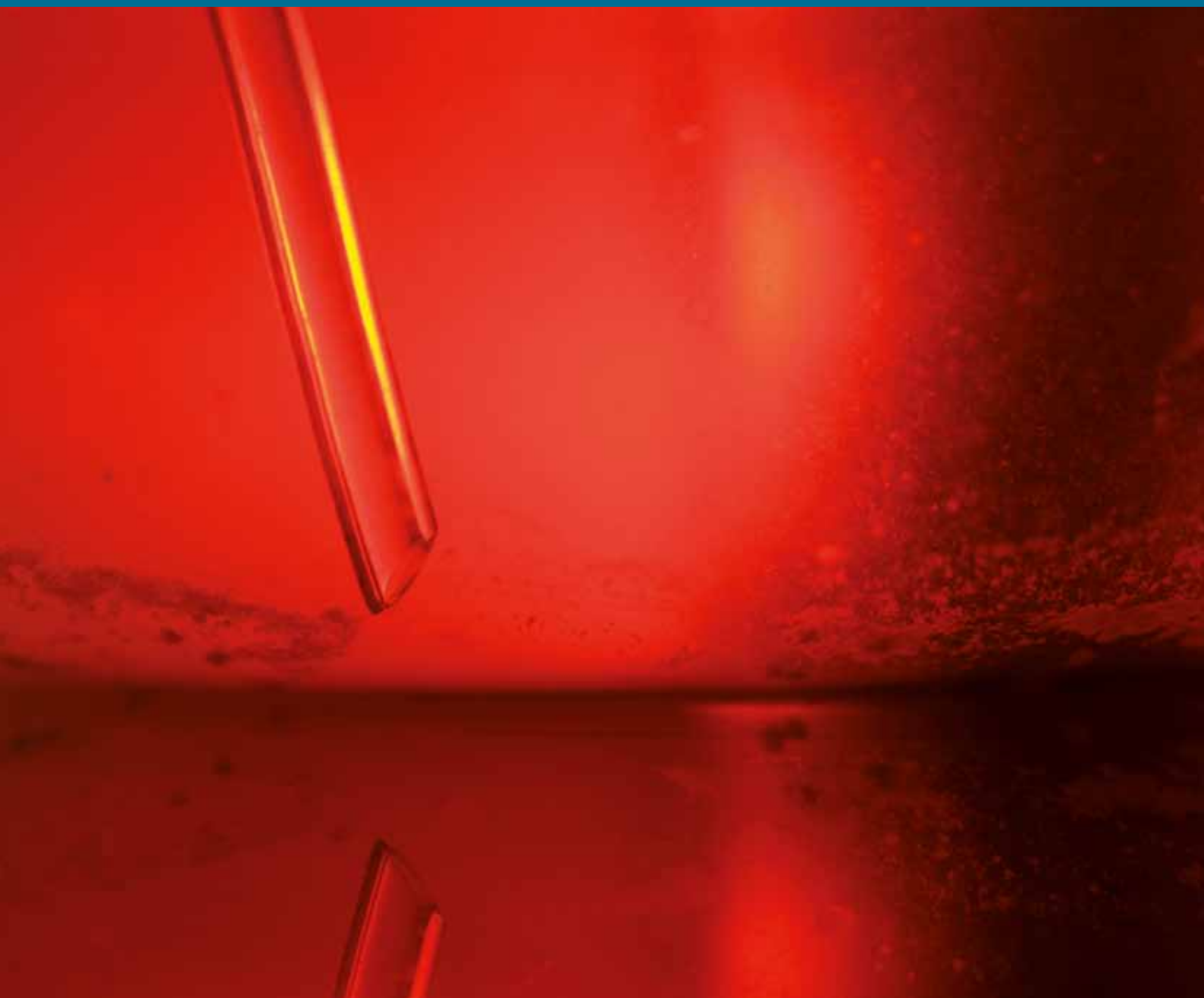
Fraunhofer

INVESTIGATIONAL BIOPHARMACEUTICALS



INVESTIGATIONAL BIOPHARMACEUTICALS

The Fraunhofer-Gesellschaft is known for its outstanding and distinctive principle: translation of scientific research into industrial applications.

The production of investigational biopharmaceuticals is a rapidly evolving business that requires up-to-date knowledge in all the disciplines involved. Almost 1000 biopharmaceuticals are currently being tested in clinical trials, and the number of candidates in pre-clinical research is orders of magnitude higher. All pre-clinical and clinical investigational medicinal products (IMPs) require the availability of drug substances in GMP (clinical use) or almost identical quality (pre-clinical use).

The collaboration of two Fraunhofer institutes from the Fraunhofer Group for Life Sciences (VLS) – Fraunhofer IGB and Fraunhofer ITEM – gives clients access to nearly every step involved in a biopharmaceutical manufacturing process – from the gene to the clinical product.

This brochure gives an introduction to the combined fields of expertise and services as well as a reference list and contact details for further information.

The Fraunhofer IGB and the Fraunhofer ITEM provide combined and synergistic expertise in cost-effective bioprocess development and corresponding analytical methods. This includes a broad range of techniques, from gene synthesis to protein expression and purification from lab scale to large scale. Production organisms can be both bacterial and mammalian cell lines. The range of services also includes optimization and scale-up of cultivation and purification processes, and maximization of yields and purity to address pharmacological safety aspects.

The GMP facility of the Fraunhofer ITEM enables manufacturing of investigational biopharmaceutical ingredients and includes aseptic fill and finish to dosage forms based on infusion bags, ampoules and vials.

Services

- Strain and cell line development
- Mammalian cell cultures
- Microbial cultivation
- Downstream processing
- GMP manufacture of investigational biopharmaceuticals
- Protein analytics
- Cell-based assays
- Formulation
- Aseptic filling
- Therapeutic proteins

STRAIN AND CELL LINE DEVELOPMENT

A large share of biopharmaceuticals currently on the market are produced by genetic engineering using various recombinant expression systems. Most of the recombinant proteins that have gained marketing approval to date are produced either in recombinant prokaryotes, mainly in *Escherichia coli (E. coli)* or, more dominantly, in recombinant mammalian cells.

We offer technologies for both – prokaryotic and mammalian systems for the development of a robust and reproducible process. This includes strain development, the optimization of fermenter processes at lab scale and the facilitated scale-up of the process to production levels.

Prokaryotic expression systems

The different microorganisms (bacteria and fungi) provide an attractive expression system for therapeutic proteins. They can usually be cultured in large quantities, inexpensively, and in a short period of time by standard fermentation techniques.

Eukaryotic expression systems

Mammalian cells have become the dominant system for the production of recombinant proteins. They offer several advantages for the expression of heterologous proteins, since transcription, translation, and post-translational modification processes are conserved among higher eukaryotes. Manufacturing recombinant proteins at industrially relevant levels requires technologies that can engineer stable, high-expressing cell lines rapidly, reproducibly, and with relative ease. Besides the conventional establishment of producer cell lines, such as the DHFR and GS systems, we offer a specialized method for fast and robust generation of mammalian producer cell lines (see next page, Special focus: Development of high-producing mammalian cell lines).

Services

Prokaryotic expression systems:

- Expression plasmid design
- Species-specific codon optimization
- Efficient regulatory sequences
- High amplification
- Batch and fed-batch fermentations
- Media design
- Facilitated scale-up
- Process optimization

Eukaryotic expression systems:

- Transient expression in 293T cells
- Stable expression in CHO-K1, BHK, and 293T cells
- Cloning in expression and targeting plasmids
- DHFR and GS expression systems in CHO cells
- Amplification with MTX or MSX
- Targeting in proprietary TagCHO cell line
- Verification of RMCE
- Suspension-adapted host cell line
- Adaptation to protein-free media
- GMP manufacture of master and working cell banks

SPECIAL FOCUS:
DEVELOPMENT OF HIGH-PRODUCING MAMMALIAN CELL LINES

The pharmaceutical market today is under intense cost and time-to-market pressure. The adherence to predetermined cost and time lines often decides about failure or success of a project. Conventional procedures for the establishment of a production cell line rely on the random integration of the gene of interest (GOI) into the genome of the host cell line. However, this approach is very time-consuming and takes up to 18 months for the establishment of a producer cell line.

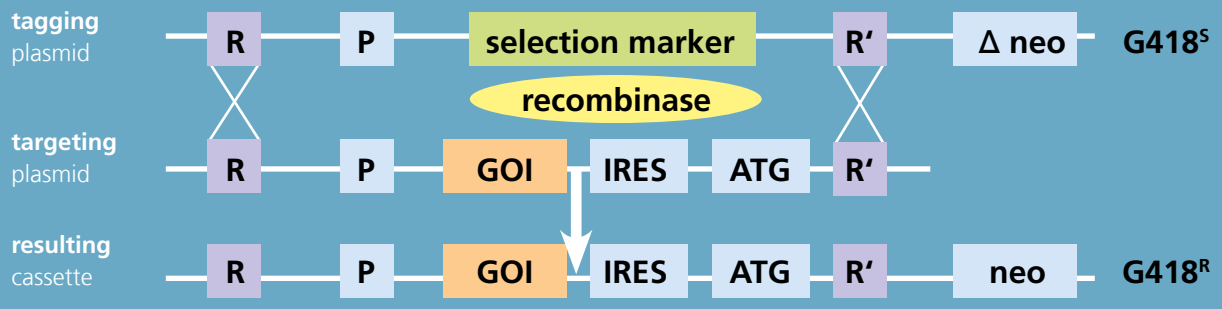
To overcome the long generation time, both institutes work in parallel on the establishment of different systems, using several cell lines (e.g. CHO, BHK and HEK). These systems (e.g. RMCE) allow the generation of mammalian cell lines leading to reproducible productions with high yields at a short generation time.

RMCE technology for establishment of high-producing CHO cells

The current development focus for biopharmaceuticals is on antibodies in CHO cells. Normally, the conventional approach for establishment of CHO producer cell lines takes up to 18 months. Using the RMCE (recombinase-mediated cassette exchange) technology, we have established a proprietary CHO cell line (TagCHO) which reduces the time required to generate a suitable production CHO cell line to 6 weeks.

TagCHO is a platform for integration of genes of choice into a defined chromosomal environment with predictable expression. It was especially designed for production of antibodies, but can also be used for other proteins. TagCHO cell lines are established by an extensive screening step in which suitable chromosomal loci are identified and marked by integration of specific recognition sequences for a recombinase enzyme by a tagging cassette. The TagCHO tagging cassette can be exchanged via a recombinase with a new targeting cassette carrying your target gene. So far, the cassette exchange has been successfully implemented for the production of human antibodies, for example IgG1 and IgG2.

Principle of recombinase-mediated cassette exchange (RMCE)



The tagging and targeting plasmids carry two different recombinase sites (R and R´). At these sites, the recombinase cuts and introduces the target sequence. After successful targeting, the resulting cassette becomes G418-resistant.

MAMMALIAN CELL CULTURES

Today, recombinant mammalian cells are mainly used for the production of complex protein APIs because of their ability to produce post-translational, human-like modifications. The focus of our Animal Cell Culture Group is on the development of robust and reliable cultivation processes, scale-up from lab to pilot scale, validation of critical parameters, and GMP manufacture of investigational therapeutic proteins.

Protein targets are mainly recombinant glycoproteins and antibodies. The group has at its disposal the technical equipment required for all common process steps.

Services

- Screening of production cell clones and cell lines
- Adaptation to suspended-culture and serum- and protein-free conditions
- GMP manufacture and storage of cell banks
- Process development for batch, fed-batch, and perfusion cultivations in lab-scale bioreactors
- Optimization of cultivation processes for high product yields
- IPC analytics of glucose, lactate, ammonia, amino acids and ions, FACS analysis, HPLC for protein and impurity quantification (SEC and IEC)
- Scale-up of batch and fed-batch cultivations up to 400 L, perfusion cultivations up to 50 L
- Development or adaptation of media suitable for fed-batch or perfusion cultivations
- Validation studies for identification and characterization of critical parameters
- GMP manufacture of clinical-grade APIs

Methods and equipment

- Cell retention devices: spin filter, alternate tangential flow (ATF), acoustic settler (BioSep), cell settler (gravitational), centrifuge
- Cultivation systems: culture flasks, spinner flasks, shake flasks, single-use bioreactor (Wave) up to 30 L, stirred-tank reactors from 2 to 400 L
- Culturing techniques: batch, repeated batch, fed-batch, perfusion, repeated perfusion
- Cell lines: CHO, BHK, HEK, Hybridoma, HL60, CAP (human amniocyte cell line), insect cells (Hi-5 and SF9)

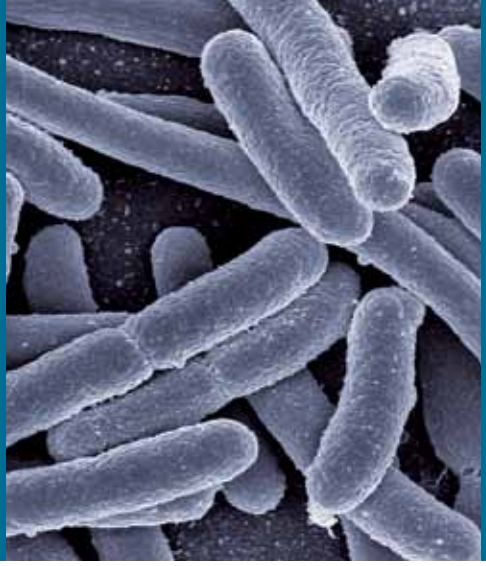
SPECIAL FOCUS:
ANTIBODY PRODUCTION PLATFORM

Since the 1990s, antibodies have emerged as important therapeutics, leading to a fast growing market. In addition to the requirement of efficient large-scale manufacturing processes, rapid production of APIs for early-stage clinical testing is a necessity to reduce costs.

Our Cell Culture Group has been placing special emphasis on the development of standardized procedures and protocols for antibody (API) production with recombinant mammalian CHO cell lines, the primary focus being on the reduction of development time.

Key elements are:

- Development of in-house media (including concentrated feed media) for broad application to different CHO cell lines
- Use of experimental design methods for rapid parallel evaluation of key fed-batch process parameters
- Standardized protocols for a range of different cell retention devices for use with perfusion cultivations
- XD™ technology for high cell densities and high antibody titers



MICROBIAL CULTIVATION

The focus in this subject area is on the development and performance of microbial cultivation processes including subsequent primary recovery. Core competences are the cultivation of microbial systems (bacteria, fungi, and yeasts) and the primary processing steps for the production of complex biologically active substances such as proteins, nucleic acids, vaccines, antibody fragments, and bacteriophages.

The portfolio includes the development of API manufacturing processes from lab scale to pilot scale including in-process and quality control, the investigation of single-unit operations from a biochemical engineering perspective, and the manufacture of APIs for research and development projects and for pre-clinical and clinical use.

Services

- GMP-compliant cell bank manufacture
- Development and scale-up of cultivation processes
- Development of batch and fed-batch strategies
- Development of processes for preparation of inclusion bodies
- GMP cultivation of microbes
- R&D tools: reaction kinetics, model development, and simulation of stationary and dynamic biological systems

Methods and equipment

- Bioreactors from laboratory scale up to 400 L
- Laboratory-scale single-use bioreactors (Wave system)
- Filtration (microfiltration, ultrafiltration, and sterile filtration)
- Centrifugation (steam-sterilizable solid-ejecting centrifuge, tubular centrifuge)
- Cell disruption (high-pressure homogenizer, chemical/enzymatic)

SPECIAL FOCUS:
PLASMID DNA (pDNA) PROCESSES

Plasmid DNA (pDNA) is the biopharmaceutical of the future for use in gene therapy and DNA vaccination. The major advantages over conventional gene therapeutic agents and vaccines are shorter time frames for production process development. This enables rapid availability for clinical trials. Moreover, compared to conventional vaccines, pDNA offers major benefits regarding aspects of patient safety. The key elements to be considered in the development of pDNA production processes are:

- Optimal vector design for efficient host replication
- Detailed process knowledge – to determine and define relevant and critical process parameters allowing robust, reproducible, and efficient production
- Validated analytics – to enable product characterization

At the Fraunhofer institutes doing biopharmaceutical contract research, these elements are considered simultaneously and are clustered into a platform technology that allows for nearly every possible construct to be produced in sufficient quantity and quality. GMP batches can generally be manufactured after a short development phase.

Key elements

- Host selection and transformation
- Model-based definition of process parameters
- Different scales of production
- Optimized cell separation

DOWNSTREAM PROCESSING

The focus here is on the development and scale-up of purification sequences for active (bio)pharmaceutical ingredients (APIs), i.e. the translation of laboratory-scale unit operations into technical scale and GMP-compliant production for clinical trials in phases I and II.

Targets have been various biopharmaceutical APIs, such as recombinant proteins (EPO, GCSF, and beta-interferon), antibodies and antibody fragments, plasmids, virus-like particles (VLPs), and phages. These products have been purified either from microbial or mammalian cells. Our Downstream Processing Group has at its disposal technical equipment required for the development and performance of all downstream process steps from lab scale to production scale.

Services

- Development and optimization of downstream processing sequences
- Development of renaturation processes
- Process scale-up to pilot or production scale
- GMP-compliant purification of biopharmaceuticals for clinical trials in phases I and II
- Validation of critical process parameters
- Validation of equipment cleaning procedures

Methods and equipment

- High-throughput technology for fast process development (HTPD)
- Protein refolding up to 1000 L scale
- Extraction with aqueous two-phase systems
- Precipitation
- Solid/liquid separation: centrifugation, cross-flow micro- and ultrafiltration (membrane area up to 6 square meters), dead-end filtration
- Chromatography: IEX, HIC, TAC, affinity, GPC, and mixed-mode
- Chromatographic equipment from lab scale to process scale: ÄKTAexplorer 10 and 100, ÄKTApilot, BioProcess, column diameters up to 30 cm
- Preparative HPLC

SPECIAL FOCUS:

PURIFICATION PLATFORMS FOR ANTIBODIES AND PLASMID DNA

Time-to-clinic and time-to-market are key factors for success. The faster the proof of principle can be provided in clinical trials, the higher the value of a candidate API. For this reason, a special focus has been placed on the development of robust standard procedures (platforms) for purification of antibodies and plasmids. Based on these platforms, only minor modifications at each purification step have to be made to develop processes for new antibodies or plasmids. Furthermore, conditions are optimized by high-throughput technology in 96-well plates for faster process development. The separation of impurities such as HCP, DNA, endotoxins, and viruses (in case of antibodies) is taken into account, as is the separation of product-related impurities such as aggregates and degradation products. All purification steps are selected and combined with a focus on high biological activity, a high level of purity, and few unit operations. Adequate analytical tests are implemented in our quality control unit.

Key elements

- Standardized purification sequences for antibodies and plasmids
- Optimized separation of plasmid isoforms, yielding a highly homogeneous (> 95% supercoiled) product
- Optimized separation of antibody aggregates and degradation products to yield > 95% homogeneous monomeric product
- GMP-compliant purification of g-quantities of antibodies and plasmids
- State-of-the-art analytics to determine residual impurities, plasmid isoforms, and antibody affinity (capillary gel electrophoresis (CGE), HPLC, real-time PCR, Biacore)
- Product characterization
- ICH stability tests

GMP MANUFACTURE OF INVESTIGATIONAL BIOPHARMACEUTICALS

Investigational medicinal products (IMPs) must be manufactured in compliance with European drug regulations and with the quality system of good manufacturing practice (GMP) to be used in clinical trials. When the drug substance, i.e. the pharmacologically active component of an IMP, is a complex biomolecule (a protein, glycoprotein, or nucleic acid), GMP quality attributes have to be taken into consideration already at the very beginning of development of the manufacturing process for the drug substance. For these kinds of drug substances the Pharmaceutical Biotechnology Division of the Fraunhofer ITEM covers the complete sequence of process development steps which are needed for an individual biopharmaceutical product candidate to progress from the idea to the clinical phase.

GMP operations can generally be initiated after approval of both generic and process-specific GMP requirements by the local and the federal regulatory authorities.

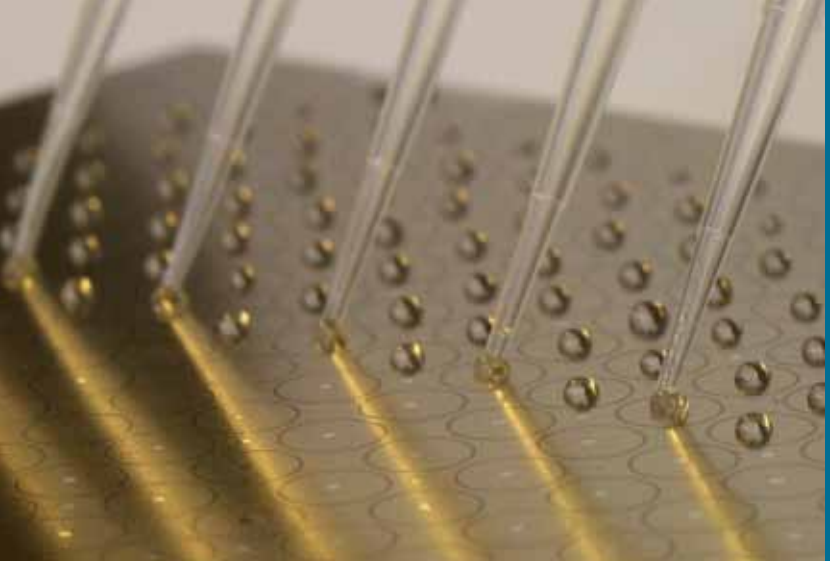
The first manufacturing license for our facilities was obtained in 1997, when the present Pharmaceutical Biotechnology Division of the Fraunhofer ITEM was still the Biochemical Engineering Division of the Gesellschaft für Biotechnologische Forschung (GBF). Since then, the GMP license has been extended several times for a wide variety of biopharmaceutical drug substances, such as recombinant and monoclonal antibodies, lymphokines, therapeutic enzymes, and nucleic acids/plasmids. The former GBF division was acquired by the Fraunhofer ITEM in 2008 and has been operated since then as the Pharmaceutical Biotechnology Division.

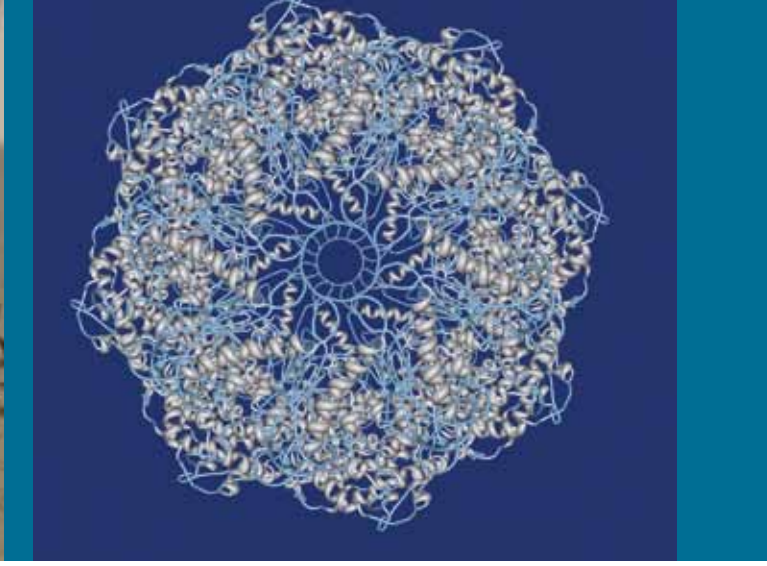
Cultivation of microbial and mammalian cells in volumes up to 400 L is performed in bioreactors equipped for operation in batch and fed-batch mode. Perfusion cultivations can be performed with up to 50 L reactor volume. Purification hardware based on process chromatography systems and membrane filtrations enable purification of biomolecules in batch sizes up to 100 g.

GMP operations with biopharmaceutical drug substances as targets must be performed in a particle-controlled environment (clean room). For such unit operations, the Fraunhofer ITEM has a comprehensive clean-room facility based on grade-B (1000), grade-C (10,000), and grade-D (100,000) clean rooms equipped with bioreactors, process-scale chromatography and filtration systems, and a fill and finish unit for infusion bags, ampoules, and vials.

Services

- GMP-compliant manufacture of master (MCB) and working cell banks (WCB) for microbial and mammalian cells in compliance with the ICH Q5D guideline
- Development and scale-up of biopharmaceutical drug substance manufacturing processes (cultivation scheme and purification sequence) based on mammalian cell cultures and microbial cultivation
- Validation of manufacturing processes for biopharmaceutical drug substances in compliance with the requirements of Annex 15 of the EU GMP Guideline
- Development and validation of analytical procedures in compliance with the ICH Q2 guideline
- Performance of stability studies for process intermediates, drug substances, and drug products in compliance with the ICH Q1A guideline
- GMP manufacture of investigational drug substances for clinical trials





PROTEIN ANALYTICS

In the past decade, the importance of mass spectrometry for protein analyses has greatly increased. It can be used both to identify a protein and to analyze its amino acid sequence. In the process, possible alterations in the side chains can be detected, such as those occurring in posttranslational modifications. In combination with different sample preparation procedures, mass spectrometry is an important tool in the repertoire of current methods for analyzing individual proteins or entire proteomes. Special requirements are stipulated for proteins prepared for medical use. Among other things, it must be possible to exactly reproduce the amino acid sequence of the relevant protein. By means of mass spectrometric analysis, it is possible to verify the sequence from both the N and the C termini (ISD). After appropriate sample preparations, which can also include a directed cleavage of the protein by proteases and the chromatographic separation of the resulting products, it is possible to verify the correct amino acid sequence with high sequence coverage (LC-MALDI-MS/MS). In this analysis, contingently present posttranslational modifications can also be investigated.

Application example

Modification of pharmaceutical proteins

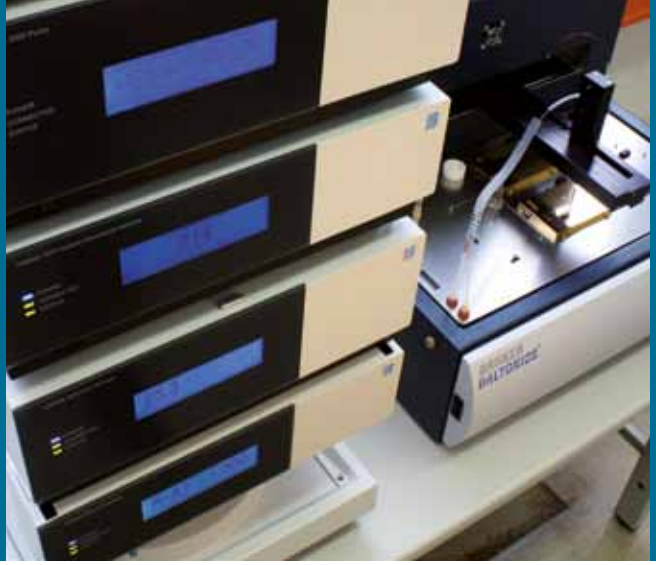
Biopharmaceutically employed active substances or diagnostics can be conjugated using chemical methods with polyethylene glycol (PEG). In this manner a coating is applied, which results in definitively reduced immunogenicity, elevated stability against proteases and retarded excretion. Thus, the active substance (or the diagnostic) can develop its action more efficiently. At the Fraunhofer IGB, mass spectrometry with MALDI-TOF/TOF was used to provide proof of a successful conjugation with appropriately modified proteins and to characterize them.

Services

- Protein identification
- Protein characterization – posttranslational modifications
- Expression analysis – proteomics
- Search for biomarkers
- Quality control of expressed proteins

Methods

- Purification or concentration of proteins by means of precipitation, filtration or chromatographic methods
- Identification of proteins via ISD analysis
- One- or two-dimensional polyacrylamide gel electrophoresis with subsequent sensitive staining of the proteins
- In-gel digestion of selected bands or spots with proteases or chemical agents
- Purification and concentration of peptides
- Reversed-phase chromatographic separation of peptide mixtures with automatic transfer of the fractions to mass spectrometric sample carriers (LC-MALDI)
- Identification of proteins via peptide mass fingerprint analysis
- Identification of proteins by means of MS/MS (PSD or CID analysis)
- *De novo* sequencing of peptides with the aid of chemical modification to improve quality
- Specific purification of modified proteins or peptides
- Identification of posttranslational modifications in the MS/MS or ISD spectra



Equipment

- **Mass spectrometer**
  - Ultraflex II MALDI-TOF/TOF
  - Finnigan LCQ DECA
  - Thermo Fisher LTQ XL
- **HPLC**
  - Ultimate 3000 nano-LC
  - Surveyor LC system
  - Agilent 1290 Infinity
- **LC-MALDI fraction collector**
  - Proteineer fc
- **Isoelectric focusing**
  - Ettan IPGphor II
- **2D electrophoresis**
  - Protean® plus Dodeca™ cell
  - Protean II xi Cell
- **Laser gel scanner**
  - Image Reader FLA-5000 Series
- **Software**
  - Bruker Compass
  - MASCOT Server
  - Xcalibur
  - Proteome Discoverer
  - Delta2D
  - Advanced Image Data Analyzer (Aida)

Key elements

- Purification and preparation of proteins
- Determination of protein purity
- Identification of proteins
- Determination of protein sequence

CELL-BASED ASSAYS

Over the past years, cell-based assays have been used more frequently especially for pharmaceutical research and drug development. Cell-based assays refer to the use of living cells as diagnostic tools. They offer a more accurate representation of the real-life model than non-cell based assays and furthermore offer the possibility to monitor the behavior of the respective cells. Insights from these cellular assays have been shown to facilitate drug discovery, saving considerable time and expense. These developments also help in the reduction of secondary screens in the future. The established cell-based assays offer a broad range of applications.

Provided by the Fraunhofer IGB:

- screening for antimicrobial and antiviral compounds
- evaluation of toxic and non-toxic substances
- detection of infectious viral contaminations
- detection of pathogen-associated microbial patterns (PAMPs)
- screening for pathogen recognition receptor agonists and antagonists

These assays allow a set of simple as well as more complex read-outs, e.g. visual or photometrical, via qPCR, or via changes in cell morphology.

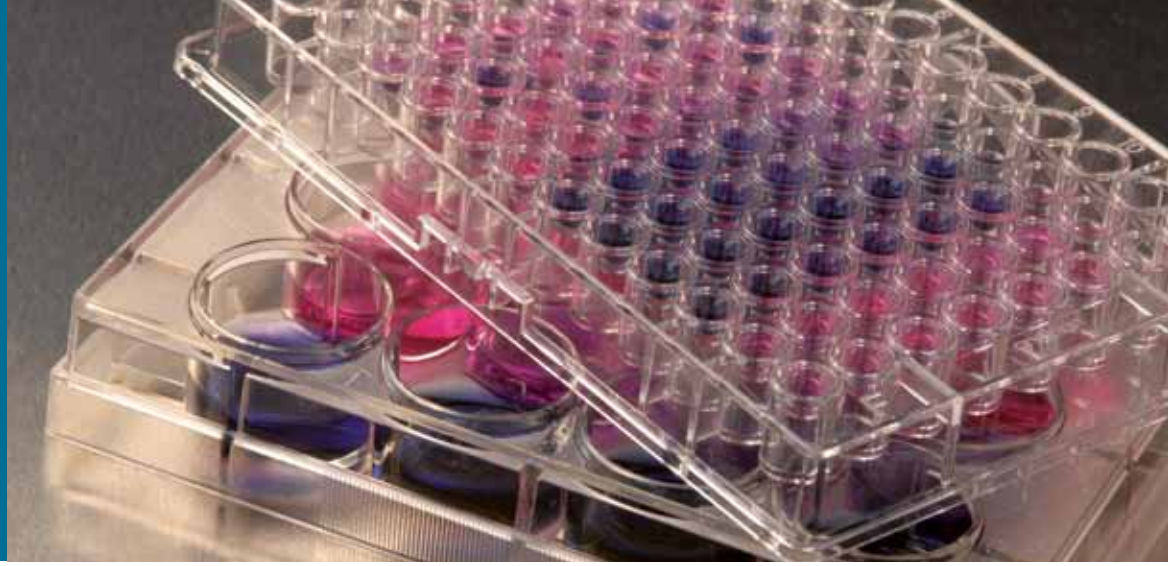
Cell-based test system for the detection of pyrogenic residues

Pyrogens – fever-producing remnants from bacteria, viruses or fungi – which enter the human bloodstream may cause sepsis. Sepsis is considered to be one of the most severe complications in hospitals, caused by the sum of life-threatening symptoms and pathophysiological changes induced by these compounds also called pathogen-associated microbial patterns (PAMPs, such as microbial remnants or isolated chemical structures, e.g. cell wall components), or by entire microorganisms. The body responds by producing endogenous mediators (cytokines), which activate inflammation cascades. In sepsis, hyperstimulation of these cascades leads to a systemic reaction that is no longer under control and may result in multiorgan failure. In order to prevent the transmission of pyrogenic residues into the human bloodstream, products must therefore be tested for the absence of pyrogenic residues. There are currently three commercially available methods for the detection of pyrogens, but they are either very costly or limited to specific pyrogens. The challenge is to provide a simple universal detection system for pyrogens at low cost.

At the Fraunhofer IGB we have developed a new, cell-based test system that allows PAMPs to be identified and differentiated via their natural pattern recognition receptors (PRRs) like the toll-like receptors (TLRs), NOD-like receptors (NLRs) or dectins coupled to a reporter gene assay. PRRs are receptors of the human immune system, which recognize components of viruses, bacteria or fungi and normally initiate cytokine response. For this assay cell lines were stably transfected with the appropriate receptor complex, expressing no other PRR receptors and containing a reporter gene, which is activated by PRR activation. Induction of the TLR receptors by a specific ligand therefore leads to the activation of the transcription factor NF-κB, which induces the expression of a reporter gene. Pyrogens present in the analyte can be detected via the expression of the reporter gene.

Specifications

- The cell-based test system allows fast and easy qualitative and quantitative detection of pyrogens without standard laboratory equipment
- It can complement or replace existing tests such as LAL and IPT



- Pyrogens can be detected in injectable drugs
- In addition, the assay enables the screening for TLR antagonists, which are increasingly used in dermatology in order to suppress immune reactions.

Validation for virological safety using the virus-protection assay (Antiviral Assay, AVA)

Pharmaceutical proteins produced by using cell cultures and medical devices derived from animal tissues must be checked for virus contaminations according to USP Pharmacopoe Europeae or ISO standards. However, viruses used in these assays pose a risk of human infection. Therefore, these assays have to be performed under strict biosafety standards up to BL2 level. Testing for antiviral activity can be performed at the Fraunhofer IGB according to GLP (Good Laboratory Practice) standards. The lab is certified for assay types involving "Cell-based assays for the determination of biological parameters". An Antiviral Assay (AVA) is routinely used for measuring the biological activity of interferons (IFN) according to GLP. The determination of the antiviral activity of interferons is based on the induction of cellular responses in cell cultures, suppressing the cytopathic effect of the infectious virus. This can be detected quantitatively using a simple and robust photometric assay. Additionally, other viral assays such as the tissue culture infectious dose50 (TCID50) and the plaque assay are carried out according to GLP.

The tissue culture infectious dose50 (TCID50) and the plaque assay

Besides the AVA described above, we perform a variety of assays to determine the titer of cytopathogenic viruses in various samples. Quality system assay capabilities to suit the different development and regulatory needs are available, from R&D level to certified GLP standards.

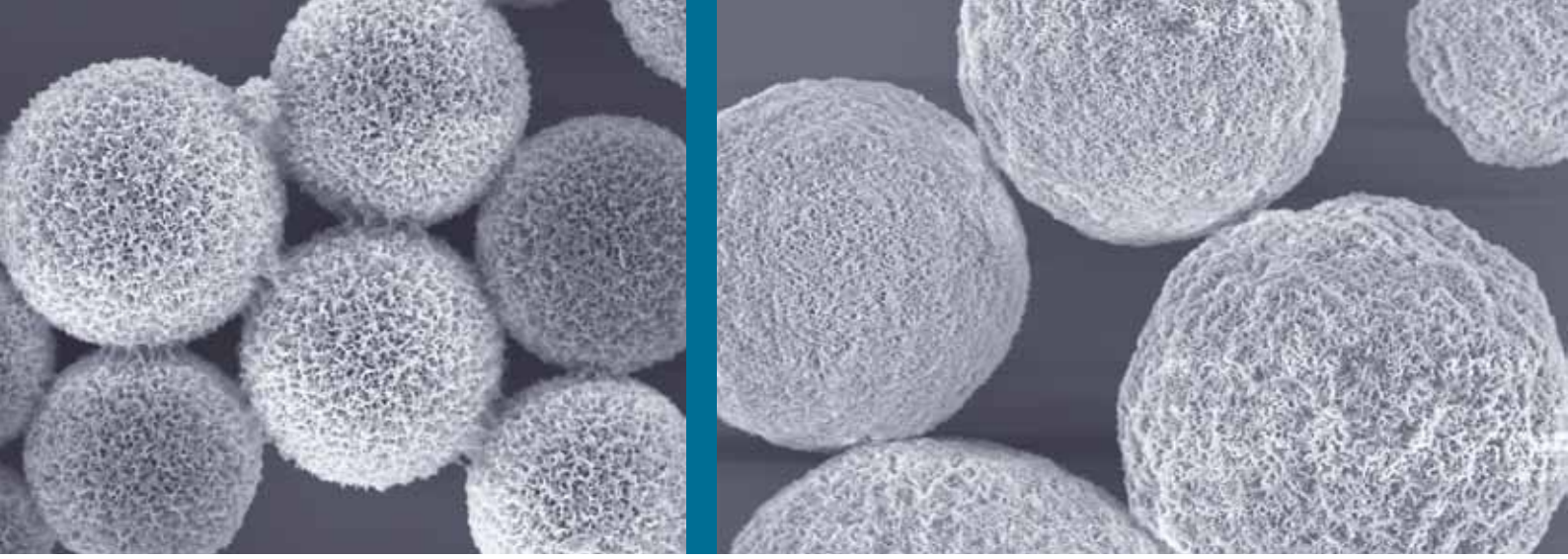
Cytopathogenic viruses can be quantified by the number of plaques or pocks they cause on susceptible cell monolayers. Using this assay, we can screen drug compounds for plaque inhibition. The tissue culture infectious dose50 (TCID50) assay viruses which have cytopathic effects (CPE) can be quantitated. Endpoint techniques are used for viruses which do not grow in culture, when 'Lethal Dose50' (LD50) or 'Infectious Dose50' (ID50) values must be calculated. They are also used in the case of viruses which are not cytopathic or do not produce plaques. We use several statistical methods for analyzing the data generated, e.g. Spearman-Karber analysis.

qRT-PCR-based virus detection

In addition to cell-based assays for detection of virus contamination, methods which determine the viral nucleic acids can be used as well. In combination with enrichment and purification steps of viral RNA/DNA from samples, highly sensitive and specific PCR (polymerase chain reaction) based methods allow the detection of viruses present in even very low amounts. With these fast and quantitative methods, large numbers of samples can be processed in parallel. Based on a specific nucleic acid sequence, the method can be adapted to each virus of interest. The combination of this detection method with cell culture can be used even for highly sensitive determination of virus activity.

Services

- Testing of recombinant proteins for virus contaminations
- Determination of the titer of cytopathogenic viruses in samples on behalf of clients
- Implementation of virus tests in the production process



FORMULATION

Drug delivery and drug targeting

A great challenge in the treatment of diseases is the targeting of active substances to the location of the disease, i.e. to a tissue or an organ. Membranes are the most important barriers here that screen the location from the drugs to be delivered. Metabolization frequently reduces the intended effect of a drugs at the target location. In addition, incorrectly distributed or modified substances in the body can result in side effects. An already tried and tested way of avoiding these disadvantages is the production of particular drug formulations by embedding the substance into a polymer shell or matrix.

Controlled release

Carrier polymer core-shell nano- and microparticles control the release of substances intended to produce a certain effect. Biodegradable compounds are of special interest, as these are completely metabolized or broken down after use in the body or in the environment. Commercially available biodegradable linear polyesters often yield insufficient properties to bind active substances. That is why new polymer matrix systems – biodegradable and biocompatible block copolymers – with improved properties and various molecular weights are being developed at the Fraunhofer IGB. By varying the molecular weight and the ratio of the hydrophilic and hydrophobic monomer units we can influence the kinetics of the release of encapsulated substances. The functional groups of which biodegradable polymers are composed determine the physical and chemical properties as well as the rate of release and breakdown.

Surface modification – efficient drug targeting

The polymer particles can additionally be functionalized on the surface for an effective targeting of drugs to a particular location in the body. At the Fraunhofer IGB, we modify the surface of nano- and microparticles using well-established coupling methods via free carboxy groups. In addition to biodegradable nano- and microparticles we develop biological-synthetic particles that simulate the conditions on the surface of the cells.

Particle technology

Core-shell particles are composite materials consisting of at least two different components. At the Fraunhofer IGB, such-particles with a minimum diameter of 30 nanometers as well as microparticles up to several 100 micrometers are produced from organic and inorganic materials. We have the possibilities of various polymerization techniques such as mini-emulsion or dispersion polymerization and subsequently loading by swelling, direct infusion of drugs by double emulsion technologies and two lab spray-drying systems.

Spray drying

Spray drying is a unique method to convert a solution, suspension or emulsion into a solid powder in a single process step, but many other processes can also be completed in a single stage: modification of particle size, agglomeration of nanoparticles, drying suspensions, further particle coating for protection against acids, for example, immobilization of liquids and solid materials in a matrix and manufacture of microcapsules. This is a widely established process in many industries such as pharmaceuticals, food, chemical or materials science. With the nano-spray dryer in our lab we can process sample amounts of up to 100 mg, fine dried particles from 300 nm to 5 µm with narrow size distribution, receiveing a high product yield of up to 90%, which is ideal for R&D feasibility studies on high-value pharmaceutical and nanomaterial formulations. On the other hand, the mini spray dryer in our lab is the perfect solution for quick,

continuous and gentle drying of heat-sensitive molecules in aqueous and organic solutions to free flowing powder, high-performance cyclone separation with high yield and minimal product loss, and easy scale-up of the process to industrial production.

Services

- Development of biocompatible and biodegradable particles
- Loading and encapsulation of particles with drugs
- Surface coating of nano- and microparticles
- Bioconjugation of biomolecules
- Development of hybrid materials
- Formulations of drug matrix systems
- Performance of feasibility studies

Equipment

- Biological and polymer analytics
- MALDI-TOF/TOF mass spectrometer (Bruker Ultraflex II)
- Thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC), and simultaneous thermoanalysis (STA)
- Gel permeation chromatography (GPC) with 4 detectors
- Microelectrophoresis (zeta potential)
- Dynamic light scattering (SLS, mastersizer, measuring range 0.1 nm to 10 µm)
- Static light scattering (SLS, mastersizer, measuring range 50 nm to 2 mm)
- Microscopy, scanning electron microscope (SEM), atomic force microscopy (AFM)

Loading of particles

- A mini- and a nano-spray dryer for the formulation of particles
- High-performance liquid chromatography (HPLC)

ASEPTIC FILLING

The final processing of biopharmaceutical investigational drug substances to investigational medicinal products (IMPs) is performed in GMP grade B (EU) clean-room suites equipped with laminar flow benches for manual filling operations and a restricted-access barrier system (RABS) for machine filling of liquid dosage forms. RABS and laminar flow benches provide clean-room grade A (EU) as well as class 100 (US) environmental conditions for aseptic processing involving open primary containments of final dosage forms.

Dosage forms are infusion bags and bottles as well as ampoules and vials, with volumes from 1 to 20 ml.

Formulation of investigational bulk drug products can be performed in volumes up to 60 L.

All aseptic filling operations are performed in compliance with Annex 1 of the EU GMP Guideline. US requirements are met as well.

Aseptic fill and finish operations have been GMP-licensed since 2010.

THERAPEUTIC PROTEINS – PROTEIN DESIGN WITH INTERFERONS

Interferons are proteins and part of our immune system. They are only synthesized when induced by viral or other challenges. They are synthesized by specialized cells, subsequently secreted, and bind to highly specific receptors on target cells. This specific interaction with the receptors causes the signal transduction to the nucleus and thus regulates the production of about 50 different genes.

Through their receptor binding interferons act pleiotropically, meaning they mediate various biological activities such as:

- antiviral, antiinfectious
- antiproliferative, cytotoxic
- immunomodulating

All these activities are of high pharmaceutical value as shown by several clinical applications in cancer and viral infections.

There are three classes of interferons: 16 subgroups of interferon-alpha, one interferon-beta and one interferon-gamma. The interferons are relatively small proteins with 143 to 172 amino-acids ranging from 19 to 45 kDa. They are modified by glycosylation. Interferon-gamma is a homodimer, while the others are monomers.

Clinical and market potential of interferons

All interferons are synthesized by genetic engineering techniques. The preferred systems for the synthesis are bacterial cells. Interferon-alpha is used for oncological indications and for the treatment of viral hepatitis. The market value is about 1 billion US$. A similar value is expected for the next years for Interferon-beta for the treatment of multiple sclerosis. Much less used (so far) is interferon-gamma, which helps in the treatment of chronic granulomatosis disease, kidney cancer and rheumaticological diseases.

Generic interferon-beta (CinnoVex)

Multiple sclerosis (MS) is the most common disease of the central nervous system. Estimates put the number of people suffering from multiple sclerosis at about 2.5 million worldwide. The only therapeutic successes achieved so far have been with interferon-beta, a protein produced naturally in the body. It slows down the progression of the illness and reduces the relapse rate. Biotechnological techniques make it possible to engineer this endogenous protein in bacterial or mammalian cells. An interferon-beta-1a, whose biotechnical engineering and production up to pilot scale was optimized at the Fraunhofer IGB in collaboration with CinnaGen Company, was approved in 2006 as biogeneric by the Iranian Food and Drug Administration (IFDA). It is produced and marketed in Iran as CinnoVex by the biotech company CinnaGen.

The Fraunhofer IGB successfully cloned the human protein into a suitable expression vector and established the production of the natural protein by a stable transfection in a mammalian cell line. The interferon-beta-1a thus obtained is glycosylated like the human protein. *In vitro* it shows a higher biological activity than interferon-beta-1b, which is produced in bacteria and is not glycosylated.

In the Fraunhofer IGB laboratories, a multi-disciplinary team developed the production of the pharmaceutical protein up to pilot scale. We have developed the fermentation process as well as downstream processing, resulting in a highly purified protein. We identified the protein by amino acid sequencing and proved its antiviral effects. The Iranian CinnaGen gave proof of its clinical effectiveness within three years, including appropriate quality control and clinical trials.

Interferon-beta with enhanced bioavailability (Soluferon®)

The hydrophobicity of the human interferon-beta molecule, however, is unwanted both in terms of technical and pharmaceutical aspects. The resultant strong tendency of aggregation, for example, involves high technical expenditure for the preparation of the protein in a pure state and affects yield, formulation, stability and bioavailability.

Therefore, at the Fraunhofer IGB we have genetically engineered an IFN-beta variant with 9 hydrophobic amino acids substituted by a hydrophilic one which is better soluble and possesses a higher bioavailability. The following amino acids were substituted by serine: Leu5, Phe8, Cys17, Leu47, Phe50, Leu106, Phe111, Leu116, and Leu120.

In the context of a worldwide valid and exclusive agreement the Vaccine Project Management GmbH (VPM) undertakes the development of the patented protein variant expressed in mammalian cells pre-clinically and clinically up to phase II. No later than after successful completion of a phase II trial, if applicable even before, VPM strives to out-license the protein variant to one of the major pharmaceutical enterprises.

VPM is a company founded as a result of an initiative of the German Federal Ministry of Education and Research (BMBF) and the German Society for Biotechnological Research (GBF), Braunschweig, for the commercialization and development of vaccines. The novel interferon-beta variant is registered as Soluferon®.

Recently, its formulation, the physical/chemical properties and the bioavailability have been examined on behalf of VPM at the Fraunhofer IGB. In addition, production processes were carried out. GMP-development of the test medication, however, is realized in cooperation with VPM.

Enhanced bioavailability

Determination of the antiviral effect in blood plasma showed a higher biological activity of Soluferon®. Soluferon® possesses a bioavailability significantly higher by the factor of 6 in comparison to conventional interferon-beta.

Soluferon® has great potential as a new drug on the world market: it is expected to have lesser side effects and an increased effectiveness. The new interferon-beta variant gives cause for hope to all multiple sclerosis patients and can possibly also be used for the treatment of other illnesses, for example virus infections or cancer.

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