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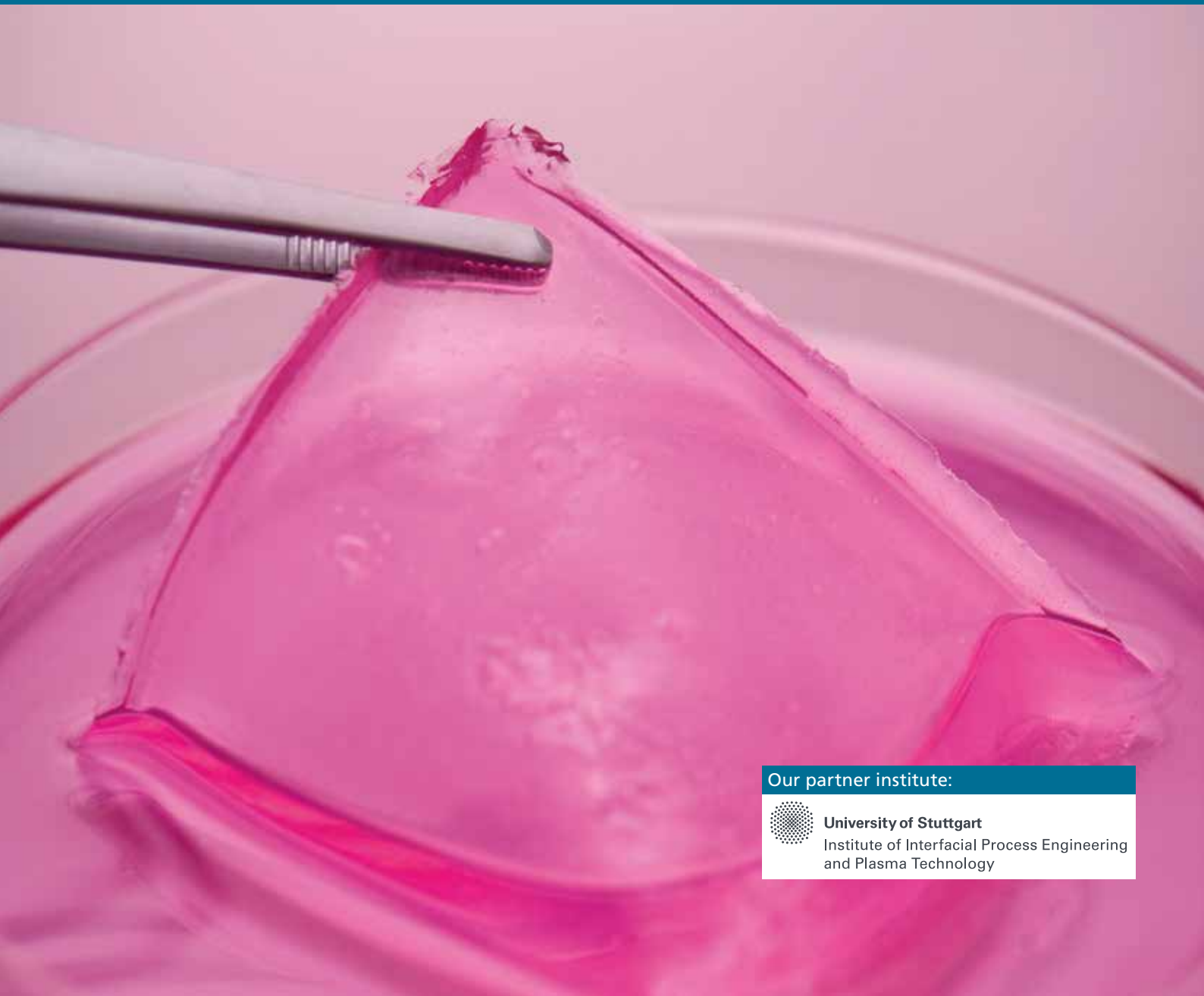
FRAUNHOFER INSTITUTE FOR INTERFACIAL ENGINEERING AND BIOTECHNOLOGY IGB

BIOMATERIALS

DEVELOPMENT, SYNTHESIS, AND CHARACTERIZATION OF MATERIALS IN DIRECT CONTACT WITH BIOLOGICAL SYSTEMS



Our partner institute:

University of Stuttgart
Institute of Interfacial Process Engineering and Plasma Technology

BIOMATERIALS – BIOCOMPATIBLE MATERIALS IN DIRECT CONTACT WITH BIOLOGICAL SYSTEMS

Biocompatible, biomimetic, bioactive

The minimum requirement for a biomaterial is that it must not damage the biomolecules or cells, for example of an organism, with which it comes into contact. This is called biocompatibility.

Frequently, more is expected of modern biomaterials. They should transmit active signals to their biological environment. For example, by acting biomimetically, i.e. by imitating and initiating naturally occurring processes. The materials can provide molecular recognition sites that serve as anchor points for coupling molecules or cells, or make available biological signaling molecules and release them at the appropriate time. Also, biomaterials can imitate the mechanical properties of a natural cell environment and thus promote the growth behavior and the differentiation of cells. In this way it is possible to produce complete artificial tissues. Besides biocompatibility, a central role is therefore played by biofunctionality in the development of modern biomaterials. The applications of the biomaterials range from dentures to suture and wound dressing material, from stents to contact lenses and from substrate material for the *in vitro* cultivation of cells to replace materials for joints, bones and soft tissue.

In this brochure we present the research activities of the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB in the area of the development of synthetic and biological biomaterials for use in contact with biological systems. We show you applications in medicine, pharmaceutics and medical technology as well as in cell culture and tissue engineering.

BIO-
COMPATIBILITY

CELL-MATERIAL-
INTERACTIONS

BIOMATERIALS AT FRAUNHOFER IGB

Development of biomaterials

At the Fraunhofer IGB, together with our partners from research and industry, we develop and characterize biomaterials on the basis of metals, ceramics, polymers and composite materials for biotechnological, diagnostic and therapeutic applications. Chemists, physicists, biologists and process engineers work hand in hand in interdisciplinary teams. Thus at our institute we develop biomaterials along the entire innovation chain (see above). Here we have set ourselves the task of adjusting the chemical and physical properties to the intended use (pp. 4–5), creating the optimum surface features for the interaction with the biological system (pp. 6–7), as well as a comprehensive chemical and biological characterization of the biomaterials (pp. 8–11).

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MATERIAL DEVELOPMENT

We develop biomaterials

- in the form of polymeric hydrogels and organic or inorganic membranes
- as a two- or three-dimensional scaffold structure for cell culture and tissue engineering
- as particulate systems that carry or release biomolecules on their surface
- in the form of surface coatings produced by plasma and wet chemical processes

Production of polymers and hydrogels

Polymerization processes

Our know-how in chemical synthesis includes all standard polymerization processes such as radical, ionic and step-growth polymerization (e.g. polycondensation) and various processes for the synthesis of polymer particles. For example, we produce polymeric biomaterials from biobased building blocks.

Crosslinking technology

At the Fraunhofer IGB we use well-established chemical and physical crosslinking technologies to produce tissue-like hydrogels. Additionally, in our laboratories we develop our own processes and biocompatible crosslinkers that are suitable, for example, for *in situ* encapsulation of cells or that simulate special properties such as the elasticity of natural tissues.

We work with photo-induced, radical crosslinking and initiator-free reactions on the basis of click chemistry. Using physical crosslinkings triggered by pH or temperature changes, we can produce customized reversible switchable networks.

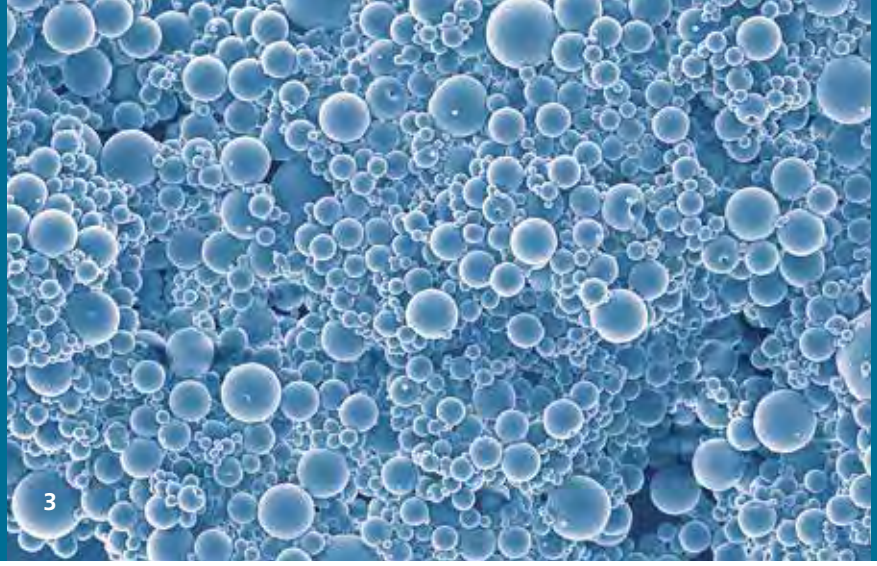
The controlled crosslinking of synthetic or biobased molecular building blocks results in hydrogels with adjustable mechanical and biological properties.

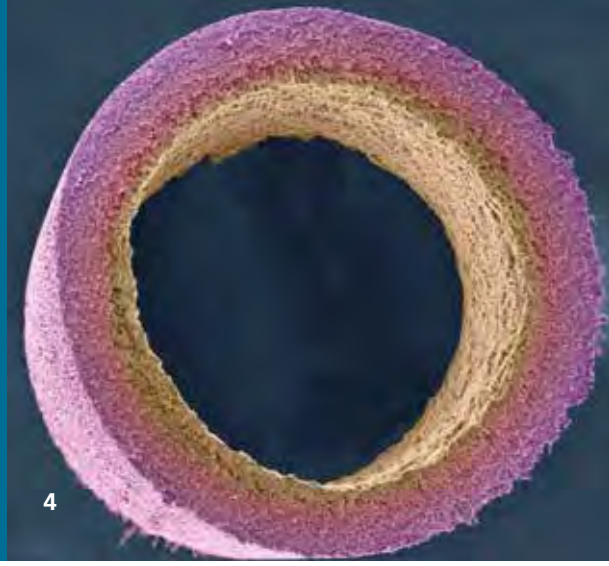
Production of biofunctional particles

Our particle systems are given their biofunctions by encapsulating bioactive components in their core or by binding them on their surface. As particulate carrier material, we synthesize inorganic or organic particle cores with diameters ranging from 30 nm to 10 µm.

Biodegradable particles

We encapsulate biological and medical factors such as growth factors or pharmaceutically active ingredients – depending on the solubility by means of water-in-oil-in-water or oil-in-water emulsions – in biodegradable particles, for example, of polylactid (PLA) or chitosan. Thus the active substances can be introduced into an aqueous environment and released there in a controlled manner. In doing so we customize the release speed of the encapsulated substances via the choice of the polymer and the degree of crosslinking of the particles.





Cell-mimetic particles

Particles that present proteins on their surface, for example signaling molecules, can to some extent act like a cell and activate other cells. For example, in the sol-gel processes we synthesized silicon oxide (SiO_x) particle cores and first of all functionalized the particle surfaces with carboxyl groups, to which we bound the protein tumor necrosis factor-α (TNFα). These particles triggered signaling cascades in certain cells that otherwise only the TNF protein anchored in the membrane of cells can trigger. If we additionally equip the particle core with a fluorescent dye, the docking of the particles to cells and the cell death triggered by the TNF particles can be followed under the microscope.

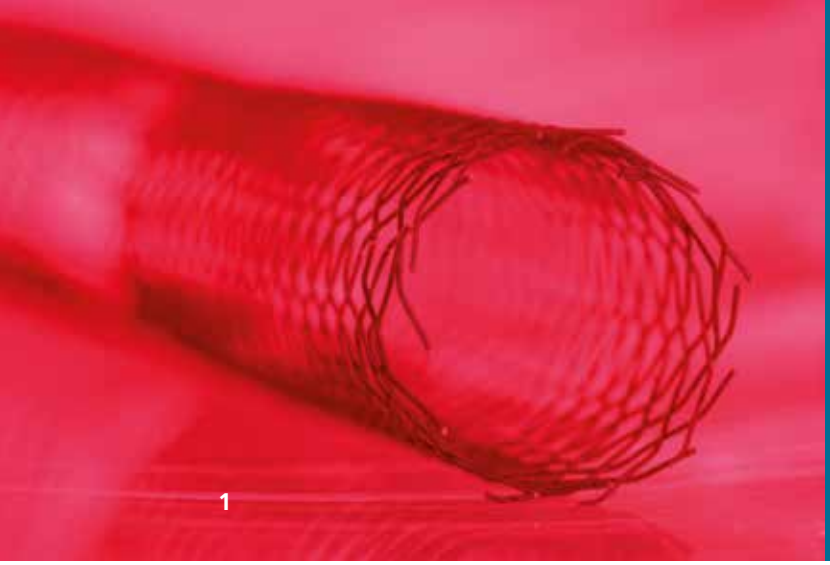
Organic-inorganic composite materials

Inorganic particles such as HAP, TCP, SiO_2 or TiO_2 are dispersed in solutions of polymers, for example in PLA, PCL or hydrogels. Various dispersion processes are available (ultrasonic, ball mill) for this purpose. Subsequently these dispersions are processed e.g. by wet-spinning, electrospinning, knife coating or extrusion and are consolidated in this process. Crosslinking is possible by means of the functionalization of the internal interfaces of the composite materials. In this way the tensile strength of hydroxylapatite, for example, can be increased by dispersion in polylactic acid.

Production of porous biomaterials (membranes, fibers, fleeces)

To build up highly porous biomaterials we use various molding and pore formation processes such as phase inversion, wet-spinning, electrospinning, freeze drying and template processes. We produce porous materials from various materials such as PLA, hydrogels, PES, PSU, PEEK and even from composite materials (PLA-HAP). On the one hand, porous biomaterials permit a good colonization with cells, as these can migrate easily in the material. On the other hand, endotoxins are selectively removed in contact with blood with the help of porous membrane fibers (cf. also examples of applications on p. 14).

1 *Synthetic hydrogel.*
2 *Biological hydrogel.*
3 *Biodegradable particles in which a growth factor was encapsulated.*
4 *Regio-selectively equipped membrane for blood purification.*



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SURFACE MODIFICATION

The interaction of a material with its environment is determined significantly by its surface chemistry and topography. Especially in the development of biomaterials – materials that are suitable for direct contact with biological systems such as body fluids, tissues or cells – the interface contributes decisively to the performance of the material. At the Fraunhofer IGB we have at our disposal a wide range of technologies and processes to customize the surfaces of materials for their field of application.

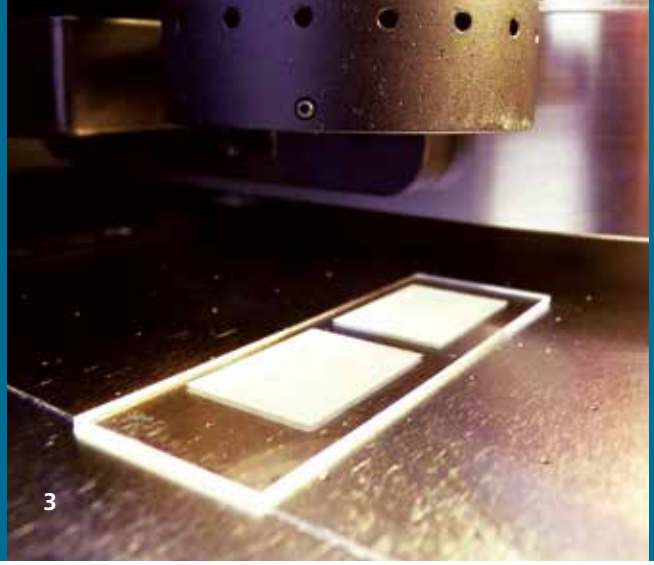
Wet-chemical processes

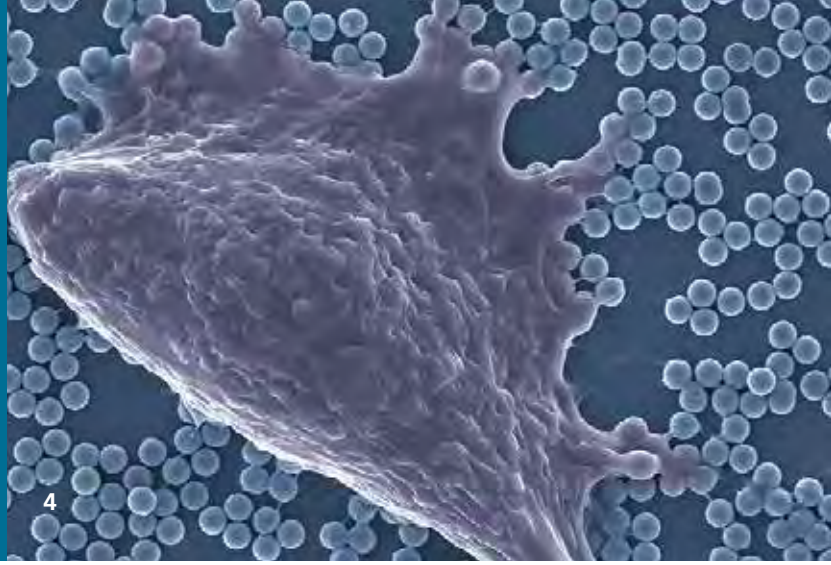
Depending on the surface chemistry of a material, available functional groups are used and biomolecules such as proteins, DNA or polysaccharides are bound covalently. First of all the biomolecules are chemically derivatized, as required. For example, surfaces of acrylate implants are coated with thiol-modified heparin in a targeted way to permit colonization with endothelial cells.

We activate materials that do not provide any functional groups as a target by means of various gas phase processes, thus introducing specific functional groups.

Gas phase processes (plasma, PVD, PECVD, CVD)

By means of gas phase processes thin layers (monolayers up to several hundred nanometer thick) can be deposited on surfaces without changing the volume characteristics of the basic material. The physical-chemical properties of the surfaces such as the surface tension, the roughness, the dynamic wetting behavior or the adhesion characteristics toward proteins or cells can be modified by changing the process parameters. For example, amino or carboxyl functions can be specifically generated on surfaces that, on the one hand, directly influence the interaction with cells or are used for wet chemical functionalization with biomolecules. Plasma processes also create thin swellable release layers that can release active substances (e.g. antibiotics on implants).





Printing processes

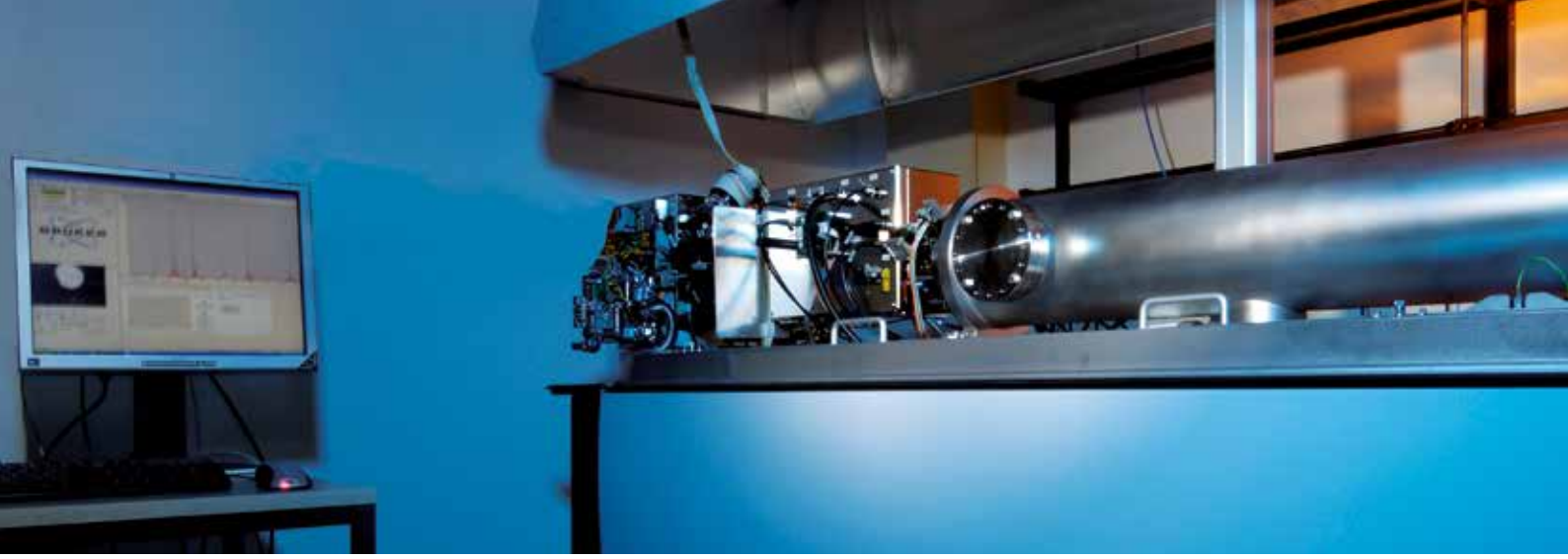
The development of biomaterials sometimes requires the flexible functionalization of surfaces, for example the coexistence of cell-adhesive and cell-rejecting areas or the combination of hydrophilic and hydrophobic areas in the production of microfluidic test systems. With the help of digital printing processes such as inkjet printing or Laser Induced Forward Transfer (LIFT), material layers can be applied to surfaces direct, i.e. without the elaborate manufacturing of masks, in any programmable patterns that are required.

At the Fraunhofer IGB we develop inkjet-suitable inks for coating surfaces with biological and biofunctional materials such as proteins or active substance-loaded, degradable particles. The high-precision inkjet printer DMP 3000 (Fujifilm Dimatix, USA) is available to produce functional layers with resolutions in the micrometer range.

Properties of the functional coatings

- biocompatible
- biofunctional
- antithrombogenic
- biodegradable
- antimicrobial
- friction-reducing
- reduces unspecific protein adsorption
- cell-attractive/cell-controlling
- releases active substances

1 *Stent coating in the plasma.*
2 *Surface modification of a rigid gas-permeable contact lens in the plasma.*
3 *Printed particle layers in the printing unit of a high-precision printer.*
4 *Cell in interaction with a microparticle-coated surface.*



PHYSICAL-CHEMICAL MATERIAL CHARACTERIZATION

At the Fraunhofer IGB we provide comprehensive characterization of biomaterials – from the physical and chemical characteristics to the properties of the material in contact with biological systems (pp. 10 – 13).

Functional characteristics

Wettability, slide characteristics, special mechanical or rheological characteristics or a specific adsorption behavior – which property should be the distinguishing feature of your biomaterial? We deal with the characterization and, building on this, together with you we develop optimization strategies.

Stability

For many applications the stability of the biomaterial is of crucial importance. On the one hand, we quantify the long-term stability of materials. We also examine the degradation behavior of resorbable materials – because, for certain applications, precisely the opposite is required. For example, suture material or fixing screws for bone fractures should, as far as possible, degrade in the body after the healing process, so that no further surgical procedure is necessary.

Every biomaterial is sterilized before use. Gamma or UV radiation, steam or ethylene oxide sterilization subject the materials to a varying degree of stress. We analyze the sterilized material and insure that, even after sterilization, the customized biomaterial still has the required stability characteristics.

Surface characteristics

The interaction of the biological system with the biomaterial is influenced decisively by the surface properties such as the topography and the chemical composition (roughness, functional groups, chemical potential, swelling capability, hardness). We examine the chemistry and physics of the interfaces with a wide range of methods. Using modern imaging processes, for example, we can represent the topography of the surface down to the sub-nanometer range and also make the chemical composition or protein adsorption visible with integrated spatial resolution.

OUR MEASURING TECHNIQUES

The measuring techniques we offer comprise methods for characterizing liquids, gels and solids with various geometries: particles, membranes, fibers, textiles, (ultra-)thin layers and smaller three-dimensional components.

Microscopic processes

- Light/fluorescence microscopy, laser-scanning microscopy (imaging of structures ~1 µm)
- Scanning electron microscopy (imaging of structures 10 nm – 10 µm)
- Infrared/Raman microscopy (demonstration of the local distribution of functional groups)
- Atomic force microscopy (visualization and measurement of surface roughness down to the atomic range)

Spectroscopic processes

- Infrared, Raman, UV, photoelectron spectroscopy, atomic absorption spectroscopy (AAS) to characterize the chemical composition of a material
- Electron spin resonance spectroscopy (ESR) to detect long-lived radicals
- X-ray photoelectron spectroscopy (XPS) for the chemical analysis of the uppermost atomic layers
- Ellipsometry and imaging ellipsometry to determine layer thicknesses < 1 nm to 1 µm, also spatially resolved

Mass spectrometry

- MALDI-TOF-MS, ESI-MS to detect and identify proteins, peptides and polymers, MALDI imaging
- ICP-MS
- GC-/LC-MS

Equipment for mechanical material characterization

Special / other analyses (particles, membranes, porous systems, polymers, fluids)

- Particle size distribution, Zeta potential
- Specific surface, DSC, TGA, contact angle measurements, tensiometry, GPC

BIOLOGICAL MATERIAL CHARACTERIZATION

The interactions at the interfaces between materials and adjacent biological systems are complex. Consequently the interaction between surfaces and the biological system can be influenced and controlled on different levels. On the molecular level surfaces can be prepared so that relevant molecules are specifically bound. This includes metabolic products, proteins and so on.

Protein adsorption

Within a few seconds first of all unspecific proteins adsorb on the surface of materials from the surrounding environment. These proteins then modify the degree of adhesion of bacteria and cells. We can equip surfaces wet-chemically or by means of plasma technology so that the protein adsorption is controlled. As a result we are in a position to control an increase or a decrease of the adsorption as well as the selective adsorption of certain proteins and their orientation relative to the surface. This, among other things, is of great importance for cell adhesion in tissue engineering.

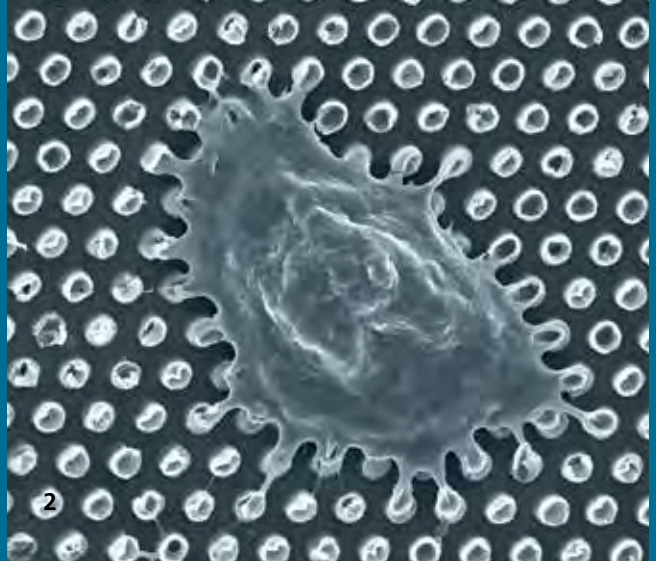
1 *Testing biocompatibility.*
2 *Primary keratinocytes on nubbed structure. The cell interacts directly with the nubs and is firmly anchored.*
3 *Human endothelial cell, grown on a 3D-printable polymer.*

Biocompatibility

In accordance with the Medical Devices Act, in order to protect patients, medical products have to be subjected to comprehensive tests before they become available on the market. One of these necessary tests is providing proof of biocompatibility. This must be guaranteed independently of whether the medical product is used for a short-term, long-term or even lasting body contact. With our many years of experience, we at the Fraunhofer IGB have established the testing of biocompatibility and cytotoxicity according to DIN EN ISO 10993-5. In order to verify the biocompatibility of materials *in vitro*, in addition to very wide range of cell lines (DIN EN ISO 10993-5) or primary cells of different tissues, we also employ three-dimensional test systems with organ-specific properties. As one of the first 3D models, we obtained accreditation for our 3D skin model on the basis of DIN EN ISO 10993.

Adhesion of microorganisms

Adjusting the adhesion of bacteria or other microorganisms on the surface of biomaterials is a decisive factor for the clinical success of implantable materials. At the Fraunhofer IGB we develop surface coatings that suppress the build-up of biofilms – actively by releasing active substances or passively by reducing the bacterial adhesion.



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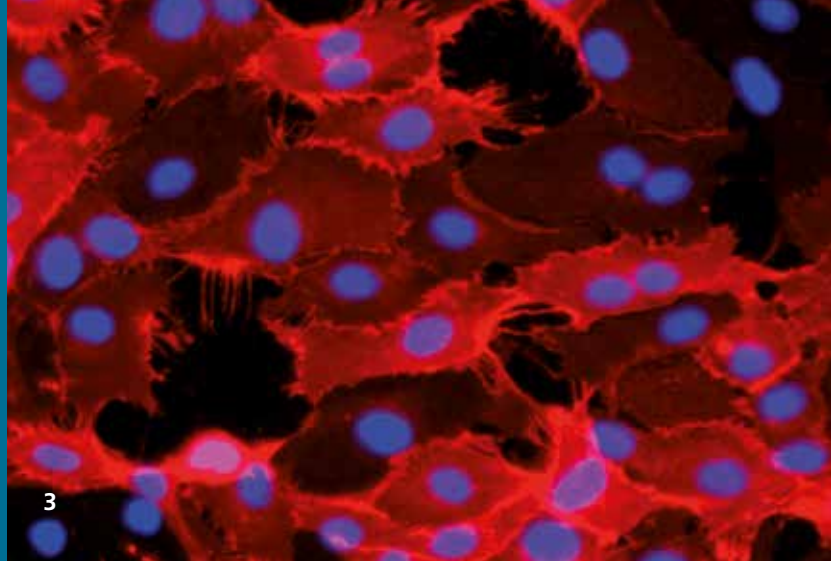

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Characterization of cell-material interactions

In vivo – which means in the natural surrounding tissue – cells are to be found in a structure, the so-called extracellular matrix, with which they communicate and interact by means of various stimuli. Different characteristics of this cell environment such as mechanical properties, charge distributions or the topography have a direct influence on the cell behavior. Biomaterials that reflect these nature-given properties to a high degree, are the basis for functional, durable and compatible products.

Our research in this area therefore pursues the following objectives:

- Understanding the interaction between biological cells and the extracellular biological matrix or non-biological material
- Characterization and optimization of the properties of materials for cell culture surfaces, implants as well as scaffold and supporting structures
- Modification of biomaterials including their characterization and application testing

Our knowledge of the interactions between cells and materials as well as our expertise in the field of cell cultivation enable us to identify the influence of the chemical composition or various surface structures of the biomaterials on cells. In addition to standardized analytical processes we also develop new methods of analysis to meet your requirements.

Tailored to the intended use of the product, for the cell type that is relevant to the application in question, we can evaluate the following parameters on the biomaterial:

- Cell morphology
- Cell adhesion
  - formation of cell-cell junctions
  - formation of the actin skeleton
- Cell-matrix interaction
  - number/character of filopodia
  - cell alignment
- Cell-differentiation
  - on the protein level
  - immunohistochemical analyses
  - evidence of released factors (e.g. inflammation mediators)
  - on the RNA level
- Hemocompatibility
  - coagulation
  - hemolysis, where applicable complement activation
- Proliferation capability
- Cell-vitality
- Cell-damage
  - morphological changes
  - membrane disintegrity
- Cell-specific parameters
  - enzyme activity

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EXAMPLES OF APPLICATION

Selective surfaces for tissue engineering

The isolation of primary cells as a pure culture and their cultivation and multiplication in the laboratory is a precondition for the production of *in vitro* tissues. Many cells dedifferentiate *in vitro*, that means they lose their characteristic markers, as cultivation on commercial cell culture substrates by no means reflects the complex microenvironment of the cells *in vivo*. By modifying individual material properties such as the topography and surface chemistry we develop carrier materials that facilitate a selective proliferation of cells or control the differentiation of cells. An example of this is a material with which we were able to enrich primary melanocytes in large quantities from human skin biopsies. To produce implants and prostheses we investigate surface modifications that reduce the adhesion and the proliferation of fibroblasts. With their rapid adhesion and proliferation these connective tissue cells represent a problem for the firm anchoring of many implants in the body, as they prevent the adhesion of tissue-specific cells.

BioRap – production of vascular structures by means of Rapid Prototyping

In an interdisciplinary project of the Fraunhofer Institutes IAP, IGB, ILT, IPA and IWM the Fraunhofer IGB is developing biofunctional coatings and biofunctional inks for the production of artificial blood vessel systems. In a specially designed bioreactor system the artificial tubular structures are colonized with a layer of human endothelial cells.

The overall aim of the project is the technical replication of soft tissue structures with Rapid Prototyping techniques such as 3D inkjet printing, stereolithography and multiphoton polymerization. The task of the Fraunhofer IAP in the consortium is the synthesis of customized basic materials for generative production techniques. The Fraunhofer Institutes ILT and IPA are responsible for the development of the combinable production process using 3D inkjet printing and laser-based polymerization techniques. The Fraunhofer IWM defines – on the basis of modeling and simulation – bio-inspired construction plans for the vessel systems and optimizes the flow characteristics.

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Optimization of implant surfaces

In the case of so-called endo-exo prostheses the artificial leg substitute is connected directly to the femur. For the patient this means more stability and better transmission of forces, however at the material emergence site there may be severe inflammatory reactions. Together with our partners we develop optimized titanium surfaces to increase the compatibility of such prostheses. The adhesion, proliferation and differentiation of primary skin cells are evaluated on the modified materials and the integration of titanium implants is analyzed *in vitro* in 3D skin models.

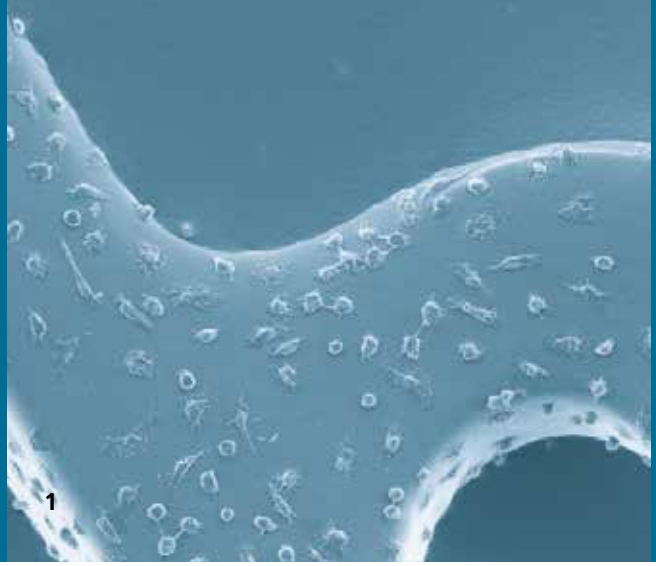
Innovative bone replacement materials

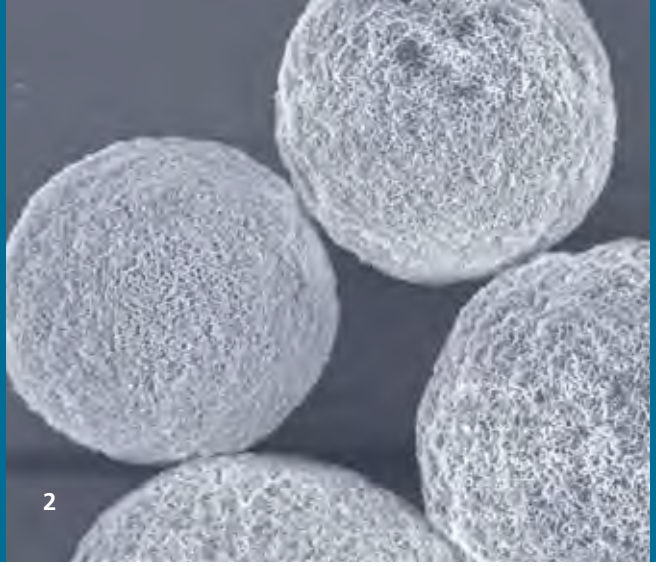
In several research projects carried out with our cooperation partners we are developing cell-populated matrices that are to be employed for bone defects in accordance with the patient's individual situation. As experts, we test a very wide range of biocompatible bone replacement materials after the application of various cell types and therapeutic FDA-approved growth factors. Here we carry out *in vitro* studies of cell-material interactions and examine the osteopromotive properties of the materials under dynamic cultivation in a bioreactor system developed in-house.

Surface modification of RGP contact lenses

To improve wearing comfort and to reduce the protein adsorption we have modified the wetting properties of rigid gas permeable contact lenses by means of plasma technology.

1 *Biological vascularized matrix (BioVaSc).*
2 *Printed polymer tubule with biofunctional coating for the supply of in vitro tissue cultures.*
3 *Skin test system.*
4 *Bioreactor components.*





Coating of stents

Stents are already widely used as vascular prostheses in the coronary area, also with injuries of the trachea (tracheal stent). After depositing an amino-parylene layer on the stent surfaces we were able to bind endothelial cell-specific anchor molecules to these layers, thus achieving a colonization of the stents with endothelial cells. This prevents the body from recognizing the material as "foreign" and being encapsulated.

Hollow fiber membranes for blood purification

A specially developed plasma process enables the production of hollow fiber membranes that permit single-stage dialysis. The hollow fibers are functionalized in such a way that the sensitive blood cells are washed unhindered through the unmodified lumens of the hollow fibers. The blood plasma, on the other hand, is filtered through the pores of the membrane. Their surface is functionalized in such a way that inflammatory endotoxins such as lipopolysaccharides (LPS) adhere to it. The new process puts considerably less strain on the patient because a substantially smaller part of the blood volume is located outside the body than in the conventional two-stage blood purification process.

Protein stabilization in dry layers

By adding stabilizing components we formulate proteins in such a way that they retain their folding and their function in dry layers. These layers are storable and can be used, for example, to transfer proteins free of any contact to a substrate, for instance an implant, by means of Laser Induced Forward Transfer (LIFT).

1 *Cell adhesion on a parylene-coated stent.*

2 *Biodegradable particles loaded with active substances.*

OUR SERVICES AT A GLANCE

At the Fraunhofer IGB we offer our partners complete development of biomaterials from developing a suitable synthesis strategy, by way of characterization to the biological evaluation. Here you benefit not only from our many years of experience, but above all from the interdisciplinary work of our research teams from the fields of biology, chemistry and process technology.

Materials development

- Advice on issues relating to the synthesis of biomaterials
- Development of customized biomaterials
- Biofunctionalization of synthetic materials

Physical-chemical material characterization

- Determination of mechanical properties
- Determination of functional properties
- Material stability and degradation behavior
- Surface analytics

Biological material characterization

- Characterization of cell-material interactions
- Biocompatibility testing
- Hemocompatibility tests

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Fraunhofer IGB

The Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB develops and optimizes processes and products in the fields of health, chemistry and process industry, as well as environment and energy. We combine the highest scientific standards with professional know-how in our competence areas – always with a view to economic efficiency and sustainability. Our strengths are offering complete solutions from the laboratory to the pilot scale. Customers also benefit from the cooperation between our five R&D departments in Stuttgart and the institute branches located in Leuna and Straubing. The constructive interplay of the various disciplines at our institute opens up new approaches in areas such as medical engineering, nanotechnology, industrial biotechnology, and environmental technology. Fraunhofer IGB is one of 69 institutes and independent research units of the Fraunhofer-Gesellschaft, Europe's leading organization for applied research.

www.igb.fraunhofer.de

Networking augments expertise

The Fraunhofer IGB works closely with the Institute for Interfacial Process Engineering and Plasma Technology IGVP of the University of Stuttgart. This permits continuity of the projects from the basic research to the application.

www.igvp.uni-stuttgart.de

Stay in contact: