production of tissue engineering products for clinical use.

Scientific and technological expertise, creditability, customer orientation as well as maintaining and increasing the competence of our co-workers on all levels give us confidence in mastering our future.

Prof. Dr. Herwig Brunner

Content





Research

Development

Service

Profile of the institute

Competencies

Network

Finances

Research and development for environment, health and technology

The Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB in Stuttgart develops and optimizes biotechnological processes and products for the environment, health and technology. In addition to contract R&D we offer our clients services in analytics and consult them introducing novel technologies. Industrial companies of different branches as well as municipal, state and governmental authorities are our customers.

Application-oriented and interdisciplinary

The success of new products needs more than ever, the interdisciplinary and constructive cooperation of science and engineering. More than 60 scientists, from different fields like chemistry, physics, biology and engineering, work together at the Fraunhofer IGB. Especially the small- and medium-size enterprises (SMEs) profit from the multidisciplinary potential of our institute.

Our aim is always the direct transfer of the research results into economic processes and products of industrial practice. We offer our clients the enormous economic and ecological potential of biotechnology; in addition, we meet the challenge of the ethical responsibility that is linked with its application.

Complete solutions, from the test tube up to pilot plants under industrial conditions are our excellence. This has been documented in numerous cases in continuous cooperation with our clients.

Competencies and business units

Fraunhofer IGB offers its clients scientific and technological competencies in three areas:

- Interface technology, material sciences, membrane and energy systems
- Molecular biotechnology and cellular systems
- Environmental biotechnology

Since 1998, two additional research groups, with topics in protein screening and biomimetic interfaces support the scientific work at Fraunhofer IGB.

A strict administration is the warrant for the actual project management and controlling.

The quality management system enabled again in the year 2002 the accreditation of various analytical laboratories according to international standards.

Our current core competencies deal with four operational units:

- New molecules for pharmaceutics and chemistry
- Organoid cell systems
- Functional materials and membranes
- Bio- and membrane processes for environment and renewable energy

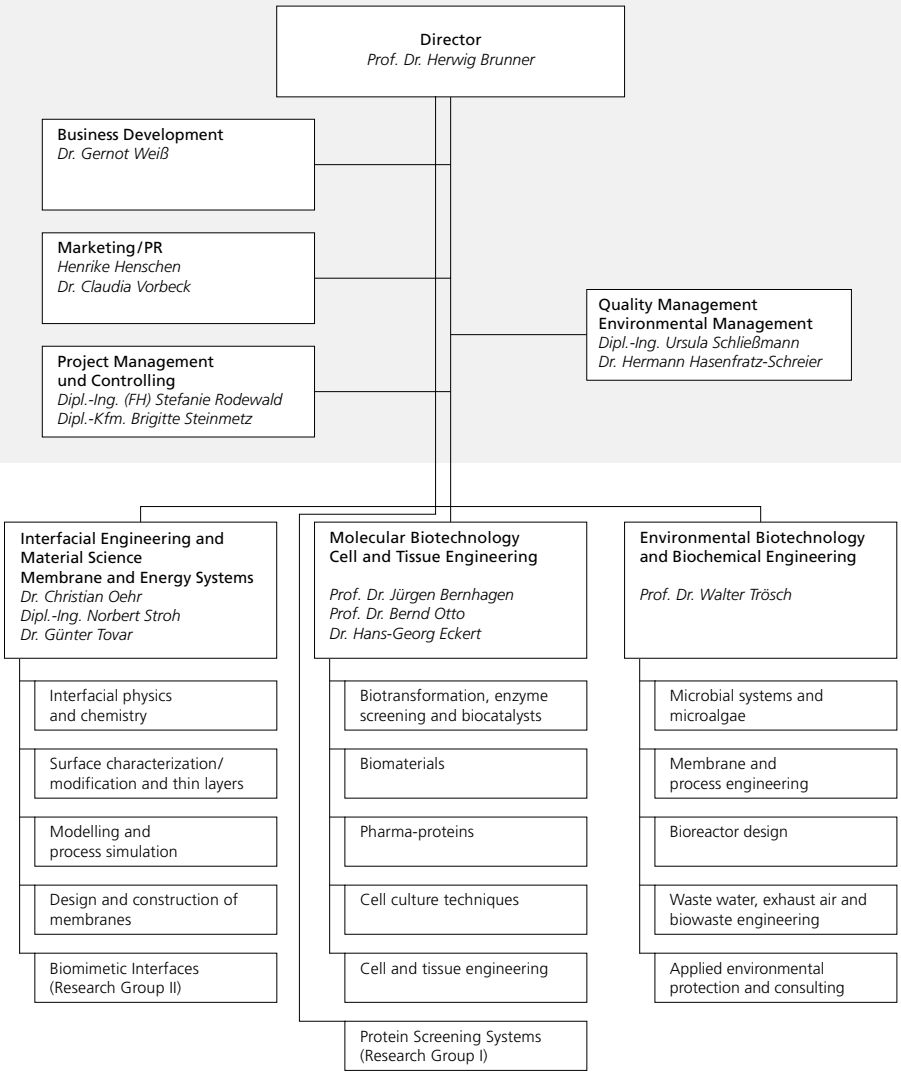
Service centers

Five service centers complete the R&D offer of Fraunhofer IGB:

- GMP services (page 107)
- Special analytical services (page 108)
- Biochemical and molecular biological analytics (page 110)
- Surface analytics (page 112)
- Consulting in waste reduction and environmental management (page 114)

Relevant industries

- Chemical, pharmaceutical and cosmetic industries
- Medical technology
- Food and beverage industries
- Construction and remediation enterprises
- Metal processing, galvanics
- Automotive industries and their suppliers
- Plant engineering
- Membrane manufacturers
- Air-conditioning industries
- Textile and leather industries
- Paper and pulp industries
- Printing plants
- Municipal, state and governmental authorities



Our offer: research – development – education – consultancy

Our R&D services range from scientific and technical basic research to the development of new applications from laboratory up to pilot plant scale.

In contract for our customers we develop new products and processes and optimize processes, e. g. to open up new application areas.

We plan and construct components and plants: Design and construction of pilot plants at Fraunhofer IGB, testing and demonstration of plants, support in transferring them into industrial use.

We offer the training of executives, engineers and scientists in seminars and workshops at Fraunhofer IGB or the contracting company.

We give you consultancy in the fields of molecular and cellular biotechnology, environmental bioengineering and bioremediation, membrane technology and interfacial engineering as well as disposal of waste and hazardous compounds.

We carry out feasibility studies and analyses of new processes and products with respect to realization, risks, competitors and economic viability.

We support you by performing patent and market surveys.

We offer consultancy in technology planning as well as support concerning patent questions and financing strategies.

Equipment

For almost 150 employees Fraunhofer IGB provides more than 5,000 square meters for laboratories, pilot plants and offices. Furthermore, Fraunhofer IGB is equipped with a modern storage for chemicals and hazardous compounds of regional significance.

Scientific infrastructure

Our central service for patent research, which is connected to worldwide literature and patent data banks, is available for internal and external inquiries.

Laboratories and technical facilities

- Biotechnical pilot plants (applications for environmental and sterile technology)
- Bioreactors of various forms and sizes (laboratory, technical and pilot scale)
- Genetic engineering laboratories and pilot plants for the production of recombinant products of the safety standard up to B2
- GMP unit (cleanrooms, separate storage room, quality control area etc.) with cell culture laboratories and pilot plant up to the safety level B2
- Isotope laboratory
- Laboratories for chemical and biochemical analysis with various chromatographic, spectroscopic and electrophoretic methods
- Plants for the production of membranes and membrane modules
- Membrane testing plants and facilities for membrane application
- Plasma installations for cleaning, pre-treatment, activation, modification and coating of surfaces
- Electron microscopes (TEM, SEM)
- Sonde microscopes (AFM, STM)
- Spectrometers for analysis of surfaces and thin layers

Accreditation

In order to extensively fulfill the requirements and needs of our customers, at some laboratories of Fraunhofer IGB a quality management system has been established. Assessors from an international recognized accreditation body have evaluated the technical competence of our staff in executing particular test procedures and checking the validation for our methods and equipment, as well as the measuring and testing equipment itself and the quality management system introduced. Fraunhofer IGB obtained accreditation according to DIN EN ISO/IEC 17025 for the following analytical methods and test procedures:

- High performance liquid chromatography (HPLC)
- Ion chromatography (IC)
- Size exclusion chromatography (SEC)
- Electron spectroscopy for chemical analysis (ESCA)
- Gas chromatography (GC, GC/MS)

With the accreditation we can guarantee the quality of our test methods, which are developed specifically for the customer's needs if no standard methods are yet available.


D4CH Deutsche Akkreditierungsstelle Chemie GmbH

Deutschen AkkreditierungsRat

Akkreditierung

Chemische und chemisch-physikalische Analytik

Further information:

- GMP services (page 107)
- Special analytical services (page 108)
- Biochemical and molecular biological analytics (page 110)
- Surface analytics (page 112)
- Consulting in waste reduction and environmental management (page 114)

Competencies and contacts

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Managing advisory committee

The managing advisory committee consults the director and participates in decision processes concerning the research and business politics of the institute.

Members in 2002:
Prof. Dr. H. Brunner, Prof. Dr. J. Bernhagen, Dr. H.-G. Eckert, Ass. U. Laitenberger, Dr. C. Oehr, Prof. Dr. B. Otto, Dipl.-Ing. N. Stroh, Prof. Dr. W. Trösch, Dr. U. Vohrer.

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PD Dr. Franz-Josef Johannes †
Automated Protein Screening Systems

On January 6th 2003, Franz-Josef Johannes deceased suddenly and completely unexpectedly. We lost an engaged scientist and a highly estimated colleague. His commitment has always been the realization of his scientific ideas and projects as well as the training of his students. Dr. Johannes will leave a large gap in our institute.

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Public Relations and Marketing
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Dr. Claudia Vorbeck
Public Relations and Marketing
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Representative figures

Personnel

At the end of the year 2002, 147 people worked at Fraunhofer IGB, more than 90 percent in R&D. The percentage of women was 46 percent.

Fraunhofer IGB is also active in the education of students from different scientific fields and of trainees for chemical laboratories and administrational fields.

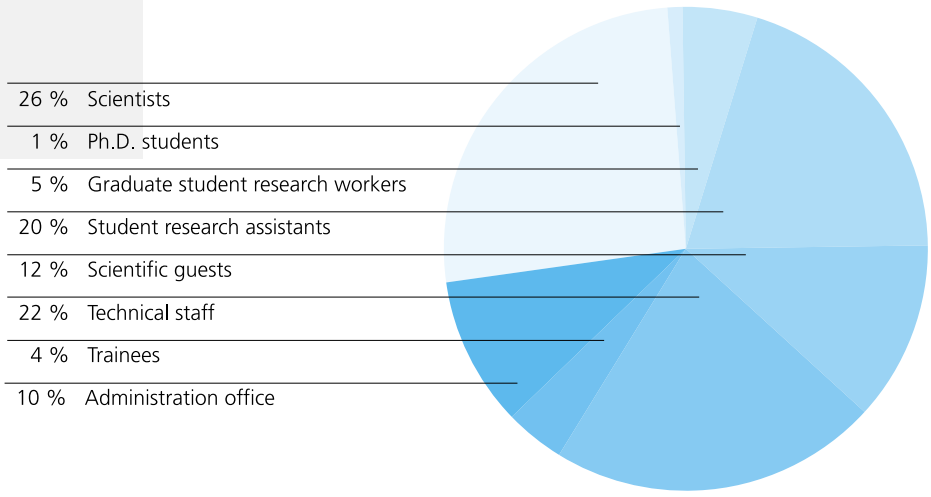
Staff members	Number
Scientists	38
Ph. D. students	1
Graduate student research workers	8
Student research assistants	30
Scientific guests	17
Technical staff	32
Trainees	6
Administration, office	15
	147

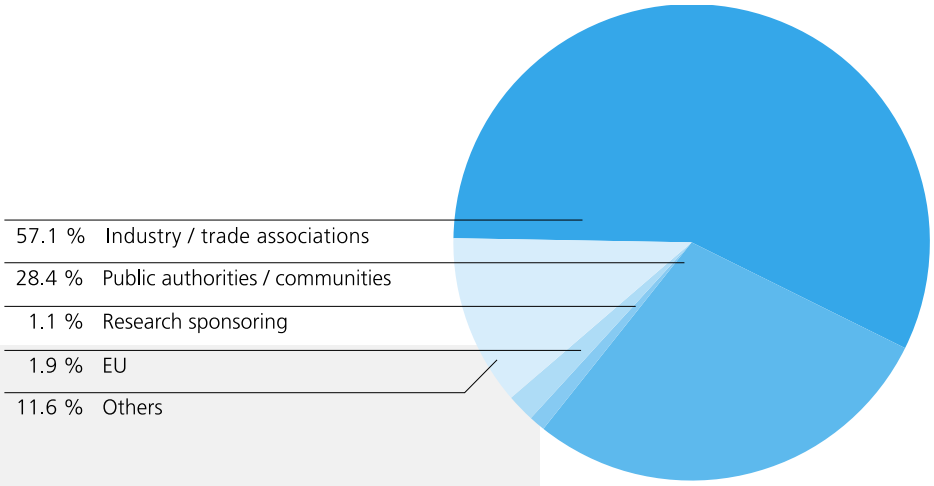
Budget

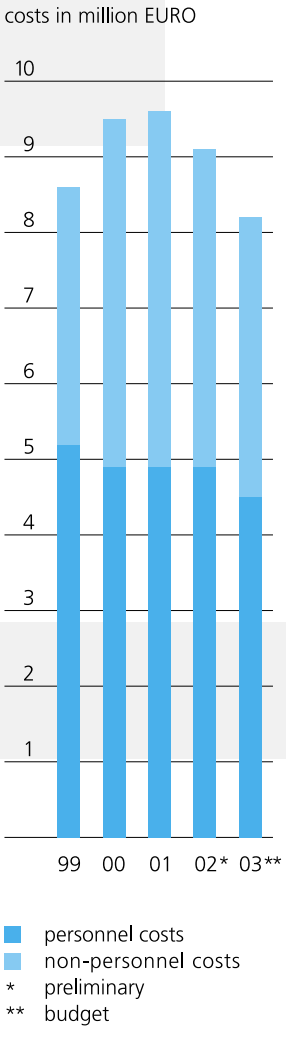
The financial structure differentiates between the institutional budget, including personnel and non-personnel costs as well as corresponding revenues, and the strategic investment budget.

Total budget for the year 2002 amounts up to 10.5 million Euro, 9.1 million Euro were spent on the institutional budget (4.9 Mio Euro on personnel costs, 4.2 Mio Euro on non-personnel costs), 1.4 Mio Euro went to strategic investments.

Governmental funding covers 18 percent of the institute's institutional budget. 57 percent of the institute's revenue is brought in by contract research and projects acquired directly from the industry.







personnel costs
non-personnel costs
* preliminary
** budget

Network with science, economy and public authorities

The integration of the Fraunhofer IGB into excellent research networks secures trend-setting scientific results for our customers. Longstanding and successful cooperation with different universities and institutes of the Max-Planck-Gesellschaft in Stuttgart and at other locations in Germany guarantee our scientific basis. Cooperation with other institutes of the Fraunhofer-Gesellschaft enables us to develop new solutions synergetically for the industrial needs.

Cooperation with universities

Basic research is a must. Therefore, the Fraunhofer IGB keeps close contacts to fellow universities. The managing director as well as several heads of department also act as university professors:

- Prof. Dr. Herwig Brunner,
  Institute for Interfacial Engineering, University of Stuttgart
- Prof. Dr. Bernd Otto,
  Chair for Molecular Biology and Protein Design, School of Veterinary Medicine Hannover
- Prof. Dr. Walter Trösch,
  Professor for Biotechnology, University of Hohenheim
- Prof. Dr. Jürgen Bernhagen,
  University Hospital RWTH Aachen

Institute for Interfacial Engineering IGVT

The Institute for Interfacial Engineering at the faculty of »Process Engineering and Technical Cybernetics«, University of Stuttgart, has been founded in 1999/2000. Its precursor, the chair of Interfacial Engineering, has already been founded in 1994, and the director since then is Prof. Dr. Herwig Brunner. The institute holds two research groups and is situated in the building of the Fraunhofer IGB, thus guaranteeing close and efficient collaboration with the Fraunhofer IGB's departments of Membrane and Energy Systems, Interfacial Engineering and Material Sciences as well as Molecular Biotechnology.

The institute´s mission is the education of a new generation of academics who are trained in biomedicine and biotechnology, closely related to their engineering background. This is realized within the graduate courses of Biomedical Process Engineering and Bioengineering and within the study programs of Technical Biology.

Chemical Surface Engineering

This research group, led by Dr. Günter Tovar, focuses on the synthesis, characterization, activation and structuring of interfacial substrates for technical, biotechnological and biomedical purposes.

Main research areas include:

- Molecular imprinting of polymeric materials for sensor surfaces
- Synthesis of polymeric particles by emulsion polymerization
- Synthesis of molecular precursors for surface functionalization
- Synthesis and characterization of self-assembled monolayers
- Basic research for the development of system components for micro- and nanobiotechnology

Biochemistry

This group mainly investigates the biomedical and pharmaceutical potential of peptides, polypeptides, and proteins. Currently, the group focuses on the biochemistry of cytokines. These are synthesized either chemically or biologically in bacterial or eucaryotic cells by recombinant methods and isolated by various state-of-the-art purification procedures. Using site-directed mutagenesis, functionally different protein variants are generated. Molecular targets of interest are the cytokine MIF, the signalling factor Jab1, the amyloid peptides and proteins such as IAPP and Aβ, respectively, as well as the cell growth proteins RBD and RaS.

In addition to potential pharmaceutical applications the aim is to utilize the proteins for specific functionalization of technical and biomimetic interfaces which in turn can be applied for the development of biosensors and biochips, thus enabling them for the further elucidation of protein/protein interactions or binding effects and pathways of molecular signal transduction and supplying molecular devices for novel screening systems.

Contact

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Personnel structure of the IGVT

Scientists	9
Ph.D. students	10
Student research assistants	6
Technical staff	6
	31

Board of trustees of the Fraunhofer IGB

The Fraunhofer institutes are consulted and advised by a board of trustees whose members come from industry, public authorities and the scientific community.

Members of the board of trustees in 2002:

- **Dr. Hans-Georg Batz**
  Roche Diagnostics GmbH
- **Prof. Dr. Armin Fiechter**
  Sigriswil, Switzerland
- **Dr. Dieter Jahn (chair)**
  BASF AG
- **MinR Gerd Heitmann**
  Ministry of Economic Affairs of the State of Baden-Württemberg
- **MinDirig Dr. Heribert Knorr**
  Ministry of Science, Research and Arts of the State of Baden-Württemberg
- **Prof. Dr. Klaus Pfizenmaier**
  University of Stuttgart
- **Prof. Dr. Ralf Riedel**
  Technical University Darmstadt
- **Dipl.-Ing. Otmar Schön**
  HYDAC Technology GmbH
- **MinR Dr. Wolfgang Stöffler**
  German Federal Ministry of Education and Research
- **Prof. Dr. Edda Töpfer-Petersen**
  School of Veterinary Medicine Hannover
- **Prof. Dr. Rolf G. Werner**
  Boehringer Ingelheim Pharma KG

Scientific-technical committee

The scientific-technical committee supports and consults the various departments of the Fraunhofer-Gesellschaft in fundamental scientific and technical questions. Members of the scientific technical committee are the institutes' managing directors as well as one elected member from each institute.

Members from Fraunhofer IGB:

- **Prof. Dr. H. Brunner**
- **Dr. U. Vohrer**

From sludge to energy: A Fraunhofer success story

Fraunhofer IGB has developed a high-performance process for the digestion of sewage sludge. About two years ago, the process was integrated into the existing disposal line of the Heidelberg municipal sewage plant, consisting of three egg-shaped fermentation towers.

The result: Today, the Heidelberg sewage plant converts sewage sludge into biogas within a far smaller area and much more quickly and effectively. This enables costs to be saved, energy to be obtained while at the same time less digested sludge has to be disposed of (also see project report, page 98).

On the occasion of the 25th anniversary of the Abwasserzweckverband (AZV) Heidelberg on 24th September 2002, the sewage plant invited interested members of the public to view the new high-performance digestion system.

Figure 1: Prominent persons at the opening of the high-performance digestion system at the Heidelberg (South) Sewage Plant.
From left: Mayor Horst Althoff (Neckargemünd), Mayor Dieter Möhrlein (Eppelheim), Chairwoman of the Regional Council Gerlinde Hämmerle, Mayor Beate Weber, the Association Chairman of the Abwasserzweckverband (AZV) Heidelberg, Prof. Dr. Raban von der Malsburg and the Director of the AZV Heidelberg, Ulrich Zwissler.

Fraunhofer IGB held a discussion with the technical manager of the AZV Heidelberg, Dipl.-Ing. Jürgen Weber. Jürgen Weber studied engineering sciences in Karlsruhe, with an emphasis on domestic water supplies.

IGB: Mr. Weber, since April 2001, there has been cooperation between the Abwasserzweckverband Heidelberg and Fraunhofer IGB. What was the initial situation and what were the problems you faced?

Weber: OK, we have a sewage plant built in 1982 on the water side and which operates in line with the latest state of the art, which means that it also biologically eliminates the nutrients phosphorus and nitrogen. On the sludge side, we had and still have three old fermentation towers, each with a capacity of 2,500 cubic meters, which were left over from the old sewage plant built in the 1960s. These fermentation towers were no longer able to process the sewage sludge produced, i. e. stabilizing and gasification.

IGB: And what did you do then...?

Weber: We organized an engineers' competition, but were not satisfied with the results. Then by chance I read an invitation from the Fraunhofer Institute. It concerned the annual seminar that your Institute holds on municipal sewage technology. I went along and listened to the philosophy of the Fraunhofer Institute. At that time there was a research project »Energy from Sewage Sludge« being carried out by the Fraunhofer Institute, which was being supported by the Baden-Württemberg Ministry of the Environment, and we simply joined it. We took our sludge to the Fraunhofer Institute, where it was examined in the technical center, in the semi-industrial system, for degradability and convertibility. The result was that a very high level of digestion could be achieved in high-performance digestion. In the final analysis, that was the deciding factor for us to cooperate with the Fraunhofer Institute and so the project, adapted to the conditions prevailing in Heidelberg, was jointly thought through, put together and then finally implemented and completed in 2002.

IGB: What experience have you since had with this method?

Weber: I have to say that it's a completely new process and there's always a degree of uncertainty in the beginning. That's how I felt too. However, I saw no other alternative. And the concerns that we had or that I had were completely dispelled. Since it's been in operation, the system has worked in a stable way, and it has a very high level of digestion – a really high level of digestion. It does not foam – in other words everything that we had hoped for and what the Fraunhofer Institute and the supplier company had guaranteed us has proved to be the case.

IGB: What perspectives, in your estimation, does this technology have?

Weber: This technology can most certainly be used in any sewage plant. The question is, what's already there and how can I integrate it? We in Heidelberg have so far had very good experiences with this concept, and I think that this solution is also suitable for sewage plants that have the same problems as us: fermentation towers that don't work and with unfavorable geometries. And here too I see a possibility of achieving a better digestion rate by integrating the high-performance digestion upstream, and also ensuring the process stability of downstream digestion.

For sewage works that do not yet have digestion, that are situated in an area of ten to twenty thousand inhabitants and that have wet sewage disposal or stabilization systems, my preferred solution would be two-stage high-performance digestion of the type that has been built in Leonberg. In such cases it can, in economic terms, be worth setting up a high-performance digestion system. You have to compare on an operating costs basis the sludge reduction, the quantity reduction and the energy use of the sewage gas with current operation. I see interesting and economical applications in particular for sewage plants that do not yet have any digestion whatsoever.

IGB: Thank you for talking to us, Mr. Weber.

Contact

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Figure 2: 24th September 2002 is open day at the Heidelberg (South) Sewage Plant: The high-performance digestion system of Fraunhofer IGB – on the right in the picture – is ceremonially inaugurated.

Experience the uniqueness of Fraunhofer research and start your European career as Marie Curie fellow.

Marie Curie Intra-European Fellowships are individual fellowships that aim at providing advanced training tailored to the researchers' individual needs in order to become independent. The duration of the fellowships is between one and two years.

Who can apply?
A researcher from EU or Associated States with at least 4 years research experience or a Ph. D. and willing to spend a mobility period working in a host institution located in another EU or Associated State, different from his/her own and from that where they have been recently active. The researcher applies »in liaison« with the host institution. Eligible host institutions are organisations active in research or research training (e. g. universities, research organisations, international organisations, enterprises, etc.).

Which research topics are supported?
Proposals from all areas of scientific and technological research of interest to the European Community are welcome and there are no priority areas.

How does it work?
The researcher applies to the Commission jointly with the host institution. If the proposal is selected, the Commission signs a contract with the host. The researcher signs an agreement/contract with the host institution.

What does the funding cover?
The most substantial part of the funding goes towards covering the expenses related to the researcher and the project. The researcher receives allowances to cover monthly living expenses, the costs related to travel and the mobility as well as a career exploratory allowance. Likewise the host will manage the contribution for expenses directly related to the execution of the project by the researcher. Additionally, the host receives contributions to overheads and management costs.

The next deadline is the 18th February 2004. It is expected that an additional call will be published with deadlines in 2005 and 2006.

(Source: European Commission)

Fascinating European research: Opportunities for SMEs (CRAFT)

Within the 6th European Research Framework a specific scheme for SMEs having a capacity to innovate but with limited research capacity is foreseen: Cooperative Research (»CRAFT«). SMEs may entrust research work to solve their particular problems to research performers (research institutes, universities etc.). Ownership of the results will rest with the SMEs.

In a Cooperative Research Project, there must be at least:

- Three independent SME participants, established in two different Member States or Associated States, of which at least one shall be established in a Member State or Associated Candidate Country
- Two RTD performers, independent from any other participant and established in two different Member States or Associated States, of which at least one shall be established in a Member State or Associated Candidate Country

In addition, some other enterprises and end-users who have a particular interest in solving specific problems or needs of the SMEs involved may participate in the project by making a contribution to its costs, under conditions ensuring they do not assume a dominant role. These enterprises and end-users must be independent from any SME participant or RTD performer.

A SME is an enterprise which:
- Has fewer than 250 employees
- Has either, an annual turnover not exceeding 40 million Euro or an annual balance-sheet total not exceeding 27 million Euro
- Conforms to the criteria of independence. An independent SME is a SME which is not owned for 25 per cent or more of the capital or the voting rights by one enterprise or jointly by several enterprises falling outside the definition of a SME

(Source: European Commission)

The Fraunhofer IGB invites you to participate in the fascinating world of European research. We are looking forward to cooperating with you.

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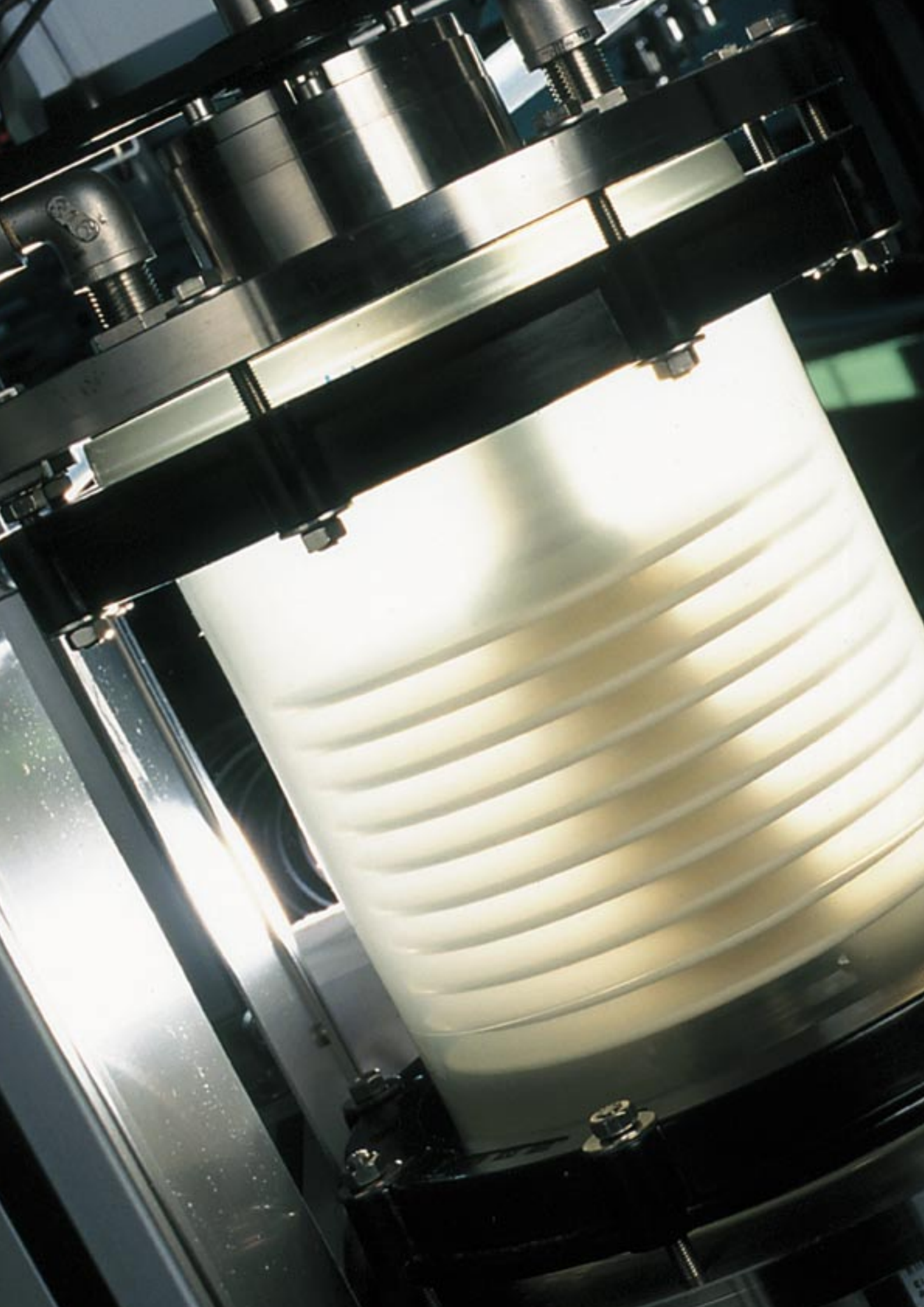
For further information on our activities within the European research programs visit our website: www.fraunhofer.igb.de

China: Guest scientists at Fraunhofer IGB

Since 1990, Fraunhofer IGB has been collaborating with China's »State Key Laboratory of Catalysis (SKLC), Dalian Institute of Chemical Physics, Chinese Academy of Sciences« in the development of inorganic membranes. Starting with Prof. Dr. Xiong, the former head of the Institute in Dalian, seven scientists have conducted membrane development projects at Fraunhofer IGB since 1990, in some cases over a period of several years. The basic research of the Chinese guest scientists has optimally supplemented our existing competences, and has contributed towards establishing ceramic membranes as an area of competence at Fraunhofer IGB. The research has been jointly published. In 2002, research cooperation was also established with the National Engineering Research Center of Membrane Technology in Dalian. We also want to continue the existing cooperation in the years ahead.

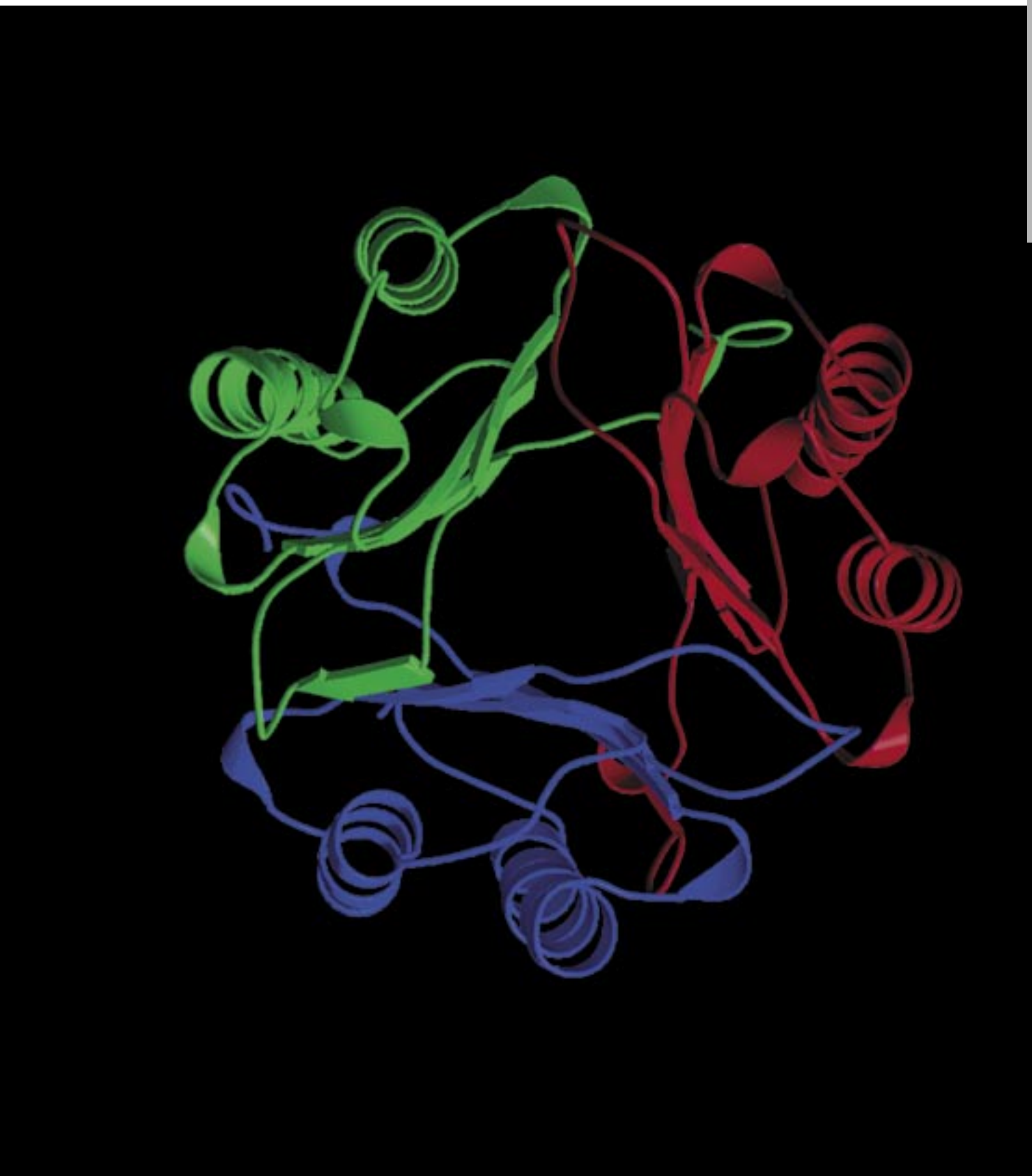
Brazil: Water, waste and energy cooperation

The cooperation between Fraunhofer IGB and its Brazilian partners has continued to develop since the cooperation contract was concluded in 2001 with the Universidade Metodista de Piracicaba (UNIMEP). The second jointly organized international »Water and Energy« workshop in Piracicaba found a very positive resonance among scientists and the public, and for the first time enabled local companies to participate. In the same period, the largest Brazilian meeting for engineers from all fields, the XXX COBENGE – Congresso Brasileiro de Ensino de Engenharia – was held at the UNIMEP, with Fraunhofer IGB staff also participating. At the present time, the cooperation is focussing on projects concerning waste water purification, refuse handling and obtaining energy from refuse. In 2002, the crucial contacts with municipal authorities and companies locally were strengthened and extended, and in 2003 it is intended to expand them still further by incorporating German industrial partners.



Research and development 2002

Molecules for pharmaceuticals and chemistry



The IGB business unit »New molecules for pharmaceuticals and chemistry« has its focus on four businesses:

- **Therapeutic agents (proteins, peptides) and screening for pharmaceutically relevant targets**
- **New diagnostics and diagnostic tools based on biochemical and biomolecular approaches**
- **Screening for new enzymes**
- **Bioprocess engineering: fermentation, scale-up, and downstream processing**

In these fields our attention is directed to molecular and cell biological methods.

Our facilities include:

- various cell culture laboratories of the safety level B1 and of GLP/GMP-like standard
- several genetic engineering laboratories of the safety standard up to B2
- a pilot plant for the production of recombinant products B1/B2 up to the 100-liter scale
- a colony picker for thinning out large amounts of bacterial and fungal colonies in microplates
- a cell sorter for the rapid separation of animal and microbial cells
- protein sequencers
- arrayer and scanner for the manufacturing of high-density microarrays (DNA, protein)
- 2D-gel electrophoresis
- a MALDI-TOF mass spectrometer for molecular weight determination of high-molecular compounds
- several workstations and systems for structural modeling and design of proteins

In a number of ongoing projects, synergistic effects are being used with our departments related to surface and interfacial technology, with the neighboring Fraunhofer IPA and the Fraunhofer »Life Sciences« institutes as well as with the University Hospital at the RWTH Aachen and the Medical University of Hannover.

Recombinant pharmaceutical proteins and targets

Genetic engineering enables to isolate individual genes from the hereditary material of organisms and to install them in host organisms, which then produce the target protein. Many disorders result from a lack or dysfunction of physiological proteins. When the correct variant of the protein is administered, the disease can be treated. On the basis of the molecular structure of the active protein it is possible to use protein design techniques for modifying the structure of the protein in order to enhance its biophysical, biological and medical properties.

The IGB has been working for about ten years on projects on the structural elucidation, recombinant production, and design of pharmaceutical proteins. For the interferons, spectacular successes have already been achieved. Present research work is concentrated on interferon agonists and antagonists, and also the cytokine MIF. A primary task here is to find out how the protein acts biologically, since knowing its mode of action is a prerequisite for the development of MIF-based strategies in medical therapy.
Furthermore, IGB investigators elucidated peptide hormones effecting the calcium metabolism and amyloid (poly)peptides [1] (see page 16, IGVT) as well as ribonucleotides with angiogenetic effects.

Current developments will be illustrated in the following research reports. The research history of interferons and MIF is outlined here as an example for the development of pharmaproteins.

Interferons – New pathways in drug development

When in 1957 Isaacs and Lindemann made their pioneering discovery of the interferons, they could not foresee what unprecedented scientific and economic career awaited these proteins of the human immune system.

At the beginning of the 1980's, the recombinant DNA technology opened the chance to produce large amounts of these proteins at reasonable prices.

During this era of euphoria the first medical indications were discovered mainly for Interferon-α. Several types of cancer could now be treated with great success. But nevertheless there were also setbacks. Most solid tumors were resistant to interferon therapy.

1 in collaboration with the Laboratory for Molecular Peptide Research of the Physiological Chemical Institute at the University of Tübingen.

Figure left page: Crystal structure of human MIF at 2.6 Å.

With the beginning of the next decade a new breakthrough in a so far untreatable disease seemed to be possible. The Interferon-β therapy led to a remarkable reduction of the relapse rate in multiple sclerosis. In 1993 Interferon-β for relapsing remitting multiple sclerosis was approved by the Food and Drug Administration (FDA) as a drug against this neurological disorder.

Today the interferons are well established on the international pharmaceutical markets. Interferon-α has its main indications in the treatment of chronic myeloid leukemia and hepatitis C. The market value is more than one billion US $. Comparable revenues are gained with Interferon-β in multiple sclerosis therapy. Interferon-γ, as the interferon with the strongest immunomodulating properties, is used in the treatment of chronic granulomatous disease and renal cell carcinoma.

At the beginning of this millennium we are facing new challenges in interferon research and therapy. Due to physical properties or structural changes during production and downstream processing of interferons these proteins have their clinical limitations. These limitations are caused by low solubility, low stability and low bioavailability. All these properties affect the pharmacokinetics of the drug and need to be improved by further research.

Additionally, clinicians are often confronted with the problem that the immune system of the patients who receive interferon therapy raises neutralizing antibodies, which is often the cause for therapy abrogation.

Since 1986 a team of qualified scientists in the Fraunhofer IGB department of Professor Bernd Otto is working on the improvement of the biophysical, biological and medical properties of the interferons using the most sophisticated genetic engineering and protein design methods. The department was honored by several research awards and filed six patents so far. Thus many properties of the proteins could be improved. For example, an Interferon-γ variant with enhanced thermal stability and another with enhanced biological activity were developed. A novel method was established at the model system Interferon-α, which allows the manufacture of more soluble proteins. By means of Hydrophobic Engineering, the hydrophobic protein Interferon-β could be manufactured as a more soluble, bioavailable variant (page 30). Patents are pending or have already been granted for the modified interferon variants as well the process to manufacture soluble proteins.

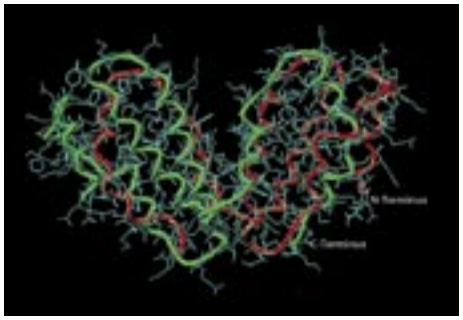


Figure 1: Dimeric structure of Interferon-γ. By insertion of disulfide bridges the variant IFN-γ-HS was stabilized thermally resulting in less aggregation tendency.

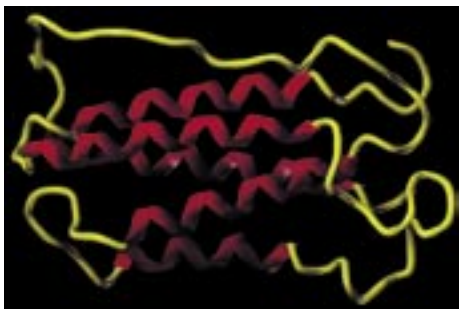


Figure 2: Interferon-α can be produced in high purity with high biological activity by a novel biosynthesis and purification strategy avoiding de- and renaturating steps.

MIF – a classical immune factor with surprising functions

One other classical immune factor is the cytokine Macrophage Migration Inhibitory Factor (MIF). MIF was first reported on over 6 decades ago (Rich and Lewis, 1932). It was then recognized that »the migration of neutrophilic leucocytes and macrophages in cultures of tuberculin-treated antigen was inhibited by antigen«, leading up to the initial conclusion that immunostimulated lymphocytes may be induced in culture by antigens to produce MIFs. 30 years later, Bloom, Bennett and David (1966) demonstrated that such soluble immune factor mediated tuberculin-dependent delayed-type hypersensitivity reactions. It is therefore that MIF and the interferons have been considered the first cytokines to be discovered.

However, it was not until 25 years later that a Macrophage Migration Inhibitory Factor was characterized at a molecular level by expression cloning from T-lymphocytes (Weiser et al., 1989). Work that led up to the surprising identification of MIF as pituitary hormone and endogenous antagonist of glucocorticoid action (Bernhagen et al., 1993; Calandra et al., 1995) then also demonstrated a broad role for MIF as proinflammatory cytokine.

To date, the biology and structure of MIF have been elucidated in large parts. However, the molecular mode of action of this factor has remained unresolved. A cytokine membrane receptor has not yet been identified. The MIF group at the Institute for Interfacial Engineering (IGVT) of the University of Stuttgart and the Fraunhofer IGB has set out to elucidate the molecular mechanism of MIF action as a prerequisite to develop MIF-based medicinal strategies. Using state-of-the-art molecular biology techniques as well as biochemical and cell biology methods, the group has begun to broadly characterize the structure activity profile of MIF. At the same time, target cell mechanisms are investigated and screening systems devised to potentially identify MIF-related molecular targets (page 36).

Target screening

Since most common diseases have been shown to be influenced by inherited variations in our genes, completion of the Human Genome Project and mapping of the human genome and genome of disease-causing organisms, new approaches in the therapy of diseases are being developed. Whereas, in the past, thousands of natural and synthetic substances were investigated for their therapeutic effect by a trial-and-error process, knowledge of the cause of diseases at the molecular level is now enabling the targeted development of new pharmaceuticals. One approach is to identify new targets – sites of action for the potential drugs –, which can be found using modern high-efficiency screening techniques. The Protein Screening Systems and Genomics research group, which receives funding at federal and state levels, successfully uses modern techniques of genome and proteome analysis to identify endogenous and pathogen-specific proteins as new target molecules for pharmaceutical agents.

Genome-wide screening systems for proteome and transcriptome analysis have been developed initially on the model organism *Candida albicans*, the most common fungal pathogen in humans. The identification of the molecular pathogenic mechanisms of this yeast is of great interest to medical therapy. The *Candida albicans* genome, completely decoded in mid-2001s, is used as an experimental system for the construction of genome-wide DNA microarrays (page 44). Within the scope of a consortium consisting of only five international members, the group works on the annotation of the *C. albicans* genome (http://genome-www.stanford.edu/fungi/Candida/docs). The comparison of pathogenic and non-pathogenic *Candida* strains at the proteome and transcriptome level may provide the molecular basis for the development of new antimycotics. By comparative protein expression analyses, pathogenicity-relevant proteins can be directly identified. Recently proteins were identified using two-dimensional gel electrophoresis, their expression is correlated with the pathogenicity of the cells (page 38).

Novel powerful software solutions and computational knowledge management is essential for the efficient analysis of enormous data volumes resulting from genome-wide experiments of genomics and proteomics. The Fraunhofer IGB is participating in the EU research project GeneStream working in the development of such bioinformatics.

The two-hybrid system is to be developed further as an established method for screening proteins. This screening method identifies protein partners on the basis of protein-protein interactions. The method is especially suitable for investigating and revealing cellular signal transmission pathways. Errors in these complex processes are frequently correlated with the emergence of diseases, such as the formation of tumors. The aim of the work is therefore to transfer the two-hybrid system to mammalian cells, so that new cellular target proteins in therapeutically significant signal transmission pathways can be identified. An essential focus of these studies is the recently discovered protein kinase Cμ (PKCμ). PKCμ appears to intervene in the programmed cell

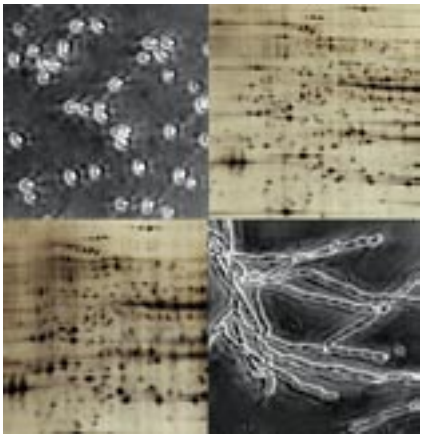


Figure 3: Comparison of cell morphology and protein expression of pathogenic (top) and non-pathogenic (bottom) strains of the yeast *Candida albicans.* Respective light microscope images and protein expressions by 2D-gel electrophoresis are shown.

death (apoptosis) induced by the tumor necrosis factor (TNF). Drug-induced inhibition of PKCμ might, therefore, represent a new approach in tumor therapy (page 40).

Diagnostic tools and agents

As well as its potential for the therapy of diseases, genome sequencing is also creating highly promising possibilities in diagnostics, since techniques based on DNA analysis can be used to detect pathogenic organisms such as viruses, bacteria and fungi, as well as inherited diseases or development of cancer, with precision, speed and efficiency. Large numbers of samples are making it increasingly necessary to automate and miniaturize the detection processes. DNA chips are ideally suited to the analysis of genes within a very small space. As well as DNA microarrays for determining transcription profiles, arrays for determining point mutations (single nucleotide polymorphisms, SNPs) are being developed at the IGB for diagnostic purposes (e. g. resistance mechanisms in *C. albicans*). Furthermore, IGB is developing DNA arrays for the diagnosis of infectious diseases and human SNPs together with SMEs as well as improved diagnostic tools for mamma-carcinomas as the coordinator of a state-funded consortium (page 46).

In the field of DNA-assisted disease diagnostics, automated/miniaturized solutions already exist for the areas of DNA amplification and DNA detection. The significance of sample preparation was only recognized at a late stage. Working together with its neighboring institute the Fraunhofer IPA, the IGB therefore focused on this objective and has developed miniaturizable and automatable methods for the rapid isolation and detection of DNA.

For this purpose, a mechanical disruption method was developed and optimized for tissues and cells from a wide variety of biological sources (IGA module). The cell lysis is furthermore realized in a special apparatus operating with pulsed electrical fields (high voltage). Since this technique does not involve chemical additives, the DNA obtained is suitable directly for PCR and for a broad diagnostic repertoire with a wide variety of probe sequences and probe lengths.

Biocatalysis and enzyme screening

Enzymes, applied as technical biocatalysts, have a tremendous impact on detergent, food and beverage industries. Also in the pharmaceutical and chemical industry they conquer new areas of application, for example the production of fine chemicals and synthons. Due to their high selectivity and specifity the use of enzymes can even be superior to synthetic chemistry. In particular biocatalysts are being more often used in the synthesis of chiral compounds. Contrary to chemical syntheses enzymes operate with no by-products – costs of increased consumption at substrate, product cleaning and waste disposal do not arise therefore. Further advantages of the enzyme catalysis are to achieve high yields under mild and environmentally friendly reaction conditions.

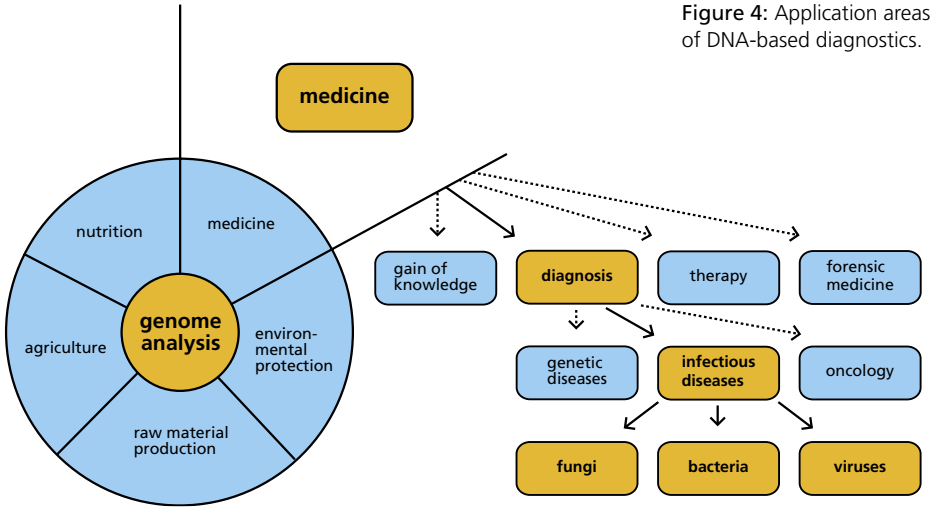


Figure 4: Application areas of DNA-based diagnostics.

The need for new enzymes for the different operational areas constantly increases, to optimize existing procedures or to enable new reactions. For this reason the Fraunhofer IGB pursues a new way in order also to use the potential of only with difficulty or non-cultivable microorganisms: The DNA of all microorganisms from different environmental samples was collected in a gene bank, which can be tested with suitable assays on the desired biocatalytic activity.
Thus we offer exclusive access to unknown enzymes for industrial customers in our »Screening Center« (page 48).

In the preparation of biological materials (e. g. chitosan), a further area of intensive work, the strategy being pursued is that of optimizing existing enzymatic or chemical processes by means of molecular-biological methods (page 50).

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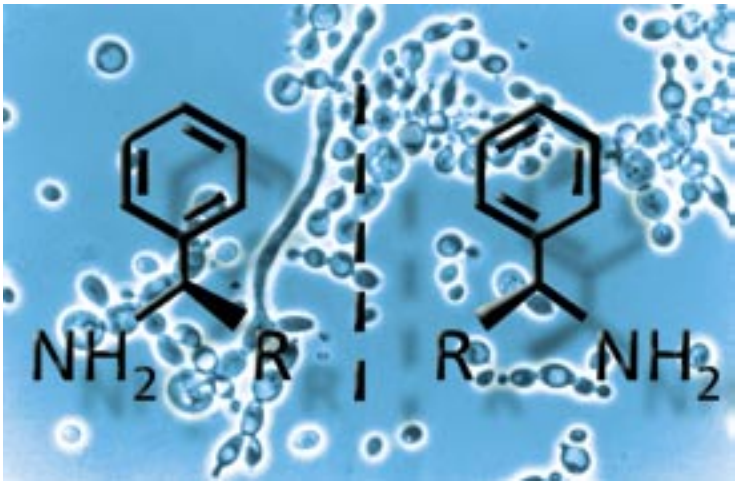

Figure 5: The two enantiomers of chiral compounds often differ in their effects. For the production of chemical or pharmaceutical products, therefore, only one of the enantiomers is used. Enzymes are highly specific and produce the desired variant in high purity.

Recombinant »second generation« Interferon-β with enhanced bioavailability

Vision

Interferon-β (IFN-β) is used for the treatment of multiple sclerosis and has a market value of more than 2 billion US $ worldwide. Recombinant human Interferon-β is produced successfully in bacterial (*E. coli*) and mammalian cells (CHO). The latter supply a glycosylated protein with an enhanced biological activity *in vitro*. Major problem, however, is the low solubility of IFN-β with respect to yield, purification, formulation, storage and bioavailability of the protein caused by its tendency to aggregate.
Using the known three-dimensional structure of murine Interferon-β, the structure of human IFN-β was therefore altered by protein design, generating a more soluble protein. For the selection of amino acids the »Hydrophobic Engineering« was used. This modeling concept enables the substitution of hydrophobic amino acids by soluble ones.

Technology: Hydrophobic engineering

The three-dimensional structure of human IFN-β was calculated using the published structure of murine IFN-β. By computer modeling we have identified areas on the surface of the IFN-β protein, which attribute to the hydrophobicity but are not essential for the biological activity. We developed IFN-β variants with single and multiple amino acid replacements by substituting up to eight hydrophobic amino acids by serine in these hydrophobic patches. As a result, the biophysical properties of the protein were improved without changes in the biological activity.

Expression in *E. coli*

One resulting new IFN-β variant with eight substituted amino acids was expressed in *E. coli* cells, purified and characterized by biophysical and biological methods. *In vitro* this new IFN-β is biologically as active as the unmodified one. However, in animal experiments it shows a highly increased pharmacokinetic stability and bioavailability as shown in Figure 2A.

Eucaryotic expression in CHO cells

Furthermore, the new variant was successfully expressed in and purified from CHO cells. The hereby obtained glycosylated IFN-β variant shows – just like the unmodified wild type IFN-β from CHO cells – tenfold enhanced biological activity compared to non-glycosylated IFN-β from bacterial cells *in vitro*.

But the new IFN-β variant from CHO cells is more soluble and can be concentrated higher than wild type IFN-β from CHO cells. *In vivo* it shows a more than fourfold increased bioavailability (Figure 2B).

Outlook

Due to its enhanced solubility this new IFN-β from CHO cells is suitable not only for the therapy of multiple sclerosis, but in addition can be applied for further innovative indications. We are seeking industrial partners for further clinical development.

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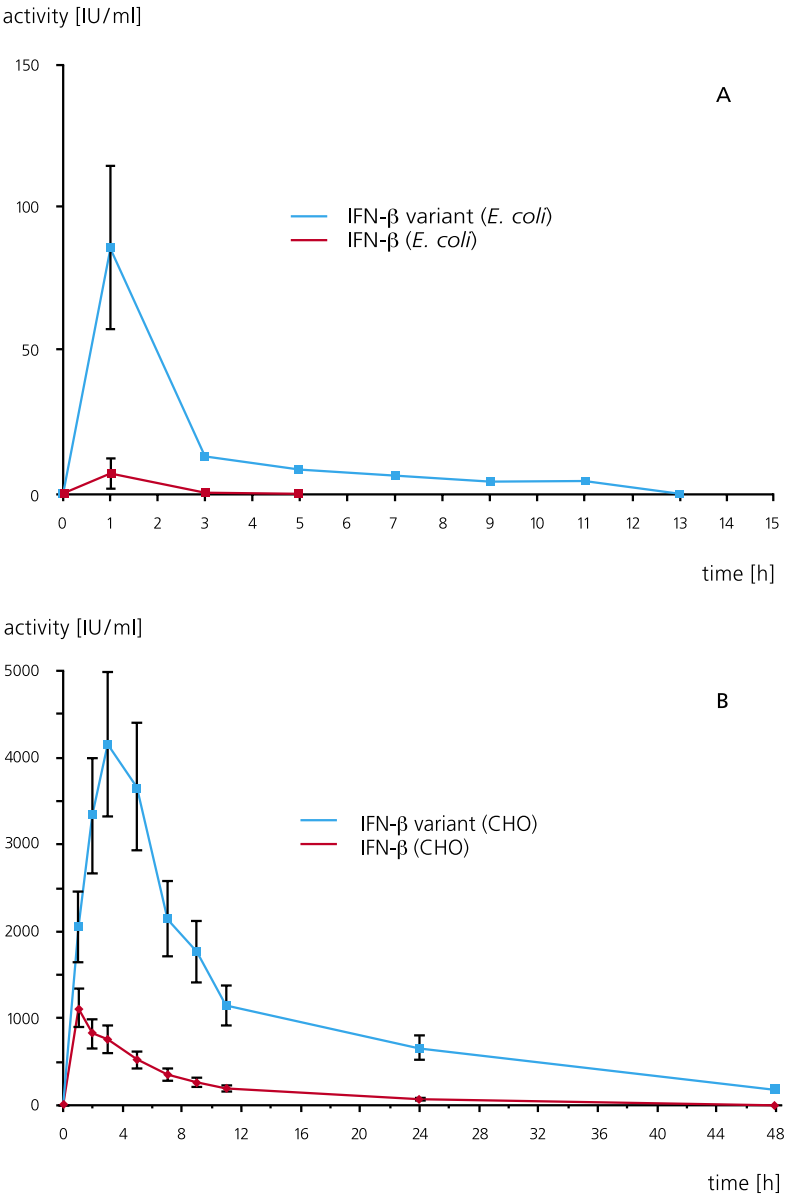
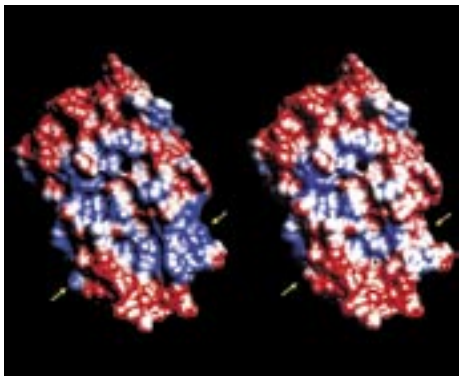


Figure 2: Pharmacokinetics in animal experiments of the new IFN-β variant (blue) from bacterial cells (top) and CHO cells (bottom) in comparison to wild type IFN-β (red). Shown is the biological activity of IFN-β in the serum after subcutaneous injection. In all experiments the same activity of IFN-β was applied (1×10^7 IU).

Figure 1: Comparison of the surfaces of wild type Interferon-β (left) and the new soluble variant with reduced hydrophobicity (right). Arrows indicate regions of substitution. Hydrophobic regions are blue, hydrophilic ones are red.



Interferon-γ variants with increased stability for new indications

Vision: to increase the therapeutic value of IFN-γ

Interferon-γ (IFN-γ) has multiple biological effects that have an influence on the human immune system. Based on these effects several therapeutic approaches were made such as idiopathic pulmonary fibrosis, kidney cancer, or chronic granulomatosis. A major disadvantage for the therapeutic application of IFN-γ is its low thermal stability and its tendency to form inactive aggregates. In contrast to most cytokines the natural occurring human IFN-γ does not possess disulfide bridges. Aim of this project was therefore to introduce stabilizing disulfide bonds by means of genetic engineering, without changing the overall structure of the protein.

Technology: directed protein design

Insertion sites for disulfide bonds with very precise geometry were calculated using computer-modelling techniques, based on data of the known three-dimensional structure of IFN-γ. Directed modification of the IFN-γ gene was used to engineer new variants.

IFN-γ with increased stability

Several different IFN-γ variants were constructed, each containing two disulfide bonds. The new variant IFN-γ-HS was strongly stabilized in respect to thermal inactivation (52 °C to 68.5 °C) compared to wild type IFN-γ and showed fewer tendency to aggregate. The biological activity was conserved. This project was awarded by the International Society for Interferon and Cytokine Research (ISICR) a research prize.

Outlook

The IFN-γ-HS variant will be tested *in vivo* to determine its pharmacokinetic behaviour. We are currently looking for a partner to transfer the project into clinical application.

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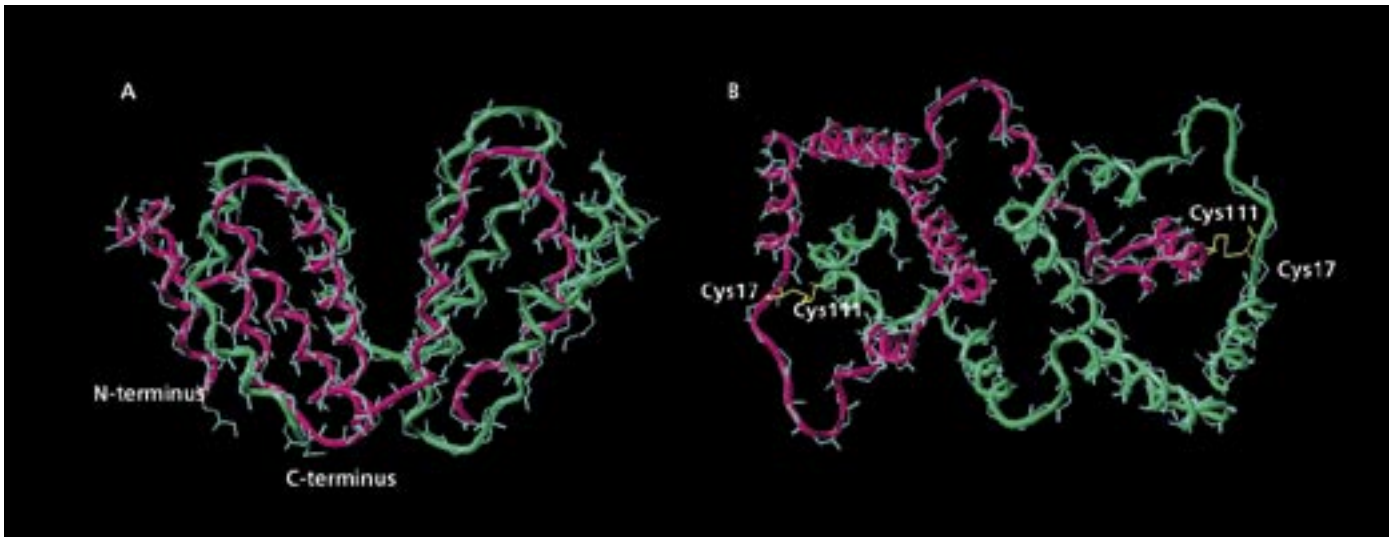
Figure 1: Structure of human Interferon-γ.
A: Wild type
B: Variant IFN-γ-HS with two disulfide bonds



Proinflammatory effects of Interferon-γ

Besides the clinically beneficial antiviral and immunomodulatory activity of Interferon-γ this cytokine is characterized by its pronounced proinflammatory properties. In many inflammatory diseases Interferon-γ is negatively associated with the course of disease. A prominent example is multiple sclerosis (MS). During the acute phases the concentration of Interferon-γ is significantly increased and the administration of Interferon-γ is worsening this disease. As an anti-inflammatory counterpart, Interferon-β is used as therapeutic drug in MS.

Screening for antagonists and agonists of Interferon-γ

Therefore, our aim is to identify substances, which selectively inhibit the Interferon-γ signalling pathway. This particular transduction pathway is well established and illustrates the variety of possible targets for an inhibition (Figure 1). Though far more participants – identified as well as unidentified ones – of that signalling network are present and important *in vivo*, a cell-based assay had to be developed. To detect all possible targets we use a cell-based assay covering the receptor-binding of the ligand, the signal transduction on its way to the nucleus (Jak-STAT-pathway) up to the biological response (e. g. the antiviral protection state). Additionally, the assay is being used for the parallel screening for agonistic substances exhibiting antiviral activities.

As important as the assay is the choice of samples. As marine microorganisms are a rich source of biologically active secondary metabolites, we test crude extracts and isolated pure substances derived from those microorganisms within the joint project »Marine Biotechnologie« (marine biotechnology) funded by the State of Lower Saxony by screening for Interferon-γ inhibiting as well as for antiviral activities. The possibility to detect low concentrated substances that might not easily be isolated is an advantage of crude extracts.

In our primary screening assay, human cells are incubated with the sample. Both, the screening for inhibiting as well as for antiviral activities is performed by infection of the cells with a lytic virus (EMCV). After stimulating the cells with Interferon-γ for virus protection, cell lysis is prevented, except when the sample contains an Interferon inhibiting agent. In the case of screening for antiviral compounds, the virus challenge without additional Interferon-γ protection results in cell lysis, unless antiviral activities in the sample prevent lysis. If cells have lysed or not, can be determined by staining the cells thus showing cell vitality (Figure 2). Because a cytotoxic sample would have the same effect as an Interferon inhibitor, we test for cytotoxicity of the samples in parallel.

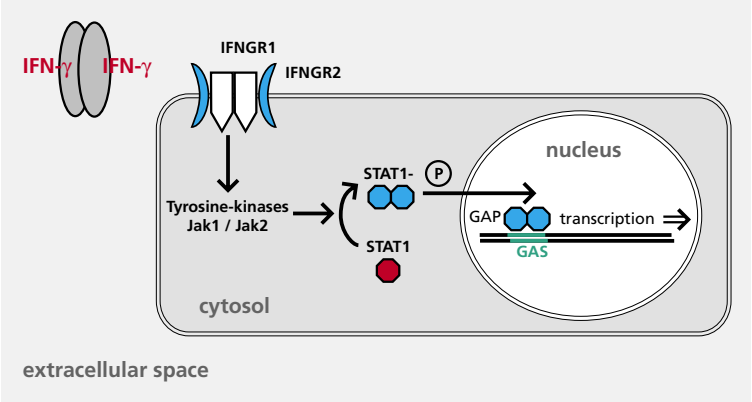


Figure 1: Interferon-γ signal transduction pathway.

Results of screening

More than 1,000 samples have been tested. Three samples inhibiting the Interferon-γ action have been identified. Searching for the mode of action we tested for the capacity to inhibit STAT1-phosphorylation and activation of an Interferon-γ-specific promotor construct as important checkpoints of this pathway. While there was no inhibition of STAT1-phosphorylation, the Interferon-γ-induced gene expression was significantly decreased. The molecular target between activation of the transcription factor and transcription of the Interferon-γ-induced genes remains to be elucidated to settle the question of specificity.

The antiviral substances found in the primary assay are being further analyzed.

Screening for endogenous effectors

While further substances are being tested with the assay mentioned above, additional screening approaches for anti-inflammatory drugs have been developed.

As one feedback mechanism the cell is turning off the Interferon signal by inducing a protein called SOCS1 (suppressor of cytokine signaling). We cloned the SOCS1-Promotor upstream of an easily detectable Luciferase gene. With this system we are able to identify substances inhibiting the Interferon-γ response.
Another cloned reporter system combines the human Interferon-β promotor with the Luciferase reporter gene. Substances able to selectively induce Interferon-β might replace the Interferon-β therapy for example in multiple sclerosis.

Outlook

The activities already found are being purified and characterized in detail to elucidate their molecular structure. The specific reporter systems will be used for the screening of the marine substance collection for the screening for endogenous active compounds. Furthermore, the screening assay as well as the collected samples could be easily expanded to screen for activators and for inhibitors in additional signal transduction pathways of medical interest.

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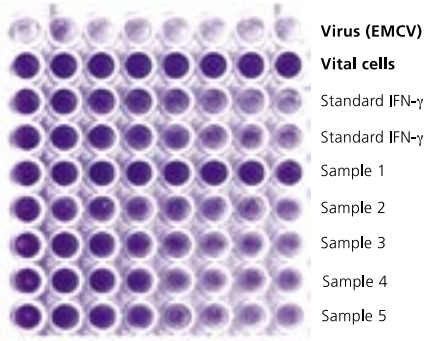

Figure 2: Antiviral assay after staining vital cells with crystal violet. Controls, standard substances and samples are tested in different concentrations to obtain qualitative as well as quantitative results.

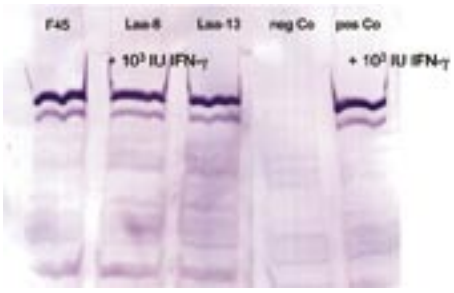

Figure 3: Western blot revealing tyrosine phosphorylation of STAT-1. Samples are stimulated with Interferon-γ after incubation with a phosphorous-specific antibody. Phosphorylation is only prevented in the negative control.

Identification and development of MIF-based small molecule drugs by way of rational drug designs

Background

The cytokine Macrophage Migration Inhibitory Factor (MIF) plays a critical role in inflammatory processes, but also has endocrine and enzymatic functions. MIF is a (co)-mediator in the occurrence of inflammatory diseases such as *asthma bronchiale*, atopic dermatitis, psoriasis, ARDS, rheumatoid arthritis, septic shock or rejection reactions following organ transplants. Based on its structure activity profile and as a possible antagonist of glucocorticoid action, the development of MIF-based treatments is of great relevance for many chronic inflammatory and immune diseases. Whereas MIF strategies based on antibodies have been pursued so far, the approach of Fraunhofer IGB concentrates on the development of possible anti-MIF small molecule drugs. In collaboration with the Institute of Interfacial Engineering IGVT at the University of Stuttgart, the transcription coactivator JAB1/CSN5 [1,2] was identified as a central mediator of the cellular MIF effects [3]. The work in this area is ongoing and will in future also be conducted in collaboration with the Department of Biochemistry and Molecular Cellular Biology of the University Hospital of Aachen. Initial results have shown that the MIF-JAB1 complex is involved in the cell cycle regulation and the AP1 transcription pathway [3]. JAB1/CSN5 and/or the MIF-JAB1 complex are molecular targets for the new MIF-based therapeutic approaches.

The task

The instrument of »rational drug design« (RDD) employed by Revotar Biopharmaceuticals AG for developing potential active agents will follow the comprehensive preparatory work on MIF. Revotar, an independent German subsidiary of the Texas Biotechnology Corporation (TBC), is setting up a »fully integrated pharmaceutical company« (FIPCO) with the aim of researching and developing innovative and highly-effective treatments with few side-effects to treat inflammatory diseases.

The approach

The task of »molecular modeling« is to identify and develop target-related low-molecular inhibitors that can be taken orally (small molecule drugs, SMD). The molecular modeling of Revotar includes supporting the complete development process of an active agent candidate by a highly-integrated data management system that enables combined access to all biological, chemical and medical data important to the substrate development. The planning, monitoring and evaluation of the substance libraries created by the combination chemistry, taking into account all available information, is linked to this. This integrative procedure is in contrast to the usual sequential research approaches that have proved to be inefficient.

Outlook

Many years of preparation relating to MIF in the basic research are finding a route into the economy in synergy with the application-orientated aims of the Fraunhofer research, with Revotar AG being an ideal partner for linking the approaches taken in the research with modern bio-informatics and rational drug design.
Due to the possibly strong side-effects of cortico-steroids, which affect young patients in particular, the development of innovative, highly-effective anti-inflammatory substances that at the same time have few side effects and can be taken orally are of major pharmaceutical and economic benefit.

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The *Candida* threat

Candida albicans is the most common fungal pathogen. In healthy individuals *Candida* might cause superficial infections of skin and mucous layers. This is more a nuisance than a dangerous infection. However, for individuals with a suppressed immune system, like patients after organ transplants, chemotherapy or AIDS, *Candida* is a serious threat. *C. albicans* holds the fourth place in causing nocosomial infections. In Germany, several thousand patients die of candidemia each year. To date there is too little medication effective against *Candida* and severe side effects or resistance against the antimycotica available has been observed. To understand the molecular mechanisms of infection thus is the basis for the development of new therapeutics not only against *Candida* infections.

Target screening

Virulence mechanisms can be identified by comparing virulent versus non-virulent strains. We have performed a direct comparison of the proteomes from a clinical isolate of *C. albicans* and a non-pathogenic derivative thereof, using 2D-gel electrophoresis. The proteins differentially expressed could be responsible for the pathogenicity of the organism. By developing a specialized solubilisation protocol we could reduce the complexity of the proteome in a way that facilitates the identification of differentially expressed proteins. Furthermore, we pursued an approach analyzing specifically cell surface proteins of *C. albicans* as the first interaction site of the pathogen with its host. In this approach cell surface proteins were specifically labeled, isolated by affinity purification and identified by peptide mass fingerprinting.
To be able to evaluate the virulence of distinct *Candida* strains and other pathogenic microorganisms, we developed an assay system based on reconstituted human skin and gut equivalents in collaboration with the Department of Cell Systems at the Fraunhofer IGB. Using these test systems we have a simple but efficient model to avoid animal experiments and to facilitate drug screening.

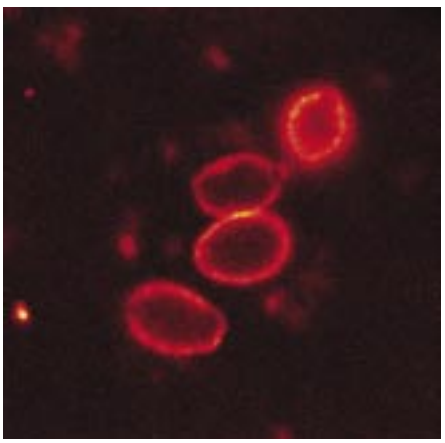

Figure 1: Surface proteins of *C. albicans* were visualized using indirect immune fluorescence. For this purpose these proteins were biotinylated. The covalently bound biotin was detected using Cy3-labeled antibodies.

Results

We were able to identify several proteins present only in the pathogenic form of *C. albicans* with the help of 2D-gel electrophoresis using a differential solubilization approach. Some of these proteins have no known homologues in non-pathogenic yeast like *S. cerevisiae* and thus could be responsible for the pathogenicity of *C. albicans*. This is currently verified by gene deletion and overexpression experiments. We already could verfify four of these proteins as being important for hyphal development. Hyphal development is essential for the virulence of *C. albicans*. Proteins essential for hyphal development thus are ideal targets for pharmaceutical development. Since these proteins are not present in humans, no side effects are to be expected from drugs acting against the *Candida* proteins. This work was awarded the Hugo-Geiger Award 2002 (M. Röhm).

Furthermore, more than 30 cell surface proteins have been identified using the above mentioned approach. These proteins are currently validated as described.

Outlook

The validation of the potential virulence proteins identified will be continued by deletion and overexpression studies. Using the *in vitro* test systems we will evaluate the effect of these mutation on the virulence of *C. albicans* and thus the value of the respective protein as a target. Furthermore, the technologies already described will also be used for studies in other human as well as plant pathogens of clinical or agricultural significance.

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Awards

Hugo-Geiger-Preis 2000:
Sequential solubilization of *C. albicans* protein using 2D-PAGE (D. Rothenstein), Development of reconstituted skin and intestine models for virulence studies of pathogens (C. Dieterich)

Hugo-Geiger-Preis 2001:
Identification of *C. albicans* cell wall protein as targets (C. Urban)

Hugo-Geiger-Preis 2002:
Target validation in *C. albicans* (M. Röhm)

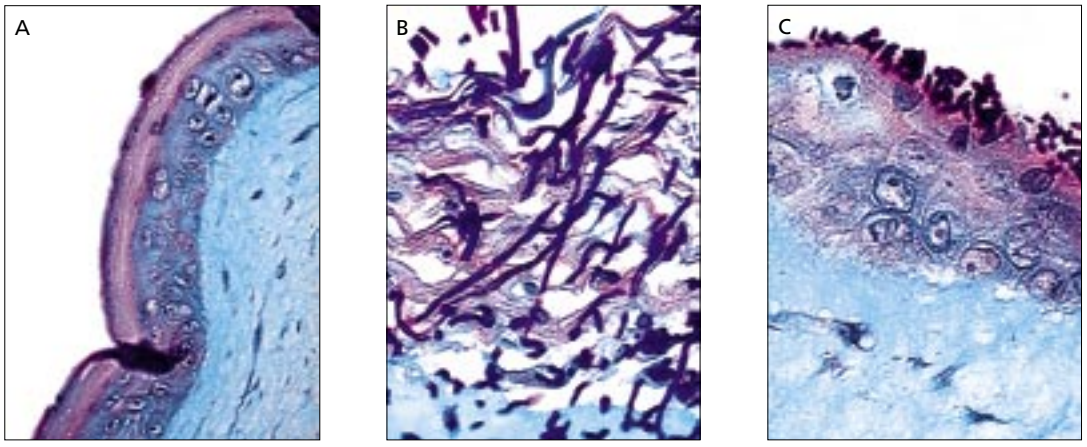

Figure 2: Infection of reconstituted skin (A) with a clinical isolate of *Candida albicans* (B), or an avirulent mutant (C). The clinical isolate penetrates the protective layer of keratinocytes and invades through the epithelial cell layers into the matrix, leading to disintegration of the model system after 48h (B). The avirulent mutants do not form hyphae and show no ability to invade into the tissue. *C. albicans* was only detected on the tissue surface.

Function of protein kinases as key molecules in cellular communication

Phosphorylation of proteins

The reversible phosphorylation of proteins plays an essential role in triggering biochemical processes. Protein kinases phosporylate their target proteins (targets) on serines/threonines and tyrosines thus influencing their activation state. Therefore, protein kinases are involved in key positions triggering the regulation of cellular signalling processes and enhanced activity of tyrosine kinase is seen e. g. in tumor cells.

Induced cell death (apoptosis) and the regulation of cell proliferation are well analyzed cellular signalling pathways regulated by protein kinases. Therefore protein kinases are important target proteins in the development of pharmaceutics e. g. for tumor therapy.

Cooperative research

The Fraunhofer IGB research group Protein Screening Systems develops and utilizes techniques to measure the activation of protein kinases: Specific antibodies recognize selectively phosphorylated amino acids which indicates the activation state of protein kinases.

Further on we use antibodies to identify tyrosine and threonine phosphorylated proteins, which enables us the detection of the complete cellular activation patterns.

Using a model enzyme called protein kinase Cμ (PKCμ) the cell specific control of different signalling pathways is analyzed. This research is performed in collaboration with the Institute of Cell Biology and Immunology at the University of Stuttgart within a common research project of the local Sonderforschungsbereich »Topology and dynamics of signal processes« using latest confocal microscopy techniques.

Activation of PKCμ

Enzyme autophosphorylation and transphosphorylation plays an essential role during the control of enzyme activity. For instance the complex three-step phosphorylation of PKCμ contributes to differential functions. We explored the mechanism of activation of PKCμ through further protein kinases of the PKC family, PKCη ad PKCε [1,4]. This seems to be regulated cell-specifically as a function of higher signal mediators. PKCη and PKCε phosphorylate PKCμ

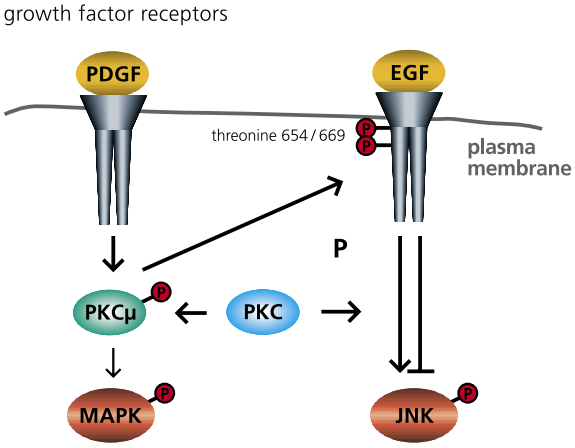

Figure 1: Schematic view of PKCμ/PKC regulated protein kinase cascades. Shown are two growth factor regulated signal pathways, which control each other through protein phosphorylation.

PDGF	Platelet Derived Growth Factor
EGF	Epidermal Growth Factor
JNK	c-jun N-terminal Kinase
MAPK	Mitogen Activated Protein Kinase

directly in the so-called activation loop leading to its complete activation. This results in enhancement of signalling pathways triggering cellular proliferation. A key enzyme, the so-called mitogen activated protein kinase, is activated which results in the phosphorylation and activation of certain transcription factors leading to cell division [2]. Interestingly through the activation of PKCμ a negative feed back loop is triggered to other signalling pathways controlled through PKCη. PKCμ is involved in a growth factor (PDGF) regulated signalling pathway triggering cell division. At the same time the enzyme also controls a signal pathway, which is induced by a different growth factor (EGF), presumably by direct phosphorylation of this receptor. Signalling via that EGF-receptor controlled pathways in turn inhibits the activation of stress-induced signal transducers like JNK.

Localization

Using confocal laser microscopy cellular localization of PKCμ was investigated [3]. This work especially is important, as this enzyme has – dependent on the cellular context – differential functions. In epithelial cells e. g. PKCμ is located at the Golgi compartment and controls the cellular logistics of transport of novel proteins. In contrast in lymphocytes, the enzyme is involved at the control of cell division and the synthesis of immune modulators like cytokines. The activation of PKCμ through both isoenzymes happens at the Golgi compartment, as both enzymes are located here.

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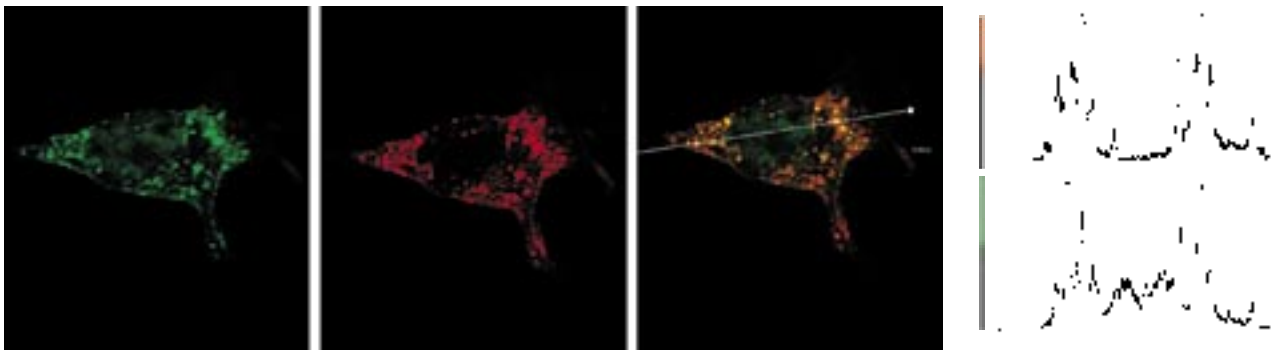

PKCμ PKCeta Overlap

Figure 2: Co-localization of PKCμ and PKCη. Shown is a confocal picture of an immunostaining after expression of both enzymes in the mammary epithelial cell line MCF7. PKCμ was expressed as a fusion protein with the green fluorescence protein. PKCη was visualized through immunohistochemical staining with respective antibodies. On the right picture fluorescence intensity is shown in red and green channels. The overlap of both channels is shown in yellow indicating co-localization.

Characterization of regio-selectively provided hollow fiber membranes via endotoxin binding studies

The initial situation

The therapeutic aphereses systems that are used today in clinical practice (systems for separating the plasma from the corpuscular elements of the blood such as erythrocytes, leukocytes and thrombocytes) use only the plasma fraction of the extracorporeal blood. The cellular blood components are first separated via a plasma separation unit. This process requires a large degree of apparatus complexity and intensive supervision by trained nursing staff. The comparatively much easier to handle haemo-perfusion systems, in which the non-fractionized blood directly perfuses the adsorber matrix, have in contrast a low degree of haemo-compatibility, because the blood cells come into direct contact with the surface structures. The use of haemo-perfusion systems would also be particularly advantageous for eliminating toxins like the endotoxins that occur in conjunction with bacterial inflammations. However, incompatibilities arise here not only due to the contact between the blood cells and the membrane surface, but there are also interactions with the surface groups that form endotoxins, which further reduces haemo-compatibility.

The aim

Within the framework of a BMBF association project with the participation of the company Gambro Dialysatoren GmbH of Hechingen, Fraunhofer IGB, the IGVT of the University of Stuttgart and the University Hospital of the RWTH Aachen, the aim is to regio-selectively provide plasmapheresis membranes for blood cleansing with a nano-scale chemically active overstructure, so as to allow the separation of endotoxins (lipopolysaccharides, LPS) from human blood in a single step. The provision of the membrane should be such that from the outside of the membrane towards the lumen, a surface provision gradient is created. The lumen side of the cell-compatible surface should remain unchanged.

The solution

In initial experiments, the adsorption of LPS was examined using the model of acrylate beads, on the surface of which there was covalent binding of oligomer arginine. The molecular structure of the oligomer arginine should be analyzed. LPS binding tests were used as part of the characterization of the LPS binding. With the aid of these tests, it is intended to show that endotoxins can be eliminated from a solution within minutes via their binding to *o*-Arg-acrylate beads. The experimental system should therefore simulate physiological conditions. In a second stage, the model should be applied to hollow fiber membranes. LPS O111:B4 was used as a commercially available reference substance for endotoxin adsorption on the *o*-Arg-acrylate beads.
In order to examine the regio-selectivity of the immobilization of the LPS-binding molecular species, special marker molecules such as gold particle-marked LPS binders should be developed during the remainder of the project.

The test principle

Endotoxins catalyze the activation of a proenzyme from »Limulus ameobocyte lysate«. The LPS test is therefore also called a Limulus-ameobocyte-lysate (LAL) test (Figure 1). The quantity of the activated enzyme is proportional to the quantity of LPS used. The activated enzyme catalyzes the cleavage of *p*-nitroanilin (*p*NA) of the colorless substrate Ac-Ile-Glu-Ala-Arg-*p*NA. The product *p*NA that is formed can be photometrically measured at 405-410 nm. There is a linear relation-

ship between the LPS concentrations used and the adsorption, so long as the concentration of LPS is between 0.1 and 1.0 EU/ml.

Results

The molecular species that bind LPS (*o*-Arg) were identified via HPLC analysis and MALDI-TOF mass spectrometry as dimeric and trimeric arginine peptides. Figure 2 shows the results of the LPS binding test on *o*-Arg-acrylate beads. If there is an optimum LPS concentration, which was set homogeneously to 100 ng/ml following optimization of the solubilization protocol, the *o*-Arg beads bind LPS quickly and efficiently. Beads and methods are also suitable for removing LPS at 37 °C.

Conclusion

The test system developed can be applied to the analysis of the LPS removal rates through hollow fiber membrane modules. These experiments are the focus of the remaining project phases.

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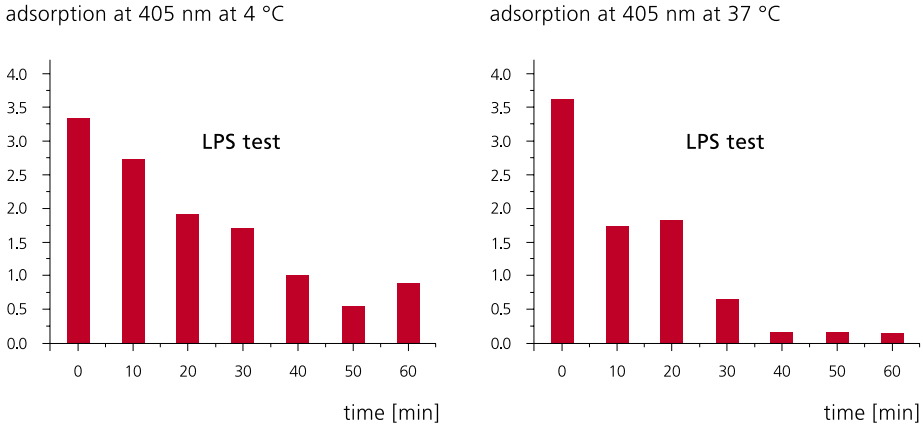

Figure 2: Decrease of the LPS concentration over time by adsorption on *o*-Arg acrylate beads. The experiments were carried out on acrylate beads at 4 °C (left) and 37 °C (right) and with a 20-µl suspension. The optimum LPS concentration was 100 ng/ml after optimized ultrasound. The *o*-Arg acrylate beads were charged in a buffer solution (PBS) with a defined concentration of LPS and incubated for various times (0-60 min). In the supernatant, the quantity of LPS not bound to the *o*-Arg acrylate beads was determined with the LAL test. The *o*-Arg acrylate beads quantity, the incubation time and the different vessels were looked at as variables. The chain lengths of the arginine residues were varied. Experiments with other oligo-arginines and commercial references produced in some cases similar but continuously worse results with regard to the kinetics and LPS removal capacity. The composition of the beads is a commercial secret of the industrial partner.

Figure 1: Picture of the marine life-form *Limulus polyphemus*.

Microarrays as a key technology

Within recent years, complete genomes of more than 800 species including the human genome have been sequenced. In order to fully benefit from the generated information, highly parallelized and miniaturized methods have been developed, including microarrays representing one of the key technologies. The basic principle of microarrays is rather simple: DNA or protein molecules are fixed in an ordered fashion onto a solid support. Then, complex samples are isolated from cells or tissues of interest, labeled and investigated using such a biochip. Biochips enable the researcher to look at thousands of genes or proteins in parallel in a single experiment. The individual DNA or protein probes act as sensors to gain information about transcription, expression or mutations of the respective gene.

Manufacturing of microarrays

Both, DNA as well as protein arrays are made in-house using contact printing methods. Printing is done by high throughput robots. In an in-house collaboration, we are developing microarrays based on nanobeads with significantly higher sensitivity and flexibility compared to conventionally used biochips. For DNA-microarrays both synthetic oligonucleotides and PCR amplicons are used as probes. Protein arrays were made by spotting antibodies or proteins. In addition to transcriptional profiling, this technology is used at the Fraunhofer IGB to detect SNPs (single nucleotide polymorphisms) in the human genome or mutations in pathogenic microorganisms associated with resistance to antibiotics. This is done by performing on chip enzyme reactions (e. g. minisequencing or arrayed primer extension APEX) as well as by allele specific hybridizations.

Projects

The Fraunhofer IGB is using successfully the DNA-microarray technology mainly for the identification of virulence mechanisms and detection of mutations associated with resistance to antimycotics in *C. albicans*. A comprehensive DNA-chip containing virtually all open reading frames of the *C. albicans* genome was established using the sequence database at Stanford University. In collaboration with the Max-Planck Institute of Molecular Genetics in Berlin a non-redundant set of about 7,000 genes was extracted from the database and used to design specific probes. This PCR based microarray is used to perform genome-wide transcriptional profiles. While focusing on the identification of virulence factors many other aspects are investigated including e. g. clinical cooperations on resistant strains. Moreover, we are member of an international consortium working in collaboration with the Sanger Centre, UK on an improved and standardized gene annotation strategy for this obligate diploid genome (http://genome-www.stanford.edu/fungi/Candida/docs/).

Specific DNA-microarrays have also been designed for instance to study cell wall dynamics in *C. albicans.* This array, containing about 120 cell wall related genes was used to investigate the yeast to hyphae transition in clinical isolates and non-pathogenic strains. The results were published recently.

In collaboration with the NMI, Reutlingen we furthermore developed protein arrays, to detect autoimmune disease in man.

Bioinformatics is essential for extracting knowledge from these comprehensive arrays and other high throughput experiments. In order to fully benefit from those data, new and highly sophisticated software tools, are being developed e. g. to correlate and structure experimental data with the available databases. The Fraunhofer IGB is participating in an EU funded project called GeneStream.

Further projects at the Fraunhofer IGB in this area include the development of diagnostic DNA-arrays to detect human pathogens or SNP´s, oligonucleotide chips to differentiate highly homologous gene family members, new fluorochromes for DNA-labeling and chip detection and others. We will realize the manifold possible applications with further cooperation partners.

Outlook

One focus will be to identify virulence mechanisms of pathogens (mainly in *C. albicans*) using transcriptional profiling. In particular host-pathogen interactions will be studied using human tissue equivalents manufactured at our institute. In addition, DNA and protein microarrays will be further improved, especially with regard to new surface technologies.

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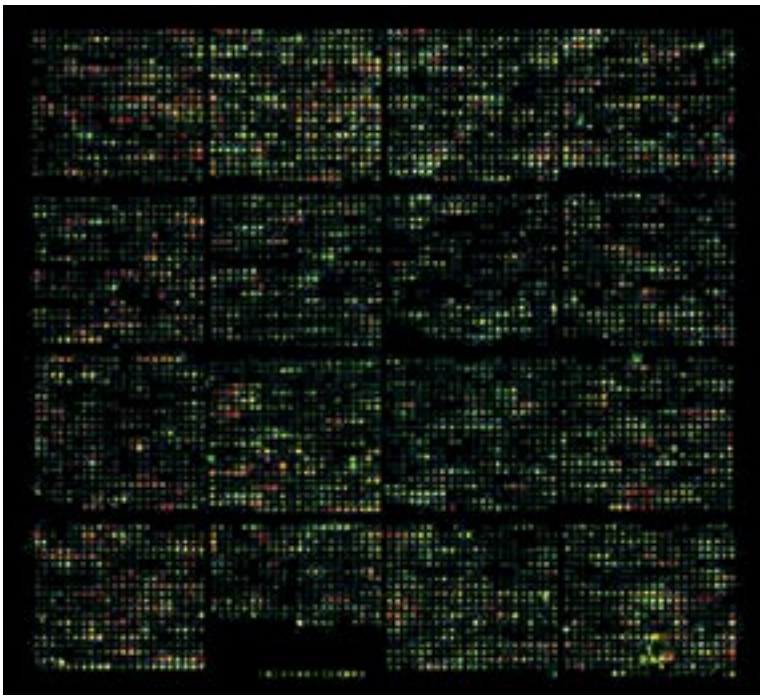

Figure 1: Transcriptional profiling of *Candida albicans*. This microarray represents 7,200 genes derived from the genomic sequence of *C. albicans*. A comparison of cells grown in the yeast form (Cy3-labeled / green) and hyphal form (Cy5-labeled / red) is shown.

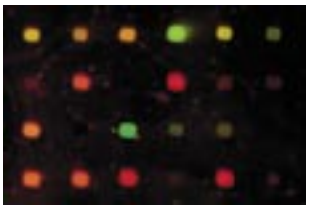

Figure 2: DNA-chip for differentiation between strongly sequence-homologous members within gene families by the introduction of mismatch positions. It was hybridized with differently marked DNA probes of two genes from the SAP family.

Simultaneous analysis of a genome

The understanding of biochemical signalling pathways triggering the organization of multicellular organisms has been enhanced enormously during the last years. Using molecular technologies thousand of genes have been identified enabling an understanding of the complex differential gene expression.
Through the availability of the sequences of complete genomes the technology of DNA-microarrays has been established. This method enables a parallel analysis of the expression of thousands of genes. With DNA-microarrays transcriptional analysis of more than 10,000 genes can be performed in parallel enabling the analysis of changes in gene expression upon cellular signalling. Using DNA-microarrays changes of gene expression between normal and pathophysiological tissue can be compared [1].

The DNA-microarray technology is regarded to be very expensive and generates a huge amount of data, needing a complex evaluation. For several pathological situations the knowledge of a limited amount of marker genes is suitable for an unequivocal characterization. For instance using whole genome-wide transcription profiling in leukemias approximately 50 genes were identified, which are characteristic and sufficient for the classification of leukemias. Accordingly changes in gene expression of selected marker genes can be used for the differential diagnostic of chronic myeloic leukemia and chronic lymphatic leukemia [2].

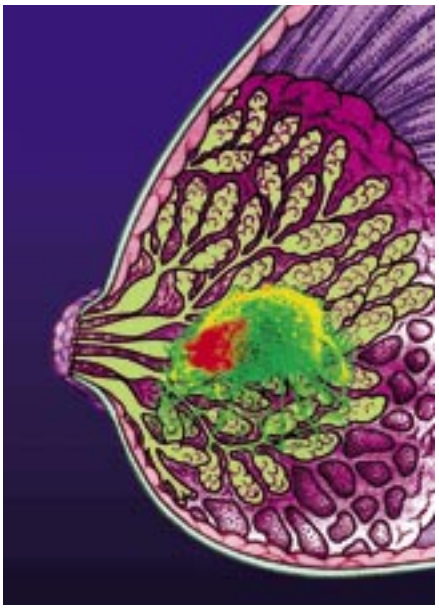

Figure 1: Each year there are approx. 50,000 novel cases of breast cancer from which approx. 18,000 are fatal. This signalizes the enormous significance for the public health system in Germany. Approximately every 9th woman comes down with breast cancer during her lifetime.

DNA-microarray as a tool for molecular biology

Together with the cooperation partner from the Robert-Bosch Hospital, Stuttgart (Prof. Knabbe) we selected approximately 200 genes, which, if expressed incorrectly, are associated with the onset of breast carcinomas. The list of genes used will be continuously updated. In recent genome studies, it has been shown that the knowledge of the expression pattern of 80 genes is sufficient enough to characterize mammary epithelial carcinomas and to give a prognosis for the patients [4]. The list of genes includes genes coding for components of estrogen signalling.
The presence of the estrogen receptor is essential for the classification and characterization of breast tumors. Antiestrogens are used for the therapy of breast carcinomas as they regulate the production of the Transforming Growth Factor (TGF), which negatively influences the growth of mammary epithelial tumors. Accordingly our DNA-microarray contains genes, which are regulated through TGF. Further features are a selection of genes, which are involved in proliferation, and the induction of apoptosis, as their expression is often changed especially in tumors. In addition cancer genes and tumor suppressor genes were included. A selection of known breast cancer marker genes like growth factor receptors, cytokeratins and transcription factors complete the list of genes used.

This DNA-microarray will be used as a molecular tool for the analysis of cellular proliferation upon overexpression of selected protein kinases.

Characterization of breast tumors

The novel developed microarray shall be used for the characterization of transcription profiles of breast tumors. The primary objective is to compare conventional tumor characterization methods with gene expression profiles to identify a typical signature, which enables the prediction about tumor progress and success of an applied therapy. It has been shown in several elegant studies for breast cancer [3] and leukemias [1] that tumors can be classified on the basis of gene expression signatures. In contrast to these studies using DNA-microarrays with several thousand genes, we will use exclusively genes of which the expression is indicative for the development of a given tumor. Figure 2 shows the raw data of an expression profile comparing a human breast tumor with an established breast tumor cell line (MCF7).

Furthermore, this DNA-microarray will be used as a molecular tool for the analysis of signal transduction pathways. For instance in cell lines of human embryonic kidney cells and of cervix carcinomas protein kinases as part of signalling pathways are overexpressed.

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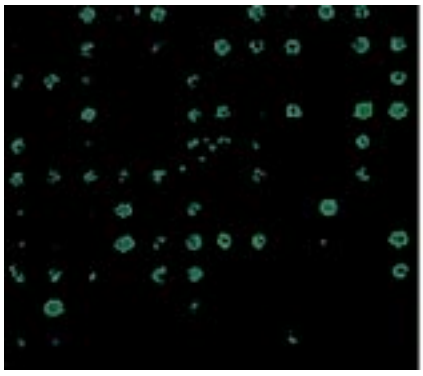
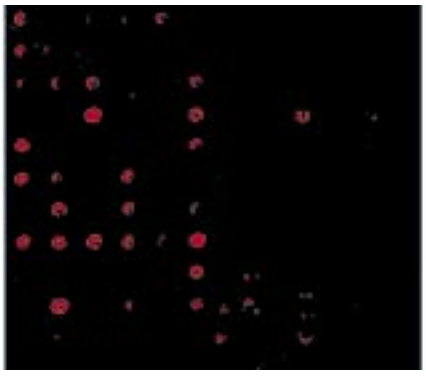
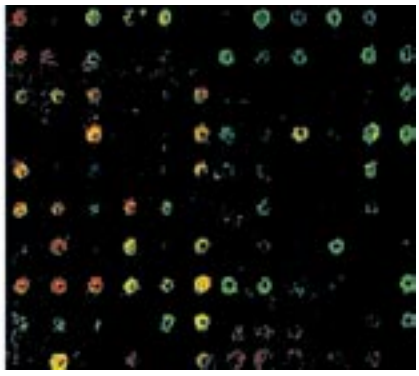


Green: These genes are expressed in MCF7 cells.



Red: These genes are expressed in the examined tumor.



Yellow: Same gene expression in both cell types.

Figure 2: Transcription profiling of a breast cancer cell line (MCF7) compared with a breast tumor.

New enzymes from soil gene libraries

Exclusive access to unknown enzymes

The Screening Center of the Fraunhofer IGB offers a procedure, with which new enzymes from non-cultivable microorganisms can be detected. The genomic DNA from all types of bacteria contained in a soil sample serves as raw material for a gene library, which is then tested by suitable assays on the desired biocatalytic activity. Thus we can offer an exclusive access to new, yet unknown enzymes for partners of chemical, pharmaceutical, food, textile and leather industries, as well as for enzyme producers.

Operational areas of technical enzymes

By means of suitable enzymes the efficient production of defined products with high enantiomeric purity is possible. However, undesirable by-products often arise with classical chemical synthesis. The environmental impact and the costs, which result from the increased consumption of substrates, product purification as well as waste disposal, can be drastically reduced by the use of appropriate enzymes. By the use of enzymes, existing procedures can be improved and even completely new products can be produced. Enzymes can also be used for the specific degradation of unwanted by-products. Biocatalysts offer a wide range of applications, resulting in a sizeable demand for enzymes that meet special requirements. These include higher stability, optimal pH or temperature ranges or new reaction mechanisms.

Figure 1: Screening Center at Fraunhofer IGB: DNA of microorganisms from soil samples is expressed in laboratory strains.

Classical methods are limited

Usually new enzymes are detected in master collections of microbial strains, by scanning on appropriate metabolic activities. Alternatively new types of bacteria from the nature have been enriched. With these procedures however, only those microorganisms can be collected, which can be grown under laboratory conditions as pure cultures or as a mixture of fewer strains. This condition applies however only to less than 5 per cent of the bacterial strains existing in nature. Therefore, the metabolic potential of the majority of microorganisms is out of reach with conventional methods.

Screening strategy opens novel potentials

With the screening strategy of the Fraunhofer IGB this shortage of the non-cultivable microorganisms can be overcome. The DNA of all microorganisms existing in a soil sample is purified, fragmented, inserted in appropriate vectors and brought into a host strain, which can be cultivated easily. Each clone of the thus gained extensive gene library contains part of the genomic DNA and expresses the proteins encoded therein. After screening with functional assays the positive clones are sequenced, followed by further characterization. Interesting hits can be subcloned into specialized vec-

tors or optimized by mutation and subsequent selection, if necessary. With this strategy enzymes can be detected, which would remain undiscovered with classical methods.

New enzymes – unknown sequences

At the Screening Center of the Fraunhofer IGB a gene library with now 50,000 individual clones was produced and examined for different metabolic activities. Using a high-throughput assay we identified 45 clones with esterase activity, four of them also convert unusual substrates. We found no sequences identical neither to another of our new clones nor to any other protein sequence so far published in the relevant databases. Therefore the gene potential of our gene libraries is sufficient diverse to discover numerous new enzymes. In addition to esterases we also found by selective screening tests amylases, proteases, phosphatases, katalases and dioxygenases. Our plan is now to further establish location-specific gene libraries and appropriate high-throughput-suited assays on technically utilizable enzyme activities according to the needs of industries.

We offer

- Screening of gene libraries for new enzymatic activities
- Subcloning, sequencing, expression and characterization of new enzymes
- Custom-made gene libraries for special requirements
- Development of new enzyme assays
- Enzyme optimization

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Echo from the industry

»The approach of the Fraunhofer IGB to enzyme screening appeared very promising to us from the beginning on. However, to develop the entire methodology would have been too expensive for a producing company. We authorized the Fraunhofer IGB with the generation and screening of the gene library. The co-operation has been excellent.«

Dr. Michael Breuer,
BASF Research Fine Chemicals and Biocatalysis

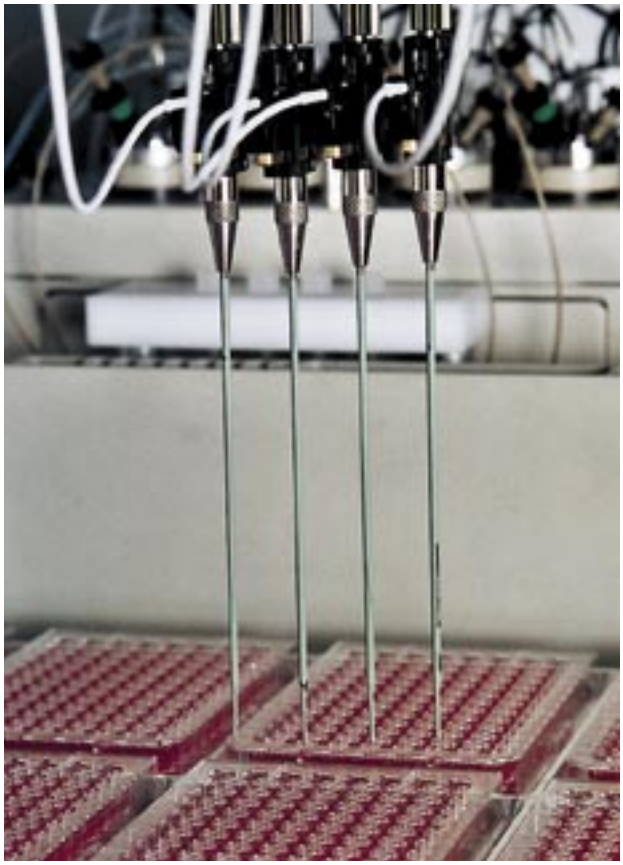

Figure 2: Automated screening with the halogenid sensor at Fraunhofer IGB/IPA.

A new chitin deacetylase for high purity chitosan

From product mixture to high purity compound

Chitin is the second most abundant biopolymer, second only to cellulose. It is a polymer of N-acetyl-glucosamine and is mainly found in insects, fungi and marine invertebrates. Source for industrial production are crab shells.

Because of its chemical and biological characteristics, chitosan, a derivate of chitin has a broad range of commercial applications. It can be used for medical purposes, e. g. regeneration on connective tissue and for cosmetic applications. As a result of its extraordinary characteristics it is also used for waste water treatment, seed coating and food production.

The main problem of the current production is the treatment of chitin with hot, concentrated soda lye. Unspecific cleavage of the polysaccharide chains may occur beside the desired deacetylation. This unwanted degradation cannot be controlled in the chemical process, resulting in mixtures of products.

High purity chitosan of a constant reproducible composition, however, is a prerequisite for medical applications. Therefore, scientists at Fraunhofer IGB have developed a biotechnological process for the mild and defined production of chitosan. This research is part of a federal project called »Marine Biotechnologie« founded by the state of Lower Saxony.

Biotechnological production

Splitting off the acetate groups of the chitin is the essential step in chitosan production. In nature, this reaction is performed by a class of enzymes called chitin deacetylases. Using molecular-biological methods we have been looking for new chitin deacetylases in marine microorganisms:

- Alignment of known chitin deacetylases on protein and DNA level
- Isolation of genomic DNA from marine microorganisms (mixed and pure cultures)
- Degenerated PCR to obtain partial sequences
- Sequencing of PCR products
- Direct sequencing of genomic DNA to obtain full length genes
- Cloning of the new gene in a suitable vector for recombinant expression in *E. coli*
- Detection and purification via an affinity tag (Strep-Tag II)
- Preparation of the enzyme in a functional form
- Characterization of the enzyme

New chitin deacetylase

Using molecular biological screening methods we obtained the sequence of a new chitin deacetylase. It shows high similarity with known enzymes. A patent is pending.

The gene has been cloned and recombinantly expressed in *E. coli*. The protein can be prepared from inclusion bodies in high yields as well as in soluble form. Protein isolated from inclusion bodies has to be renaturated to get an active protein.

Experiments have shown the enzymatic activity of the recombinant protein. Optimal conditions, e. g. for pH, temperature and buffers have been characterized. Soluble chitin oligomers can be deacetylated by the enzyme as well as solid chitin, e. g. from crab shells. The degree of deacetylation is up to 90 per cent. Main goal is the biotechnological use of the enzyme.

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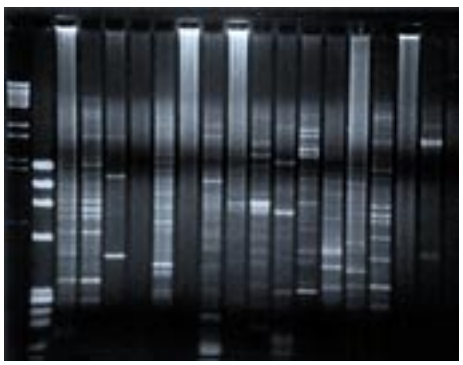
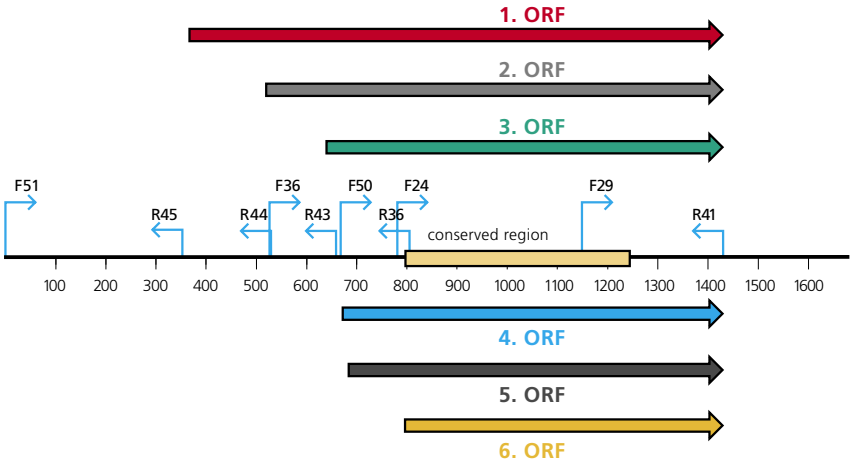


Figure 1: Degenerated PCR for the screening for new chitin deacetylases.

Figure 2: Schematic depiction of the new chitin deacetylase (ORF = open reading frame).



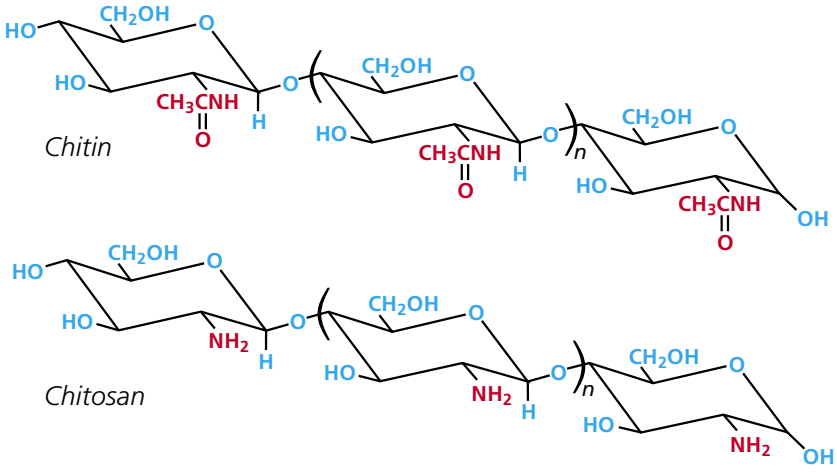
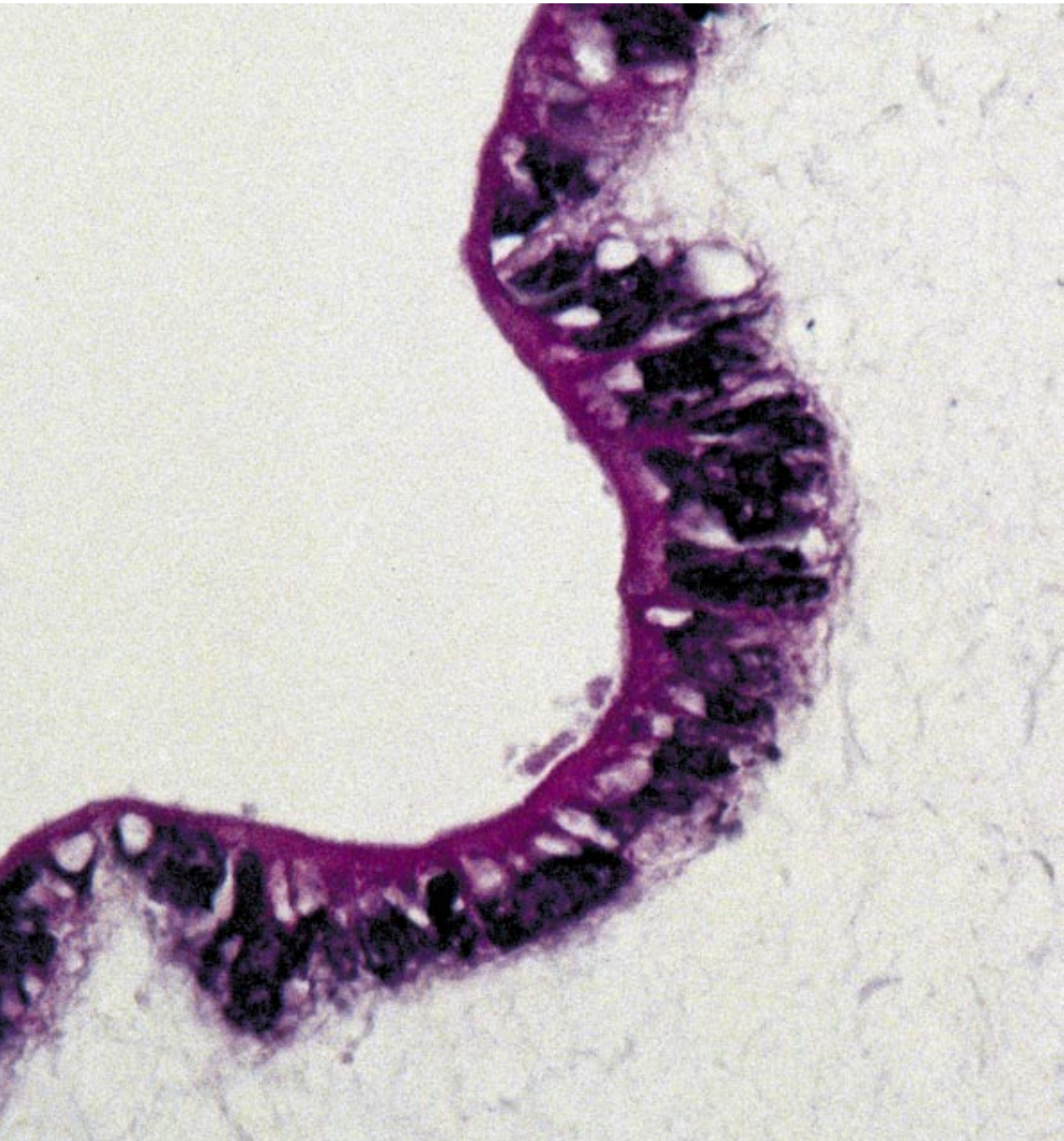


Figure 3: Chitosan can be produced by deacetylation of chitin.

Organoid cell systems



Since 1985 the Department of Cell Systems at the Fraunhofer IGB is engaged with isolation and *in vitro* culture of primary cells of different origin. Using a cell matrix component particularly developed for this purpose the morphogenesis of tissues and organs could be simulated in three-dimensional cell cultures.

Based on this, a three-dimensional skin equivalent was developed, which was awarded with the »Josef von Fraunhofer prize« in the year 2000. In the meantime, following different aims, this test system could be developed further and characterized in detail. In addition, a broad experience in the three-dimensional culture of diverse other primary cell types is available.

A special challenge exists in offering specifically designed three-dimensional cell culture systems according to the requirements of our cooperation partners. On one hand, such systems represent the basis for scientific projects; on the other hand they can be used for development of diverse products up to marketable industrial applications. Future steps of the department will intensify its competences particularly in automation and high-throughput systems. Additionally, an increasingly important factor will be the availability of techniques, which ensure an efficient and stable genetic modification of primary cell populations.

Besides the work with differentiated cells, future focus will involve research and development projects with adult stem cell populations. With the assistance of the three-dimensional systems specified above, it is intended to establish an optimal cell culture environment to support *in vitro* culture of adult stem cells, in order to give a better access to this attractive cell population for experimental work. In this context, mesenchymal and hematopoietic stem cells appear to be of particular interest, but also the isolation, characterization and culture of epithelial stem cells from skin and intestine are prominent topics in current and planned projects.

Tissue engineering products and production in compliance with GMP

Based on this technology platform, the department has begun to open two new operational fields in the last three years: process development for »tissue engineering« products and pilot scale production of such products for application in clinical phase I/II studies. With regard to this, the department acts as a competent partner for high-quality research services and offers small enterprises and highly specialized clinical institutions without own infrastructure

Figure 1: Crypts of porcine intestine, histological section (630-fold).

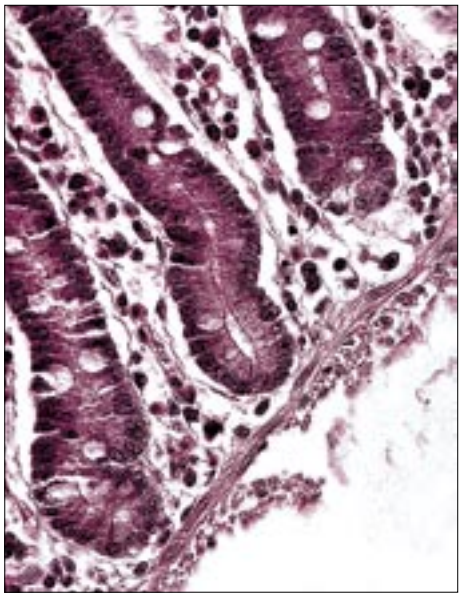


Figure 2: Diagram of intestinal crypts. Stem cells are located at position 4 from the base of the crypt.

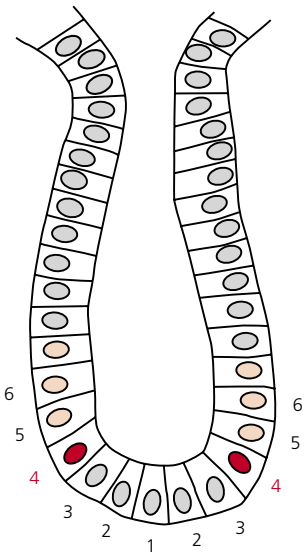


Figure on the left:
Intestinal model.

the unusual possibility for outsourcing whole projects in research and development up to the production of specimens for clinical evaluation. In this context Fraunhofer IGB serves as a contract manufacturer according to the German Drug Law (»Arzneimittelgesetz«; AMG). For this purpose a clean room facility has been established and opened in September 2002. Within this facility class 100 clean rooms equipped for cell culture work are available. Appropriate areas for quality control, separate storage space, working places for documentation and an archive are also included. The establishment of a quality assurance system and the availability of experienced and particularly trained personnel complete the requirements and permit contract process development and contract manufacturing of investigational cell-based drugs in a limited number.

Process development for three-dimensional cartilage cell cultures and for the manufacturing of collagen as basic component of the cell matrix in compliance with the guidelines for Good Manufacturing Practice (GMP) was finalized in the fourth quarter of 2002. Subsequently an application for manufacturing authorization according to §13 AMG for human cartilage cell transplants was filed at the responsible local authorities. Starting from this experience and supported by the existing documentation it is planned to extend the manufacturing repertoire in 2003 for the classical method of cartilage defect therapy, the so-called »Autologous Chondrocyte Transplantation« (ACT).

Cell processing and production of drug specialities

In the future the department plans to realize an extension apart from the tissue engineering field towards projects on process engineering and contract manufacturing in cell and gene therapy. In particular the production of gene therapy drugs for clinical phase I/II trials is heavily restricted by several guidelines and production capacities for such drug specialities are a major bottleneck in the field. For this business the department has a referable experience and will establish a broad inventory of tools. This will include equipment for experimental and clinical cell separation, for production of genetically modified haematological transplants, for *in vitro* differentiation of clinically attractive cell populations, for production of technical or clinically relevant cell banks and for the manufacturing of pre-clinically and clinically applicable gene transfer vehicles (in particular retroviral vectors).

In 2002 the main strategy of the Cell Systems Department at Fraunhofer IGB was redefined. As a new component the process engineering tradition inherent in the institute will be efficiently defined towards the needs and applications of new disciplines in biomedicine. The specialization on GMP process engineering within »tissue engineering« and the cell and gene therapy derived from the competence of many years in bioprocess engineering and is extended by profound knowledge in questions of regulatory affairs for the manufacturing of clinically applicable material. Thereby the experience of the department evolves in relation to changed requirements in applied research and development. In that way a so far not considered clientele of project and cooperation partners is addressed. These considerations thereby ideally comply with the goals of Fraunhofer IGB and of the »Life Sciences« network of the Fraunhofer-Gesellschaft.

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Figure 3: Qualified personnel in the GMP production unit.

Three-dimensional organoid cell cultures as test systems

Since 1985 the Fraunhofer IGB is developing *in vitro* test systems using cells of different origin. Main topics are isolation, characterization, cultivation and functionalization of primary cells.

Starting from this platform, the department has specialized in working on three-dimensional cell culture model systems. These models, for example human skin or cornea equivalents, exhibit organ-specific characteristics and thereby qualify in an outstanding manner for the analysis of scientific questions. Furthermore the department uses this expertise for the characterization and evaluation of interesting substances from the pharmaceutical, chemical and cosmetic industries.

A large number of different projects were carried out and finished on this basis in the past years. Synergistic effects with main topics of other departments of the institute, as for example plasma treatment of textiles to increase biocompatibility, were used as well as customized analytical services provided by a DIN/ISO and GLP certified service laboratory present in the institute. The following compilation shows an overview of specific cell lines, primary cells and model systems the department is working with.

Cell lines

CaCo (human colon carcinoma), CCRF-CEM (human T-cell leukemia), HaCaT (human keratinocytes), HT 1080 (human fibrosarcoma), HeLa (human cervix carcinoma), Hey (human kidney cell carcinoma), HL-60 (human premyeloic leukemia), HT29 (human colon carcinoma), K 562 (human chronic leukemia), KG 1 (human acute myeloid leukemia), Lovo (human colon carcinoma), MCF 7 (human breast cancer), MelJuso (human melanoma cells), SK Mel 30 (human melanoma cells), TE 1 (humane erythrocyte leukemia), TE 671 (human rhabdomyosarcoma), U 937 (human lymphoma), C3H10 T1/2 (murine embryonal cells), FDCP-1 (murine bone marrow stem cells), FDCP-Mix cl. A4 (murine bone marrow stem cells), L929 (murine fibroblasts), MS 5 (murine stromal cells), NIH 3T3 (murine fibroblasts), WEHI 3B (murine myeloid leukemia), WEHI 3B (murine myeloid leukaemia).

Primary cells

- Hematopoietic stem cells (human, porcine, murine)
- Mesenchymal stem cells (human, porcine, murine)
- Endothelial cells (human, porcine) from aorta, cornea, skin, umbilical cord, adrenal gland
- Epithelial cells (human, porcine) from bladder, cornea, skin, trachea;
- Chondrocytes (human, porcine)
- Hepatocytes (human, porcine, murine)
- Keratinocytes (human, porcine)
- Fibroblasts (organ specific; human, porcine, murine)

Three-dimensional organoid cell culture model systems and examples for applications:

- Cornea (human, porcine) – cornea model as alternative to Draize test
- Intestine (porcine) – intestine model as test system for studies of metabolism
- Skin (human, porcine) – skin equivalent as test system for wound healing studies, for investigation of the neo-angiogenesis, for »substance screening« procedures
- Cartilage (human, porcine) – cartilage equivalent as test system for studies of metabolism and differentiation
- Liver (human, porcine, murine) – liver cell cultures for studies of metabolism, approaches in system-biology

Primary cell culture model systems can be designed towards a given application. This and the extension of such models by specifically functionalized cells or cell populations opens the door for a wide variety of applications.

A special attribute of the above described *in vitro* cell culture systems is that single cells or whole cell populations constituting the model have outstanding properties for genetic manipulation. This aspect is going to be increasingly utilized in future for establishment of new systems and/or their specific functionalization.

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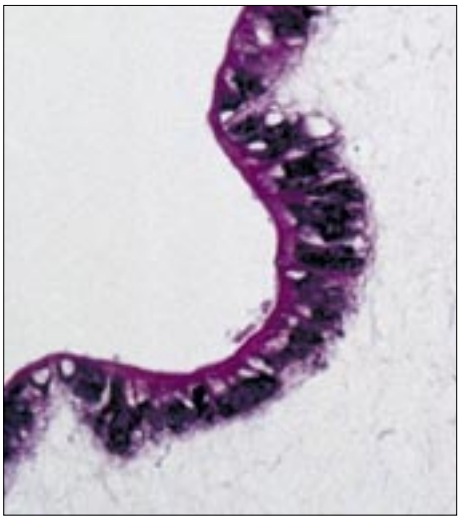
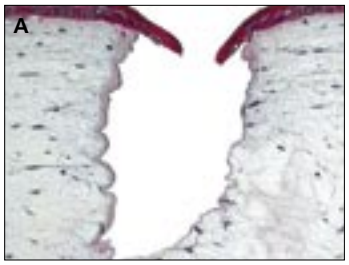


Figure 1: Intestine model.

Figure 2: Cross-section of a three-dimensional cornea equivalent.

Skin model for studies in wound healing

In the last year the skin model specified above was optimized for studies in wound healing. Precise and highly reproducible wounding was established by laser technology. Due to their regeneration ability, cultivated keratinocytes proliferate after injury. The analysis of suitable cellular and molecular parameters in a defined time related manner leads to precise and presentable kinetics. These kinetics can be used to characterize certain agents active in wound healing. Methods as for example confocal microscopy analysis to estimate the number of cultured cell layers built up in the epidermis can confirm quality of skin equivalents before and after an experimental test procedure.



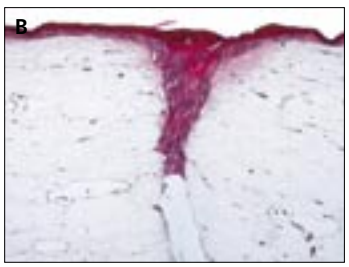


Figure 3: Wound healing at three-dimensional skin equivalent (100x).
A: 3 h after injury
B: 72 h after injury

Process development for »tissue engineering« products

»Tissue engineering« (TE) is a new discipline in the biomedicine, which describes biotechnological procedures using primary cells for the regeneration of tissues and organs. Common examples of *in vitro* cultured TE products are skin or cartilage transplants.

Process engineering

Basically two main topics have to be covered during process engineering for a TE product: First, the experimental establishment and verification of the procedure leading to the desired product and the concordance of the procedure to common pharmaceutical standards. Second, it is necessary to legalize medical application in context of clinical studies and thus later in patient care. The most currently available TE products are so-called patient-specific drugs. Cells are taken from a patient or a donor and are manipulated in the laboratory so that they can be used for therapy of specific diseases. The department of cell systems has demonstrated its proficiency for both issues of process development in the last years.

Process development

Many years of experience in isolation, characterization and *in vitro* culture of human primary cells and in the establishment of three-dimensional cell cultures confirms the department as a competent cooperation partner for the first part. In addition an excellent knowledge is present about cell culture matrices, in particular about the application of collagens. Moreover the scientific development of *in vitro* manipulated transplantable material includes, besides an excellent documentation, also biocompatibility studies and application in suitable animal models.

GMP and pharmaceutical standards

The second part is mainly a process of adjustment of a developed procedure to the current GMP guidelines. This requires a current knowledge of the interpretation of above-mentioned guidelines for the special scope of »tissue engineering«. The goal of this step is usually defined in obtaining a so-called manufacturing authorization according to European pharmaceutical standards. Reaching this goal is accompanied with standardization and validation of the process and an extraordinarily intensive documentation. Thus a simply production process is normally covered by several hundred specific hierarchically structured and authorized documents which are also necessary if marketing authorization on an European level is intended.

The following points are part of a successful process development:

- Definition and qualification of starting materials and the contractual obligation and, if necessary, auditing of the designated suppliers
- The qualification and validation of plants and equipment used during production
- Design of training documents and courses for personnel
- Generation of standard operating procedures (SOPs) and pre-designed protocols for production and quality control and appropriate related documents
- Determining specifications to describe the quality of the products
- The definition of in-process and end-product controls
- The warranty of the biological safety of the product by suitable methods demonstrated to be state of the art in science and technology
- Nomination of suitable external service providers for example for biosafety testing and contractual obligation and auditing of this laboratories

- Coordinating final release procedures; generation and storage of pilot and retention samples
- Process validation
- Archiving of documentation and preparation for know how transfer to a pharmaceutical company

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Cartilage transplants

In 2003 a project on the development of human cartilage equivalents for the cartilage defect repair was finalized with the application for manufacturing authorization at the responsible German authorities. The application file covers the GMP compliant manufacturing of both cartilage transplants and collagen, which is used as a cell matrix. The process development carried out for this project thereby includes both main sections mentioned above.

Figure 1: Sorting and separation of specific cell populations from tissues and organs with a FACS-Vantage.

Figure 2: Cartilage transplant, four weeks after implantation in mini pig.

Figure 3: Cartilage cell matrix one year after transplantation.

Manufacturing of investigational medicinal products according to GMP guidelines

The manufacturing of investigational medicinal products (IMPs) in »tissue engineering« (TE) and cell and somatic gene therapy is heavily regulated by several different laws and guidelines. In principle GMP guidelines that are part of the German Drug Law (§54) apply.

The adaptation of a manufacturing process to this rules, as already mentioned in the last chapter of this report, is an essential component of each process development in the biomedical disciplines specified above. It represents one of the important milestones in the value chain for substances and therapeutic agents manufactured on the basis of living cells. At the same time this represents the transition from preclinical to clinical research and is finalized with the production of the clinical samples.

Contract manufacturing

For TE, cell and gene therapy it is unusual that all steps during drug development are done by the same unit. With regard to this the Fraunhofer IGB offers the validation of a manufacturing process and the production of investigational drugs in a pilot scale to cooperation partners and customers. This can be split up from the respective process development as a separate service for realization of clinical trials. In this context, the Fraunhofer IGB acts as a contract manufacturer (according to the German Drug Law).

Further competencies

Related to the established technology platform for primary cell culture, long lasting expertise and excellent equipment display the opportunity to carry out also complex cell processing procedures. This complies also with the competencies and technologies of other departments of the institute; likewise those of the DIN/ISO and GLP certified analytical service laboratory (HPLC, GCMS/MS, GC, pyrolysis-GCMS) and the departments for Molecular Biotechnology (chip technology, quantitative PCR) and Interfacial Engineering and Material Sciences (scanning electron microscopy, specialized surface analytics).

GMP compatible clean room facility

For operating as a contract manufacturer the Department of Cell Systems runs a clean room facility (~150 m^2) with separate units for production and quality control. A so-called »Site master file« describes the plant, which is registered for operations at safety level 2 according to the German Gene Technology Law. The production facility has adequate air conditioning specifying for class C and B clean rooms. In the B area two cell culture working places classified A in B are established (*class* 100, US nomenclature). The plant and the equipment have been qualified retrospectively or prospectively and have been validated for specific production processes; the monitoring of relevant equipment occurs by computer-assisted technology and is connected to the central technical management unit. The department operates GMP manufacturing with a staff of seven particularly trained co-workers (including »qualified« persons according to the German Drug Law) and runs a quality management system as defined in chapter 1 of »EC Guideline to Good Manufacturing Practices for Medicinal Products and Active Pharmaceutical Ingredients«.

In the last year two audits by local authorities have been past and final technical improvements regarding the air conditioning have been established.

The equipment was extended by a closed system for sterility testing. A documented revalidation process completed both processes. For the next two years the focus has been defined being the manufacturing of specific gene therapeutics.

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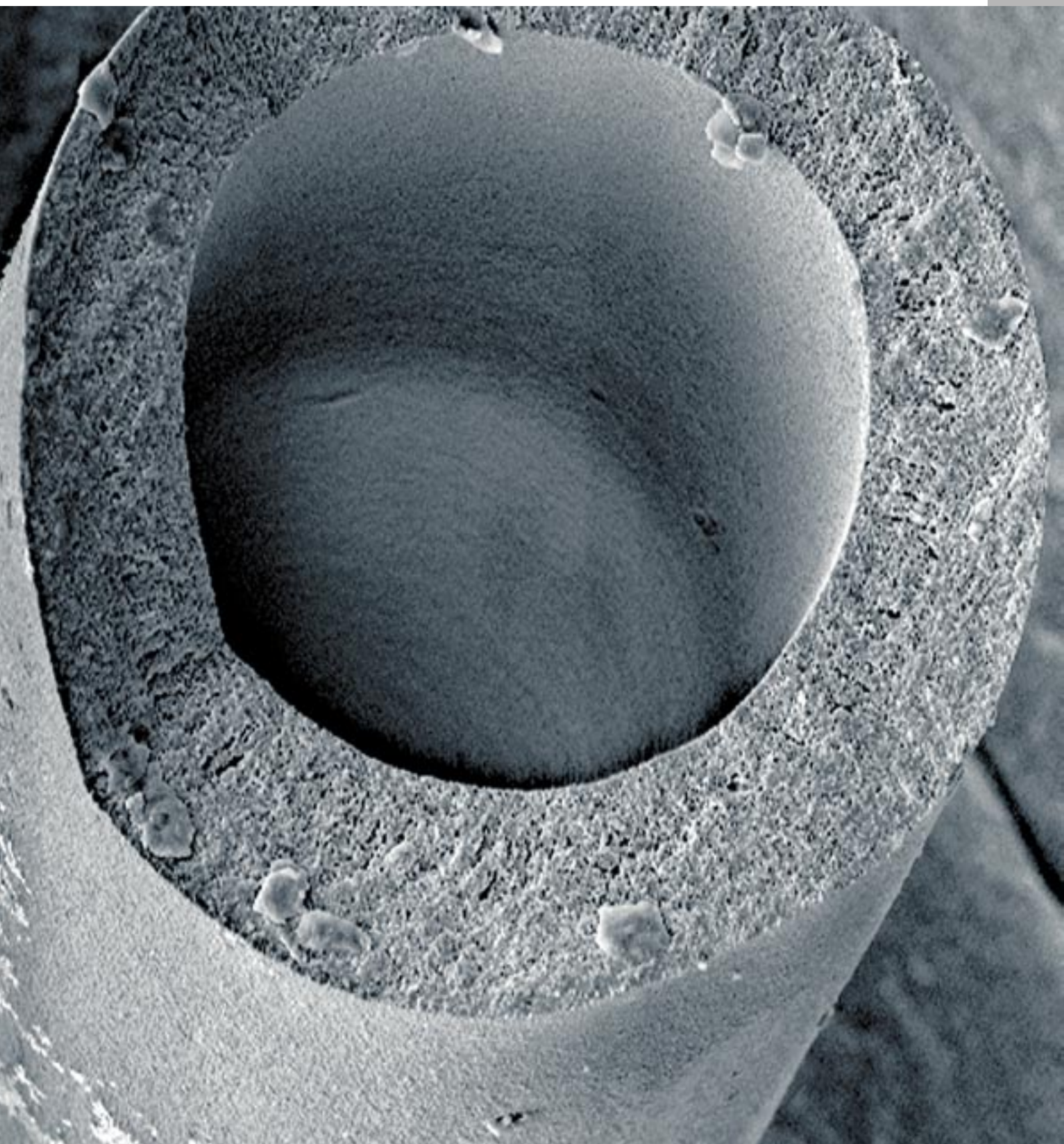
Figure 1: Manufacture of cartilage transplant in clean room.

Figure 2: Liquid culture incubator with fluorescense detecting system (Bactec 9120) for automatic sterility testing.

Figure 3: Qualified personnel in the GMP manufacture area.

Functional materials and membranes



In our business unit »Functional materials and membranes« we put our focus on three different fields:

- **Interface engineering of membranes, textiles, non-wovens and polymers**
- **Biomaterials and biomimetic interfaces (including nanobiotechnology)**
- **Development of inorganic and polymeric membranes and design of novel membrane modules for (bio-)technical separation processes**

Tailor-made surfaces

Increasing demand on industrially applied materials is often related to their surfaces, that is, their interface in relation to other materials and media. Often, different apparently non-compatible properties of the materials and their surfaces are requested. The material should be for example distortable, but its surface hard and rigid. Or, a synthetic material should show only a few polar groups in its matrix – and thus, only absorb a small amount of water – but its surface should be wettable. Such a way to look at a problem requires a thorough characterization of the surface with surface-analytical methods and the know-how of different surface-modification and coating techniques. On this basis, the Fraunhofer IGB is finding specific solutions for individual problems. The analytical techniques are shown in detail in chapter Surface Analytics (page 112).

Advantages of plasma treatment

Plasmas are ionized gases and vapors, which contain a large amount of electronically excited particles as well as ions, electrons and radicals. These particles react with surfaces of various substrate materials in contact with plasmas, and depending on the process to break down (cleaning, etching), to functionalize, or coat these substrate materials.
Low-temperature plasmas are generated at low pressures. In contrast to the wet-chemical processes only a small amount of chemicals is needed in the plasma modification process and, thus, has economical and environmental advantages. Another advantage in plasma processing is that chemically inert materials can be modified, and this modification can be restricted to the proper surface. When using wet-chemical processes comparable results can only be achieved by using aggressive baths.

Surface preparation

At the Fraunhofer IGB gas-phase processes, as well as effective wet-chemical processes are applied (Table 1). With these techniques, surfaces can either be etched (cleaned), or chemical functions can be generated resulting for example in enhanced wettability. Even thin layers can be polymerized thereon (e.g. scratch resistance layers).

Sterilization with plasma

Not only organic materials can be removed and thus the surfaces cleaned, but also bacterial cells and spores can be inactivated with plasmas. Thus plasma procedures can be considered as an alternative to formaldehyde- or ethene oxide sterilization.

Functionalization of surfaces

The application possibilities of many workpieces can be enlarged considerably if satisfactory volume characteristics of a material can be combined with specific new surface characteristics. This applies in particular to products with a highly specific surface such as membranes, fleece, and textiles. We have succeeded for example in making the pores inside of commercially available polysulfone based microfiltration membranes hydrophilic. Materials can also be made hydrophobic if their water absorption ability is to be reduced. An example for application

Table 1: Characteristics of materials which can be modified through plasma processes (excerpt).

Properties	Affected quantity	Applications
Mechanic	Scratch resistance, roughness, stress friction	Wear resistance
Transport	Diffusion coefficient and solubility for gases, liquids and ions	Membranes, corrosion resistance diffusion barriers
Interfacial	Surface tension	Wetting
Chemical	Cross linking, specific functions	Solvent resistance, immobilization of cells and grafting of peptides
Electrical	Specific electrical resistance, dielectrical constant	Electrical board, dielectrical films, condensator films
Optical	Refractive index, absorption coefficient	Antireflective films, transparent films and optical filters
Biological	Biocompatibility	Implants

is the hydrophobic finish of textiles. To paint, glue or to use polymers in composite materials, chemical functions have to be generated on their surfaces. These, on the other hand, allow an interaction with the varnish, the glue, or another working material. Beside polyolefins, perfluorohydrocarbons (PTFE, FEP), and polysulfone also provide material surfaces where the surface energy is increased by plasma treatment.

Coating

Glow discharges lead to film deposition under certain conditions. Due to their high cross-linking, these coatings are chemically and mechanically very stable. Modification by a monolayer coating is sufficient enough for several technical interfaces (e. g. as adhesion mediators for gluing processes and as spacer molecules for enzyme immobilization). In other cases, however, the desired effect can be realized only by thin layers, e. g. by corrosion resistant or scratch resistant layers developed at the Fraunhofer IGB. In cooperation together with other Fraunhofer institutes a coating has been developed, which increases the adhesion of copper to polyimide drastically. This achievement is very important for flexible imprinted circuit boards.

Functional separation membranes

A similar challenge was the plasma-chemical deposition of permselective layers on membranes for the separation. In order to prepare those membranes, a carrier-structure consisting of ceramic, metallic or polymer filters is used respectively. In this case plasma-chemical methods offer the advantage compared to wet-chemical procedures, as the pores of the carrier do not get clogged and very homogeneous layer depositions can be achieved.

Engineering

The up-scaling from laboratory experiments to pilot plant and further on the engineering to industrial scale is one of the main challenges in applied research. Surface engineering demands considerable effort to be invested in developing a technical process. Our know-how and excellent equipment and operational possibilities are available for industrial challenges. For example, the Fraunhofer IGB has installed a multi-chamber plasma apparatus for various surface treatments on foils, textiles, fleeces, and membranes. In addition, Fraunhofer IGB is involved in several national and European-wide initiatives as a path primer for the development of the plasma technology.

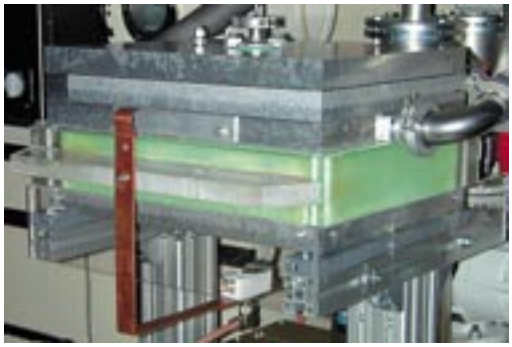


Figure 1: Plasma reactor for the treatment of materials in DIN A3 scale.

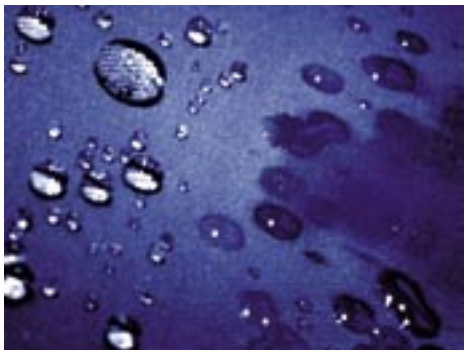


Figure 2: Hydrophobic finish of textile tissue (cotton/polyester).
Right: untreated
Left: after plasma treatment

Biomaterials and biomimetic interfaces

Interfaces can interact with biological systems in a highly complex way. For example blood coagulates in contact with certain materials, proteins are denatured on hydrophobic surfaces. Interactions between biological systems and boundary surfaces play an important role within many areas of industrial interest, in particular in medicine and medical technology, as well as for clean or sterile production conditions.

A main focus of research at the Fraunhofer IGB is using in-house material-oriented as well as biochemical basic research for the development of either bio-transforming (bioactive, biocompatible or bioinert) materials – here summarized as biomaterials – or biomimetic interfaces for the application in medical and biotechnologies. A further area of interest for such biofunctional interfaces is biosensorics.

Biocompatible surfaces

In interaction with biological systems a high quality demand for interfaces is required. Thus, the contact surfaces have to be characterized and prepared in respect to their geometric (roughness, specific surface, porosity) and their energetic properties (surface tension). We develop interfacial chemical properties by various analytical and preparative techniques in order to be able to offer materials according to desired quality standards, e.g. biocompatibility. The European Society for Biological Materials defines biocompatibility as »the ability of a biological material to show the suitable reaction in a specific application«. This means, the surfaces of the materials in contact with biological systems are prepared in such a way that the nonspecific protein adsorption, often called »fouling«, has to be minimized and desired biologically effector functions have to be coupled by conserving their activity.

Polymers are used as basic materials in the institute. They are not only characterized by small weight and moderate costs, but particularly in the plastic shaping they satisfy all the demands, and they are structurable down to the micrometer range. Finally also an argument for polymers is the simple disposal possibilities for polymeric one-way items used to avoid risks of infection (medicine) or cross-contamination (diagnostics). Thus procedures are developed at Fraunhofer IGB, which provide standard, technical as well as high-performance plastics with novel boundary surface characteristics. The latter are highly attractive for medical, diagnostic and pharmaceutical applications.

Biomimetic interfaces

In contrast to bioadaptive materials (biomaterials), with biomimetic interfaces functionalized materials are developed, which mimic the molecular recognition reactions of biological surfaces found in nature.

Self-assembly is the key to design and control material surface properties on a molecular level. The Fraunhofer IGB approach is: surface-active molecules or nanoparticles are designed and synthesized and then assembled (Self-Assembled Monolayers, SAM) and covalently bound to surfaces. The elements are then coupled as modules with technical materials such as polymers, or silicone wafers in order to provide these with the desired biological recognition reactions. Thus, the new functionalized materials combine the durability and longevity of synthetic systems with the high specificity of biological materials.

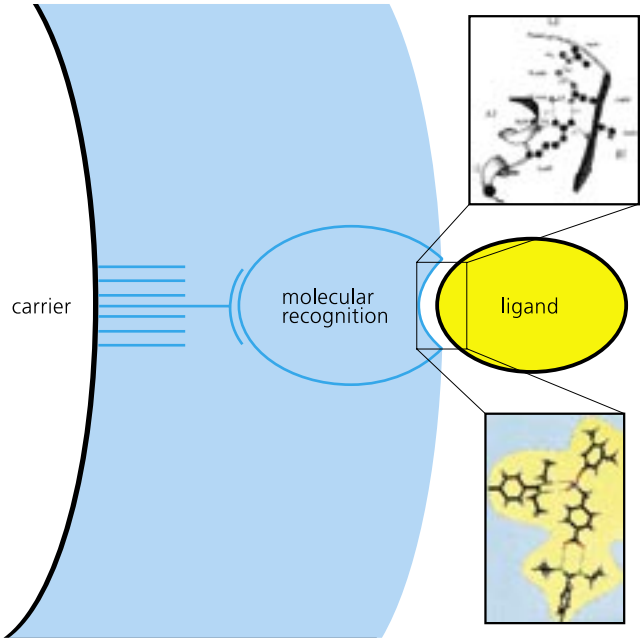


Figure 3: Surfaces of technical carrier structures are functionalized by directed immobilization of protein receptors or molecularly imprinted polymers for molecular recognition sites.

Nanobiotechnology

The described aspects of biocompatibility as well as the trend to advance into ever smaller dimensions and to utilize the acquired knowledge, lead to nanotechnology by progressive miniaturization and efficiency increase, and especially to nanobiotechnology. Hand in hand molecular biology and nanotechnology go together as trend-setting research areas ideally with the new interdisciplinary IGB business area nanobiotechnology. The institute – aware of the challenges – is already successfully positioned in this subject area and has acquired several funded projects.

Nanobiotechnology is concerned with the development, function and the production of biologically functional units on the nanometer scale. These are arranged in a further step to components, which can take over important tasks e.g. the diagnostics and therapy of diseases. By its special system properties, the intelligent interaction of such single components in complex systems again opens various application possibilities in biotechnology and biomedicine.

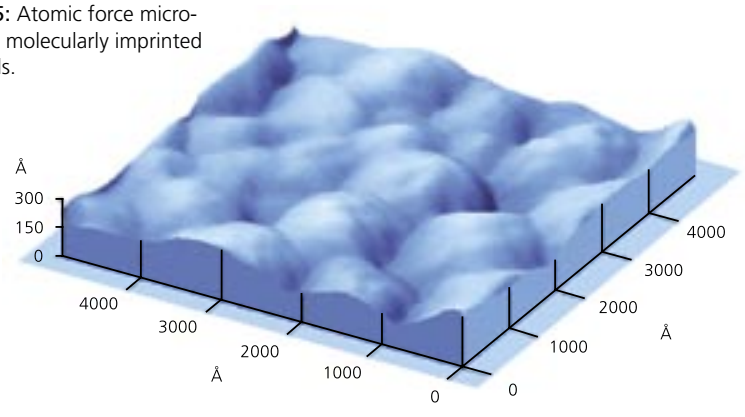
Interfaces win automatically an outstanding significance in nanobiotechnology: the smaller systems become available the more interfaces and surfaces per mass material are present. In addition the interaction of the nanoscaled units and thus the characteristics of the functional systems get dominated especially by boundary surface effects. The Fraunhofer IGB has, therefore, chosen to tailor interfaces for nanobiotechnological functional systems as a trend-setting goal.

Applications

- Development of interfaces with proteophobic effect
- Tests for plaque formation and plaque minimization in the dental area
- Interface coating for optimal cultivation of mammalian cells (e.g. with growth factors)
- Immobilization of antimicrobial substances
- Development of biosensor surfaces
- Development of biofunctional membranes for molecular separation processes
- Development of drug release and drug delivery systems
- Development of bioactive surfaces as components of new micro- and nanobiotechnological tools, e.g. for proteomics
- Alternative sterilization method for microbially contaminated interfaces such as paper and packaging materials
- Investigation of contamination by bacteria and spores in clean room facilities
- Sterile engineering: optimizing of cleansing and sterilization properties of manufacture equipment and equipment components in the food and pharmaceutical industry

Figure 4: Fluorescent nanoparticles in micrometer scale on a chip, observed with a fluorescence scanner.

Figure 5: Atomic force microscopy of molecularly imprinted microgels.



Technical membranes: tools for material separation

Today, the use of synthetic membranes to separate liquid or gaseous mixtures has obtained increasing technical and commercial significance. Membrane separation processes can be applied as a unit operation or integrated in many processes. They typically feature low investment and operating costs and provide an ecologically beneficial solution of a given separation task.

Due to their broad applicability in various fields, technical membranes have to fulfill very different requirements concerning their structure and transport qualities. At present, synthetic polymer membranes are dominant in most industrial applications. However, due to intensive development efforts also here at the Fraunhofer IGB, ceramic membranes prove to be a better alternative in many application fields. The applicability of membrane technology ranges from seawater desalination, waste water/sewage treatment, and numerous separation problems in chemical, pharmacological and food and beverage industries, up to medical applications, e. g. the artificial kidney. In order to apply a membrane process as effectively and efficiently as possible, the entire system, consisting of membrane, module plant and procedures has to be adapted to the given separation task.

Inorganic membranes

The Fraunhofer IGB can rely on over 25-year experience in the area of membrane technology. The focus of development has changed clearly compared to earlier years. With the conventional membrane procedures, like the filtration with polymeric membranes, it is not so much the search for new materials, but the improvement of process security the focus of attention by use of mechanically stable and well regenerable membranes. For these membrane procedures the inorganic membranes will play a prominent role in the future. Due to the possibility of their economical production and high charge density, the just developed ceramic hollow fiber and capillary membranes can be seen as a strong competition to the still prevailing polymeric membranes.

New impulses

There were hardly new developments with charged membranes for electrodialysis in the past years. This may change in the near future, since many developments of high interest have been accomplished for the fuel cell, which could be transferred to electrodialysis procedures.
Future developments are predominantly determined by necessary functionality. This begins with the introduction of certain surface properties, like hydrophility, hydrophobicity or bactericide surfaces over the coupling of molecular recognition sites. Thus for example the introduction of chiral selectors permits the separation from racemic mixtures. In addition, apart from the pure mate-

Technical membranes
Application for air, gases and vapors

- Production of inert gases like pure nitrogen
- Oxygen concentration
- Natural gas purification
- Separation of acid gases
- Upgrading of biogas concentration levels
- Sterile humidifying and de-humidifying
- Particle and bacteria filtration
- Recycling of solvent vapors

Figure 6: Different geometries of ceramic membranes: tubular membranes, cartridges, tubular and flat multichannel elements, capillaries.

rial separation also the development of membrane reactors for liquid media is possible by immobilization of biocatalysts and enzymes. The membrane surfaces can as well be prepared for the immobilization of living cells such as hepatocytes.

Outlook

At the Fraunhofer IGB the further development of membrane technology will focus on the development of mechanically stable and chemically resistant membranes with improved selectivity and permeability properties. Coherently, the development of modular systems and the construction of pilot plants for miscellaneous applications and separation tasks respectively will be intensified. In addition the development of inorganic membranes is to be regarded as top ranking.

Separation of fluid mixtures with non-porous membranes

Hydrophilic membranes	Organophilic membranes
Dehydration of organic fluids	*Separation of organic fluids*
– solvent upgrading	– separation of pollutants from waste water
– solvent recycling	– recycling of feed
– azetropic splitting	– azetropic splitting
Separation of effluents from the reaction	*Separation of end-products in chemical and biochemical processing reactions*
– equilibrium shifting	– continuous reactions
– increasing of yield	

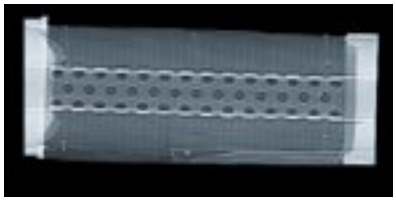


Figure 7a (left): The 60 m² membrane module cartridge with hollow fibers. Potting is demonstrated by X-ray computer tomogram.

Figure 7b (right): Membrane module with 60 m² membrane area.

Services

Interface engineering
- Surface analysis and characterization
- Surface preparation, such as cleaning, etching, grafting, coating, functionalization, and enhancing of the hydrophilicity or hydrophobicity
- Apparatus and process development: special tailoring of apparatus configuration to material forms, material properties and treatment variations

Biomaterials and biomimetic interfaces
- Characterization of biomaterials
- Biofunctionalization of interfaces

Membrane technology
- Development of organic and inorganic membranes for filtration processes, electrochemical membrane processes, membrane reactors, and contactors
- Development of membrane modules for technical, medical and biotechnical applications
- Development of membrane and hybrid separation processes
- Modeling and simulation of membrane processes
- Development, planning and construction of pilot and prototype plants, as well as plants for membrane manufacture and characterization

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Protein analysis by Fourier transform infrared spectroscopy

The protein lysozyme is well known for its antimicrobial effectiveness. Since a release of this active substance is not desired for many applications, immobilization of lysozyme on different material surfaces retaining its antimicrobial activity is a recent research objective. An important requirement is to maintain the activity i.e. native secondary and tertiary structure of the protein.

Sensitive detection of secondary structures

Analytical methods to determine the protein structure are e.g. X-ray diffraction analysis, which requires the preparation of crystalline material, and the NMR-spectroscopy, which is limited to the measurement of low mass proteins. The Fourier transform infrared (FTIR) spectroscopy provides no 3D structural information, but is very sensitive to the secondary structure, in particular the fraction of α-helix and β-sheet structures can be determined.
The absorption band at 1700 to 1600 wavenumbers (amide I-absorption) is associated to the carbonylic stretching vibration of the peptid bonding. These functional groups form inter- and intramolecular hydrogen bondings which create the typical secondary protein structures. Structural changes in the environment of these groups lead therefore to measurable shifts of the correspondend excitation energies, which leads to changes in the absorption bandshape.

Lysozyme in aqueous solution

The measurement of aqueous solutions in transmission is difficult, because water strongly absorbs in the relevant infrared spectral region. Therefore very thin liquid films with reproducible layer thicknesses must be used, since the determination of the absorption bands of the protein is done via difference formation of the protein solution spectra and the pure solvent spectra. For the transmission measurements a special flow through liquid cell with CaF_2 windows was used (liquid volume of approx. 5 microliters).
The shape of amide I-absorption bands as a function of the secondary structure for three proteins with well-known portions of α-helix and β-sheet structures is shown. The determination of the secondary structure of unknown proteins is performed by using a protein spectrum library with bandshapes of proteins with well-known secondary structures and analyzing on this base the unknown spectra by a multivariants procedure.

Table 1 shows literature values for α-helix and β-sheet fractions of the three proteins. The values in parentheses correspond to the own measurements of a similar lysozyme in aqueous solution, which were determined by the procedure described above. Taking the method error into account a good agreement with literature values is obtained.

protein	α-helix [%]	β-sheet [%]
lysozyme (chicken egg white)	34.1 (35.3)	6.2 (3.4)
albumin (bovine)	67.6	0
ubiquitin	15.8	30.3

Table 1: Secondary structure of proteins, literature values and own results (in parentheses).

Immobilized lysozyme

For first immobilization experiments the lysozyme was allowed to adsorb to silica gel from an aqueous solution. The arising changes in secondary structure of the protein were infrared spectroscopically pursued. Suitable measuring techniques for surface-modified powder are the ATR (attenuated total reflection) and the DRIFT (diffuse reflexion) technique.
Determination of the α-helix and β-sheet fractions in immobilized proteins by bandshape analysis of the measured infrared spectra similarly to the procedure for solution spectra is not possible, since the immobilization leads to an additional broadening of the absorption bands. A comparison of the bandshapes of dissolved and immobilized protein spectra can however give qualitative informations about changes in secondary protein structures. In the present example the immobilized lysozyme shows a clear broadening/shift of the absorption band toward lower wavenumbers. This can be assigned to a reduction of α-helix structures and also to an increase of β-sheet and disordered structures.

Quick and precise determination of secondary structures

The FTIR spectroscopy is valuable for the determination of secondary structures of proteins. Special advantages of the IR spectroscopy compared to other analytical methods are, apart from easy sample preparation, a fast execution and analysis of the measurements. It is therefore an effective tool for quality control or sample screening.

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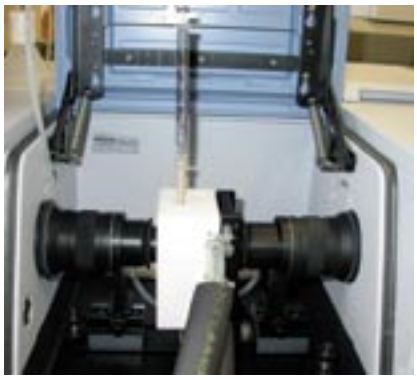

Figure 1: Temperature controlled cell for aqueous protein solutions.

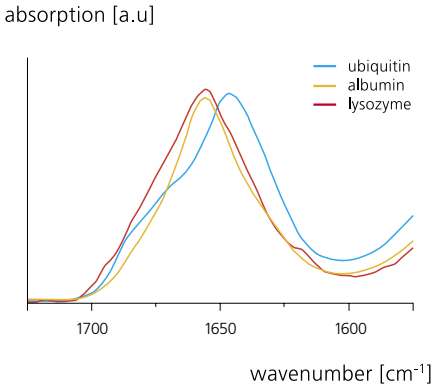

Figure 2: Amide I bands of different proteins in aqueous solutions.

Figure 3: Diamant ATR unit.

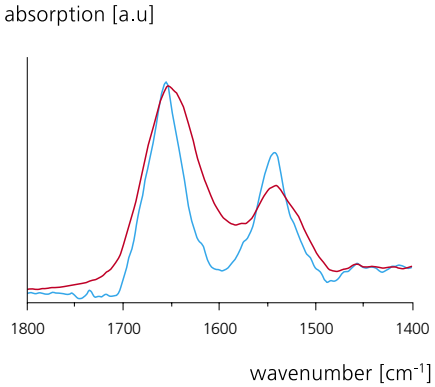

Figure 4: Amide I and II absorptions of dissolved (blue) and immobilized (red) lysozyme.

Plasmachemical microstructuring of polyolefines with carboxylic groups

Initial situation

Polymeric substrate materials like polystyrene (PS), polycarbonate (PC) or cyclic olefins (COC) are getting more attention besides silica and glass for the preparation of reaction vessels, optical slides, microfluidic components or microtiterplates in applications like medical diagnostics and pharmaceutical drug screening. Actually the market of transparent polymeric chips is demanding the availability of modified surfaces with a well defined array of wettable areas or special chemical functionalities. These modified areas are starting points to graft bioactive molecules, for instance DNA-oligomers and proteins or polar nonionic oligomers to prevent the unspecific adsorption of proteins. The rather expensive biomolecules are applied for testing of many different classes of substances. Therefore the requirement of analyte volume miniaturization and parallel screening techniques are driving forces for chemical structuring of polymeric chip surfaces in the micrometer range.

Object slides consisting of glass or silica can be modified with well established wet chemical methods. Polymeric materials are relatively inert, not solvent stable and can be modified only with highly aggressive solvents.

New technology: Dry chemical structuring

Fraunhofer IGB has developed a new technique for dry chemical structuring of polymeric surfaces on the principle of plasma glow discharge surface modification in combination with a suitable structured mask. This technique is characterized by a low energy consumption and a negligible amount of chemicals. The procedure consists of only a few processing steps, in contrast to conventional lithographic structuring methods. Also wetting problems do not play a role as they do in printing techniques. This technique provides the possibility of tailoring the chemical and topographical surface properties from ultrahydrophobic to hydrophilic or to functionalize areas of choice in the µm range with a chemical group of defined density.

Optimization of plasma parameters

The basic process parameters for structured plasma surface modification are investigated using a parallel plate reactor including an appropriate structured mask (Figure 1). As a precursor acrylic acid is introduced into the plasma. The goal of a stable functional layer in the nanometer scale with a maximal density of carboxylic groups is accomplished by variation of plasma intensity and time, precursor partial pressure and flow.

Results

Chemical microstructures have been realized on a variety of polymeric materials (Table 1). As an example, the built-up of an array of carboxylic groups on a polypropylene foil is presented (Figures 1-3). This array of monofunctionality is the starting point for the preparation of parallel microreactors with a volume of only some picoliters each. The functional density, determined with a suitable fluorescent probe is > 6 carboxylic groups/nm^2.

Manufacturing of initial batches

These samples of topographical and chemical microstructures are the first step for biochemical preparations in medical diagnostic kits, DNA-, protein- or cell biochips. The upscaling of the plasma modification process in a multi-chamber reactor offers the semicontinuous functionalization of polymers in pilot scale or in batch processing. On this basis the surface modification step can be adapted into a mass production line of »Lab on a Chip« systems.

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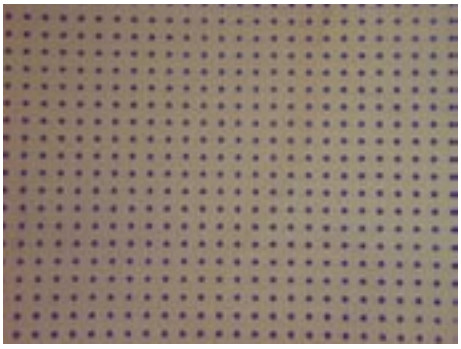

Figure 1: Array of areas, plasma-modified with carboxylic groups (Ø 0.7 mm) and stained with thionine acetate.

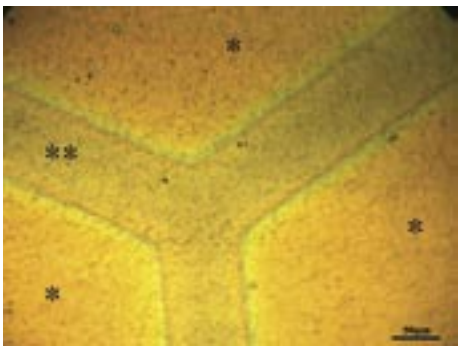

Figure 2: Light microscopy of a polypropylene foil, plasma-modified with a functional layer of carboxylic groups in the open areas (*). Non-modified areas are covered by the catwork of the structured mask (**), scale 50 µm.

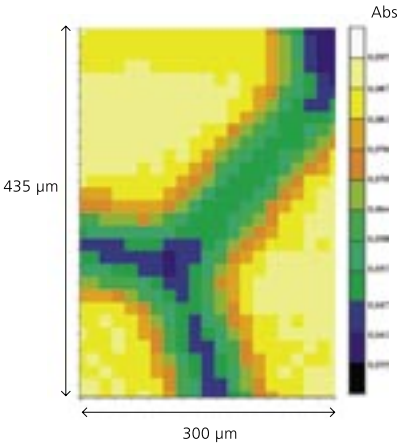

Figure 3: Local IR-absorption distribution in the 1720 cm^{-1} region, yellow areas represent presence of carboxylic groups, due to open areas of the mask used during plasma functionalization, dark areas where covered by the catwork of the structured mask.

	Functionality			
Polymer type	nitril-	amino-	carboxyl-	microstructured
Polyethylene			+	
Polypropylene	+	+	+	+
Polycarbonate		+		+
Polystyrene		+	+	+
Fluorpolymer	+	+	+	+
Silicone		+		+
Polyimide	+			

Table 1: Spectrum of plasma-technically functionalized surfaces developed at Fraunhofer IGB.

The bioactivity of cell-membrane proteins

The investigation of the bioactivity of cell-membrane proteins is the key for understanding and controlling cell-cell interaction. At present, two methods are used: The experiment is performed with the soluble form of the protein, which is often an oversimplification of the real biological situation, or recombinant cells are used carrying the target protein with a high density on their surfaces. Such cells are then brought together with the cell lines of interest. These cell-cell assays, however, are hardly clear due to the complexity of possible interactions.

Cell analogous hybrid systems – NANOCYTES®

In a project supported by the BMBF the Fraunhofer IGB, in cooperation with the Institute for Cell Biology and Immunology of the University of Stuttgart, develops particle carrier systems for membrane proteins. The objective is the immobilization of the proteins with conservation of their *in vivo* bioactivity and thus to simulate cell-cell interaction (Figure 1).
Such cell analogous hybrid systems – called NANOCYTES® – proved to be valuable tools for cell biology and immunology. Especially, the presentation of cytokines of the tumor necrosis factor family (TNF) linked to particle surfaces is in the focus of our research, because they offer a high potential in diagnostic and therapeutic applications, especially for cancer research and treatment.

Synthesis and characterization of NANOCYTES®

Silica particles with various diameters ranging from 100 nm to 1 µm were synthesized by a base catalyzed hydrolysis and condensation of silanes (Figure 2). To obtain particles with fluorescent activity, condensation was carried out in the presence of labeled silanes. Then, e. g. amino groups were covalently coupled to the particle surface by reaction with functional organosilanes. These surface groups are reacted with a heterobifunctional reagent (e. g. sulfo-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate). Thereby a surface is obtained, which selectively and covalently binds only proteins with free thiol groups at their surface domains. By this strategy recombinant TNF-α with free cystein groups at the N-terminus was immobilized on the particle surface and the particles were then characterized by physico-chemical and biological methods.

Bioactivity of NANOCYTES®

The most important feature of the NANOCYTES®, their bioactivity, was investigated in cell assays by fluorescence microscopy (Figure 3). Cells were used for this assay carrying the specific receptor for membrane bound TNF. Soluble TNF variants do not show any response with these cells. Intracellular proteins, which bind to the TNF receptor are labeled with green fluorescent protein (GFP) by recombinant methods. Prior to the experiment TNF modified particles are labeled with a red fluorescence marker. Under observation by confocal microscopy TNF-functionalized particles were transferred into the cell culture. After 15 minutes incubation time the TNF-NANOCYTES® specifically bind to the cells (Figure 3A). A signal transduction is triggered, which leads to apoptosis

within 40 minutes (Figure 3C). This experiment shows the proof-of-principle: The biohybrid particles exhibit a bioactivity, which comparably was only observed with cell membrane bound TNF so far.

Applications

In combination with additional cell-specific targeting functions various applications can be realized *in vivo*, like the purging of tumor cells with NANOCYTES®. Furthermore, functional NANOCYTES® are interesting tools for basic and clinical research. In contract or cooperation we can provide tailor-made NANOCYTES® with individual functionalizations.

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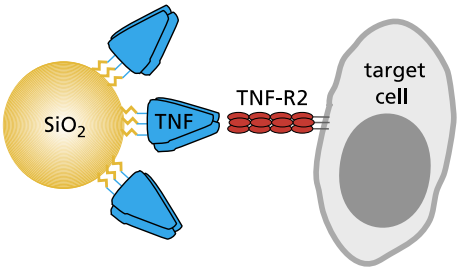


Figure 1: Model of the bioactivity of NANOCYTES®: Nanoparticles are modified with a bioactive cytokine (TNF) and by binding the TNF-receptor of the target cell a signal transduction is started.

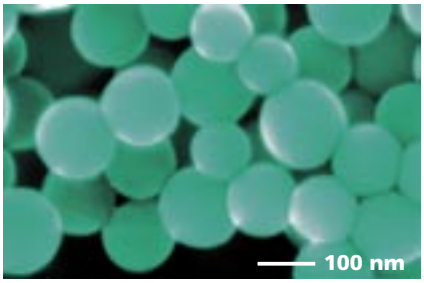
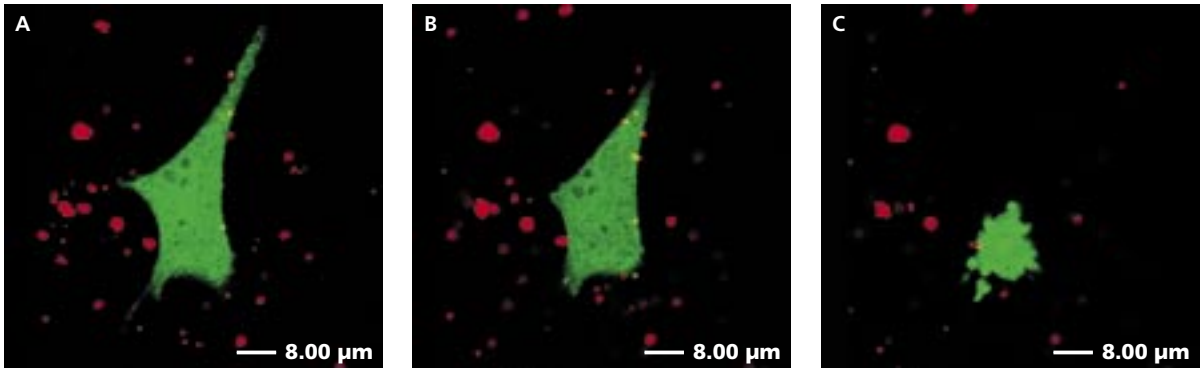


Figure 2: The size of the particles was investigated by scanning electron microscopy and the diameter was determined to 100 nm.

Figure 3: Bioactivity of NANOCYTES®: The binding of TNF-functionalized particles (red) to the target cell is investigated by confocal microscopy (yellow as overlay). Proteins (green) are recruited and signal transduction is started, which finally leads to the intended apoptosis (controlled cell death).
A: after 15 min
B: after 30 min
C: after 40 min



The advantage of biomimetic nanoparticles

Molecularly imprinted nanoparticles open a completely new way for the development of synthetic affinity receptors for the use in biotechnological applications. The great gain compared to biological molecules is the higher stability of the synthetic binding matrices. As the nanoparticles are very finely distributed in a solution, they can be used directly from solution in dispersion or as ultrathin layers for further affinity reactions.
The nanoparticles open also an attractive way for the flexible preparation as functional surface coatings, as they can easily be deposited to form a dense layer. Molecular imprinting is a technology that generates specific binding sites in a polymer network via template polymerization. The binding sites are then used for molecular recognition processes analogous to the antibody-antigen reaction, but with a fully synthetic system. A problem for the use of the imprinted polymers as biosensors in the past was their external shape. So far, particles were prepared by synthesizing a polymer network monolith, followed by grinding and sieving. Irregularly shaped microparticles can thus be obtained. Nevertheless, the known synthetic strategies lead to polymers with high selectivities, mainly usable for chromatographic applications. Our aim is to open the attractive principle of molecular imprinting for a wider field of applications.

Innovative production process

The spherical particles possess clearly favorable properties for the use as synthetic receptors. In the group of Biomimetic Interfaces at the Fraunhofer IGB, we apply miniemulsion polymerization for the synthesis of molecularly imprinted particles in a nanometer range. This method allows a thermodynamic control of the preparation conditions and is thus optimally suitable for the preparation of synthetic receptor constructs. The particles are obtained in a one-step reaction and are easily purified by ultrafiltration.

Molecular imprinting and application

The synthesis of highly crosslinked polymer networks, e. g. poly(methacrylacid)-co-(ethylen-glycol-dimethacrylat) in the presence of different amino acid derivatives as molecular templates leads to well defined nanoscaled spherical particles. The stable colloids are synthesized without coagulation in quantitative yields (98 ± 2 per cent). Then our broad analytical spectrum is ready for the characterization of the nanoparticles (surface tension measurements, gravimetrical measurements, dynamic light scattering, transmission electron microscopy, scanning electron microscopy, ^{1}H- and ^{13}C-CP-MAS-NMR as well as gas adsorption experiments by the method of Brunauer-Emmet-Teller).
The range of the particle sizes of the synthesized colloids is from 50 and 300 nm (Figure 1). Thus the nanoscaled imprinted particles are excellent products for affinity reactions as they provide an immense specific surface easily accessible for the ligand molecules.
The recognition and binding properties are the most important feature of our new material. ^{1}H-NMR, UV-measurements and especially microcalorimetry proofed the specific interaction between the synthetic receptor and a variety of ligands (Figure 2). Our new and proprietary technology opens the way to elaborate completely new applications of biomimetic receptors in the field of bioanalytics, diagnostics as well as in purification processes.

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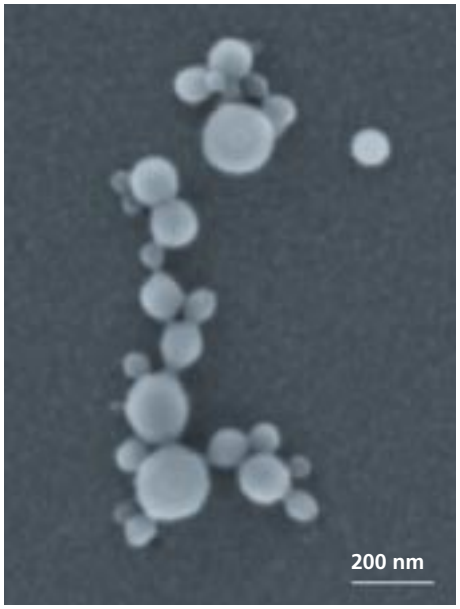

Figure 1: Scanning electron microscopy of molecularly imprinted nanospheres.

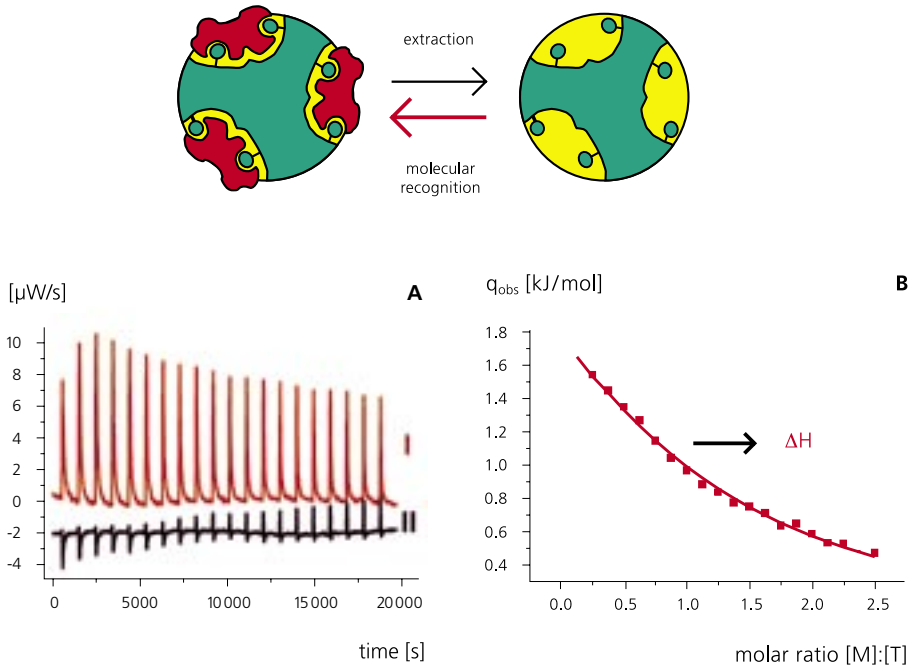

Figure 2: Direct monitoring of the molecular recognition of a chiral template molecule with molecularly imprinted nanospheres using micro-calorimetry.

New composite membranes for highly specific separations in life sciences

Product purification in downstream processing

Specific separation of a single compound from a mixture is a key issue in downstream processing as well as in chemical sensing. Separation of racemic mixtures is for example of increasing importance in the pharmaceutical industry. Often the two enantiomers of a chiral substance possess completely contrary properties. But also the purification of products (amino acids) from biosynthesis processes requires the highest purity as possible. Depending on the application and scale, today a variety of complex separation techniques exist, which are associated with high financial and manned effort. Our approach described below is an efficient and easy alternative compared to the established methods for separation of small quantities of specific substances, like preparative chromatography or common solid phase extraction.

The composite membrane: a highly selective sandwich structure

The focus of our studies concentrates on a new composite membrane which consists of two commercial polymer membrane disks with a thin selective layer of molecularly imprinted nanospheres in between (Figure 1). The nanoparticles have a diameter of about 100 nm to 220 nm. In the outer shell of the spherical nanoparticles molecular imprints are accessible which bind the target molecule from the mixture to be treated according to the principle of molecular recognition while the mixture is running through the composite membrane. Important advantages of our modular approach in relation to processes producing directly molecularly imprinted or functionalized membranes are the simple fabrication of the composite membrane and the very high density of imprints. Within the scope of our work at first the basic feasibility of this approach was evaluated. We concentrated on the theoretical and experimental investigation of the hydrodynamic aspects within the selective nanoparticle layer and their influencing parameters. Furthermore, first experiments have successfully shown the separation quality of this new composite membrane.

Manufacture und characterization

The nanospheres were synthesized by miniemulsion polymerization. This polymerization corresponds to a suspension polymerization using strong shear forces to transform the oil droplets to nanodroplets in the size range of the later nanoparticles. The nanodroplets serve as nanoreactors, thereby a nearly 1:1 copy of nanodroplets to solid nanospheres is realized. The imprinting effect was made by adding a chiral amino acid derivative to the reaction mixture as a molecular template. After the polymerization the template is removed by extraction.

Polyamide membranes with a diameter of 44 mm and a pore size of 0.1 µm were used as support and cover of the composite membrane. For the coating of the support membrane the so-called dead-end cake filtration technique is used. A 50 ml suspension of 0.05 g extracted nanospheres in ultrapure water was filled in an ultrafiltration cell with the installed support membrane. The suspension was pressed through the support membrane by nitrogen at a constant pressure of 0.14 bar thereby depositing the particles onto the surface of the membrane. After drying the coated membrane, the nanoparticle layer was covered by a second membrane and clotted at the borders with conventional glue. The pressure loss, the temperature, and the mass flow through the membrane were measured online during the coating procedure (Figure 2).

New technology

The results of our present studies indicate the feasibility of our approach. In relation to common separation processes this new composite membrane works as a combination of solid phase extraction and an affinity membrane process. Molecularly imprinted polymers available today can be used as stationary phase in HPLC columns. Our nanoparticle approach allows realizing similar separation performance and binding capacities under normal lab conditions without the technical and material complexity of HPLC equipment. If higher flow rates or a higher throughput are necessary, this can be achieved by numbering-up, i. e. by the parallel use of more than one composite membrane.

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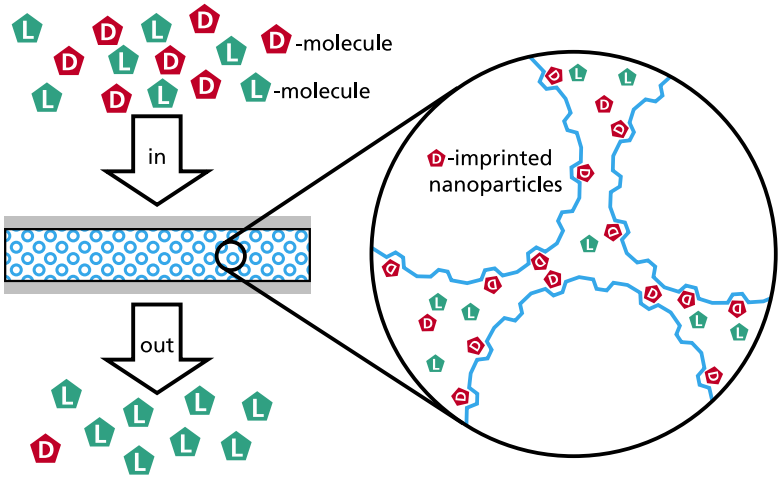
Figure 1: Functional principle and set-up of the composite membrane.



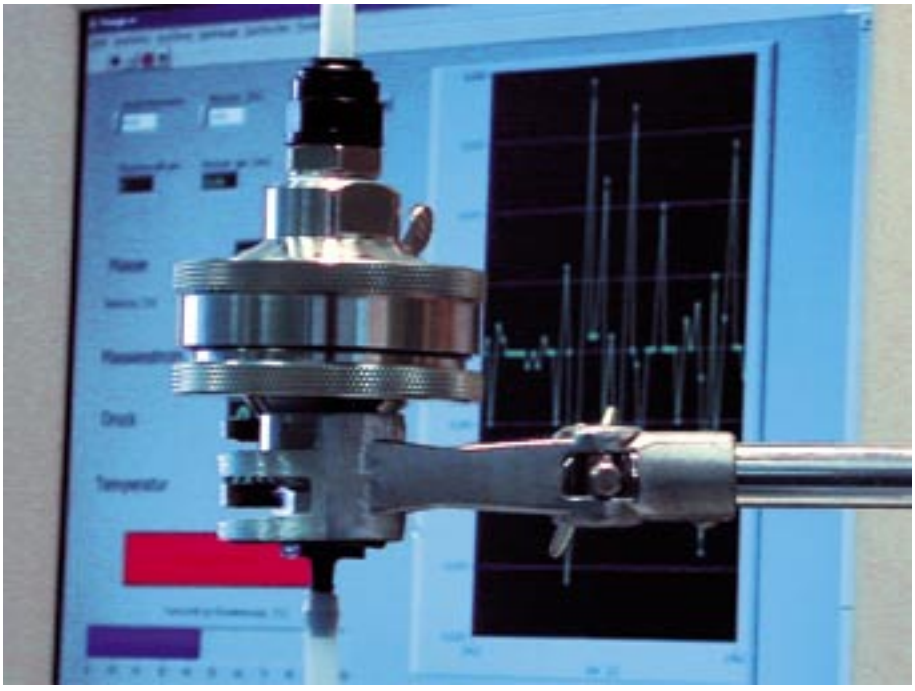


Figure 2: Experimental set-up for the characterization of the composite membrane and the measurement of its hydrodynamic properties.

Development of »bucky paper« for the use as artificial muscles

Nanotubes with outstanding properties

Soon after the discovery of carbon nanotubes by Sumio Iijima (1991), it became clear that they have unusual characteristics. Their high current carrying capacity, estimated 1 billion ampere per square centimeter, makes them interesting for electrical applications. The high field emission at low activation voltages (1-3 V) enables a new generation of flat screens. The tensile strength is 13 times greater compared to Kevlar and 21 times compared to the best steel – with much less weight. The thermal conductivity is twice as high as in pure diamond and the thermal stability is approximately 750 °C in air and up to 2800 °C in vacuum and therefore higher than in all commonly known materials. A further property, the mechanical expansion under applied low voltage (1-5 V), predestinated these materials for the use as artificial muscles.

Meanwhile, it is known that carbon nanotubes exist in single walled (SWNT) as well as in multi walled (MWNT) geometries, which influence their mechanical properties. Their length differs from micrometers up to centimeters and accordingly they are electrically conducting or semi-conducting. The manufacture process (arc discharge, CVD or laser ablation) as well as the purification are determining factors for the obtained quality of the material.

Objective and approach

Light, low voltage electromechanical actors with high expansion and high forces are needed in almost all sectors of technology especially in medical technology. Within the framework of an internal research project, Fraunhofer IGB and Fraunhofer TEG work together on the development of actors based on carbon nanotube sheets, called »bucky paper«.

To develop an actuator on the basis of bucky paper fulfilling industrial requirements, the manufacture and the quality of bucky paper must be optimized (IGB). In addition, the material must be tested as well as adapted to a prototype actor (TEG). The optimization of the bucky paper is done in close cooperation with the Max-Planck Institute (MPI) in Stuttgart.

Manufacturing, characterization and optimization of bucky paper

An experimental set-up was realized which allows the measurement of the electromechanical properties of the produced bucky papers. In collaboration with the MPI, bucky papers made out of different starting materials could be manufactured and analytically characterized. Figure 1 shows SEM-images of bucky paper manufactured from different starting materials. Whereas the material A shows a lot of particles (amorphous carbon), the material B shows almost solely carbon nanotubes.

With ESCA as well as EDX-analysis, the chemical composition could be investigated and the concomitance of elements from the catalyst used during the production could be verified. These methods are necessary for the optimization of the purification steps.

The analysis of the adsorption-/desorption isotherms allows the calculation of the BET-surface as well as the pore-size-distribution. Both are necessary to understand the alteration of the material during the purification processes. Figure 2 shows the pore-size-distribution of the raw material as well as with an oxidative treated material.

The plots indicate that the oxidative treatment opens the ends of the carbon nanotubes and therefore also the inner surface (pore size < 10 Å) can be accessed.

To optimize the actuation properties of the bucky paper also the thickness, the alignment of the carbon nanotubes, the used electrolyte as well as the assembly (e. g. multi layer system) were varied. Thereby, the properties like response time, maximum displacement and force as well as the switching frequency could be improved compared to the earliest materials under investigation.

Outlook

Due to the postulated properties, there is an enormous interest in an industrial actor made out of carbon nanotubes. But within the experiments it could be shown that also basic research experiments have to be conducted for a better understanding of the mechanisms of the electromechanical actuation and consequently for the optimization of the bucky paper. Nevertheless, the results obtained so far indicate that first prototypes will be available very soon and that the long time stability and the optimization to special applications can be completed.

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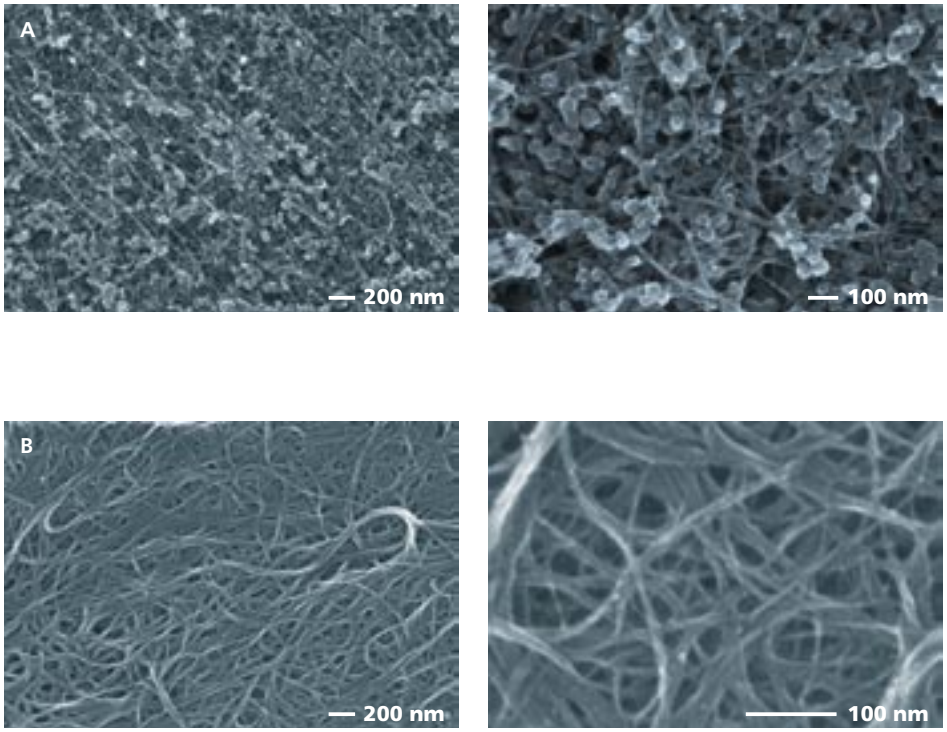

Figure 1: Scanning electron microscopy (SEM) images of bucky paper (instrument: LEO 1530 VP).
A: starting material type I
B: starting material type II

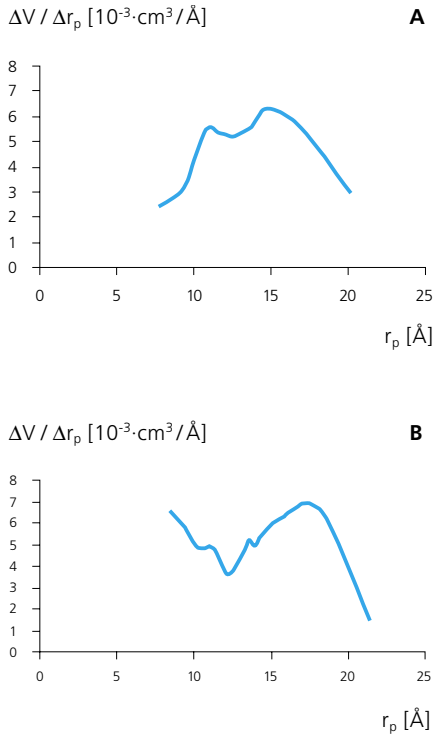

Figure 2: Pore-size-distribution calculated by means of adsorption-/ desorption isotherms.
A: raw material
B: material treated with an oxidative

Soldering

Microelectronic devices are generally mounted on boards by soldering. The soldered joints have to meet increasing requirements due to the demand for ecologically friendly lead-free packaging – from 1st January 2006 on lead must be excluded from electronic devices – or enhanced thermal loads (e. g. in automotive industry). For the reliability of an electronic assembly, most of all the shear strength of the solder connection is crucial. Hence, reliable soldered joints are merely obtained when the surface can be wetted by the solder.

Conducting boards made of copper, which are predominantly used, exhibit oxidic layers of several nanometers beside adsorption layers on the surface impeding the wetting of solders (Figure 1A). Therefore, the surface is generally pretreated prior to soldering. The most used procedure to prepare the boards for soldering is »hot air solder leveling« (HASL). The conducting boards are coated with an eutectic Sn-Pb layer to provide a solderable surface finish. Lead-free alternatives are coatings of chemical nickel/gold, chemical tin or organic passivation layers on Cu surfaces. Furthermore, fluxes are needed during soldering in order to remove residual oxidic layers from the board and prevent the oxidation of the (lead-free) solder.

Cleaning and passivation in one step

Recently, investigations at the Fraunhofer IGB showed that the cleaning of metal surfaces and the removal of oxidized layers on metals like copper, nickel, and iron are effective using plasma technique with gases enabling reduction and ablation [1]. However, the re-oxidation of copper with a 1 nm thick closed oxidic surface layer occurs within a microsecond. Hence, cleaning from oxides is only effective if the Cu surfaces might be passivated immediately.

Therefore, a low-pressure plasma process was developed at the Fraunhofer IGB within a Fraunhofer SEF project and applied for a patent, which enables the direct passivation of plasma-cleaned Cu surfaces by coating with an ultra-thin amorphous hydrocarbon (a-C:H) layer [2]. This passivation layer was proved to act like an effective barrier against oxygen permeation, thus delaying the formation of the oxidic surface layer on copper, which inhibits wetting of solders, for about 10 days (Figure 2). Within this period, the surface finish enables soldering at temperatures above 150 °C due to the thermal decomposition of the a-C:H layer. Thus, the solder is able to wet the copper surface and form a reliable solder connection by alloying with Cu (Figure 1B). If the soldering is carried out at widely oxygen-free excess pressure conditions (like for example in reflow soldering), even the use of fluxes can be omitted by soldering with lead-free bumps, which further reduces environmental load.

Thus, a one-step-procedure for cleaning and passivation of solderable copper surfaces is available omitting the use of wet-chemical processes and fluxes and enabling the application of lead-free solders.

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2 SEF »Bleifreies Packaging«, Teilprojekt II »Kontaktmetallurgie und Benetzungs-dynamik«, IZM/IGB-Abschlussbericht, 2002.

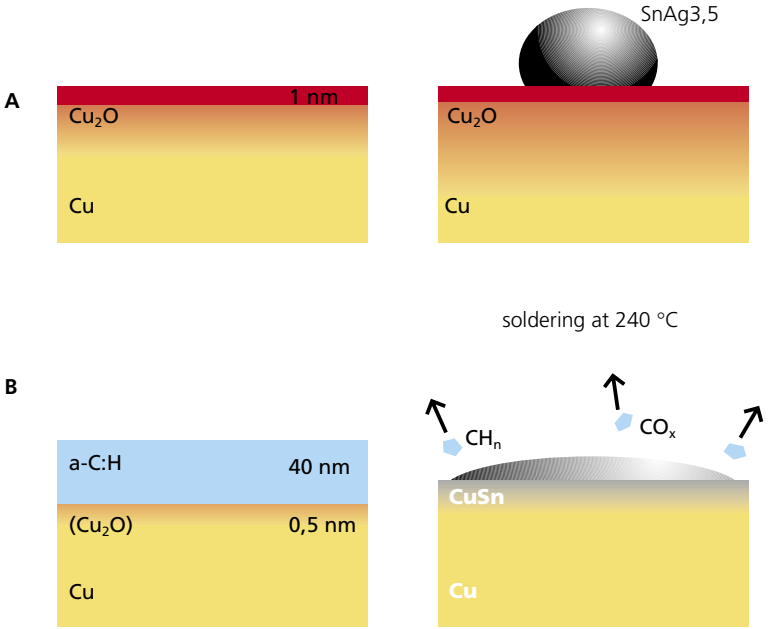

Figure 1: Soldering on a copper surface.
A: The oxidic surface layer impedes wetting of the solder.
B: The a-C:H passivation layer inhibits the oxidation of the Cu surface and enables wetting of the solder.

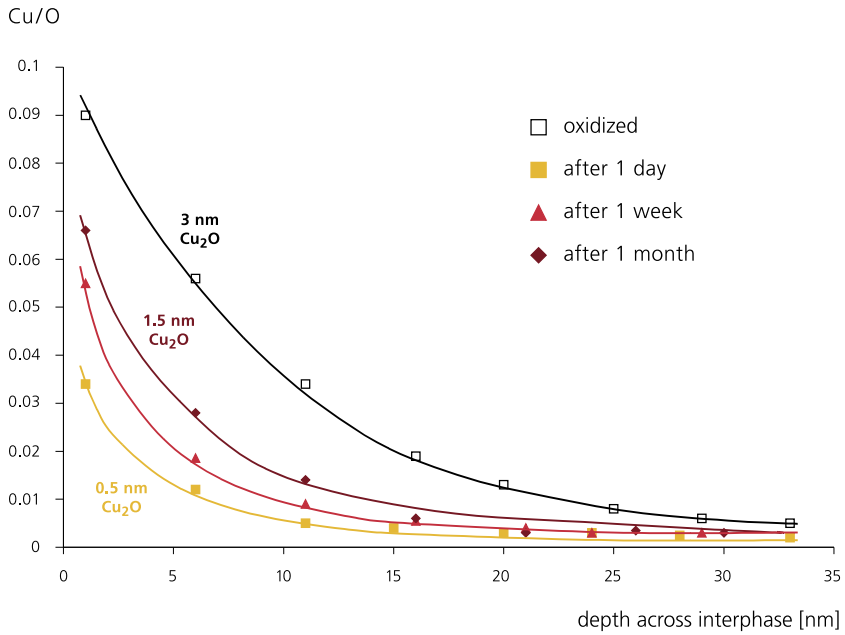

Figure 2: Analysis of the a-C:H/Cu interface by Auger electron spectroscopy. After plasma-assisted cleaning and passivation, only a low oxygen content in the interphase is detected, which increases with storage at air slowly. Without plasma cleaning a distinct O/Cu ratio is present.

Ceramic capillaries, metal membranes and their applications

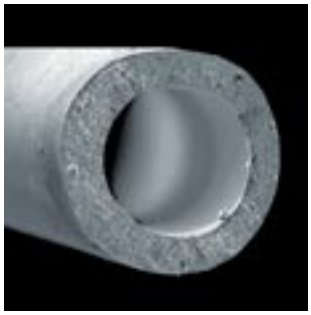

Figure 1: Ceramic capillary, Lyocell spinning process.

Figure 2: Ceramic capillary, polymeric spinning process.

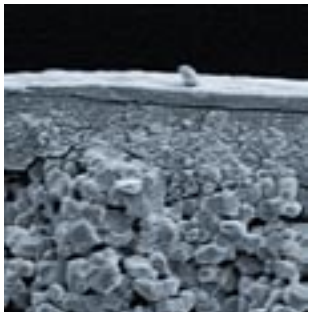

Figure 3: α-Al_2O_3 capillary coated with dense Pd layer (layer thickness ~ 0.6 µm) on γ-Al_2O_3 layer (layer thickness ~ 3 µm).

Commercially available ceramic membranes

In general, ceramic materials are chemically, thermally and mechanically stable. Thus ceramic membranes find frequent use in liquid filtration in food, chemical and pharmaceutical industries, as well as in the bioengineering processes. Different geometries of ceramic membranes have been produced worldwide, e.g. plate, disk, tube, multichannel and honeycomb. However, the commercially available ceramic membranes are relatively heavy and their fabrication is rather expensive, with membrane areas rarely higher than 1,000 m^2/m^3. Ceramic capillaries produced at the Fraunhofer IGB allow these advantages in combination with high packing densities, both compact and light-weighted.

Cost-efficient production process

Fraunhofer IGB develops a cost-efficient process for the continuous production of ceramic capillaries via a phase inversion process. Ceramic powders are dispersed within organic binder systems. The resulting slurries are extruded through annular nozzles into aqueous baths. In this way, capillaries can be obtained from oxides, nitrides, carbides or even metals. The membrane characteristics can be tuned through the slurry composition, the spinning process parameters and the sintering procedures.

α-Al_2O_3 capillary membranes

Typical α-Al_2O_3 capillaries have an outer diameter ranging between 0.5-2.0 mm, while the wall thickness is between 0.05-0.2 mm. So far, capillaries with a pore size of 0.2-1.0 mm have been produced with a porosity of 25-70 per cent. Another characteristic of these membranes is a well-defined pore size distribution. The bending strength of the capillaries reaches values up to 250 MPa indicating an excellent mechanical stability. Altogether the characteristics render these porous capillaries perfect basic materials for asymmetric membranes, which can be obtained by depositing further selective layers. Figure 1 and Figure 2 show SEM images for typical capillaries with different characteristics.

Modules with α-Al_2O_3-capillaries can be used directly in the filtration of solutions, emulsions or other heterogeneous fluids.

Metal membranes

Fraunhofer IGB is also engaged in the development of capillaries for ultrafiltration or nanofiltration, and gas separation. For this purpose, additional selective layers can be deposited on α-Al_2O_3-capillaries.

An efficient process has been developed to deposit thin dense metal membranes on α-Al_2O_3-capillaries for hydrogen separation and purification. Figure 3 shows a capillary coated with a Pd layer by electroless plating technique. The hydrogen separation properties of these capillaries are tested with H_2 and N_2 at high temperatures. For instance, at 430 °C, hydrogen permeance stays over 10 $m^3/m^2 \cdot h \cdot bar$ and the ideal separation factor $\alpha(H_2/N_2)$ over 1,000 (Figure 4) during running for over 800 hours. Such hydrogen separation properties are adequate for gas separation applications in petrochemistry, fuel cell technology or for membrane catalysis in the chemical processes.

Hence the concept of very thin metal membranes on porous ceramic capillaries is not only economically sound but also technologically feasible. Currently, both the material and the process technology are being developed at Fraunhofer IGB.

Chemically and thermally durable modules involving the search for suitable potting materials and the engineering of appropriate potting techniques and module designs is one focus. Modules with 100 cm^2 and higher separation areas have been built up with gas-tight ceramic potting. Figure 5 displays a sample module, coated with Pd on the outer surface of ceramic capillaries.

Applications and perspectives

Ceramic capillary modules provide an optimum solution with respect to size, weight and contact surface, in ultrafiltration and nanofiltration, in gas separation and in chemical reaction technology.

The automotive industry is expected to be a broad field of application for compact capillary membrane modules. The efficient removal of NO_x from the air ventilated into the passenger compartment is still unresolved. Likewise, powerful catalytic burners are lacking for the reduction of soot emissions in the exhaust gas of diesel engines.

The *in situ* reforming of fossil fuels or bio-alcohol and the subsequent conversion of CO into H_2 via water-gas-shift reaction are key processes in the automotive fuel cell technology. In the future, Fraunhofer IGB will focus on the development and engineering of membrane modules for specific processes and of membrane processes.

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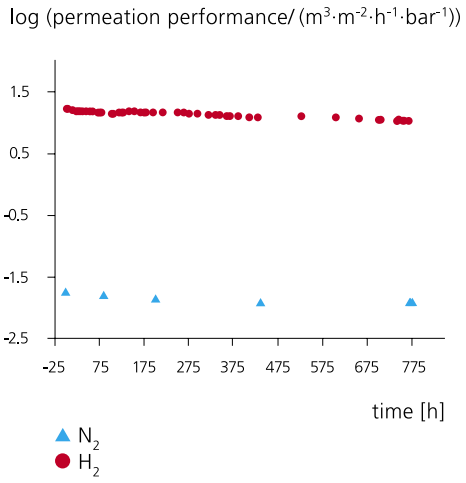

Figure 4: H_2 permeability of Pd coated Al_2O_3 capillaries (layer thickness ~ 2 µm).

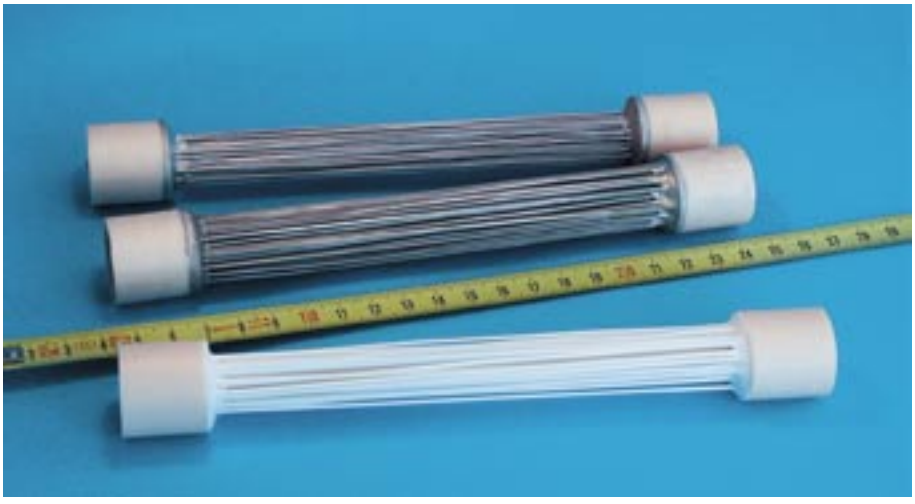

Figure 5: Purely ceramic module of Al_2O_3 capillaries with Pd coating (membrane area ~ 0.1 m^2).

Capillary fuel cell systems (C-PEM-FC)

Fuel cells: advantages and use

In relation to conventional generation of current, fuel cells are characterized by the fact that they can convert chemical energy from fuels such as hydrogen, methane gas and methanol directly into electrical energy. Concerning their efficiency (overall efficiency more than 90 per cent) and pollutant emission (decrease about of factor 10-1,000) they are superior to every other transformation technology. Further advantages are their modular construction, their noiseless operation and the fast reactivity on load changes. Since they contain no mobile parts they are very wear resistant and guarantee a high service life with less maintenance. So they are predetermined for decentral applications and for the use in less developed countries. Possible applications range from peripheral small apparatuses (mobile phones), over the energy production within the private sector up to the production of process energy in industrial applications and for energy production in commercial power stations.

Flat geometry versus capillary

Fuel cells today are mostly formed in single, plane cells of so-called flat geometry, which are fixed together in bigger »stacks«. Built in this way they reach a performance density of about 1 kW per liter. A disadvantage is that they require a lot of material, because in the stacks every single cell has to be separated by so-called bipolar plates to warrant the gas supply.

A technical and economical alternative is the capillary proton exchange membrane fuel cell (C-PEM-FC). Built in a modular construction, they cover every range of performance.

Capillary fuel cell with many advantages

Core of this system is the capillary fuel cell, as shown in Figure 1. The diameters of the individual capillaries are approximately 1.5 mm, the length approximately 15-20 cm. These elements are set within frameworks, with which the gas supply and the current demand are ensured. A fuel cell system with a defined performance can be achieved by stacking these frameworks.

By the use of the capillary geometry a 3-6 times higher performance density is attained compared to fuel cells in flat geometry. The material usage is very low; thereby the operational readiness is shortened. It is possible to produce such capillary-fuel cells for a price under 200 Euro per kW, with a large margin by high numbers of items. The feasibility of the described capillary-fuel cell is proven at prototypes. At present a semiautomatic pilot manufacturing equipment is at work.

Outlook

Predominantly the flat geometry fuel cells are being considered in all domains, where fuel cells are or will be in operation. The advantages of the capillary fuel cell regarding full automatable, continuous fabrication, low material usage and high power density are so evident and that the growth potential can be classified compared to the common flat geometry at present as extraordinary. Experts estimate that in the next five years a geometry change of the fuel cells will take place in favor of the capillary and tube geometry.

The chairman of the executive management board of RWE AG assumes that up to the year 2015, 65 TWh electric current will be won by fuel cells. This means that the generation of power by

fuel cells will boom. With the above mentioned price per kW, the capillary fuel cell will cost definitely less compared to other competition products. Altogether it is to be expected that the fuel cell market will develop to standard geometry, with the goal of later standardization. Therefore capillary fuel cells await excellent chances.

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Figure 1: Single capillary fuel cells, which can be assembled as modules.

Humidification of air with sterile water

Objective

Sterile air with a certain humidity is used frequently for diverse applications:

- Living and office rooms
- Stocks and showrooms, stores for books, museums
- Rooms where paper is manufactured, stored or printed
- Clean rooms for semiconductor production, sterile banks, laboratories and surgery rooms
- Manufacturing plants for special drugs or sterile devices

Despite this enormous market potential to date only very few devices for the humidification of air satisfying the sterility requirement are offered. Generally, these devices operate so uneconomically that they are used only if sterility is strictly necessary.

New patented process for humidification

A process and device for the humidification of air was developed and patented at the Fraunhofer IGB (patent No. DE 19919441, US 6474628, page 104). The process is based on the one hand on the application of membrane technology and on the other hand on special surface characteristics of certain porous materials.

Principle of function and modular concept

Before its entry into air the water used for the humidification is forced through a tubular membrane, which retains microorganisms and their fragments, i. e. it works like a sterile filter. After sterilization the water is evaporated by means of a hydrophilic, porous, disk-shaped body (page 105, Figure 4), e. g. a foam-like ceramic disk (Figure 1 and 2).

The disk-like shape of the humidification unit offers great advantages for the technical realization of the process: The tubular membrane, which sterilizes the water, can easily be built directly into the disk or between two disks (Figure 3) thus disposing the large surface of the disk for diffusion. Due to capillary forces the sterile water spreads over the internal surface of the disk, and is evaporated when air is flowing through the pores of the disk.

The disk geometry makes the integration of such a humidification device into the commonly used canal systems of air conditioners a simple task. By two-dimensional parallel interconnecting, the units can be assembled to very large scales in a modular manner.

The heat needed for evaporation is supplied by the air, the water and if necessary also by the environment. Thus an additional cooling of the air can be achieved. The attainable temperature reduction is a characteristic of the respective arrangement; it can be influenced within the given physical boundaries and can be beneficially used in the total concept of an air conditioning system.

Individual design

Owing to the variation options, such as the slab thickness of the porous material, the porosity, and the pore size, this new humidification concept fulfills a broad spectrum of requirements in air conditioning technology, thus opening up safe and simple low-maintainance solutions for motor vehicles and private households as well as for clean rooms in hospitals, food production and the electronics industry. If necessary, additional steps can be taken to avoid the entry of microorganisms with the feed-air – the state of the art offers sufficient possibilities.

Advantages of the membrane humidification process

- No entry of microorganisms and their fragments from the water reservoir into the air
- No aerosol formation in the admissible performance range
- Due to short diffusion length and tortuous paths, water vapour is well »mixed« with the air within the disk
- Very short length of mixing for the water vapour with the air
- Minimum water consumption, by control of the water supply; no water surplus
- Energetic more favourable than e. g. evaporation of the water at 100 °C
- Simple process engineering and scale-up by modular construction
- Cleaning of the membrane surfaces and heat sterilization of the system are possible
- Re-tooling possibility of old air conditioning systems

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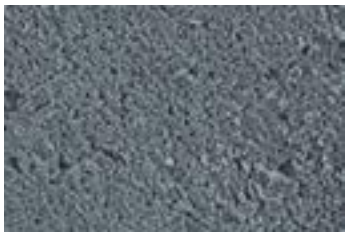


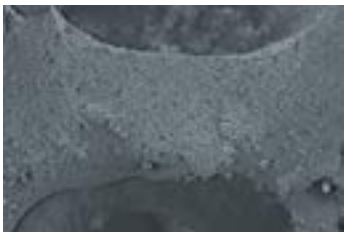


Figure 1 and 2:
SEM images of the macro-porous ceramic foam.

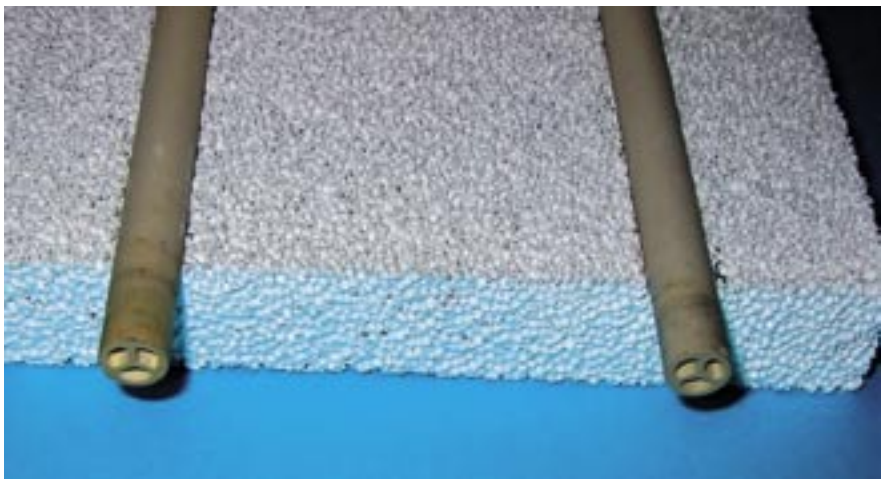


Figure 3: Humidification unit consisting of ceramic tubular membranes and two disks of ceramic foam.

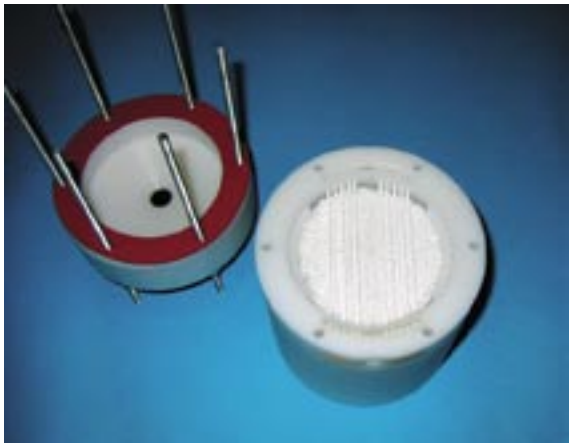
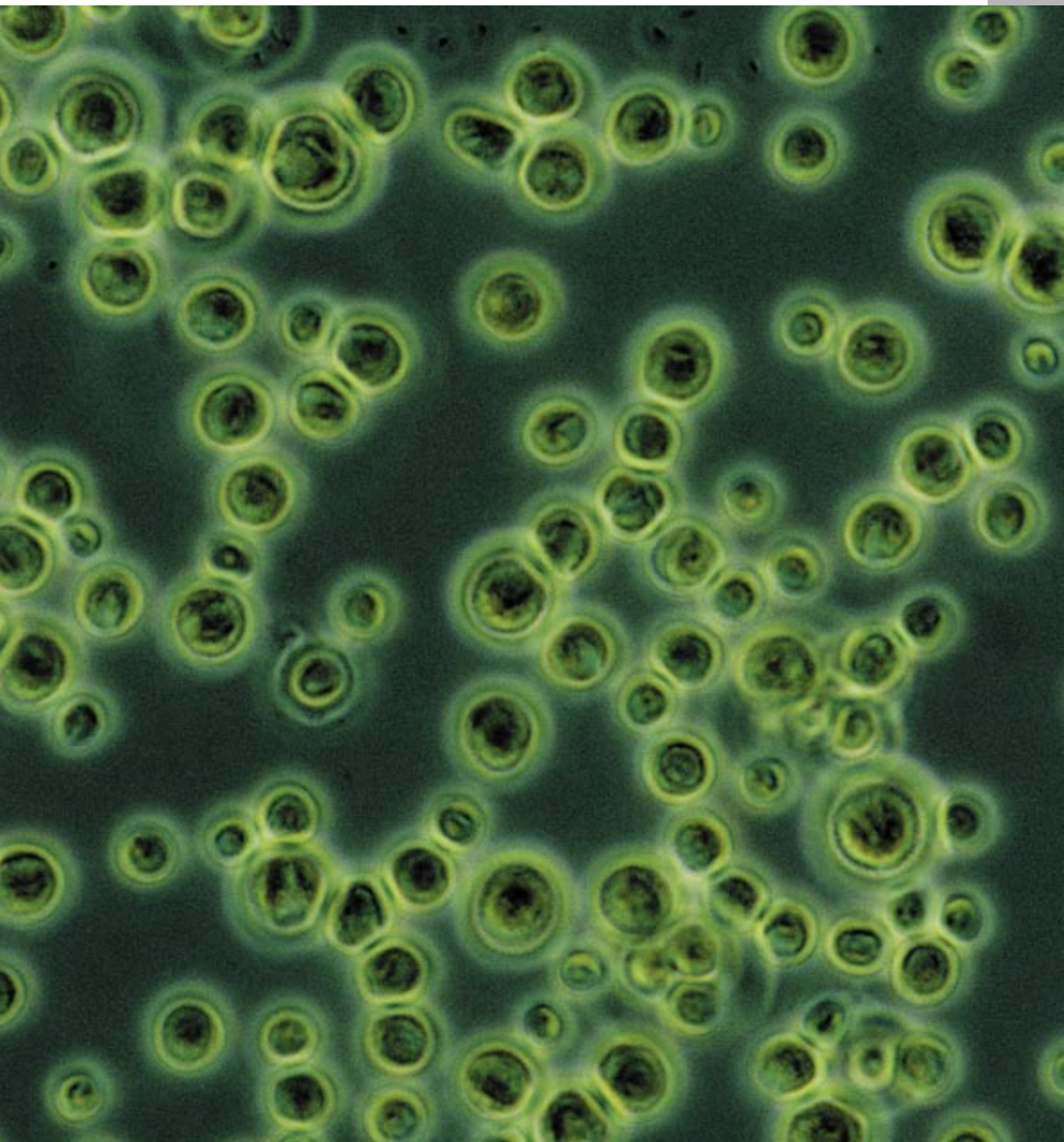


Figure 4: Prototype of a medical humidification device with polymeric capillary membranes and ceramic foam.

Bio- and membrane processes for environment and renewable energy



The business unit »Bio- and membrane processes for environment and renewable energy« includes the following business segments:

- **Recycling and conversion of waste and residual materials**
- **Industrial and municipal waste water purification, water recycling and water management**
- **Bioremediation and biotreatment**
- **Production of chemicals and energy using carbondioxide consuming microalgae**

The environmental and energy sector depend to a high degree on the conditions, which are set by national and international policy. Here in the last years sustainable changes have been adopted.
In the field of waste conversion, the German technical guidelines for urban disposal sites are of great importance. Accordingly, starting from the year 2005, waste materials containing more than five per cent organic dry matter may not be deposited. The new valuation criteria for wastes to be deposited permit explicitly mechanical-biological procedures as an alternative to incineration. The German Law for Soil Protection and the Regulation for the Application of Fertilizers impose restrictions on landlords and municipal waste disposal economies to avoid nitrogen and phosphorous emissions – a good reason for the utilization of liquid manure, sewage sludge, biowaste etc. after the concepts developed at the Fraunhofer IGB. These rely on the Recycling Law, which demands the utilization of organic carbonic waste as biogas and nitrogen as ammonium salts. Impacts on the environmental sector has also the Power Supply Law, which guarantees a fixed price from regenerative sources, when the energy is fed back to the main supply. This improves the cost effectiveness of the regenerative energy produced from organic waste.

The goal of the Federal Government to reduce CO_2 emission 25 per cent by 2005 (on the basis of 1990) will play an important role in this discussion. Regenerative biotechnological procedures like the production of resources by fast growing microalgae can help to reach these goals.

Important impulses for water management result from the adaptation of the municipal sewage treatment plants to the requirements of the EU Drinking and Waste Water Directive that will be valid up to 2005. Stricter environmental directives impose the effluent producing industries to reduce their water consumption. A possible way is the cleaning of waste water by means of the recovery of auxiliary process substances, these again are either recycled or undergo another way of disposal. The Fraunhofer IGB has an excellent expertise for solutions in industrial and municipal waste water treatment.

Environmental biotechnology – nature is the model

In nature, processing of energy and the strategy of substance handling is a circuit technology of highest perfection. Biological energy production is realized by conversion of solar radiation in photosynthesis. There, hydrogen generated by enzymatically catalysed splitting of water is bound to carbon (C), the central element of life, to build up energy-rich carbohydrates.

These energy-rich substances produced by photosynthetic activities are the source and reservoirs of energy of all energy-consuming biological reactions (i.e. oxidations). The final products of the biological energy use are CO_2 and H_2O leading to perfectly closed elemental circuits. Something similar is valid for all other essential elements of living nature as for example phosphorus (P) or nitrogen (N).

In such a way, biosystems are coupled in a global biozoenosis, so that they are mutually stabilizing each other in a dynamic balance. Therefore, in nature uninfluenced by human beings, wastes and by-products do not exist.

Although the biological circuit strategy presented above should be the model for a sustainable industrial production in order to reduce existing global and local environmental damages or the prevention of future impacts, biotechnology is used only in water purification or bioremediation. In particular, this is because biologically catalysed processing is a cheap technique compared to thermal/chemical processes for the removal of harmful substances from industrial or municipal wastewaters.

Figure Left page: Microalgae *Chorella vulgaris*, light microscopic image.

Sustainable biotechnological processes

The elementary bioreactions for the elimination of C, N and P have been adequately investigated in the scope of municipal and industrial wastewater recycling. The demands on R&D are the following areas:

- Specific planning for new or projected sewage plants based on measured parameters
- Replacement of aeration tanks by high-performance reactors (fixed-bed circulation reactors, fluidized bed reactors, high cell density reactors by microfiltration)
- Integration of recycling techniques instead of disposal (e. g. ammonia recycling instead of nitrification/denitrification)
- Integration of biological treatment steps into industrial production processes

A significant higher need in R&D for sustainable processes is the mineralization of pollutants, especially of those with a high xenobiotic character. »Xenobiotics« remain often in the usual mixed population present at the contamination site, since this does not develop so easily the necessary degradation pathways. One speaks also of the »persistence« of these compounds. Also natural substances behave in such a manner, if they occur in unnaturally high (toxic) concentrations. The elimination of such environmental chemicals requires a special technical effort and/or specially adapted biological systems. The Fraunhofer IGB offers both the know-how for the development of special biology for the degradation of a broad spectrum of environmentally hazardous substances and the know-how to transfer this special biology to technical application effectively.

The basic condition, in order to be able to establish specific biological degradation mechanisms for pollutants, lies in the knowledge of the causes for its persistence in the environment. If these are known, procedures can be developed to enrich microorganisms with special degradation capacities for pollutants and to increase their biological effect in the specific surroundings.

Usually, above all, three reasons are responsible for persistence: the xenobiotic character, the toxicity and the lacking bioavailability. Many polluting materials possess more or less distinctly two or all three of these characteristics. If the persistence causes are known in detail, microorganisms with specific degrading capacities, i. e. with the aid of more different, particularly designed strategies for the problem, can be enriched.

Thus, scientists at the Fraunhofer IGB succeeded to enrich and isolate bacteria, which are able to degrade or metabolize pollutants like chlorinated hydrocarbons, highly toxic substances (e. g. formaldehyde, cyanide) or biologically hardly available, water insoluble substances (e. g. polycyclic aromatic hydrocarbons, PAH). The process development is also coordinated with the pollutants and the requirements of the pollutant degrading microorganisms.

The special knowledge for the elimination of persistent compounds from groundwater and soil are now increasingly necessary for the elimination of drugs and endocrinal activities from waste water. These substances occur within the range of small and smallest concentrations only – the biodegradation of such low concentrated substrates, however, is reduced and, below a certain residual concentration, does not take place at all. Especially for these applications therefore, new solutions for acute environmental problems can be achieved by a combination of membrane-supported concentrating techniques with special biologies.

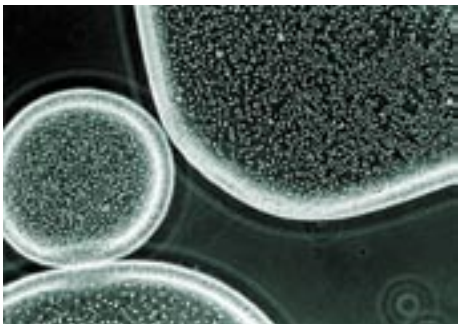


Figure 1: PAH degrading mycobacteria growing on the surface of droplets formed of a solvent in a biphasic culture system.

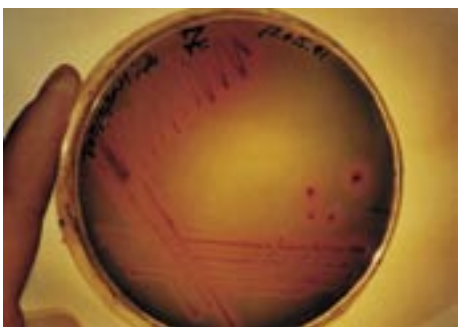


Figure 2: The new bacterial strain KS7D grows with the cyanide complex Prussian Blue as sole iron source, as seen from the decoloration of the agar.

Figure 3: Two-step high-performance plant for the digestion of sewage sludge in Leonberg.

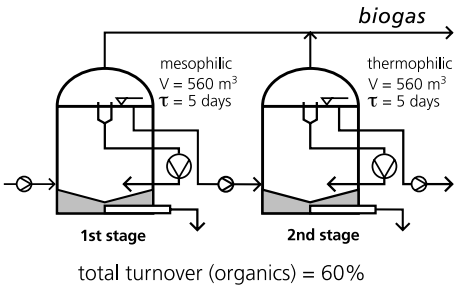


Figure 4: Diagram of two-step high-performance sewage plant in Leonberg.

Substance recycling: generation of biogas

Compared to add on-technologies, biotechnological processes can be established economically and ecologically advantageous for the substance recycling along with waste water or waste cleaning. The center of interest hereby is the use of anaerobic or substance producing anaerobic biocatalysts (microorganisms). The best-known substance recycling process of organic natural products, no matter, in which composition they exist, is the recovery of biogas.

By using that anaerobic part of the microbial food chain, as an end product of the digestion of natural materials a mixture of CO_2 and CH_4 known as biogas is released. Hereby, the methane part of the gas varies between 50-70 per cent depending on the composition of the waste material.

Biogas anaerobic technique has already been used for a long time in technical dimensions, for example for the digestion of sewage sludge or of manure. In order to achieve a break-through for the digestion of organic wastes of municipal or industrial production, a digestion technique with a complete or nearly complete conversion of natural compounds has to be created and realized instead of stabilization in order to compete with waste incineration. The Fraunhofer IGB is currently working on this complete digestion.

Complete digestion has to be specifically realized for each waste type, because of the different composition of the main waste streams as manure, bio-waste, sewage sludge and by-products of agroindustrial or food industries. Also, appropriate processes have to be developed waste-specifically. But as shown by many experiments, the digestion kinetics of the different waste streams follow a similar plan. Therefore, concerning the process design, high digestion rates can only be realized by reactor cascades coupled with membrane-based separation technologies. Additionally, the complete mineralization of organic waste as modelled in nature can only be achieved by a combined one-after-the-other application of anaerobic and aerobic treatments.

The decomposition of the organic fraction of some waste streams could already be increased significantly by the application of a two-step high-rate digestion process developed by the Fraunhofer IGB. The digestion rate of organic fractions of different wastes in a pure anaerobic cascade amounts to the following:

municipal solid waste	~ 80 %
sewage sludge	~ 70 %
municipal organic waste (separately collected)	> 92 %

The recycling of valuable substances from sewage sludges has been enlarged for an additional component, the nitrogen. By integration of a ceramic microfiltration unit in a two-stage digestion process (methane-cascade) the degradation efficiency could be increased enormously so that finally more than 70 per cent of the organic compounds of the sewage sludge are converted into biogas. This extremely high carbon digestion grade defines a filtrate with high nitrogen concentrations of about 2 kg/m³. Under the anaerobic process conditions the nitrogen compound is simply ammonia and by that it is possible to recover this value product by stripping techniques.

The application of the recovered substance as artificial fertilizer is appreciated.

The recycling of phosphor can be realized by prezipitation techniques but should be improved to an artificial fertilizer compound conditioned for excellent take up of plants.

The Fraunhofer IGB is currently working on a further increase of the degradation efficiency.

Recycling with increased sustainment

Assuming an economical product recovery, the order of sustainability of a recycling process increases with molecular weight and complexity of the recycling product. For example, lactic acid represents a substance with increasing importance as raw material for different chemical product types (the biologically decomposable polylactides, solvents). Lactic acid (lactate) can easily be produced biotechnically from lactose (milk sugar) by lactic acid bacteria, whereby only a maximum of 6 per cent of the substrate energy will be lost by means of conversion.

In whey – a by-product of cheese production – lactose represents a waste product. By biotechnical conversion of lactose to lactic acid combined with efficient product recovery in form of a 95 per cent water purification performance, a production process and environmental conservation are combined in a perfect way.

Here at the Fraunhofer IGB, the well-known biotechnical lactic acid production process was adapted to whey utilization so that lactic acid can be produced with reduced costs compared to world market prices. With this, the central requirement for the economical production of polylactides on the basis of lactic acid has been fulfilled.

As food processing industries are generating a lot of other by-products with a single and consistent composition (comparable to whey), well-known and new conversion products (e. g. propionic acid, acetone, butanol etc.) as bulk- or final chemicals may be produced biotechnologically, combining product recycling with environmental conservation in an »eco-economic« way.

The recycling of valuable substances for preserving or gaining clean water, the most important nutritive component for living beings, is turning the focus of our research activities more and more. The concept of Fraunhofer IGB aims at the recycling of components from waste- and rainwater by bioprocesses and integrated membrane separation steps mainly based on ceramic micro- and ultrafiltration. Urban water management on the basis of state of the art technologies of industrialized countries cannot be financed by developing countries as fast as they are needed by the population.

Thus a demand for cheap and sustainable quality techniques exists to fulfill the requirements of the World Health Organization for the drinking water supply in such countries. Decentralized water saving urban infrastructure techniques have to be developed, to be standardized in modules and produced in high quantities to reduce costs. Under the initiative »Decentralized urban infrastructure techniques« supported by the German Ministry of Education and Research the technology of the future is developed and demonstrated in Germany (DEUS 21) and in Brazil (Piracicaba).

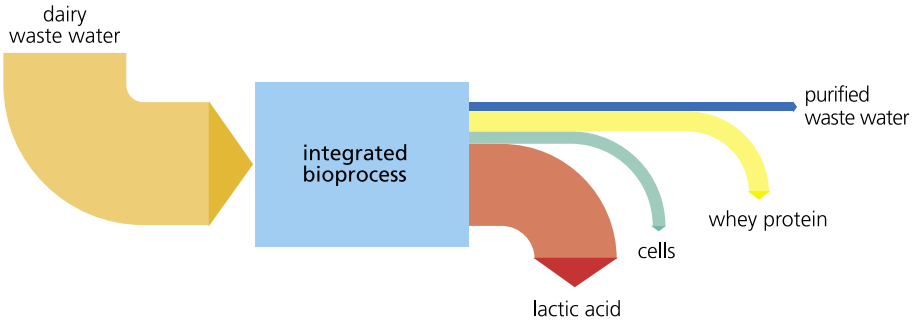


Figure 5: Schematic display of an integrated bioprocess such as the production of value products from dairy effluents.

Services

- Production of bulk chemicals and energy from waste, scale-up of bio-conversions to technical scale with optimized bioreactors
- Modern methods of waste water treatment: development of reactor systems in a modular way, test plants in technical scale and optimization of plants
- Cost-effective optimization of existing sewage plants by system analysis and specific planning
- Development of microbial systems for the degradation of hazardous compounds
- Development of techniques for remediation and treatment of contaminated wastes, soil, water, and air using biological degradation potentials
- Evaluation of the environmental relevance of remediation of organic chemical compounds and their metabolites
- Anaerobic and aerobic degradation tests
- Eco-balancing of processes according to a biological model

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Figure 6: Aeration tank of a sewage plant. Fraunhofer IGB optimizes and expands the capacity of existing plants by systematic analysis and specific measurements.

Microbiological investigations for inactivating of microorganisms in cooling lubricants

Introduction

Cooling lubricants (CL) are used in the metal-working industry, in order to attain a cooling of the tool at high working speeds during processing of the workpieces (e. g. lathing, drilling, milling and grinding). Microorganisms such as bacteria and fungi can contaminate the CL during the course of application. Thus the CL is damaged and the personnel are exposed to a hazardous situation by an increased germ level. Therefore additives with biocide and fungicide activities are added to the CL. Thereby additional costs arise and for the personnel preventive measures are to be met. The biocides must be changed in certain time intervals as microorganisms develop resistance.
The Fraunhofer IGB accomplished fundamental investigations on behalf of DaimlerChrysler for the realization of a procedure, with which the microorganism level in CL is reduced by thermal inactivation.

In biosciences, pharmaceutical and food industries processes have been developed for the thermal inactivation of microorganisms thus preventing their proliferation. Such a treatment usually needs no additives. There are already continuously working procedures, which require only short treatment times by a suitable mode of operation.

Mathematical evaluation of inactivation

The temporal decrease of a microbial population with thermal inactivation takes place approximately after kinetics of 1st order. The temporal change of the cell number N of the surviving microorganisms can be described thus after equation (1)

$$\frac{dN}{dt} = -k \cdot N \qquad (1)$$

with k as the killing constant and t as time.

The solution of this simple differential equation results in

$$\ln \frac{N}{N_0} = -k \cdot t \qquad (2)$$

with N_0 as starting germ number.

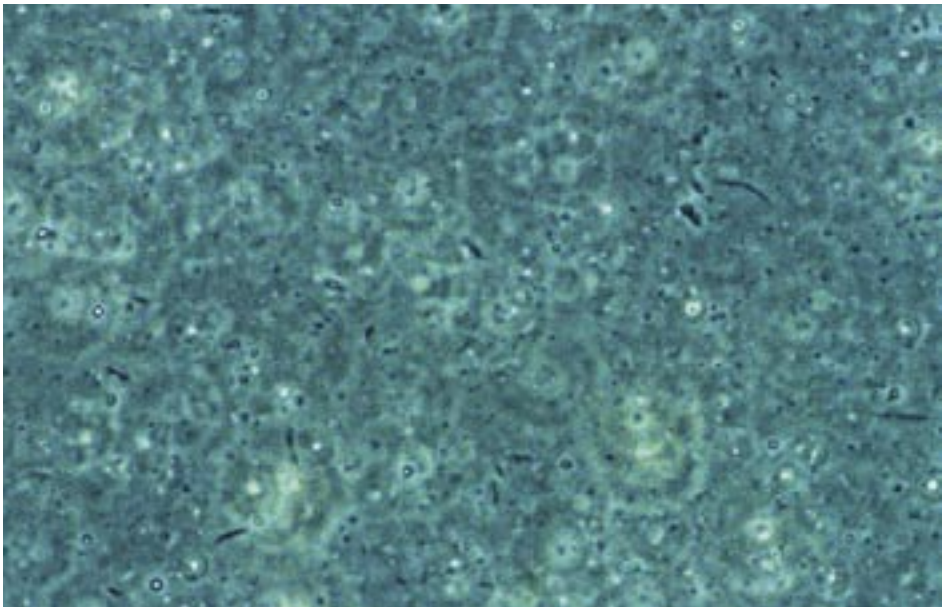

Figure 1: Micrography of microorganisms in cooling lubricants. Vegetative cells have a long shape. Magnification 1,000 fold in phase contrast.

The killing constant k is for certain microorganism and defined conditions, a function of temperature and can be shown as an Arrhenius equation

$$k = k_0 \cdot \exp(-\frac{E}{R \cdot T}) \qquad (3)$$

with constants k_0, an activating energy E, the general gas constant R and absolute temperature T.

The logarithm of equation (3) gives

$$\ln k = -\frac{E}{R} \cdot \frac{1}{T} + \ln k_0 \qquad (4)$$

for the variables ln k and 1/T with the slope -E/R and point of intersection $\ln k_0$ on the k-axis (Arrhenius diagram). The Arrhenius diagram describes the sterilization kinetics as a function of sterilization temperature and thus enables the determination of process parameters.

Results

Microbiological investigations have shown, that there are various microorganisms such as bacteria and fungi contaminating the CL. Most of the bacteria occurring in CL are spore-forming organisms. Figure 1 shows a microscopic picture of isolated microorganisms from the CL.

Sufficiently high cell numbers at the beginning of the experiment are required when investigating the sterilization kinetics. Therefore, the microbial cells had to be concentrated prior to the experiments. This was performed either by isolation of the microorganisms from the CL and following cultivation in shaking flasks or bioreactors or, as second approach, by centrifugation of sufficiently large quantities of contaminated CL.

The number of microorganisms and spores in the CL can be reduced by means of thermal inactivation. In Figure 2 the decrease of microorganisms for different temperatures is shown as a function of time in a single batch experiment. The reduction takes place in two steps: at the beginning more slowly, later faster. Figure 3 shows the determination of the sterilization kinetics after equation 4 as an Arrhenius diagram.

The short temperature overload does not impair the function of the CL.
The patent for this procedure is pending.
The procedure will soon be tested under industrial conditions at DaimlerChrysler.

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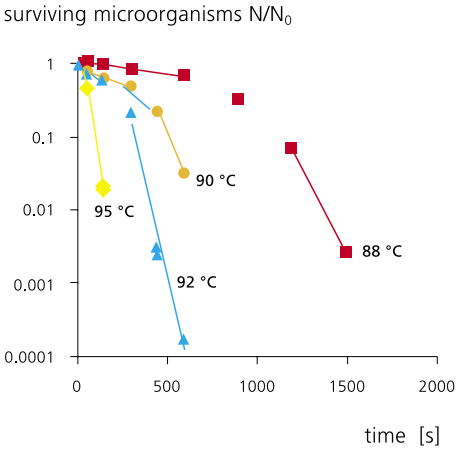

Figure 2: Representation of sterilization vs time during treatment of mixed population isolated from cooling lubricants. Temperature 88, 90, 92 and 95 °C, respectively.

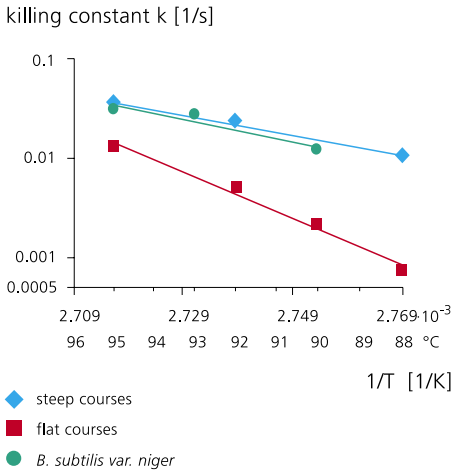

Figure 3: Arrhenius diagram of the data from Figure 2 referring to equation (4).

High-performance digestion – Sewage sludge treatment with a profit

The initial situation

In waste water sewage plants, significant quantities of sludge are produced, for example 330,000 tons of dry matter per year in Baden-Württemberg [1]. Sludge is increasingly being produced in sewage plants as a result of the biological nutrient removal which is generally applied.

The following actualities are at present causing a problem for the operators of sewage plants:

- Due to the presence of heavy metals and organic pollutants, as well as existing hygienic concerns, sewage sludge dispersion in agriculture and in landscaping cannot be supported [1,2].
- Deposition via landfill will be possible until mid-2005 at the latest following the new »Technische Anleitung (TA) Siedlungsabfall« (German technical directive for municipal waste); in general, solid contents of over 80 per cent are demanded here.

Since for these reasons sludge incineration will politically be given absolute priority in the future, the local authorities will have to get used to clear changes in their operating costs and additional investments.

With the high-performance digestion method developed at Fraunhofer IGB the sludge can be stabilized and dewatered much better. The process is also of great interest when conventional plants are being extended. Biogas is gained, with the amount of sludge being reduced at the same time.

Modern high-performance digestion

In the high-performance digestion developed by Fraunhofer IGB and its industrial partner, the so-called Schwarting-Uhde method, mixed reactors are used and operated with a relatively short retention time and high organic loading rates. These anaerobic plants can also be integrated into existing sewage plants. The process has been put into practice in, for example, Leonberg, Tauberbischofsheim and Heidelberg.

Improved degradation and less sludge

The sewage plant of Heidelberg has a capacity of 360,000 (number of inhabitants). Significant problems arose in operation of the standard digestion (Figure 1), which as a result produced a sludge that is very difficult to de-water. In the meantime a new fermentation plant has been put into operation, which consists of a high-performance digestion (1st stage) and an old fermentation tower (2nd stage) (Figure 2). Fraunhofer IGB gave scientific assistance in planning and commissioning.

Installing the high-performance digestion upstream resulted in significantly increased decomposition performance and improved sludge stabilization. This was apparent from enormous savings in flocculation media and a small amount of resulting sludge. In total, 726 less tons of sludge had to be disposed of in the first year of operation. Almost 60,000 Euros were saved in operating costs in the same period as a result of this measure.

Figure 1: Standard sludge digestion of the Heidelberg sewage plant.

High-performance digestion to produce energy

The high-performance digestion in Heidelberg was operated according to the preceding investigations of Fraunhofer IGB. Thus an organic loading rate of 10 kilograms of volatile solids per cubic meter and day was achieved. Degradation was significantly improved.

The amount of methane produced with the entire digestion system could be significantly increased in comparison with operation without a high-performance stage, and resulted in a corresponding financial profit for the plant operator: Biogas is provided as an energy carrier to the municipal utilities plants, which produce electricity and thermal energy from it.

Gaining ammonium from the filtrate water

The de-watering of the sludge produces a sludge water that contains significant quantities of ammonium. This must be removed from the sludge water in order to meet legal requirements. Normally, it is fed back into the aerobic activated sludge tank in order to achieve this, but this results in a significant increase in ammonium levels there, and a greater oxygen requirement in the nitrification. Innovative solutions for removing the ammonium from the sludge water are therefore required.

In an ongoing research project at Fraunhofer IGB, the sludge water is filtered and ammonium is then obtained with qualified process technology. Current investigations are dealing in particular with improving the process with regard to economy. Thus the knowledge gained leads to the design and operation of a pilot plant.

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2 »Umweltplan Baden-Württemberg«. Hrsg. Ministerium für Umwelt und Verkehr Baden-Württemberg. (May 2001)

Figure 2:
High-performance digestion installed upstream.

Microalgae provide raw materials

Microalgae and cyanobacteria produce, via photosynthesis, a variety of substances that are of interest for industrial applications, for example fatty acids, carotenoids and phycobili proteins (pigments) and vitamins. They are potential suppliers for food supplements, pharmaceuticals, animal feed and regenerative energy and are therefore an ecologically and economically sustainable alternative and supplement to animal and chemical raw materials. A photobioreactor – in which high biomass concentrations and at the same time high productivity are obtained by utilizing the sun as an energy source – is required for the economical mass production of algae, which in turn is a prerequisite for its economical use. Fraunhofer IGB has developed an airlift reactor with baffles (FPA reactor), with which the preconditions are created for the commercial mass production of microalgae outdoors [1].

A new type of photobioreactor

The FPA reactor works on the principle of an airlift loop reactor. By virtue of its low layer thickness and targeted flow in the reactor, it improves the light supply to all algal cells via static mixers. The photobioreactor is manufactured from plastic film in the form of two half-shells by way of a deep-drawing method, these half-shells then being welded together. Between the static mixers, rising gas bubbles create turbulence in which the algae are transported at defined short intervals out of the non-illuminated reactor zone and into the light at the reactor surface. The average light intensity that is evenly distributed to all algal cells in the reactor can be varied via the distance between individual FPA reactors outdoors. This also enables algal cultures of higher cell densities to be supplied with sufficient light, e. g. *Phaeodactylum tricornutum*, a brackish water alga that contains the poly-unsaturated fatty acid eicosapentaenoic acid accounting for up to five per cent of its biomass content. With this alga, cell densities of up to 15 grams dry weight per liter (g $DW \cdot l^{-1}$) and biomass productivities of 0.33 $OD \cdot h^{1}$ (OD, optical density) were achieved in the FPA reactor.

Astaxanthin production with *Haematococcus pluvialis* outdoors

Haematococcus pluvialis SAG 192.80 is a single-cell freshwater alga, which accumulates in a two-stage process the ketocarotenoid astaxanthin up to five per cent of its biomass. Astaxanthin can be used both as a pigment in the aquaculture (e. g. as the pinkish color of salmonid flesh) and, by virtue of its strong antioxidative effect, in food supplements (nutraceuticals) or cosmetic products.

In autumn 2002, biomass growth rates of up to 0.25 g $DW \cdot l^{-1} \cdot d^{-1}$ with cell concentrations of up to 2.5 g $DW \cdot l^{-1}$ were achieved outdoors (the Institute's site in Stuttgart) in the newly developed FPA reactor. These are the highest values achieved so far for *Haematococcus pluvialis* [2,3], attributable to the favorable light distribution in the photobioreactor. The formation of astaxanthin is induced by high light intensities (direct sunlight), a lack of nutrients or inductors such as acetate and NaCl. If these factors are taken into account in the batch

process, the cell weight increases again by a factor of three to four, and at the same time the intracellular astaxanthin content reaches up to five per cent of the cell dry weight.
Outdoors, biomass concentrations of *Haematococcus pluvialis* of up to 10 g DW·l^{-1} were achieved in the FPA reactor. This high cell density fulfills an important prerequisite for industrial astaxanthin production.

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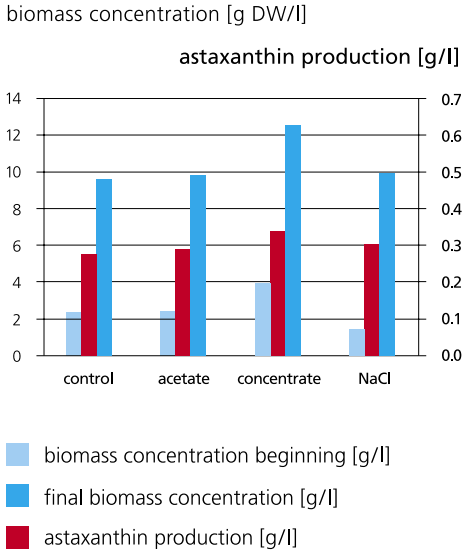

Figure 1: Biomass increase and astaxanthin accumulation of *Haematococcus pluvialis* under outdoor conditions with various inductors and cell concentrations at the start of the experiment.

Figure 2: Outdoor facility for the mass cultivation of *Haematococcus pluvialis*.
A: Photobioreactors set up in an east-west direction, with green-growing algae.
B: Photobioreactors set up in a north-south direction are exposed to direct sunlight. The *H. pluvialis* cells now accumulate the red pigment astaxanthin.

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2 Olaizola M. (2000) Commercial production of astaxanthin from *Haematococcus pluvialis* using 25.000 liter photobioreactors. J. Applied Phycology 12: 499-506

3 Boussiba S., Bing W., Yuan J.-P., Zarka A., Chen F. (1999) Changes in pigment profile in the green alga *Haematococcus pluvialis* exposed to environmental stresses. Biotechnology Letters 21: 601-604

Patents 2002

CA 2,096,532

US 09/674,655

AU 746005

US 6433868

US 6474628

DE 19919441

DE 10112863

In 2002, six Fraunhofer IGB patents were granted in Germany, Europe and oversea countries. They demonstrate our innovative strength and international competitiveness.

New human recombinant gamma-interferon
(excerpt from European patent)

»The invention provides a new polypeptide (referred to in the following text as gamma-interferon C-10L) containing 134 amino acids instead of 144. Amino acids 1 to 132 correspond to those of natural gamma-interferon. The first amino acid in position 0, is additionally present and was determined by amino acid sequencing. The amino acid in position 133 is leucine instead of glutamine. Surprisingly, it was found that the gamma-interferon C10L had a 4-fold higher activity than the complete 143 amino acid gamma interferon. Gamma-interferon C-10L has, in addition, a 24-fold higher activity, than normal gamma-interferon, as anti-proliferative acitivity, on human WISH cells. In contrast to previously known gamma-interferon, and because of its higher activity and expression rate, this shortened form makes available a gamma-interferon that allows lower and more target oriented doses to be used for therapeutic purposes.«

CA 2,096,532 (application number)
Böhm, Otto, Slodowski

A development of IGB department
Genetic Engineering, Hannover.

Electrical, integrated isolation, purification and detection of nucleic acids
(excerpt from application)

»The technical problem on which the present invention is based, lies in the task of providing a cost-effective and simple procedure for cell dissociation and nucleic acid isolation that permits the highly specific preparation, from any biotic and/or abiotic sample material, of particularly pure nucleic acids in a single step already during the dissociation of the sample. The invention solves this problem by providing a procedure for isolating nucleic acids from a sample, with the sample being dissociated under the influence of at least one electric field and the nucleic acids related being brought into contact with a nucleic acid-affine material in such a way that at least part of the nucleic acid binds to the nucleic acid-affine material.«

US 09/674,655 (application number)
AU 746005
Bernhagen, Brunner, Elkin, Geiger, Tovar, Vitzthum

A development of IGB departments
Molecular Biotechnology and Interfacial Engineering.

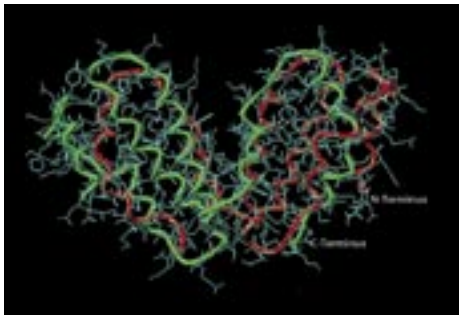

Figure 1: Interferon-γ, dimeric structure.

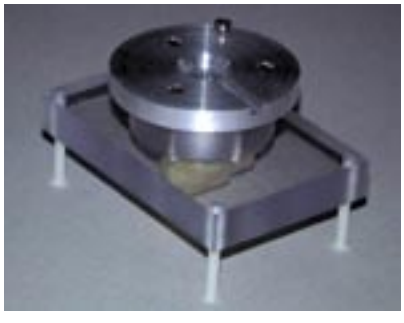

Figure 2: Prototype of a high-voltage cell for physical cell disruption.

Method for differential spectroscopic measurements
(excerpt from German patent)

»The invention relates to a process for the differential spectroscopical and differential fluorometric determination of the interaction of substances with the aid of a microtitration plate and to the use of a microtitration plate for the differential spectroscopic and differential fluorometric determination of the interaction of substances. The invention provides that two different substances are arranged separated from each other in vertically superimposed relationship in a region characterised as the reference region, whereas the mixture of the substances is situated in a sample region of the microtitration plate and can be analysed at the same time.«

US 6433868
Bernhagen, Brunner, Vitzthum

A development of IGB department
Molecular Biotechnology.

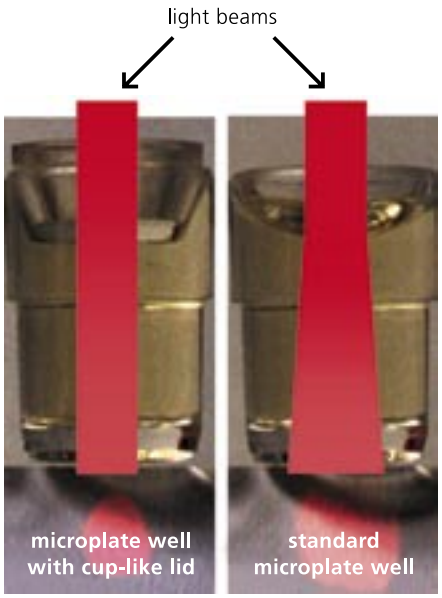

Figure 3: Effects of meniscus formation on pathlength and refraction of light beams.

Air humidification
(excerpt from German patent)

»The invention provides an air humidifier, comprising a membrane contactor, in which the membrane contactor has at least one tube membrane embedded in a hydrophilic, porous body. The invention provides for the water, which may contain microbes, to be passed through at least one tube membrane, that is to say a membrane, which is of tubular or hose-like form, into a hydrophilic, porous body. In the hydrophilic, porous body the water passes through the pores of the membrane into the body. According to the invention, it is now envisaged that the pores of the membrane will be sufficiently small for it to be impossible for either microorganism or fragments of lysed microorganisms or larger molecules, which may have a toxic or allergenic action, to pass through the membrane. The tube membrane therefore acts, as it were, as a filter for undesirable constituents of the water. The water leaving the tube membrane is therefore sterile. This water passes into the pores of the porous body, where it is dispersed by capillary forces over the entire inner and upper area of the body. The air, which is to be humidified is passed through the porous body, where it can take up sterile water over a relative large surface area.«

US 6474628
DE 19919441
Stroh, Walitza

A development of IGB department
Membrane and Energy Systems.

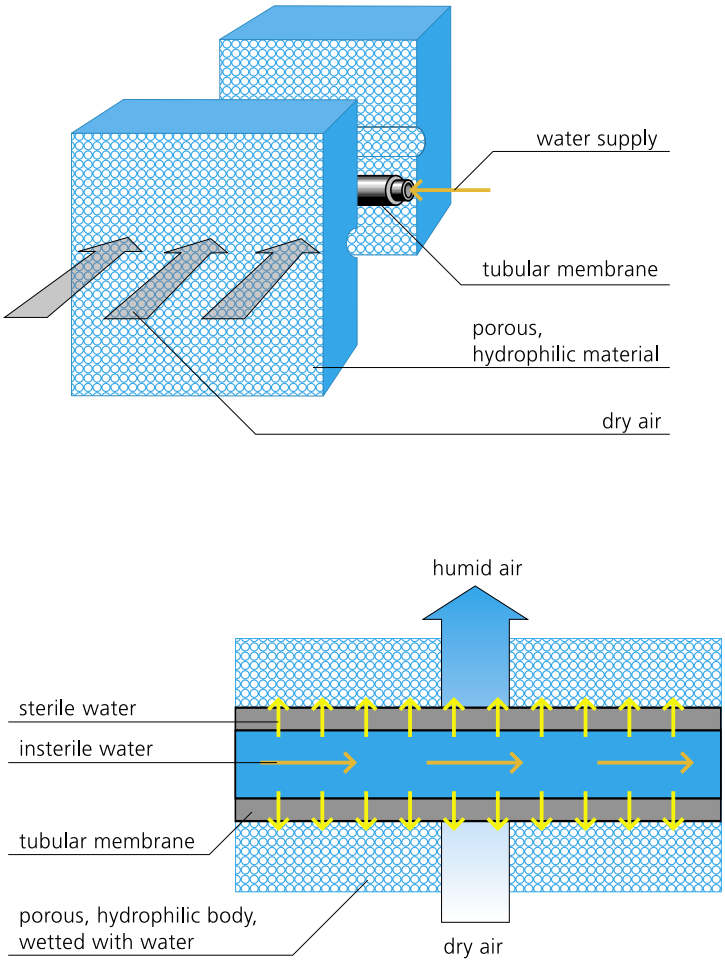

Figure 4: Humidification of air with sterile water. A humidification unit consists of a tubular membrane for filtering the water and hydrophilic porous disks for evaporation.

Interested in more? Please contact!

Dr. Gernot Weiß
Business Development
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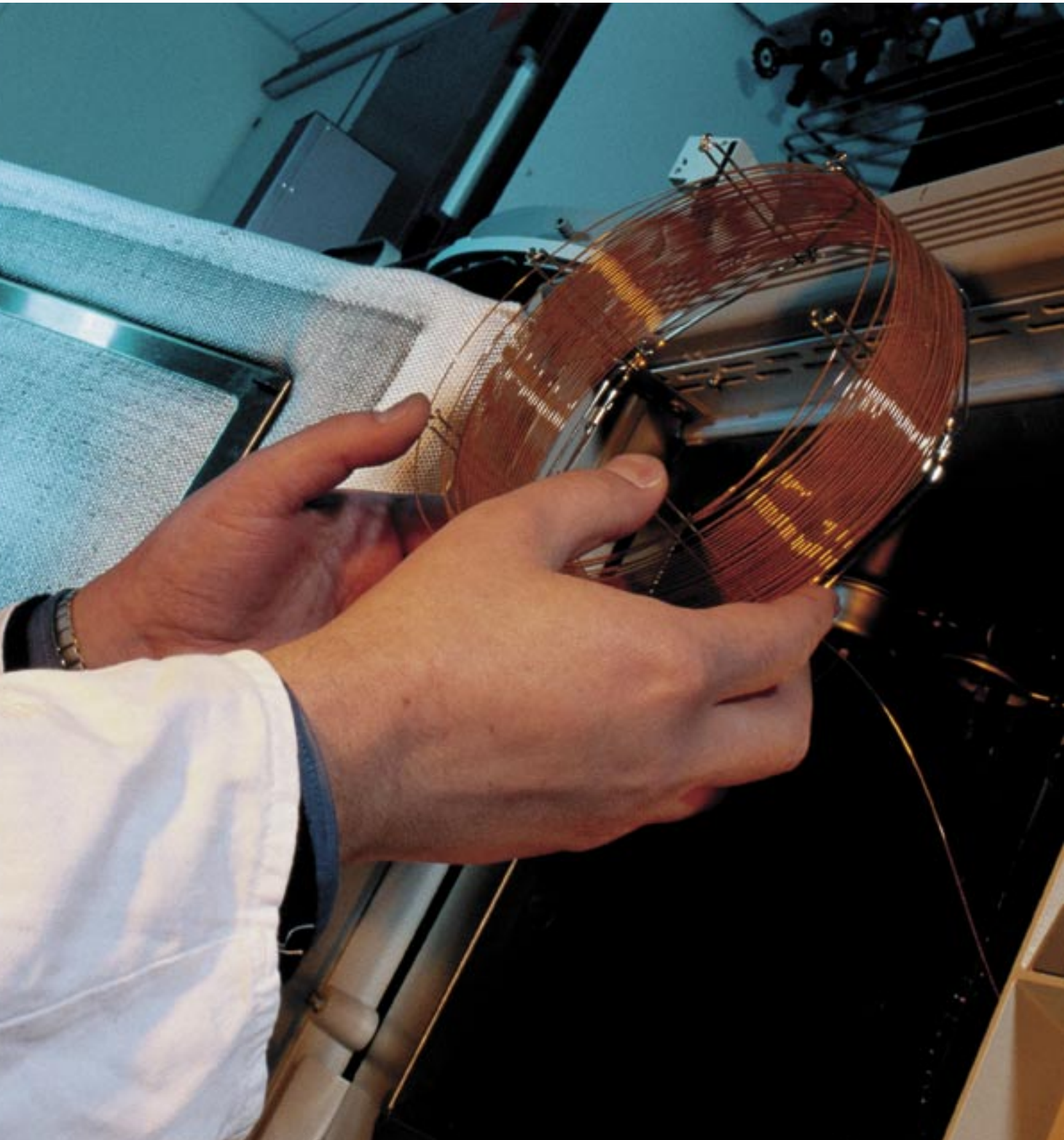
Services



Fraunhofer IGB – your competent partner for manufacturing Investigational Medicinal Products (IMPs)

Fraunhofer IGB is a competent partner for the development of novel cell based therapeutics. Our qualification is built on more than ten years of experience in primary cell culture technologies, three dimensional organoid cell culture, tissue engineering as well as cell and gene therapy. Within a network of scientists, engineers and clinicians the institute is focussing on the role of a mediator between preclinical research and clinical application. With a strong knowledge in process development and GMP compliant process engineering we are well prepared to support your product pipeline.

Manufacturing of Investigational Medicinal Products (IMPs) according to current »Good Manufacturing Practices«

Fraunhofer IGB offers services in development and manufacturing of specialized cell preparations according to the EU guidelines of current »Good Manufacturing Practices« (GMP). We provide a fully established GMP unit on 150 square meters. The production facilities include clean rooms of different classification up to A in B work places (equivalent to US *class* 100) and are registered for work with genetically modified organisms up to biosafety level 2 (according to the German Gene Law). Quality control laboratory and storage rooms are completely separated from the production unit.

Quality management

We guarantee quality of our products by running a well established quality management system. Tools are comprehensive quality control on starting materials, intermediate, bulk and end products, a complete process and product documentation according to European guidelines, qualification, process validation and monitoring, training of personnel and regular self inspections.

Our location – your advantage

At our location we provide an outstanding synergy evolved by the integration of several specialized research and service units, which you can utilize in your interest: GLP certified analytical laboratories (e. g. for HPLC analysis), Molecular Biotechnology (e. g. for chip technologies and quantitative PCR), Interface Engineering (e. g. for SEM, surface analytic). Make use of our know-how for process development and GMP compatible manufacturing of tissue engineering products and cell and gene therapeutics.

GMP compliant production – continuous production processes, trained personnel and modern clean rooms guarantee for safety and quality of our products.

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Special analytical services

The Analytical Services group of the Fraunhofer IGB offers physico-chemical analytical services for internal use as well as for external clients from industry, municipal authorities, universities and research institutes.

One of our main activities is the development of new analytical methods for specific problems of the customer, especially if regulated or standardized processes do not yet exist. Accompanying chemical analytics and evaluation of processes contribute to the optimization of products and production processes. This is also achieved by interdisciplinary cooperation within the Fraunhofer IGB.

Modern technical equipment and motivated qualified staff guarantee a prompt and careful handling of the project as well as a high quality.

Our analytical methods are oriented to GLP, DIN/ISO and EN guidelines.

Our services

- Environmental analytics, e.g. water, waste water, soil, sewage sludge, hazardous waste, air and textiles
- Analysis of residues, e.g. detection of production residues and environmental chemicals
- Quality control, e.g. of pharmaceutical drugs
- Trace analysis of organic contamination of surfaces
- Food analysis
- Analyses accompanying clinical tests and production processes
- Degasifying studies, e.g. of insulating glass panes, electronic devices, light bulbs

Ion chromatography (IC).

Equipment

- Gas chromatography (GC)
- High-performance liquid chromatography (HPLC)
- Ion chromatography (IC)
- Size exclusion chromatography (SEC)
- Mass spectrometry (MS) (GC-MS/MS, LC-MS/MS, MALDI-ToF-MS)
- Atom absorption spectrometry (AAS)
- Atom emission spectrometry (ICP-AES)
- Total organic carbon (TOC)
- Elementary analysis (C, H, N, O, S)

Accreditation according to EN ISO / IEC 17025

The testing methods liquid chromatography (high-performance liquid chromatography HPLC, ion chromatography IC, size exclusion chromatography SEC) and gas chromatography (GC, GC / MS) have successfully been accredited by an international recognized accreditation body.

Contact

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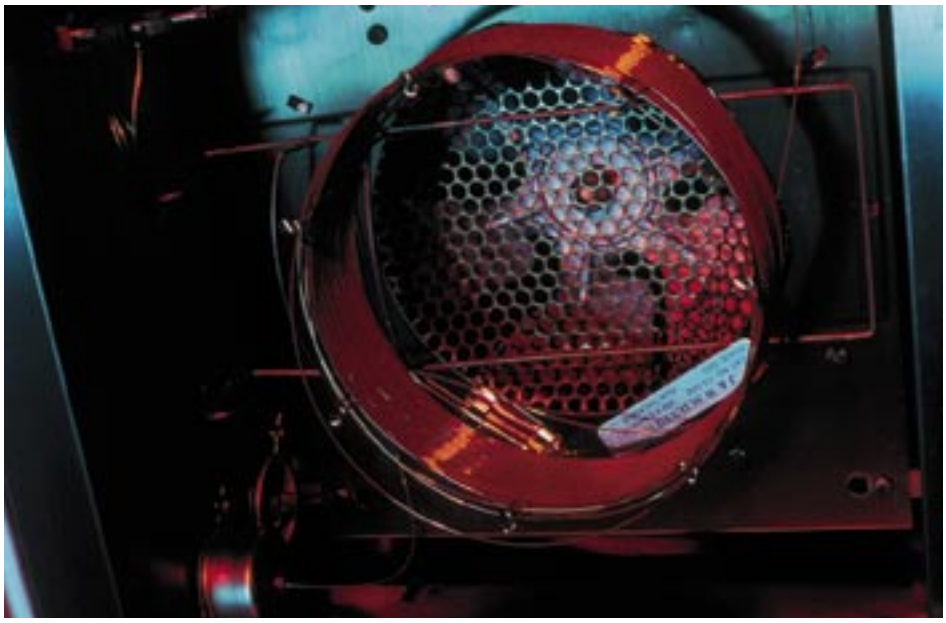


Gas chromatography (GC).

Biochemical and molecular biological analytics

Proteomics, proteins, peptides, DNA

Many questions in life sciences require knowledge of the protein sequences. At the Fraunhofer IGB proteins are isolated, purified and analyzed by N-terminal or internal sequencing (Edman degradation) or by either of two types of mass spectrometry (MALDI-TOF-MS, ESI-MS/MS).

With the help of the protein sequence information, cDNA libraries are screened in order to isolate the appropriate gene. The gene of interest may be cloned and expressed in a specific bacterial host.

These complex technologies require high material and operating costs. Furthermore, a high quality standard can only be achieved with specifically trained staff. This is also true for the synthesis of peptides. Therefore, the analytical service group of the Fraunhofer IGB offers you high quality services around proteins and nucleic acids.

Your benefit

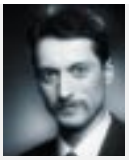
The Fraunhofer IGB offers for internal use and external clients complete analytical and biochemical services including synthesis of proteins and peptides. From this basis we expand our services to different well established DNA sequencing and cloning strategies.

Target for MALDI-TOF-MS.

Offered services »from protein to gene«

- Purification of enzymes and pharmaproteins by LC/FPLC, »narrow-bore«-HPLC from lab scale to pilot scale
- SDS-PAGE and 2D-electrophoresis
- In-gel digestion
- Enzymatic and chemical dissociation of proteins and subsequent purification of the obtained peptides by HPLC (peptide mapping)
- N-terminal and internal sequencing of proteins and peptides
- Analysis of peptides by HPLC, amino acid analysis and MALDI-ToF-MS/peptide mass fingerprint
- *De novo* sequencing of peptides by LC-MS/MS and peptide fragment mass fingerprinting
- Post-translational modification by GC-MS (sugars), LC-MS and ion chromatography with pulsed amperometric detection
- Data base search
- Localization and analysis of conserved sequences in protein families for the synthesis of primers
- Screening of cDNA banks for the cloning of genes
- PCR-based nucleic acid analytics
- Cloning in different expression systems
- DNA sequencing
- Preparation of cDNA banks
- Chemical synthesis of peptides, polypeptides and small proteins
- *In vitro* translation

Contact



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Dr. Hans Weber
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Protein extracts of a *Candida albicans* culture have been separated according to their iso-electrical point and molcular weight and have been made visible through a silver staining.

Surface analytics

The material testing becomes more and more important as reliable characterization of surfaces is pivotal for their possible use in technical products. This is especially the case for methods enabling the investigation of structure, surface characteristics and the coating of workpieces in detail.

The Fraunhofer IGB is offering a wide range of analytical methods for the characterization of chemical, physical and morphological features of surfaces and interfaces as well as thin films and liquids.

Our most modern technical equipment is available for internal projects as well as for external clients from industry, public and municipal authorities.

Services

Chemical analysis

- Electron spectroscopy for chemical analysis (ESCA), multipoint, imaging, valence band
- Auger electron spectroscopy (AES), deep profile
- Energy dispersive X-ray fluorescence analysis (EDX)
- MIR- and NIR-spectroscopy, limited total reflection (IR-ATR)
- Infrared microscopy
- Fluorescence spectroscopy

Topography / morphology

- Light microscopy (LM)
- Scanning electron microscopy (SEM)
- High-resolution field emission scanning electron microscopy (FE-SEM)
- Atomic force microscopy (AFM)
- Scanning tunnel microscopy (STM)

Surface tension

- Static and dynamic contact angle, various methods of measurement
- Tensiometry according to Wilhelmy or du Noüy, dynamic bubble-pressure method, dynamic drop-volume method

Specific surface

- Adsorption/desorption curves (BET surfaces, pore distribution)

Figure 1: Field emission scanning electron microscope (FE-SEM) with energy disperse X-ray fluorescence spetroscopy (EDX).

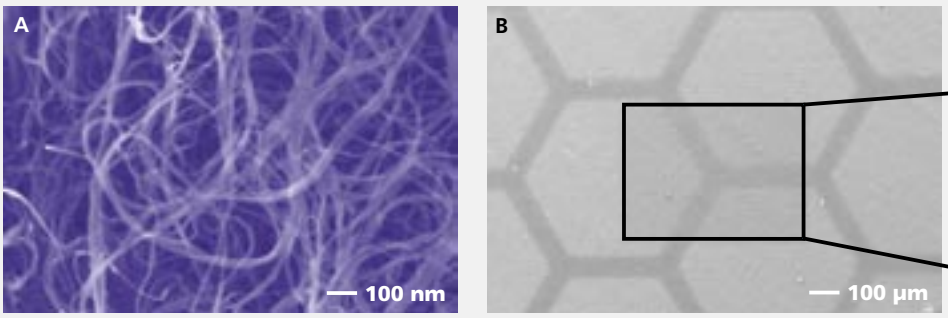

Figure 2: A: Singled Wall Carbon Nanotubes (SWNT), FE-SEM-Image. **B:** Structured coating of polypropylene with acrylic acid plasma. (A TEM-Grid was used as coating mask.), FE-SEM-Image. **C:** EDX-Map of the oxygen signal, experimental set-up LEO 1530 VP.

We characterize

surfaces of workpieces of

- solid materials,
- thin layers,
- powders and
- liquids

consisting of

- polymers,
- ceramics, glass and
- metals.

With these methods the following characteristics can be obtained:

- wettability
- adsorption
- corrosion
- film thickness
- adhesion
- purity
- roughness
- chemical composition
- electrical surface resistance
- electrical charging effects

By cooperations with various analytical laboratories, we can even provide analyses we do not have on hand warranting our own quality standards.

Accreditation

In order to guarantee our clients the best available quality, and thus to fulfill their requirements at the highest level, a quality management system was introduced in the Fraunhofer IGB laboratories. Linked to that, the analysis procedure electron spectroscopy for chemical analysis (ESCA) has been accredited by an international recognized accreditation body according to DIN EN ISO/IEC 17025.

Contact

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Figure 3: ESCA – electron spectroscopy for chemical analysis – enables the exact determination of the chemical composition of a surface (Kratos, Axis Ultra).

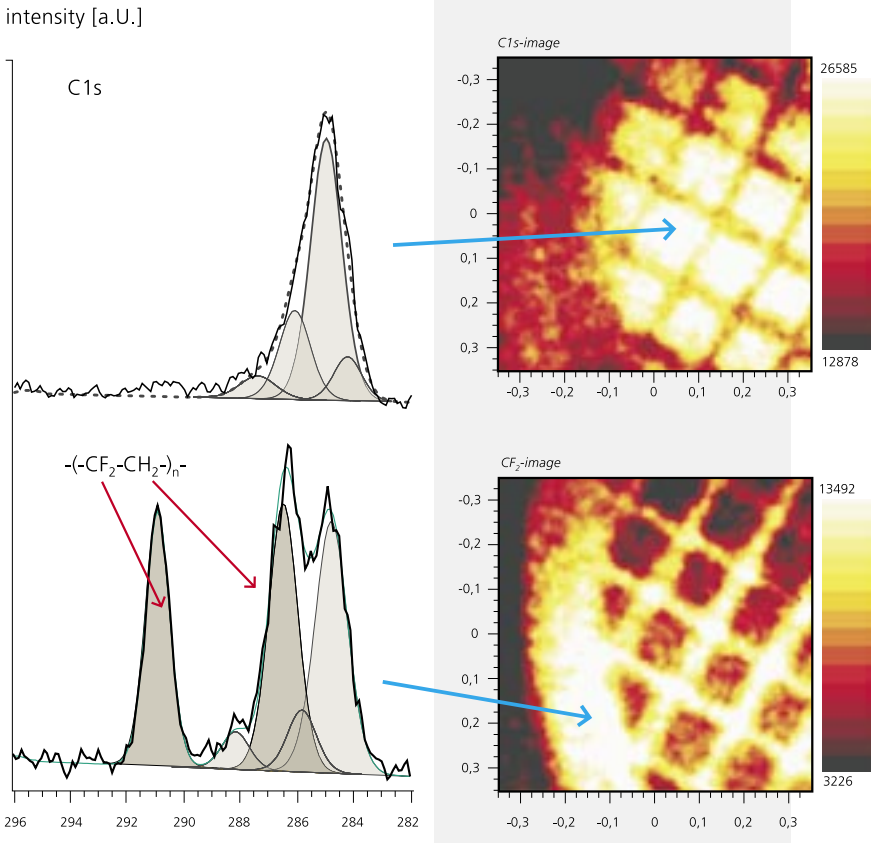

Figure 4: ESCA-C1s spectra and ESCA images of structurized coated PVDF.

Consulting in waste reduction and environmental management

A steadily growing number of manufacturing companies have realized the economic cost-effective benefits resulting from controlled use of chemicals and environmentally friendly treatment or disposal of waste and waste water.

Environmental management in combination with quality management is an excellent means to optimize the use of resources and to reduce costs. The Fraunhofer IGB can show you how to integrate the methods of environmental/quality management in your company and can help you to carry out the so-called »Legal Compliance« review in order to fulfill all relevant environmental regulations.

Furthermore, we can help you with specific consulting regarding the particular environmental aspects of your company, e.g. waste management, storage and disposal of waste materials.

Our know-how

The Fraunhofer IGB can solve your problems concerning environmental management. An analysis of the actual condition in environmental protection is the basis for an operational environmental management system. We examine whether the plants and the assigned technologies correspond to the state of the art and whether the legal requirements are fulfilled. Raw material and energy-saving potentials are assessed in form of an environmental balance.

Together with the company management the specified condition is defined and the environmental policy of the enterprise is formulated. The comparison of the being and actual condition shows the weak points. In cooperation with a project team of the enterprise we discuss solutions and concepts. We have also an access to the broad know-how of other Fraunhofer specialists.

The Fraunhofer IGB will inform you about the conditions of the »Best Available Technology«.

Subsequently, we compile an environmental program for the improvement of operational environmental protection. The environmental management system supports the continuous improvement process. In addition in the form of interface plans organizational structures and responsibilities are specified for the tasks in the operational environmental protection organization. Methods and work instructions are developed for environmental relevant processes.
An effective and vivid environmental management system can be achieved by the inclusion of the co-workers of the enterprise into operational process. We can also support you with certifying according to DIN/EN/ISO 14001 or with validating after the EEC ecological audit regulation EMAS (eco management and audit scheme).

Environmental management systems

Environmental management systems are available for enterprises, which would like to obtain a systematic and continuous evaluation and improvement of their environmental efforts, such as DIN/EN/ISO 14001 or EMAS II. The sole responsibility of the enterprises for environmental protection is placed under the motto »sustainable development«. The main idea of the EEC environmental audit regulation is »preventing, the decrease and, as far as possible, the removal of the environmental impact from its origin, on the basis of the causer pays principle as well as a good management of the resources of and the use of clean or cleaner technologies.«

Services

Hazardous compounds

- Analysis of internal company matters concerning hazardous wastes and environmental problems
- Management/handling of hazardous compounds – implementation of relevant guidelines in your company
- Laboratory ventilation – energy-saving by estimated use
- Analysis and evaluation of the working environment
- Environmentally friendly design of chemical reactions

Waste

- Waste management: internal company concepts and waste balances
- Conversion of LAGA waste index to EEC waste index
- Consultation in the processing/ treatment and temporary storage of hazardous wastes

Waste water

- UV-photochemical activated wet oxidation (H_2O_2, ozone) of waste water containing organic compounds
- Chemical elimination and treatment of hazardous compounds

Environment

- Serviceable guidance in the establishment of an environmental and quality management system or integration in an already existing quality management system

Contact

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Fraunhofer IGB is equipped with a modern storage for chemicals and hazardous substances of regional significance.


Dipl.-Biol. Marc Röhm
Dipl.-Biochem. Christian Schmalz
Fraunhofer IGB
Dipl.-Ing. Ines Westphal
Fraunhofer IBMT

Events

Trade fairs

Awards

Names, dates, events 2002

Cooperations

Outlook 2003/2004

Publications

Marc Röhm's diploma thesis was awarded the 1st prize.

Two Hugo Geiger prizes 2002 go to Marc Röhm and Christian Schmalz

The first 2002 Hugo Geiger prize of the Fraunhofer-Gesellschaft went to the up-and-coming biologist Marc Röhm from Fraunhofer IGB. Röhm has investigated four proteins that are responsible for the development of the hyphae in the pathogenic fungus *Candida albicans*. If we know the molecular basis of the infection mechanisms, it is possible in the long term to develop specific and more compatible medicines against fungal infection that block in a targeted way the proteins of the pathogen that are responsible for its virulence. Previously used active agents kill the fungus or limit its growth, but at the same time make it increasingly resistant. Marc Röhm's diploma thesis is part of a research project for investigating the proteome of *Candida albicans*, to which the research group »Automated Protein Screening Systems« of Fraunhofer IGB has devoted itself since 1998 (see page 38 for the project report).

Christian Schmalz, an up-and-coming chemist from Fraunhofer IGB in Hannover, was awarded a second prize. With the aid of a genetically engineered enzyme, a chitin deacetylase, he succeeded in making the manufacture of the popular natural substance chitosan, a breakdown product from the chitin exoskeletons of prawns, more pure and mild than before. To do this, he first of all isolated in samples taken from the sea bed genes from enzymes out of bacteria which are known to break down chitin. Via homologous sequences of different known genes, he has identified a new chitin deacetylase and cloned it in bacteria cells. The new enzyme can supply chitosan in a highly pure form without harming the environment. A patent application has been lodged for the gene (see project report on page 50).

With the Hugo Geiger prize, the Bavarian state government recognizes excellent and application-orientated diploma and doctorate theses in biological sciences. The work must be directly related to a Fraunhofer Institute. The prize was awarded for the first time in 1999. The person after whom the prize was named, Hugo Geiger, was patron at the founding event of the Fraunhofer-Gesellschaft on 26th March 1949.

Christian Schmalz: Winner of the 2nd Huger Geiger prize in 2002.

The EMTEC prize goes to Mirjam Kilgus

On 12th July 2002, Mirjam Kilgus was awarded the EMTEC Magnetics prize, worth 500 Euros, for her thesis on »metallic membranes for hydrogen separation« at Fraunhofer IGB. Very pure hydrogen is required for supplying fuel cells in order to prevent a decrease in efficiency and in order to increase the life of the cells. Metal membranes, particularly palladium membranes, are regarded as ideal separation systems for obtaining such high-purity hydrogen. As part of her degree thesis, Mirjam Kilgus investigated the production of impervious palladium/copper coats on porous hollow fiber carriers α-Al_2O_3. These thin coats display good stability and separation capacity. The economical arrangement of thin membrane coats on ceramic hollow fibers covers a wide range of applications in the production of pure hydrogen for both stationary and mobile fuel cell applications, as well as for applications in the chemicals industry.

Girls get a taste of science

The Germany-wide »Girls' Day« on 25th April 2002 promoted by the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung) offered female students between 15 and 18 years insights into the working world of science and research. 74 female students from 15 grammar schools came to the Fraunhofer Institutes Center (IZS) in Stuttgart. In the biological laboratory of Fraunhofer IGB, eight girls familiarized themselves with molecular biology working methods under the title »Making genetic make-up visible«: Experiments and information were used as potential recruiting tools for a career as a scientist. One female student summarized that »no lengthy presentations, but active participation« was the most interesting thing. The feedback was extremely positive: 88 per cent found the Girls' Day at least good, and of these as many as 44 per cent felt that it was very good. Half of the girls spontaneously expressed an interest in a work experience placement at the Fraunhofer-Gesellschaft.

Female student using a pipette in the biological laboratory of Fraunhofer IGB.

The biologist Dr. Christiane Buta from Fraunhofer IGB informs female students about the working world laboratory.

Trade fairs and exhibitions

Medtec Exhibition and Conference 2002
Europe's Premier Event for the Medical Device Industry
5-7 March 2002,
Stuttgart

IFAT 2002
13th International Trade Fair for Environment, Waste Water and Waste Disposal:
Water, Sewage, Refuse and Recycling
13-17 May 2002,
Munich

Erde 2.0
Exhibition of the Ministry of Environmental Affairs and Traffic of the State of Baden-Württemberg
15 June - 28 July 2002,
Stuttgart

Bio & Business 2002
Biotechnology Baden-Württemberg towards the World Market
27 June 2002,
Heidelberg

BioDigital 2002
International Trade Fair and Congress for Biotechnology, Bioinformatics and Microarrays
9-11 October 2002,
Freiburg im Breisgau

Workshops and seminars at Fraunhofer IGB

7th Meeting for Municipal Waste and Waste Water Treatment »Technologies for the Future«
7 March 2002,
Fraunhofer Institutes Center, Stuttgart

9th Symposium Trends in Membrane Technology:
»Gas Separation with Membrane Processes«
29-30 April 2002,
Fraunhofer Institutes Center, Stuttgart

GuTec mbH – Systems for environmentally-friendly decontamination

GuTec mbH, a company for environmentally-friendly technologies, develops mobile systems for the removal of hazardous compounds from industrial sewage.

Organic hazardous compounds, heavy metals and radioactivity are removed both, economically and in an environmentally friendly way, with the aid of bio-adsorbents from chemically modified bran. In each case, the concentration of the contaminants fell below the target values of the environmental and water authorities.

In addition to the development and construction of mobile decontamination systems, GuTec mbH offers its clients licenses and consultation services concerning all aspects of removal of hazardous compounds. In July 2002, the company was entered in the commercial register of the town of Mosbach in the Odenwald region. The managing director is Dr. Günther Mann.

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E-Mail: GUTecmbH@aol.com

Upcoming trade fairs and exhibitions

2nd International Symposium on Molecular Diagnostics and Skin Gene Therapy
27-29 March 2003,
Düsseldorf

ACHEMA 2003
27th International Exhibition-Congress on Chemical Engineering, Environmental Protection and Biotechnology
19-24 May 2003,
Frankfurt/Main

Innovationsbörse Gesundheitstechnologien
22 May 2003,
Stuttgart

Strategies for Organ Repair International Conference
1-2 July 2003,
Erlangen

BioTechnica 2003
International Trade Fair for Biotechnology
7-9 October 2003,
Hannover

Envitec 2004
International Trade Fair Environmental Technology and Services
17-19 February 2004,
Düsseldorf

K 2004
International Trade Fair Plastics and Rubber
20-27 October 2004,
Düsseldorf

Upcoming workshops and seminars at Fraunhofer IGB

8th Meeting for Municipal Waste and Waste Water Treatment »Technologies for the Future«
10 April 2003,
Fraunhofer Institutes Center, Stuttgart

10th Symposium »Trends in Membrane Technology«
Date will be announced,
Fraunhofer Institutes Center, Stuttgart

Details may be subject to alterations.

Get further information here:

www.igb.fraunhofer.de

03

Scientific cooperations

Cooperations with universities

Escola de Engenharia de Piracicaba (EEP), Brazil

Escola Superior de Agricultura »Luiz de Queiroz« (ESALQ), Brazil

Harvard Medical School, Boston, MA, USA

Ludwig Institute for Cancer Research, Stockholm, Sweden

Pennsylvania State University, University Park, PA, USA

Technical University of Darmstadt

Technical University of Munich

School of Veterinary Medicine of Hannover

Universidade Metodista de Piracicaba (UNIMEP), Brazil

University of Kassel

University of Gießen

University of Hohenheim

University of Nürnberg-Erlangen

University of Regensburg

University of Stuttgart

University of Tübingen

University of Würzburg

University of Amsterdam, NL

University of Bratislava, Slowak Republic

University of Kent, UK

University of Nottingham Medical School, UK

University of Pittsburgh, PA, USA

Cooperations with Fraunhofer Institutes

Fraunhofer Institute for Applied Polymer Research IAP, Teltow

Fraunhofer Institute for Applied Materials Research IFAM, Bremen

Fraunhofer Institute for Manufacturing Engineering and Automation IPA, Stuttgart

Fraunhofer Institute for Silicate Research ISC, Würzburg

Fraunhofer Institute for Solar Energy Systems ISE, Freiburg

Fraunhofer Institute for Systems and Innovation Research ISI, Karlsruhe

Fraunhofer Institute for Environmental, Safety and Energy Technology IUSE (UMSICHT), Oberhausen

Fraunhofer Institute for Process Engineering and Packaging IVV, Freising

Fraunhofer Institute for Reliability and Microintegration IZM, Berlin

Fraunhofer Life Sciences Alliance, in cooperation with the Fraunhofer Institute for Biomedical Engineering IBMT, St. Ingbert, the Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, and the Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover

Fraunhofer Alliance for Cleaning Technology, in cooperation with Fraunhofer FEP (Electron and Plasma Technology, Dresden), Fraunhofer ICT (Chemical Technology, Pfinztal), Fraunhofer ILT (Laser Technology, Aachen), Fraunhofer IPA, Fraunhofer IPK (Production Systems and Design Technology, Berlin) Fraunhofer IST (Thin Films and Surface Engineering, Braunschweig), Fraunhofer IWS (Material and Beam Technology, Dresden)

Fraunhofer Alliance Polymer Surfaces (POLO), in cooperation with the Fraunhofer Institutes FEP (Electron and Plasma Technology, Dresden), IAP (Applied Polymer Research, Golm), IFAM, IPA, ISC and IVV

WISA Project »Development of novel inorganic membranes«, in cooperation with the Fraunhofer Institute for Ceramic Technologies and Sintered Materials IKTS, Dresden, the Fraunhofer ISC and the Fraunhofer Institute for Mechanics of Materials IWM, Freiburg

Cooperations with research organizations

Bundesanstalt für Materialforschung und -prüfung (BAM)

Dalian Institute of Chemical Physics, Dalian, China

Deutsches Krebsforschungszentrum, Heidelberg

Deutsches Zentrum für Biomaterialien und Organersatz, Stuttgart-Tübingen

Gesellschaft für Biotechnologische Forschung GBF, Braunschweig

Imperial Cancer Research Council (JCRF), London

Institut für Textilchemie ITC, Denkendorf

Institut für Textil- und Verfahrenstechnik ITV, Denkendorf

Max-Planck-Institut für Biochemie, Martinsried

Max-Planck-Institut für Festkörperforschung, Stuttgart

Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Golm

Max-Planck-Institut für Metallforschung, Stuttgart

Max-Planck-Institut für Molekulare Physiologie, Dortmund

Max-Planck-Institut für Polymerforschung, Mainz

Naturwissenschaftlich-Medizinisches Institut NMI, Reutlingen

Niedersächsisches Institut für Peptidforschung, Hannover

Stanford University Department of Chemical Engineering

Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Cooperations with hospitals

Blutspendezentrale, Katharinenhospital, Stuttgart

Charité Berlin

Katharinenhospital, Stuttgart

Marienhospital, Stuttgart

Olgahospital, Stuttgart

Orthopädische Klinik, Aachen

Robert-Bosch-Krankenhaus, Stuttgart

Universitätsklinik, München

Universitätsklinik, Tübingen

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Bundesministerium für Bildung und Forschung (BMBF), BioChance, Nachhaltige Bioproduktion,
Evaluation Committee

COST Action 527 of the EU: Plasma Polymers and Related Materials,
Management Committee, Vice Chairman

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Senatskommission für Grundsatzfragen der Gentechnik, Consultant

Deutsche Gesellschaft für Chemische Technik und Biotechnologie e. V. (DECHEMA):
Interdisziplinärer Arbeitskreis,
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Member;
Fachausschuss »Sicherheitstechnik«,
Member;
Fachausschuss »Elektrostatic Charging«,
Member;
Fachausschuss »Environmental biotechnology«,
Member

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Scientific Board

European Interferon Meeting Committee, Hannover,
Scientific Board

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Journal of Biomaterials Science, Polymer Edition (VSP, Utrecht, Tokyo),
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Supervisory Board

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Vakuum in Forschung und Praxis, WILEY VCH Verlag GmbH,
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Vereinigung für Allgemeine und Angewandte Mikrobiologie e. V. (VAAM):
Council Biotransformation, Member;
Council Umweltmikrobiologie, Member

Lectures and seminars

Brunner, H.
»Management von Forschung und Entwicklung in der Biotechnologie«,
University of Stuttgart

Brunner, H., Oehr, Ch., Tovar, G.
»Membran- und Grenzflächenverfahrenstechnik in der Biomedizin und Biotechnologie«,
University of Stuttgart

Brunner, J., Tovar, G.
»Medizinische Verfahrenstechnik«,
University of Stuttgart

Bryniok, D.
»Ausgewählte Kapitel der Mikrobiologie«,
University of Stuttgart

Johannes, F.-J. †
Practical course »Zellbiologie«,
University of Stuttgart

Johannes, F.-J. †
»Grundlagen der Zellbiologie«,
University of Stuttgart

Otto, B.
»Biochemie und Genetik der Interferone«,
School of Medicine Hannover

Otto, B.
Graduate seminar »Charakterisierung von regulatorischen Peptiden und ihrer Zielproteine«,
School of Medicine Hannover

Otto, B., Zakaria, H.
Ph.D. course »Grundlagen der Molekularbiologie«,
School of Veterinary Medicine Hannover

Otto, B., Zakaria, H.
Graduate seminar »Molekularbiologie und Proteindesign«,
School of Veterinary Medicine Hannover

Rupp, S.
Practical course biochemistry for biology and chemistry students,
University of Stuttgart

Rupp, S.
»Moderne Methoden in der Biochemie«,
University of Stuttgart

Sternad, W.
»Automatisierungstechnik«,
University of Hohenheim

Trösch, W.
»Umweltbiotechnologie und Nachhaltigkeit«,
University of Hohenheim

Trösch, W.
»Wasser-, Abwasser- und Abfallbehandlung«,
University of Hohenheim

Zakaria, H.
»Bioinformatik I«,
University for Applied Sciences Oldenburg/Ostfriesland/Wilhelmshaven

Ph.D. and diploma theses, student research studies

Ph.D. theses

Degen, J.
Entwicklung eines Photobioreaktors mit verbesserter Lichtausnutzung für Mikroalgen
University of Hohenheim

Flieger, O.
Untersuchungen zum unkonventionellen Sekretionsweg des Zytokins Makrophagen-migrationsinhibierender Faktor (MIF)
University of Stuttgart

Hoffmann, C.
Selbstorganisierende Organosilansysteme zur Funktionalisierung oxidischer Siliziumoberflächen für die gerichtete Peptid- und Proteinimmobilisierung
University of Stuttgart

Holler, S.
Reinigung kommunaler Abwässer in einem Membranbioreaktor mit hoher Zelldichte
University of Stuttgart

Rottmann, M.
Untersuchungen zur Identifizierung von Komponenten morphogenetischer Signaltransduktionswege in dem humanpathogenen Pilz *Candida albicans*
University of Stuttgart

Diploma theses

Echard, A.
Untersuchung eines energieoptimierten Filters in der kommunalen Abwasserreinigung
Institut Polytechnique de l' Université d'Orléans, Ecole Supérieure de l' Energie et des Matériaux

Fünfzig, H.
Zellzyklusabhängige Lokalisation des MIF-Jab1-Komplexes
University of Stuttgart

Gugg, J.
Eliminierung von Knarzgeräuschen von Kunststoffbauteilen im Innenausstattungsbereich von Kraftfahrzeugen durch Veränderung der Oberflächeneigenschaften mittels Plasmabehandlung
Technical University of Dresden

Kilgus, M.
Metallische Membranen für die Wasserstoffabtrennung
University of Applied Sciences Offenburg

Knecht, S.
Mikrostrukturierte Anlagerung biofunktionalisierter Nanopartikel mittels Photolithographie, Mikrokontaktstempeln und Mikroarrayer auf Glas-, Silizium- und Goldoberflächen
University of Stuttgart

Lang, R.
Verfahrenstechnische Untersuchungen an einem Festbett-Umlauffilter zum Einsatz im Bereich der kommunalen Abwasserreinigung
University of Applied Sciences Mannheim

Laug, A.
Anaerober Abbau von Abwässern mittels eines Festbett-Umlaufreaktors
University of Applied Sciences Hamburg

Lebreton, M.
Délétion du gène YWP1 de *Candida albicans,* un micro-organisme pathogène pour l´homme
Ecole Polytechnique Universitaire de Lille

Lenain, E.
Biologische Abreicherung von Schwermetallen in Kühlschmierstoffen
Ecole Nationale Superiéure de Chimie de Lille

Popp, A.
Untersuchungen zum anaeroben Abbau und zur Stickstoff-Elimination von synthetischen Abwässern mittels Membranbioreaktoren
University of Applied Sciences Mannheim

Spotka, T.
Beitrag zur Entwicklung eines Membranbioreaktors zur kommunalen Abwasserreinigung – Untersuchungen zum Sauerstofftransport
Technical University of Berlin

Steitz, B.
Präparation und Charakterisierung von ultradünnen Schichten aus biofunktionalisierten Nanopartikeln mittels der Wellenleiterspektroskopie, Ellipsometrie und Rasterkraftmikroskopie
Technical University of Berlin

Storn, V.
Untersuchungen zur Synthese molekular geprägter Nanopartikel mittels Miniemulsionspolymerisation unter Verwendung der Monomere Styrol, Methacrylsäureamid und Ethylmethacrylsäureamid
University of Applied Scienes Albstadt-Sigmaringen

Thiele, M.
Klonierung und Charakterisierung von MIF-Penetratin-Fusionsproteinen
University of Stuttgart

Thomas, T.
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University of Hannover

Wind, A.
Etablierung eines Protokolls zur Verwendung neuartiger Fluoreszenzfarbstoffe in der DNA-Array-Technologie
University of Applied Scienes Aachen

Zeller. K.
Detektion von Punktmutationen im humanpathogenen Pilz *Candida albicans* mittels DNA-Chiptechnologie
Europe University of Applied Scienes Fresenius

Student research studies

Merz, I.
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University of Stuttgart

Pfeiffer, J.
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University of Applied Sciences Heilbronn

Schneider, C.
Untersuchung organischer Passivierungsschichten auf Kupfer zum bleifreien Löten
University of Stuttgart

Wieland, A.-M.
Anreicherung der Dihydroliponamiddehydrogenase aus Katzenhaien
University of Stuttgart

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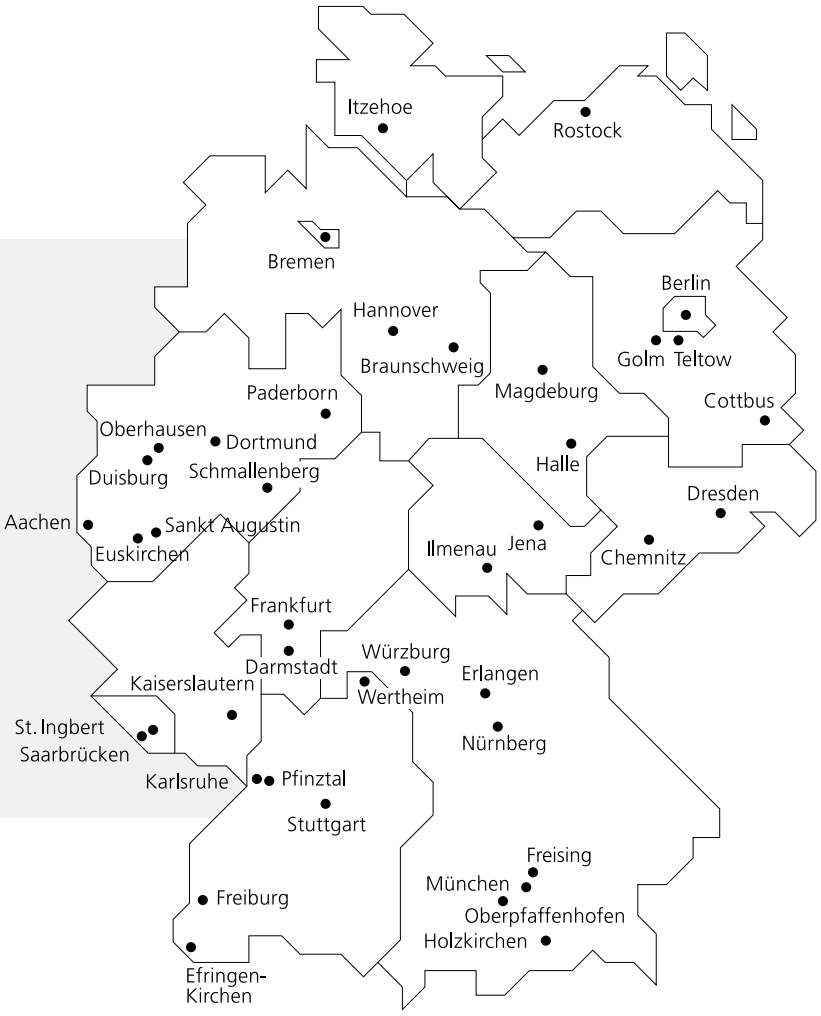

Locations of the research facilities in Germany.

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By train: At Stuttgart main station take city rail (S-Bahn) lines 1, 2 or 3 direction Stuttgart-Vaihingen. Leave at stop »Universität« and take exit »Wohngebiet Schranne/Endelbang/Nobelstraße«. Follow the signs »Fraunhofer-Gesellschaft«. The walking distance is about 600 m. You can also take the bus lines 84 or 92 up to stop »Nobelstraße«. From Stuttgart main station you will need about 20 minutes, the walking time is about 10 minutes.

By air: At Stuttgart airport take the city rail (S-Bahn) lines S2 or S3 towards Stuttgart-Vaihingen / Main Station. Leave at stop »Universität« then walk or go by bus as described above. Or you take a taxi, the distance is about 16 km, driving time 20 minutes.

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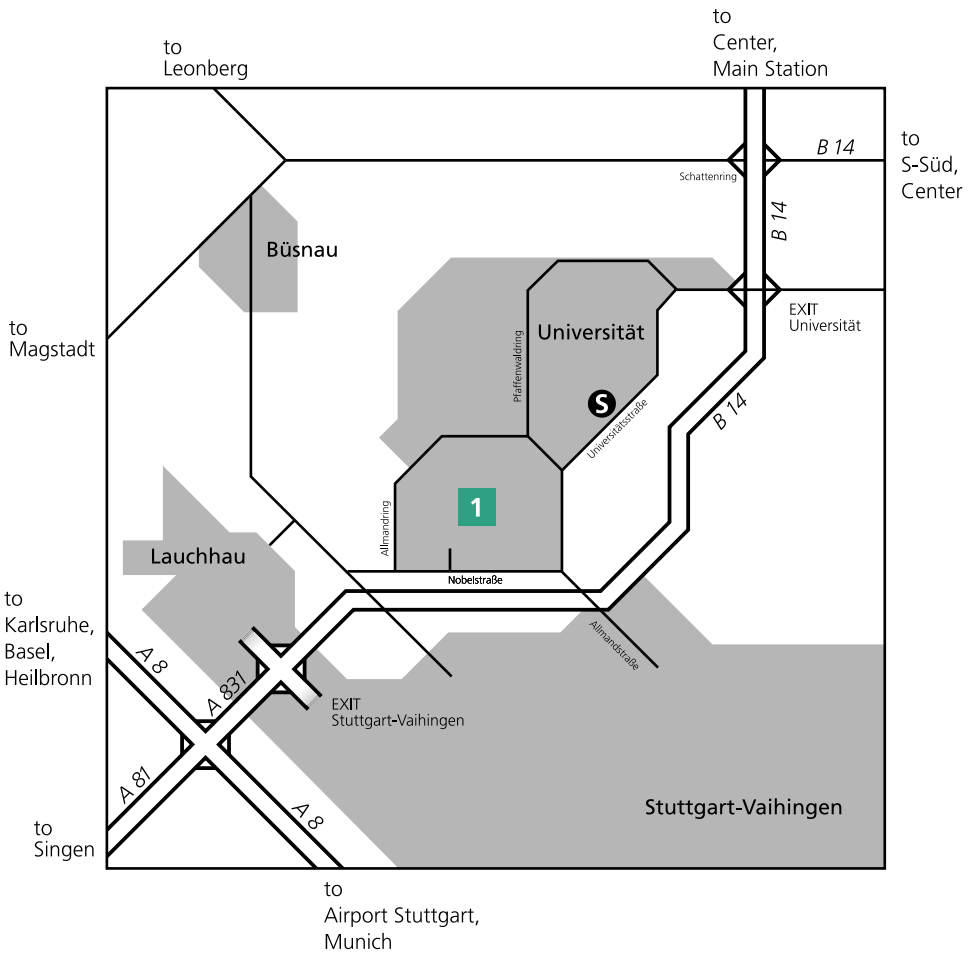

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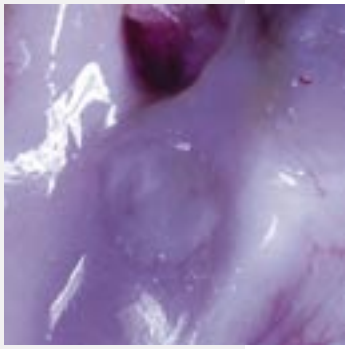
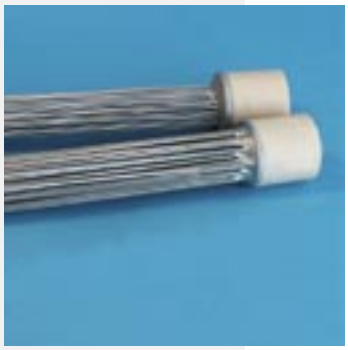
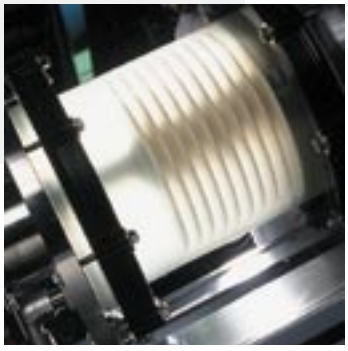


Cartilage transplant, four weeks after implantation in mini pig. At Fraunhofer IGB three-dimensional cell structures are developed as test systems or as tissue engineering products. For the latter, a production has been established in compliance with GMP guidelines.



Cartridges made of ceramic capillary membranes for gas separation (area 0,1 m^2, 2 µm palladium coating, temperature stable up to 600 °C). These modified membranes have separation coefficients for hydrogen of bigger than 1 000 and thus are ideal for the separation/purification of hydrogen. These membrane modules are especially useful for high temperature applications or for the filtration with high solvent loads.



Rotating disk filter for energy-saving filtration of waste water. The filter therefore is suited for the application in urban sewage treatment.