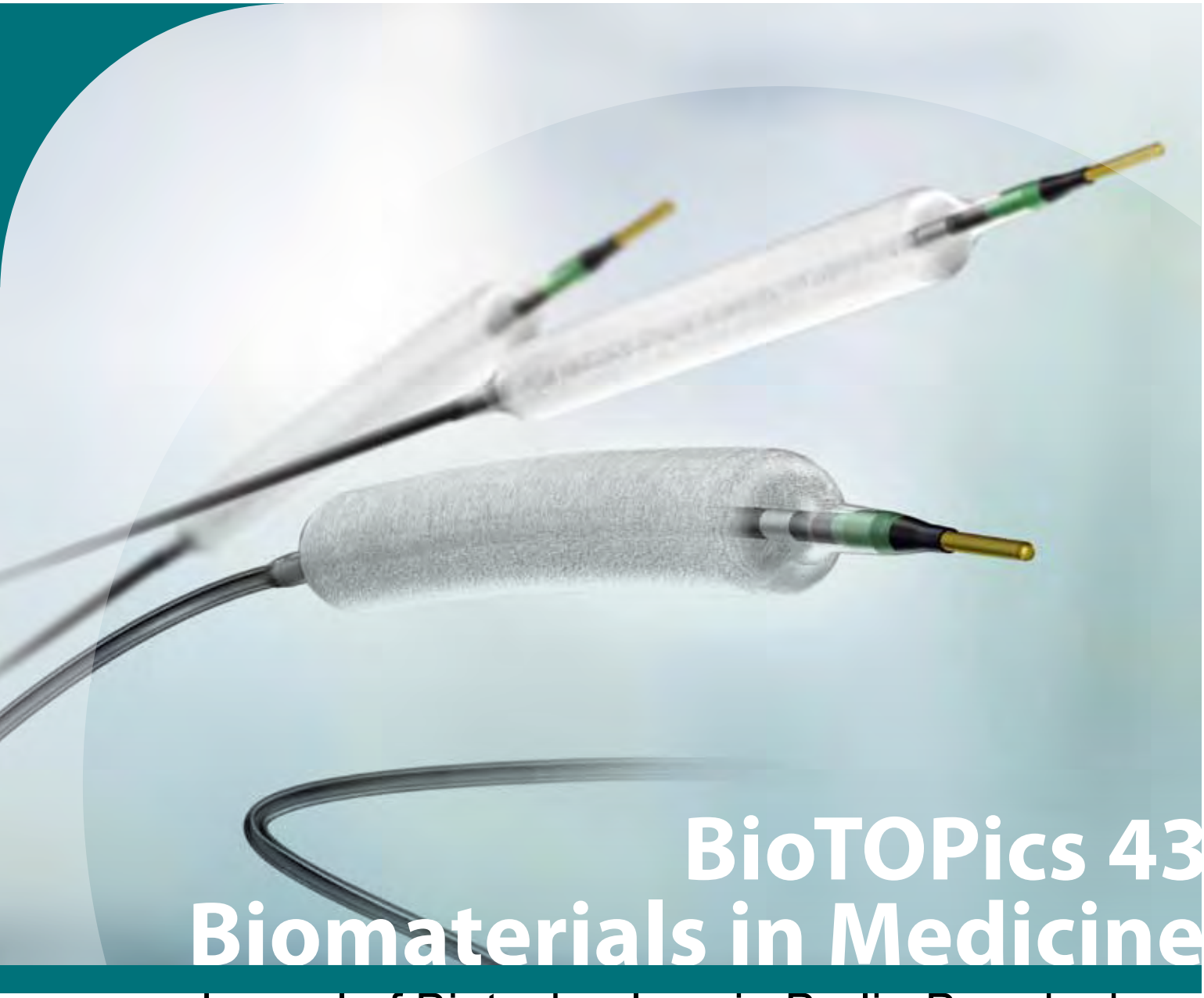

BioTOPics 43
Biomaterials in Medicine

Journal of Biotechnology in Berlin-Brandenburg

THE GERMAN CAPITAL REGION

excellence in life sciences & healthcare

Content

Publisher: TSB Innovationsagentur Berlin GmbH
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Editor: Yvonne Küchler
Design & Production: supiran.de
Translation: Textbüro Reul GmbH · Frankfurt
Photos/Figures: BioTOP or Authors. Titel: B. Braun

Biotechnologically Improved Biomaterials from Berlin and Brandenburg

For decades, biomaterials have been an integral part of medical practice. The biologization of biomaterials, however, is a relatively new trend. Increasingly, biomolecules are used for drug delivery, medical products are functionalised using biomolecules, and innovative biomaterials are created based on models from nature. Berlin-Brandenburg is positioned excellently in the material sciences field and is rapidly becoming a pacemaker for the newest generation of innovative biomaterials.

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This is the first edition of BioTOPics that presents itself in the new layout of the healthcare industry cluster Health Capital Berlin-Brandenburg. This cluster promotes cooperation between the region's players in industry and science across different subsectors to further enhance the internationally leading role of Berlin-Brandenburg in the life sciences and medicine.

It therefore seemed apposite to investigate an interdisciplinary and intersectoral theme on the interface between biotechnology and medical engineering in more detail in this issue under the title "Biomaterials in Medicine". A great deal is happening in this field in the German Capital Region. Scientists at acclaimed institutions like the Max Planck Institute of Colloids and Interfaces, the Fraunhofer Institute for Applied Polymer Research, the Centre for Biomaterial Development of the HZG in Teltow, and Charité – Universitätsmedizin Berlin are setting international standards in this field.

The outstanding networking between science and industry in the region permits swift translation of the latest research findings into marketable products. Alongside the big players in industry, small and mid-sized companies which often emerged as university spin-offs play a major role in these trailblazing activities.

Biomaterials are by definition materials which are used in medicine for therapeutic or diagnostic purposes and thereby come into direct contact with the body. In view of the rapidly growing importance of new biomaterials and the outstanding competencies in this field in Berlin and Brandenburg, we have decided to focus specifically on research and development in the following key areas:

- Biomaterials of biogenic origin
- Biomimetic biomaterials, i.e. materials guided by natural models
- Biomaterials functionalised with biomolecules
- Biomaterials for drug delivery and drug targeting.

This selection already provides an indication of the wide range of medical products which Berlin-Brandenburg can provide in the coming years for the benefit of patients.

Dr. Kai Bindseil

TSB Technologiestiftung Berlin

New Study: Surface Technologies in Berlin-Brandenburg

Intelligente Oberflächen – Innovationen aus Wissenschaft und Wirtschaft in Berlin-Brandenburg" (Intelligent Surfaces – Innovations from Science and Industry in Berlin-Brandenburg) is the title of a study which was published by Technologiestiftung in January 2012. It analysed the research and manufacturing capabilities of some 350 players in science and industry in the region who are engaged in surface engineering.

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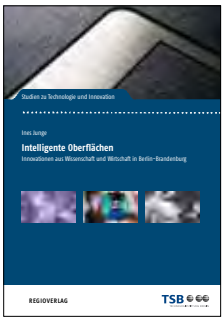
The study shows that Berlin-Brandenburg offers a very broad and detailed competency profile in this field. Surface technologies play an important role in a wide range of sectors and applications. The spectrum of applications includes innovations in the life sciences and medicine, for example biologised and biocompatible material surfaces with cell or tissue contact, (bio)analytics and diagnostics, nanobiotechnology and the integration of biological signal transmission into surfaces.

It also became clear that surface engineering is a multidisciplinary field in the region – from bioengineering through thin layer technologies in photonics, photovoltaics and electronics to the broad range of technologies in process engineering, component coating and layer analytics. The region offers considerable potential for interdisciplinary cooperation projects and synergies in these areas.

In addition to details of the individual disciplines, the authors of the study and the Technologiestiftung present the following core recommendations for the development of the surface technology sector in Berlin-Brandenburg:

- "Innovations in the form of new combinations" (Schumpeter) frequently emerge from the interfaces between disciplines. When building new research alliances, the surface technology capabilities of potential partners from other disciplines should therefore also be investigated and incorporated.

Especially in the case of entirely new surface technologies whose potential applications have not yet been fully explored, it is advisable to also include the creative sector which is strongly represented in Berlin in the development of application scenarios. This applies above all for the integration of innovative surface characteristics in "everyday products" like textiles, mobile devices or healthcare products.



Intelligente Oberflächen – Innovationen aus Wissenschaft und Wirtschaft in Berlin-Brandenburg
Junge, I.; Hammel, C. (Ed.), Regioverlag, Berlin, 2012, ISBN 978-3-929273-83-0

The study recommends the following measures to promote the development of surface engineering competencies in the biosciences:

- Improvement of the visibility and enhancement of the region's strong capabilities in the field of "materials in cell contact" and more intense networking between materials engineering and medicine;
- Promotion of basic research on the interface between electronics and biomolecule-based signals because this is a field in which surface technologies represent a special enabling technology for biosensorics.

Technologiestiftung Berlin will support all members of the regional innovation clusters in the promotion and further development of surface technologies in Berlin-Brandenburg – from the joint organisation of workshops and projects to the identification of suitable cooperation partners.

Centre for Biomaterial Development · Helmholtz-Zentrum Geesthacht · Teltow

Helmholtz Virtual Institute „Multifunctional Materials for Medicine“

Modern medicine is exploring novel strategies for curative therapies like minimally-invasive treatment procedures, approaches for inducing endogenous regeneration, and targeted drug delivery, which are based on innovative multifunctional polymeric biomaterials. Since protein-biomaterial surface interactions can critically affect the performance of such materials in a biological environment, the strategic goal of the new Helmholtz Virtual Institute „Multifunctional Materials for Medicine“ is to gain a comprehensive understanding of these complex processes in order to control the application-relevant protein adsorption behaviour in the future. The activities of this Helmholtz Virtual Institute initiative are integrated into the regional cluster “Healthcare Industry Berlin-Brandenburg – Health Capital”.

Dr. Christian Wischke
Junior group leader at HVI

Dr. Karl Kratz
Member of HVI

Prof. Dr. Friedrich Jung
Member of HVI

Prof. Dr. Andreas Lendlein
Spokesperson of the HVI

Helmholtz Virtual Institutes (HVIs) are structural projects funded by the Helmholtz Association of German Research Centres (HGF). HVIs are aiming to establish new research partnerships within the scientific community and promote the performance of German universities. Such HVIs bring together the key competencies of one or more Helmholtz Centres with those of universities and associated academic and industrial partners to create centres of scientific excellence, which are internationally competitive and form an ideal instrument to prepare the way for larger collaborative networks such as the Helmholtz Alliances.

The HVI ”Multifunctional Materials for Medicine“ is coordinated by the ”Centre for Biomaterial Development“ at the Helmholtz-Zentrum Geesthacht/Campus Teltow (HZG-Teltow) and is one of twelve new HVI initiatives, which were successfully evaluated in a highly competitive process in July 2011. In addition to HZG-Teltow, the “Institute for Soft Matter and Functional Materials” at the Helmholtz-Zentrum Berlin (HZB) and the “Institute of Chemistry and Biochemistry” of Freie Universität Berlin (FUB, university with Excellence status) and the university hospital Charité as the university partners are the Berlin-Brandenburg institutions involved in this virtual institute. Further participating institutions of the HVI are the Albert-Ludwigs-Universität Freiburg (also university with Excellence status) as a national core partner and the international partners Harvard University, Materials Research Science and Engineering Centre, (Cambridge, MA, USA), University of Tokyo, Centre for NanoBio Integration, (Tokyo, Japan) and Sichuan University, National Centre for Biomaterials (Chengdu, China). The industrial partners are mivenion GmbH (Berlin) and Fresenius Medical Care Deutschland GmbH (Bad Homburg).

The HVI „Multifunctional Materials for Medicine“ is supported financially by the Initiative and Networking Fund of the HGF for a five-year period and has its own executive and management structure. The Executive Board includes the spokesperson Prof. Dr. Andreas Lendlein (HZG) and the deputy spokespersons Prof. Dr. Matthias Ballauff (HZB) and Prof. Dr. Rainer Haag (FUB/FUB-Charité). The HVI will strongly promote young scientists so that they can progress the field in the future. Since the research topics of the HVI are highly interdisciplinary, spanning a wide range from biomaterial sciences through biointerface analysis to medicine, a coordinated multidisciplinary educational PhD program will be established, in which the Dahlem Research School (DRS) “Biomedical Sciences” of the FUB will act as the head organization. Specific support is also given to junior group leaders, who are strongly involved in the research projects, can raise research grants from the HVI (flexible funds) and will receive travel grants for internships in the labs of the international partners, e.g. at Harvard University (Prof. Weitz), Sichuan University (Prof. Gu), and the University of Tokyo (Prof. Kataoka). Furthermore, all junior research

group leaders of the HVI will have the opportunity to participate in a mentored teaching program at the FUB.

The research focus of the HVI addresses protein adsorption, which occurs instantly upon material contact with body fluids like blood or after insertion of macroscopic implants as well as micro- or nanoscaled particles in the body. This process is highly important for material performance in complex biological environments, but the processes involved are as yet poorly understood and typically cannot be well controlled. Figure 1 illustrates that polymer samples recovered from the body after implantation bear layers of proteins and cells on their surfaces. An example of high clinical relevance is the adsorption of potentially undesired proteins to cardiovascular implants, which can be a first step in a cascade of biochemical and cellular processes, which can result in serious adverse events such as myocardial infarction or stroke.

A second example of high in vivo relevance is the unspecific or specific plasma protein adsorption on novel molecular probes for fluorescence imaging in diagnostic applications (e.g. in vivo imaging system for rheumatoid arthritis [Xiralite®], mivenion GmbH), which can influence the targeting functionality (Figure 2).

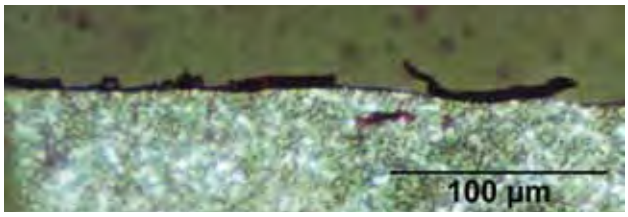


Figure 1: The adsorption of proteins as a layer on the surface of polymeric implants can strongly effect their performance. Source: HZG 2011

Extracorporeal devices which contact blood outside the body (e.g. haemodialysis systems for removal of toxin from blood, Fresenius Medical Care GmbH) frequently fail due to unspecific protein adsorption or inefficient elimination of selected blood components. This is of major social importance given the efforts in cost reduction worldwide and the expected continuous increase in patients treated with extracorporeal blood filtration.

Using its own executive and management structure, the joint key competencies of the different partners of the HVI can be organized very efficiently with HZG-Teltow as the coordinating institute. The issue of protein-polymer interaction can now be comprehensively addressed within the HVI „Multifunctional Materials for Medicine“ by making the required know-how and excellent infrastructure available to all partners. Furthermore, the interinstitutional research activities of the partners will be coordinated by the HVI and performed in close cooperation across the centres, also encompassing the needs, capabilities and results of the associated partners.

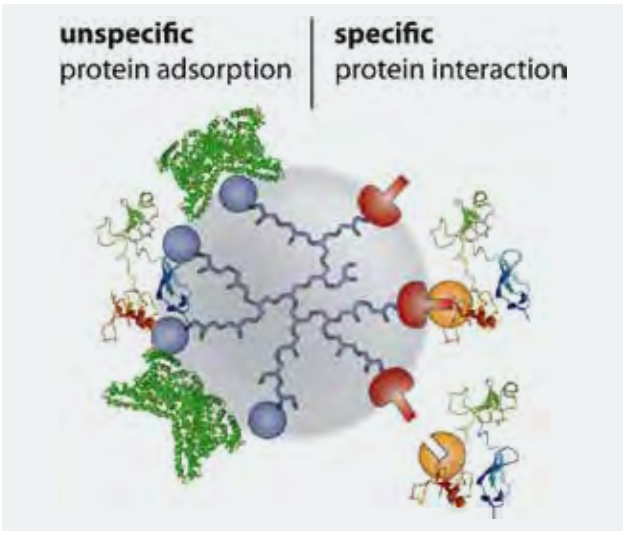


Figure 2: The adsorption of proteins to multifunctional biomaterials can be unspecific or specifically tailored. In some cases, the adsorption of specific proteins may be useful to target polymeric carriers to a tissue of interest. Dr. Wiedekind and Prof. Haag (FUB) are acknowledged for providing this scheme.

The Centre for Biomaterial Development (HZG-Teltow) will contribute its acknowledged expertise in the synthesis and characterization of medical grade polymer-based biomaterials and the comprehensive infrastructure “Biomedizintechnikum” as required for the development, processing, structural and in vitro evaluation as well as the large-scale synthesis of biomaterials. In addition, the HZG runs the clinical translation centre “Berlin-Brandenburg Centre for Regenerative Therapies (BCRT)” together with the Charité, which will be available for the evaluation of multifunctional materials in different clinical disciplines and the future translation of potential products.

The Institute for Soft Matter and Functional Materials of the HZB, which operates large scale facilities to study the structure and function of matter, a research reactor as a neutron source (BER II), and the electron storage ring (BESSY II), will contribute its expertise in biointerface analysis at the HZB. As one of only a few research institutions worldwide, the HZB offers the possibility to use advanced methods like experiments with neutrons, e.g. for small angle neutron

scattering (Figure 3). Furthermore, highly focused synchrotron radiation with a broad range of energies is assessable as well as its novel combination with established analytical methods.

The "Institute of Chemistry and Biochemistry" of FUB, which is interlinked with the internal research platform "Functional Materials at the Nanoscale", contributes their comprehensive expertise in protein analytics represented by the FUB "Bioanalytic Core Facility Protein Characterization". In addition, the clinical researchers at FUB-Charité participating in this initiative work on the Campus Benjamin Franklin in the fields of gastroenterology, inflammation, nephrology, and pain treatment. They contribute a broad range of experimental methods, as well as relevant in vitro and in vivo (animal) models.

Figure 3: The new TOF-neutron reflectometer BioRef is designed for simultaneous in-situ investigation of biologically relevant solid-liquid interfaces with neutron and infrared beams. Prof. Ballauff is acknowledged for providing this picture (HZB).

The HVI "Multifunctional Materials for Medicine" will be a nucleus to establish larger activities and comprehensive academic as well as additional industrial partnerships in the Berlin-Brandenburg region. It will be strongly interlinked with the established or planned activities of the regional coordinated cluster Health Capital, including the research platform "Functional Materials at the Nanoscale" at the FUB, the "Innovation Nucleus for Biomedical Materials" Berlin-Brandenburg, the translational research centre BCRT, the extension of Campus Teltow by the Biomedizintechnikum II (opened in December 2011), the construction of the Biomedizintechnikum III (start of construction in 2012) as well as the incubator KITZ (Kompetenz-Innovations-Technologie-Zentrum, Teltow).

As a centre of excellence, this HVI will substantially increase the international visibility and standing of the Berlin-Brandenburg region in health research.

Helmholtz Virtual Institute (HVI)
Multifunctional Materials for Medicine

Core Institutions

- Helmholtz–Zentrum Geesthacht (HZG-Teltow), Coordinating Centre
  HVI spokesperson: Prof. Dr. Andreas Lendlein
- Helmholtz-Zentrum Berlin (HZB)
  HVI deputy spokesperson: Prof. Dr. Matthias Ballauff
- Freie Universität Berlin (FUB/FUB-Charité)
  HVI deputy spokesperson: Prof. Dr. Rainer Haag
- Albert-Ludwigs-Universität Freiburg
  Prof. Dr. Prasad Shastri

International Partners

- Harvard University, Materials Research Science and Engineering Centre, Cambridge (MA, USA)
- The University of Tokyo, Centre for NanoBio Integration, Tokyo (Japan)
- Sichuan University, National Centre for Biomaterials, Chengdu (China)

Industrial Partners

- mivenion GmbH, Berlin
- Fresenius Medical Care Deutschland GmbH, Bad Homburg

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Centre for Biomaterial Development and Berlin-Brandenburg Centre for Regenerative Therapie

Biomaterials in Regenerative Medicine

Regenerative Therapies require biomaterials with properties and functions tailored to the demands of a specific application. Especially in biomaterial induced auto-regeneration, multifunctional polymer-based biomaterials are of high relevance. The cooperation of scientists from different disciplines is essential in order to perform research and development of biomaterials directed to clinical applications and products. Here, fundamental research for polymer based biomaterials meets application-motivated science aiming at translation of the gained knowledge into products and clinical applications.

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Regenerative therapies aim to restore the functions of damaged or removed tissues and organs, and can be classified into three distinct approaches: somatic cell therapy (i.e. implantation of (stem)cells triggering the regeneration), tissue engineering (i.e. implantation of tissues grown outside the body), and induced endogenous regeneration (i.e. induction of regenerative processes without applying cells). Biomaterials are applied in all three approaches. They are essential in induced auto-regeneration. Here, a biomaterial temporarily provides a structural function. Used as implant, cells settle on and in the material and replace it over time by functional neo-tissue while the implant degrades. If the material can be easily removed or is applied extracorporeal, e.g. as wound cover or as cell culture system, the material does not need to be readily degradable.

Medical applications require specific combinations of properties and functions of biomaterials. Therefore, these material properties and functions (e.g. mechanical properties, thermal transitions, degradation behavior, or the biological interactions with cells)[1] need to be controlled and tailored. On the molecular level, this can be achived by controlling e.g. the monomer composition and sequence structure of polymer chains. Acrylonitrile-based copolymers have been developed in this way, to obtain cell – and tissue – specific materials e.g. selectively supporting the growth of keratinocytes, in order to initiate the regeneration of critical skin wounds and prevent scarring,[2] and Poly(n-butyl acrylate) networks with tailored mechanical properties were designed e.g. as model substrates for mechano-responsive cells.[3]

Materials inspired by nature

In biomimetic approaches, material design is inspired by nature. Materials based on biopolymers derived from the extracellular matrix are one example. They are of high interest for regenerative medicine, since they can structurally and functionally mimic the biological environment of cells. Such materials can attract a large amount of water, resulting in the formation of hydrogels. A challenge for biopolymer-based hydrogels is the reproducibility of their material properties, which is counteracted by batch-to-batch variation of biopolymers from natural resources and the biopolymer-inherent self-organization. Recent examples for successful strategies in biopolymer-based materials are materials based on gelatin[4] and hyaluronic acid[5], which are explored exemplarily for bone regeneration. Figure 1 shows the growth of mesenchymal stem cells on a gelatin-based material, demonstrating the suitability of this material in a key step of the regenerative process.

Other than material's inherent properties, functions of materials such as the cell-material interactions shown above are observed under certain conditions only, e.g. when being implanted and being in contact to a tissue or body fluids, or if heated to body temperature. Functions may furthermore require specific processing or programming of the material. Shape-memory polymers (SMP) show this exemplarily. SMPs are polymer networks, which can be deformed from a permanent shape into a temporary shape and are fixed in this shape ("programming"). Responsivity to an external stimulus such as heat, light, or magnetic fields leads to a self sufficient active movement of

the polymer network through which the original shape is recovered (shape-memory effect, SME). The recovery of the permanent shape is enabled by recoiling of the chain segments of the network and is entropy driven.

The SME can be important in minimally invasive surgery, e.g. for fixation of an implant, and complex movements have also been realized.[6,7] Incorporation of hydrolytically cleavable groups into the polymer chain segments gives a SMP with hydrolytic degradability. An example is a multiblock copolymer synthesized from poly(ε-caprolactone) and poly(p-dioxanone) building blocks named PDC, which in addition to the SME and the hydrolytic degradability was even capable to induce angiogenesis (Figure 2).

The number of functionalities incorporated in one material system was raised to three in an SMP combining hydrolytic degradability and drug release. This multifunctional material was realized by loading the degradable SMP matrix with drugs (Figure 3).

These given results are examples for the progress and potential of fundamental research in the field, which is the basis for a technology driven, application motivated research, which finally should enable the knowledge-based design of biomaterials and the translation of the fundamental findings into products and clinical applications. In order to develop and utilize the full potential of regenerative therapies as key to the treatment of medical conditions, which cannot be cured today, therapies and products have to be translated into the clinic and be brought onto the market to have a measurable input. Therefore, key steps of the development need to be defined, and feedback mechanisms guiding the research have to be established. Figure 4 comprises the development chain of polymer-based biomaterials and medical devices thereof, which has been implemented at the Centre for Biomaterial Development of the Helmholtz-Zentrum Geesthacht (HZG) in Teltow and the HZG-Charité translational research centre Berlin-Brandenburg Centre for Regenerative Therapies (BCRT). The BCRT is co-funded by the Bundesministerium für Bildung und Forschung (BMBF), the states of Brandenburg and Berlin, and the Helmholtz Association, and was evaluated as internationally outstanding centre for translational research in the year 2010. At every step of the development chain, a feedback mechanism ensures a knowledge gain for improving the material and/or device investigated, but also fundamentally increases the understanding on how to approach new projects.

Cooperation networks for Regenerative Medicine in the Berlin-Brandenburg Region

The potential economic prospects of regenerative medicine and its impact on society can not be overestimated. In Berlin and Brandenburg, many key players in this field from universities, research institutes, clinics, and industry are localized in close vicinity, thereby forming a critical mass, capable of establishing the full development and production chain. Research and development projects therefore have an ideal environment in the capital region. This is one of the reasons why additionally to the activities of individual research groups, institutes, and industry, there are several large-scale cooperation projects driving the research and development of polymer-based biomaterials for regenerative medicine in the capital region and beyond. In the Innovation Nucleus "Polymers for Biomedicine in Berlin-Brandenburg" Poly4Bio BB (HZG, FU Berlin and BAM), which is part of the innovation strategy of the states of Berlin and Brandenburg, new polymeric materials for bioanalytics, pharmaceutics, and medicine are generated, validated, and transferred into products. The Helmholtz Virtual Institute (HVI), in which HZG, Helmholtz-Zentrum Berlin (HZB) and the FU Berlin are cooperating, is described in detail in another article in this volume. A further substantial cooperation project is the Portfolio topic „Technology and Medicine - Multimodal

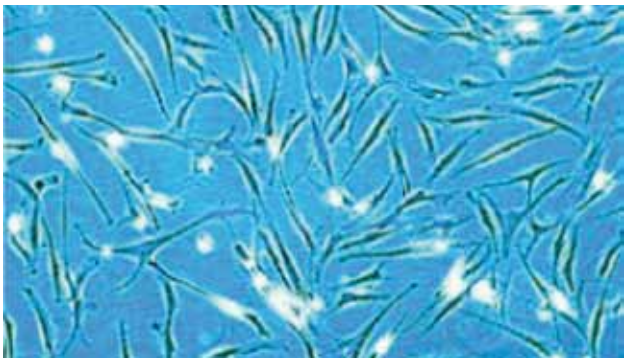

Figure 1: Morphological appearance of MSCs on gelatin-based networks, which maintained typical spindle shape (magnification: x100)

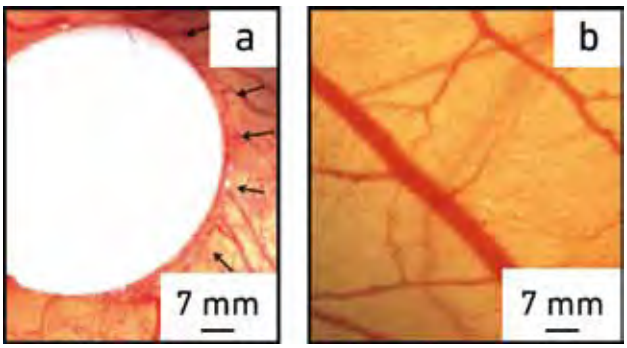

Figure 2: Angeniogenesis induced by a multiblock copolymer (PDC) consisting of poly(ε-caprolactone) and poly(p-dioxanone) building blocks. HET-CAM test a) with PDC b) without material taken after 48 hours of incubation at 37.0±0.5 °C with 65.0±7.5% relative humidity.

Imaging for Elucidating the In-vivo-Fate of Polymeric Biomaterials", which will start in the beginning of 2012. In this project of the Helmholtz Association, the six Helmholtz centres HZG, HZB, Helmholtz-Zentrum Dresden-Rossendorf, Karslruher Institut für Technologie, Forschungszentrum Jülich, and GSI Helmholtz-Zentrum für Schwerionenforschung will collaborate with the university partners FU Berlin, Heinrich-Heine-Universität Düsseldorf and the RWTH Aachen University. These interdisciplinary collaborations combining research in chemistry and material sciences with biomedical characterization and applications, as well as translational activities form the basis for new diagnostics, therapies, and medical devices. As such, they are a cornerstone for the sustainable growth of science and industry in Berlin and Brandenburg.

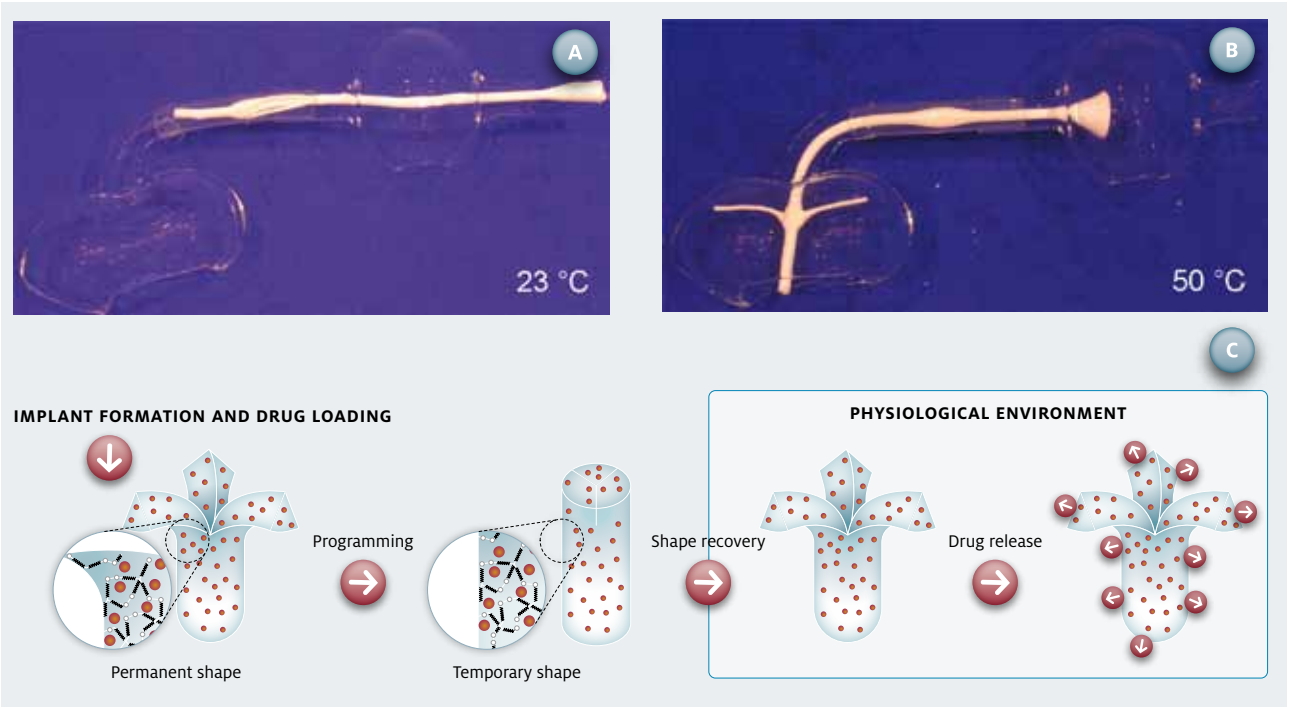

Figure 3: A degradable, drug releasing material with shape-memory effect can e.g. be suitable for a temporarily needed device such as a ureter stent, for which a demonstration object is shown here. **a)** The stent can be placed in the compact temporary shape by introducing it through the bladder and the ureter up to the kidney (the demonstration object shows bladder and kidney as glass, and the ureter as PE tube). **b)** Rinsing with warm water triggers the SME, fixating the stent. **c)** Schematic overview of the concept of programming, shape-recovery, and drug release for a drug-releasing SMP.

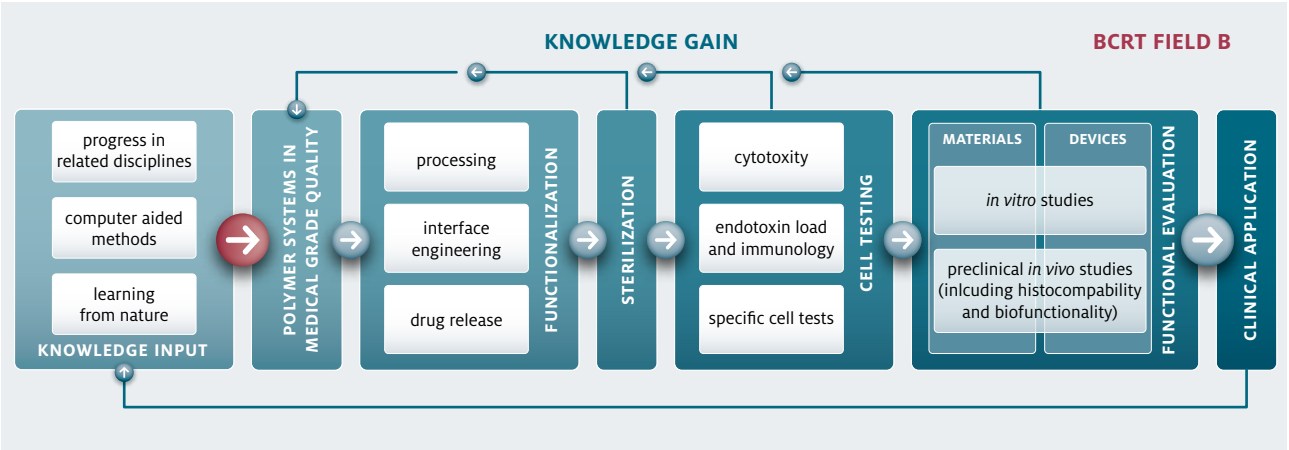

Figure 4: Development chain for polymer-based biomaterials and medical devices thereof.

Sources:

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Figure 4: Adapted from Expert Review of Medical Devices, Sep 2011, Vol. 8, No. 5, pp. 533-537 with permission of Expert Reviews Ltd.

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AutoTissue GmbH

Decellularization of Biological Heart Valves

Commonly used heart valve implants are either of mechanical or biological origin, whereby both have unique disadvantages. During recent years, tissue engineering (TE) techniques made the creation of functional tissues with specific characteristics possible. Recent investigations have led to the development of cell-free valves like the Matrix P plus N®. These valves consist only of the extra-cellular matrix (ECM), and their major advantage is that they do not calcify in vivo and possess remodelling and growth potential. Ten years of experience in patients with excellent results have now been achieved.

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Artificial heart valves: Functions, disadvantages and recent developments

Heart valves direct the blood flow through and from the heart. Failure of these valves results in severe complications which make a surgical reconstruction or replacement necessary. Currently different types of mechanical and biological heart valves are available, but all of them have severe disadvantages, i.e. the need for antithrombotic therapy throughout the patient's life in the case of mechanical heart valves and premature degeneration and calcification in the case of biological heart valves. In the field of biological heart valves, human allografts are often used. However, immunological reactions have been observed, and allografts are not available in sufficient amounts.

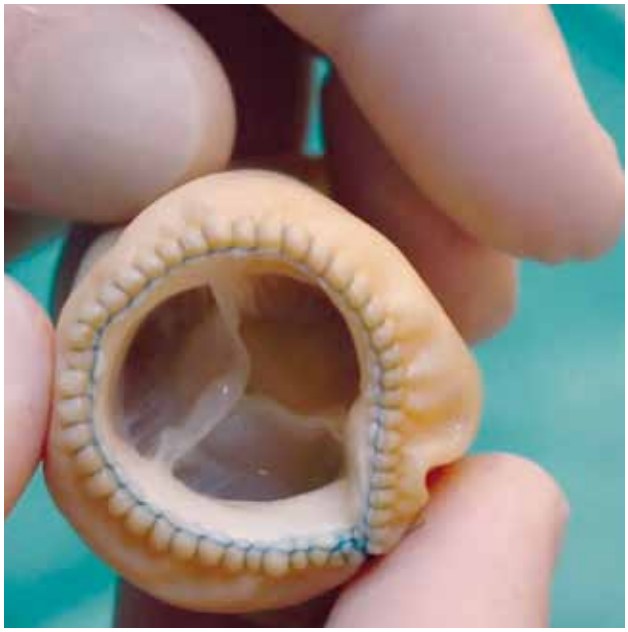

Figure: Matrix P plus cell free pulmonary heart valve

Other biological heart valves are of xenogenic origin, like the frequently used porcine heart valves. Today all porcine heart valves used in clinical practice are tanned with glutaraldehyde to increase tissue strength and to minimize immunological reactions due to porcine cellular components present in heart valves. However, these heart valves often show strong calcification which limits their life span in patients. Recent developments therefore have focussed on techniques to extract all cellular components from the tissue to limit the immunological responses of the body and to achieve an almost cell-free ECM. Most studies performed so far used detergents like sodium dodecyl sulfate (SDS) or trypsin in combination with a calcium-chelating agent to remove cells from heart valves.

However, animal investigations revealed a limited suitability of most of these protocols and only very few have gained clinical approval. One of these techniques is based on the decellularization of heart valves using deoxycholic acid (DOA). DOA efficiently removes cellular components without affecting the collagen structure or the ECM. It is a naturally occurring bile acid which has been used in biochemistry to isolate membrane proteins for a long time. Due to these characteristics, DOA is also suitable to remove virtually all cellular components from the tissue. One major advantage of the clinical use of DOA is its low toxicological potential.

Based on DOA, the medical device company AutoTissue GmbH has patented a tissue processing technique for manufacturing cell-free heart valves that is approved worldwide. Based on this process, the company has developed the first completely cell-free xenogenic pulmonary heart valves, the Matrix P and Matrix P plus N which have gained Europe-wide registration and certification. These valves have already been used throughout Europe in different surgical centres, and more than 10 years of clinical experience have been achieved with excellent results.

Human BioSciences GmbH

Biologically Active Products for Wound Healing and Cosmetics

Biologically active products can make the treatment of complex and chronic wounds more effective, but they also offer many options in cosmetics and aesthetic dermatology for correcting individual defects, counteracting ageing processes and preventing wound formation. Collagen-based wound treatment products are especially important to modern skin regeneration technologies because they combine bioactive action mechanisms with moist wound treatment. The company Human BioSciences GmbH specialises in the development of collagen-based cosmetic and wound treatment products.

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Biologically active products in cosmetics

Skin ageing is characterised by a loss of vitality, elasticity and volume as well as slackening and wrinkle formation. In addition to external environmental factors (e.g. UV radiation) genetically determined factors like the hormone level are responsible for skin ageing. With rising age, hereditary or intrinsic skin ageing leads to a decrease in the number of cells and of intercellular substance, while also impairing cell function. Water can consequently no longer be bound adequately, the skin tonus weakens, and skin as well as mimic wrinkles begin to form.

Preventive strategies focus on hydration (moisturizers, emollients or vitamins). Active therapeutic strategies aim to foster epidermal regeneration, collagen neogenesis and the reformation of blood vessels, for example by chemical, mechanical or pharmacological activation. Passive therapeutic procedures aim to replace and compensate. Compensation is the aim of a range of established biologically active products like fillers, botulinum toxin and fibroblast transplantation. Among the great variety of fillers, in particular collagen, hyaluronic acid and autogenous fat stand out due to their biological activity. The good resorbability of these bioactive preparations plays an important role. The demographic development towards an ageing society increases the demand for biologically active cosmetic products in the field of chronic wound prevention.

The problem of chronic wounds

It has been demonstrated that the natural ageing process promotes the occurrence of chronic wounds. In old age, the protective upper skin layer becomes increasingly thinner and both cell growth and cell renewal slow down. Especially the elderly are consequently affected by chronic wounds. Other causes include defective care and nutrition, a weak immune system, infections and metabolic as well as vascular diseases (Diabetes, CVI, PAOD). The most frequent chronic wounds include ulcus cruris (open leg), decubitus (pressure ulcers) as well as diabetic foot syndrome (DFS). With more than four million patients affected, chronic wounds are a major health burden in Germany. In modern wound therapy using hydroactive wound dressings, the key aim is to preserve a moist environment. In addition, bioactive wound dressings can influence the pathological wound milieu actively, for example by modulation of the tissue-depleting matrix metalloproteinases (MMP).

The biology of wound healing

Wound healing is controlled by a complex system of interactions between the cells and between cells and macromolecules. Polypeptide-based differentiation and growth factors play a vital role in wound healing processes in almost all tissues. The cells required for wound healing are attracted chemotactically. Differentiation factors induce the transformation of undifferentiated stem or progenitor cells into differentiated cells. Growth factors then stimulate cell division and the synthesis of extracellular matrix (ECM), so that new tissue can develop.

Following tissue damage involving subepithelial tissue, injury of the blood vessels results in the exposure of collagen fibres in the blood vessel walls. This is followed by specific bonding of blood platelets to collagen and the consequent platelet aggregation. Blood platelets on the wound edge activated in this way discharge bioactive factors like the platelet-derived growth factor (PDGF) and platelet-derived endothelial cell growth factor (PD-ECGF). In the course of only a few hours following the trauma, cells from the environment of the wound release the additional transforming growth factors α (TGF-α) and -β1 (TGF-β1). After neutrophil granulocytes have agglomerated in the wound, the macrophage level in the wound rises. They phagocytise damaged structures and release additional PDGF, TGF-α and -β1, whereby the migration of connective tissue cells is initiated.

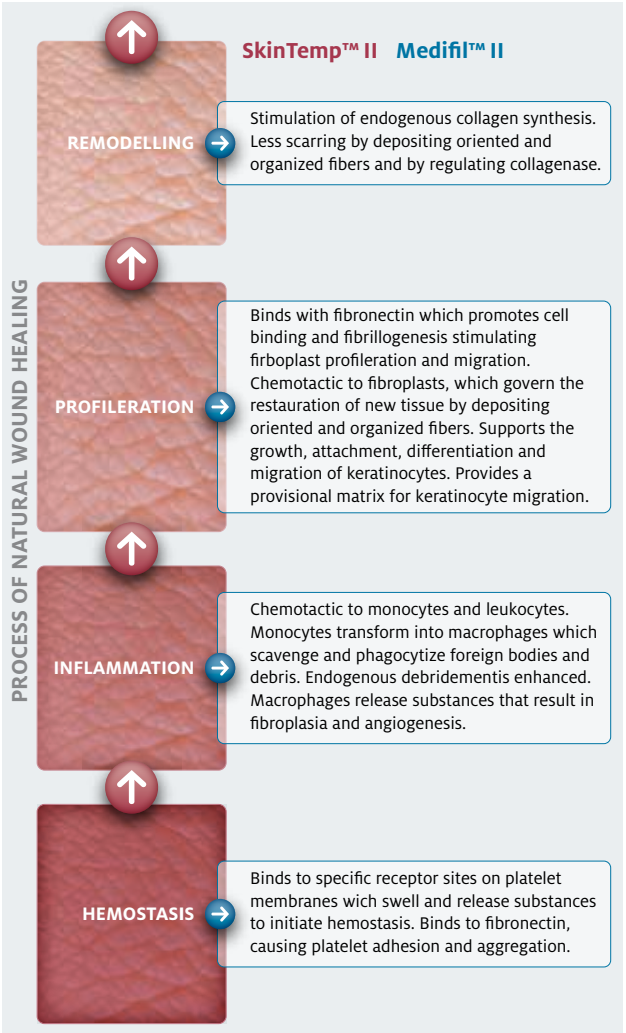
Bioactive collagen matrix SkinTemp (Source: Human BioSciences GmbH)

Biologically active products in wound treatment

Differentiation and growth factors are involved in every wound healing process. The bioactive wound products available today generally aim to reinforce the targeted action of suitable growth factors or to protect the endogenous presence of these factors in the wound by depletion of pathological proteinases (MMP) so as to support and accelerate tissue regeneration actively. Synthetically produced recombinant human PDGF is now the first commercially available human growth factor for application in a range of wound indications.

A fundamental problem in the use of growth factors for wound regeneration, however, is their application, which often requires bonding to a carrier substance (e.g. collagen in the form of sponges, membranes or gels). The use of platelet-rich plasma (PRP) is another strategy for preserving high PDGF concentrations in the wound. Blood platelets play a central role in wound healing and release a high concentration of bioactive substances into the wound. But the preparation of the PRP from autologous blood is very complex and causes additional expense.

A far more economical, but nonetheless effective strategy is to use native collagen-based wound treatment products. The delivery of a bioactive collagen matrix into the wound leads to increased platelet aggregation and activation. Fibrillary collagen is also responsible for the chemotactical attraction and activation of skin fibroblasts and keratinocytes. Native collagen fibres form the bridge for the migration of these regenerative cells and promote the resulting synthesis of growth factors and endogenous collagen. These processes are the precondition for accelerated reepithelization.



Bio-engineered collagen: mode of action. As an example of the great wound healing potential of biologically active products, the graph presents the modulation of the cellular healing processes by collagen-based wound dressings (Source: Human BioSciences GmbH).

In addition, pathological hyperactivity of proteases (MMP) results in the depletion of endogenously synthesized growth factors and collagen fibres. An important strategy of bioactive wound treatment products is therefore to influence the pathological wound milieu positively by inhibiting excessive MMP and protecting the endogenous growth factors. In addition to synthetic wound dressings with protease inhibitor coatings, in particular collagen-based wound dressings are highly effective in this regard due to their substrate inhibition.

Fraunhofer Institute for Applied Polymer Research (IAP) · Potsdam

Polymeric Biomaterials – Implants with an Interdisciplinary History

The Fraunhofer IAP specializes in the targeted development of sustainable processes and materials based on natural and synthetic polymers. The group "Functional polymers for biomedical engineering" is led by Dr. Joachim Storsberg. Together with medical experts, he is performing biomaterials research and development. He was awarded with the Joseph-von-Fraunhofer Prize in 2010 in the field of medical sciences for the successful development of an biomimetic artificial cornea.

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What are biomaterials?

Broadly speaking, biomaterials are synthetic or non-living materials used in diagnostic or therapeutic applications in medicine. They are in direct contact with the living tissue (biological environment) and display physical, chemical and biological interaction with the living biological system. Biopolymers are polymers produced by living organisms – they are not necessarily biomaterials. Biopolymers can become biomaterials if they are used for therapeutic or diagnostic purposes and therefore come into direct contact with biological tissue of the body. Biopolymers are often used to modify biomaterials and give them special functions.

Most biomaterials are of artificial origin, which means they are not biopolymers. From the materials point of view, they can be subdivided into inorganic (e. g. metals like titanium, gold, alloys, compounds like hydroxylapatite, etc.) and organic (polymers like poly(meth)acrylates, polyolefins, silicones, etc. and biopolymers such as proteins, carbohydrates, polylactide, etc.) materials.

Surfaces and interfaces of biomaterials – The critical area

Often, common polymers do not display the specific function desired for use as a biomaterial. Their mechanical and physical properties are very good, but they lack the biological compatibility necessary to meet the demands of the tissue. The part of a biomaterial interacting with the tissue is its surface. The surface of the material is therefore adapted to the specific function by intelligent chemical and physicochemical modification, often also by "nanotechnological" methods. The living tissue consequently comes in contact with this "bio-adapted" interface. Rejection by the body is a frequent problem with implants. The interface between the implant and living tissue is therefore a crucial domain of biomaterials, and special focus and care must be given on how to design this very important area to achieve the best positive interaction of implant and living tissue. Understanding the control of surface properties and functions is the key to the successful use of biomaterials in medicine.

Biomaterials – Talking with living tissue

Polymers for implantable medical devices have to meet different specific challenges. Especially implants used in ophthalmology must fulfil specific requirements as regards their physical, chemical and biological properties. In addition, polymers are modified on the surface – the interfacial part interacting and "communicating" with the living tissue. To enable this communication, active polymers such as special proteins (for example special amino acid sequences – or more complex proteins such as growth factors) are bonded to the surface to initiate cell adhesion and proliferation so as to provide integration in the surrounding tissue. By doing so, living cells from the tissue start to "talk" with the artificial implant. Also, the intrinsic properties of the polymer material itself influence the interaction with living tissue and can therefore be used for intelligent implant design.

Interdisciplinary research on biomaterials

Regarding the numerous factors to be considered, it is obvious that a successful development can only be achieved within an interdisciplinary team consisting, among others, of chemists, biologists, physicists, engineers, physicians, health professionals and surgeons. Biomaterials for implantable applications in the human eye are used e.g. as intraocular lenses to substitute the vitreous and cornea. As one of many successful examples of biomaterials development, the research towards an artificial cornea will be described here.

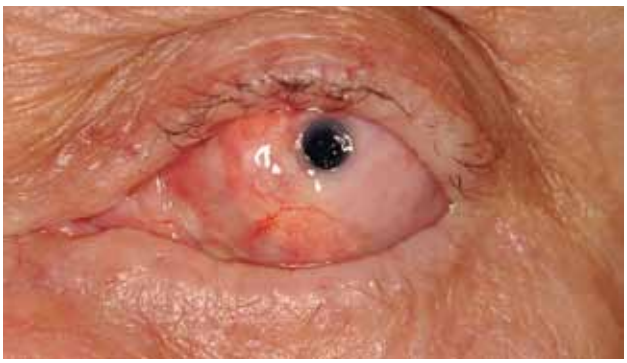
Artificial cornea – An example of an implant with very special and differentiated functionalities

The cornea of our eyes is the window to the world. Compared to a photographic camera, it is the "front" lens. If the cornea becomes

opaque – the reasons can be multiple in nature, e.g. systemic diseases, infections, accidents, chemical burns, etc. – this will result in a loss of vision, unless corrected by a corneal transplant. In cases where all known medical treatments and procedures have failed, i.e. so called "ultima ratio" patients, an artificial cornea (also called keratoprosthesis) is the last hope of restoring vision. Indications [1] for a keratoprosthesis can include ocular pemphigoid, Fuchs-Stevens-Johnson Syndrome, Lyell-Syndrome, serious chemical burns and burns, serious xerosis/xerophtalmia, leucoma adhaerens, mucolipidosis type IV, trachoma, corneal opacification caused by non-removed silicon oil filling as well as recurring cornea rejection reactions.

Artificial cornea (keratoprosthesis) (Source: Fraunhofer IAP/Armin Okulla)

Different functionalities in one and the same implant are required to fulfil different biological interactions [2]. The "interface" to the tissue, the skirt (haptic), is of such nature that tissue will stick on it, thus providing a good base for cell attachment, proliferation and healing. When implanted to the eye, this part has to grow into the existing tissue. The optical part of the artificial cornea has to display two different surfaces. The posterior optic of the artificial cornea is of a nature preventing the adsorption of proteins and cell growth, thus guaranteeing that the optical part stays clear. It is very obvious that this part has to stay clear and transparent at all times. The anterior side should be hydrophilic to provide a smooth surface, and cells should also not stick on it. What nature does without any problem is a challenge to do by man. So there are many tasks, functions and factors that have to be realized on a small piece of polymer material.



Implanted cornea (keratoprosthesis) (Source: Fraunhofer IAP/Augenklinik Martin-Luther Universität Halle (Saale); already published in J. Storsberg, K. Kobuch, G. Duncker, S. Sel, Deutsche Zeitschrift Klinische Forschung 2011, 5/6, 58-61)

Modification of biomaterials towards the desired functionality

The individual steps for the tailored modification of the biomaterial can be summarized as follows: Starting with an hydrophobic polymer material, which has to be – and stay – transparent, of course (this makes implants for the eye different from others), the biomaterial was treated in its haptic part and on the surface of the anterior optic with a plasma in order to activate the surface. The haptics was treated with alternating layers of polyelectrolytes to build up an "adhesive" layer for a protein used to promote cell adhesion and proliferation. The optical part was treated with a special monomer that was polymerized to form an interpenetrating polymer network on the optical surface. In contact with water (tear fluid) this polymer turns into a hydrogel, thus providing a wettable surface. On the haptics surface, a cytokine (a protein known as a growth factor) is adsorbed. Cells coming in contact with this protein-modified surface get a "signal" which tells them to stick on and proliferate, thus forming a tissue around the implant to keep it mechanically fixed in the existing tissue. Due to the nature of the material on the posterior side and the hydrogel on the anterior side of the optical sector, cells stop at this interface. So, this artificial implant gives back the function of the human cornea to the patient – whose vision is restored.

[1] K. Hille, *Keratoprothesen – Klinische Aspekte*, Opththalmologe 2002, 99, 523-531
[2] J. Storsberg , K. Kobuch, G. Duncker, S. Sel, *Künstliche Augenhornhaut: Biomaterialentwicklung eines ophthalmologischen Implantats mit biomimetischen Funktionalitäten*, Deutsche Zeitschrift für Klinische Forschung 2011, 5/6, 58-61

Technische Universität Berlin · Department of Chemistry

Micro- and Nanopatterned Biomaterials: Fundamental Research for Tissue Engineering Applications

How can we prevent inflammatory reactions to implant materials? How to improve the integration of scaffolds into the human body? Can biomaterials and nanomaterials science help us predict the bodily response to biointerfaces? What lessons can we learn from Nature when it comes to hierarchical organization at several length scales? Will we soon be able to control the biological responses from the single protein receptor binding, via complex formation, subcellular organization and morphological adaptation to microscopic surface patterns all the way to macroscopic functioning of a biocompatible construct?

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These are questions that our group – the "Lensen Lab" – are attempting to tackle. The Lensen Lab has developed a toolbox of tailor-made biomaterials that are patternable in 2D and 3D and at different length scales, have tunable physicochemical properties, specific degradability profiles and all the characteristics of a desired biomaterial. Being a chemist by training, I have built up a young international research team that is experienced in in-vitro work with cell cultures, e.g. assessing the cytocompatibility of any new material, investigating the cell adhesion, morphology and migration behaviour of living cells on the patterned biointerfaces, and investigating the invasion of 3-dimensional scaffolds by living tissue.

The research group focuses on 3 main themes:

1. Synthesis of novel biomaterials, e.g. hydrogels
2. Development of new and easily applicable micro- and nanopatterning methodologies
3. Understanding how the patterns influence the response of living cells.

The ultimate goal is to be able to conceive the optimal biointerface, where the size, geometry and periodicity of the surface pattern evoke the desired density of adherent cells which have a healthy morphology, merrily proliferate, actively explore the surface and migrate in a constructive manner, so that scaffold invasion, for example, is aided.

To achieve this objective, the Lensen Lab fabricates and investigates model surfaces, i.e. micro- and nanopatterned biomaterials, first to understand and then to control cellular behaviour. The fundamental insight that is gained by examining model systems will be of direct relevance to many biomedical applications, i.e. not only in tissue engineering (e.g. improving the acceptance and integration of implants) and tissue regeneration (e.g. repair of bone or brain tissue; faster wound healing), but also in diagnostics and biosensor development.

Synthesis of novel biomaterials

In the field of synthesizing novel biomaterials, the Lensen Lab has synthesized a library of linear and star-shaped pre-polymer building blocks that can be photochemically cross-linked to form elastomeric materials. The group has exploited a polyethylene glycol (PEG) material that has the properties of a hydrogel, so that it is insoluble in water after cross-linking, but can absorb large quantities of water. This material is of particular interest for tissue engineering applications since the resulting hydrogels resemble biological tissue to a great extent, due to their high water content (up to 95%) and the stiffness of the bulk gels that is similar to that of human tissue.

Development of new and easily applicable, micro- and nanopatterning methodologies

The Lensen Lab has taken soft lithography methods such as micromoulding, replica moulding, nanoimprint lithography and microcontact printing as an inspiration to conceive unique, new patterning methods. For example, a patented "secondary mould approach" method using a fluoropolymer (PFPE) and the PEG-based hydrogel has been devised to physically pattern the biomaterials' surfaces with topographic micro- and nanostructures. In a second procedure, the physically patterned replica is further modified to yield chemically and elastically patterned hybrid gels, which are topographically smooth. These patterns are introduced by filling the grooves of the mould with a liquid precursor formulation that has slightly different chemical and mechanical properties. The very versatile surface patterning method that has been invented for this purpose is called FIMIC (Fill-Moulding In Capillaries). The patterned biomaterials are characterized by "Atomic Force Microscopy (AFM)" in terms of surface topography and elasticity. Also (subtle) chemical contrasts are recognized by this scanning probe method. Moreover, a number of ways to produce nanopatterned gold templates have been developed,

for example using gold nanoparticles that can be immobilized in periodic and aperiodic, micrometer-sized patterns. These gold templates serve as anchoring points to bind proteins and other biofunctional molecules with ultimate nanoscopic precision and control.

How the micro- and nanopatterns influence the response of living cells

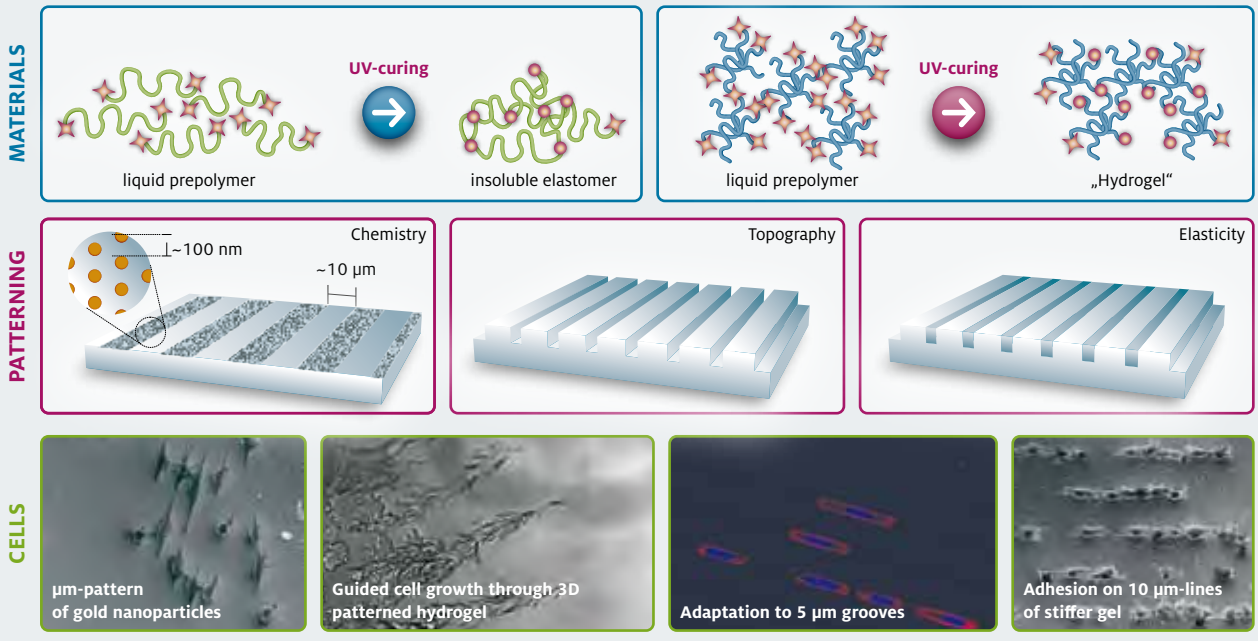
In vitro, cell adhesion to artificial surfaces, e.g. biomaterials, critically depends on the chemical, physical and mechanical characteristics of the material. Consequently, the administration of defined surface patterns of topography, chemistry and elasticity allows us to manipulate cellular responses to our biomaterials. Cell adhesion and spreading are monitored by live cell imaging, and the cell morphology is observed by optical and electron microscopy. To elucidate subcellular processes such as focal adhesion complexes that are built up from protein assemblies, highly specific immunological staining methods (using fluorescently labelled antibodies) are employed, and the stained cells are investigated in great detail by means of fluorescence microscopy. Finally, the process of mechanotransduction, i.e. the sensing of the substrate's stiffness, is investigated by time lapse microscopy in order to understand the dynamics of cell migration in response to stiffness patterns.

PEG-based hydrogels: The challenges for biomedical applications

For biomedical applications (e.g. tissue engineering or biosensors for diagnostics), cell adhesion is often undesired since it can lead to inflammation reactions and eventual failure of the implant or device. To suppress the unwanted cell adhesion, the implant material or biosensor device can be coated with a 'stealth' material that shields the underlying substrate. Polyethylene glycol (PEG) is a very well established and widely used biomaterial for this purpose. PEG has been shown to prevent non-specific protein adsorption (NSPA) very effectively, as well as the adhesion of prokaryotic and eukaryotic cells.

Nevertheless, the Lensen Lab has found that PEG is only cell-repellent if the surface is smooth and of homogeneous stiffness: On our physically and elastically patterned PEG-gels cells do adhere. Since PEG is generally well known for its anti-adhesive behaviour and commonly used for biomedical applications, it is important to take into consideration what our study has shown: Physical and mechanical surface properties can impede the anti-adhesive characteristics of PEG.

On the other hand, it also opens up new opportunities for biomimetic material design which does not rely on biochemical surface functionalization to manipulate cellular responses.



Fotos: Dipl.-Biotechnol. Vera Schulte

Max Planck Institute of Colloids and Interfaces · Department of Biomaterials

Bone Healing from a Materials Science Perspective

Bone healing is a complex regenerative process which culminates in full restoration of the bone's original characteristics and structure. A callus forms around the fracture to stabilise the broken bone. In the course of healing, a continuous formation and remodelling of different tissues occurs. However, bone healing is not always successful – but the process can be supported by biochemical or mechanical intervention and by the introduction of synthetic scaffolds.

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Material characterisation of bone tissue

The characterisation of bone tissue before, during and after bone fracture healing provides an important contribution to understanding the fundamental biological processes in bones. In the Department of Biomaterials at the Max Planck Institute of Colloids and Interfaces (MPI-KG) in Potsdam, scientists from different disciplines are investigating the material characteristics of bones at the micro- and nanometre level. These studies are implemented in cooperation with the team of Prof. Dr. Georg N. Duda at the Julius Wolff Institute of the Charité in Berlin.

In addition to histological examinations which reveal the spatial and sequential distribution of different tissue types in the callus, it is essential to evaluate and understand the characteristics of these materials. The examination of the spatial distribution and time sequence of structures in the callus tissue during healing is implemented at the MPI-KG using electron microscopy, light microscopy and X-ray scattering techniques. In addition, the mechanical properties of the tissue are determined, e.g. by nanoindentation.

The researchers at the MPI-KG were able to show that bone healing involves a tissue formation process that is similar to original bone formation. In large animals like sheep, for example, cortical bone grows very quickly in a sequence of two successive steps. First, an unstructured bone is formed, which is subsequently expanded and ultimately replaced by better structured bone. This structured bone has significantly improved mechanical properties compared to the material initially deposited. Interestingly, a similar process was recently also observed during callus formation in a healing experiment in sheep (Figure 1a). This finding led to the conclusion that bone healing requires an intermediate step. The bone is first deposited with less aligned collagen fibres as the foundation for the bone-forming cells (osteoblasts) (Figure 1b). On the surface thereby created, the osteoblasts can synthesize strongly aligned lamellar tissue by coordinated action (Figure 1c).

The healing of bone defects of critical size where the cells themselves are no longer able to close the defect can be supported by scaffolds. Here, it is of special interest how a scaffold influences the tissue generated during healing with regard to its structure and mechanical properties. Appropriate architectural design of the scaffold enables the formation of a structured fibrous network in the bone defect and is therefore the precondition for bridging the fracture site. Investigating bone fracture healing with materials science methods provides insights on the role of surfaces, on which cells can arrange themselves to form aligned tissue. Either the bone itself synthesizes suitable material and thereby creates surfaces on which mechanically competent structures can grow, or scaffolds are introduced to form synthetic surfaces for aligned bone formation.

Computer simulation of bone healing

To influence the bone healing process positively, it is necessary to understand how the process is controlled. The mechanical stimuli exerted by the muscles on the fracture site provide a decisive controlling factor. These stimuli exert local influence on the development of the stem cells which migrate into the callus.

The basic idea is that a strong mechanical stimulus leads to cartilage forming cells, whereas a weak stimulus permits the direct formation of bone. Computer simulations are excellently suited to subject ideas about the control of bone healing to critical scrutiny. A hypothesis is formulated first as to which mechanical stimuli in terms of quality and quantity lead to a special type of cell development. This hypothesis is implemented in a computer model. The sequence of the computer programme permits predictions about how the spatial distribu-

tion of the different tissues changes over the healing cycle. These predictions at tissue level can then be compared with experimental histological sections. The results from experimental material characterisation form an essential component of the computer model.

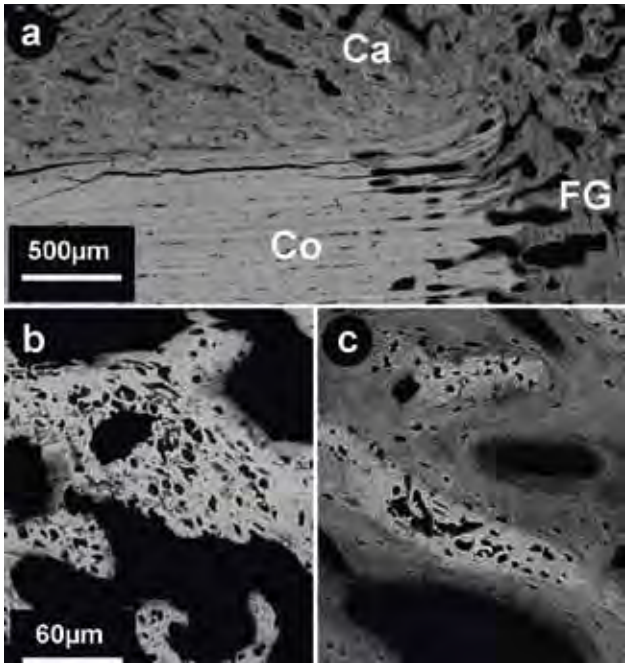

Figure 1: Electron microscopy images of bone femurs from sheep at different stages of bone healing: (a) cortex (Co), callus (Ca), fracture gap (FG), 9 weeks after start of healing. (b) The magnification of a comparable sample two weeks after the fracture event shows a poorly aligned bone. (c) Magnification of a comparable region 9 weeks after healing began shows the more developed callus. The alignment of the bone lamellae (less mineralized, dark grey areas) is parallel to the surface of the bone areas formed first (highly mineralised, bright areas). Kerschnitzki et al. Cells Tissues Organs 2011;194:119-123

The different stages of the healing process described by the computer model under careful selection of the model parameters conform to the experimental histological findings. Simulations can help answer questions like, for example, from where the stem cells migrate into the callus and from where the formation of new blood vessels begins. Stem cells and blood supply are indispensable preconditions of the healing process. The simulations have shown that the best conformity with the experiment is achieved based on the assumption that the periosteum – i.e. the bone skin on the outer side of the bone – is the main source of stem cells and blood supply.

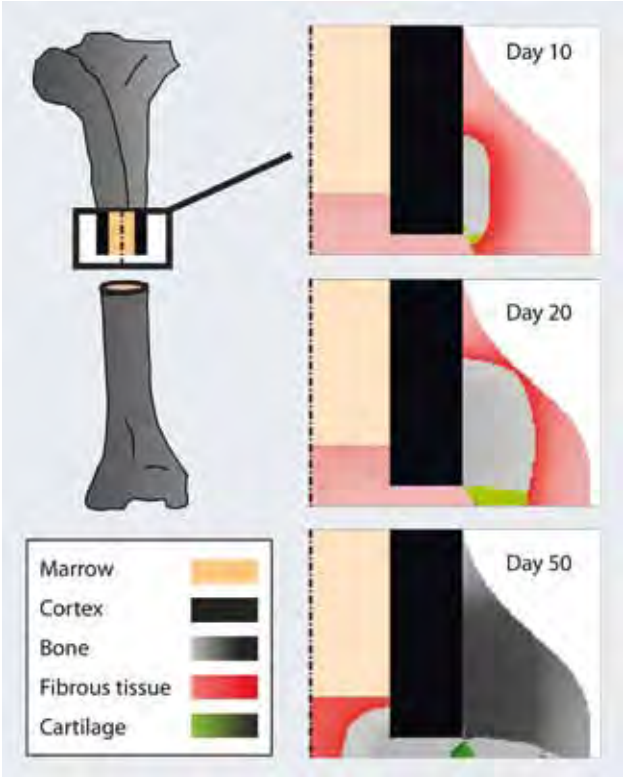

Figure 2: Computer simulation of the healing of a tubular bone. The simulation is based on an experiment with sheep where the bone was dissected (osteotomy) and the healing process studied. The simulation images only show the upper right portion of the dissected bone respectively (in black) along with the predicted distribution of the different tissues at three points in time after the dissection of the bone.

Julius Wolff Institute and Berlin-Brandenburg Centre for Regenerative Therapies · Charité – Universitätsmedizin Berlin

Biologised Implants for Bone Healing

Bone healing disorders remain a major problem in orthopaedic care and trauma surgery. Up to 30 per cent of patients with a tibia shaft fracture experience post-surgery complications. And the numbers are increasing, since the rising average age of the population can be expected to result in an increase in the number of complications following musculoskeletal injuries. In addition to improving the methods of surgical intervention, new therapeutic procedures to stimulate biological healing are currently being developed to confront that trend.

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Although bones restore their original structure and properties following injury without generating inferior scar tissue, clinical experience shows that complications can arise in the treatment of bone fractures. In five to 30 per cent of cases, delayed fracture healing, non-unions and infections occur. In addition, the trauma and operation may result in problems like blood loss, injuries to vessels and nerves and even compartment syndrome. These complications seriously impair patient health and also present a major socioeconomic burden.

Such bone healing disorders can be caused by mechanical factors such as unbalanced loads or insufficient stabilisation. Complex biological factors like impaired vascularisation and soft tissue damage which delay fracture healing may also be contributory causes.

Biological stimulation of healing

Current research in this field therefore focuses on the use of substances which stimulate healing. They include growth hormones and parathyroid hormone as well as a range of growth factors. The latter are endogenous factors which influence different cellular processes, from the development to the maintenance and regeneration of tissues and organs. Since no factors have as yet been identified which act on bone cells only, local application is essential when using such substances to stimulate bone healing so as to prevent undesired side-effects in other organs.

The local application of factors to influence bone healing

For more than ten years, the research group headed by Britt Wildemann at the Julius Wolff Institute of the Charité has studied the possibilities for improving bone healing by local application of factors. This research addresses two key themes: infection prophylaxis and the promotion of healing. Antibiotics are used for infection prevention, while factors stimulating the bone metabolism are applied to promote healing. To be effective, these substances must be applied locally, directly at the site of the fracture or defect. For two reasons: on the one hand, the fracture causes a destruction of blood vessels, so that systemically applied factors do not reach the target location in sufficient concentration. Secondly, local application permits use of a reduced dose and prevents systemic distribution of the factors which could otherwise cause undesired side-effects in other tissues.

To be eligible for clinical use, an application system must fulfil the following criteria:

- It must be biocompatible.
- It must display adequate release kinetics.
- It may not interfere in the healing processes, and
- It should not necessitate further intervention.

Application system based on a PDLLA coating

The application system for local and controlled application of factors developed by the group is based on an implant coating with the polymer poly(D,L-lactide) – in short: PDLLA. In cold condition, metal implants can be coated with a varnish-type PDLLA coating under inclusion of sensitive substances like proteins. The coated osteosynthesis material therefore serves as a stabilisation system and local drug carrier at the same time. Several studies have confirmed a high mechanical stability of the PDLLA coating on implants, while microbiological tests have confirmed its sterility. The adhesion of microorganisms was reduced significantly by the PDLLA coating and a good biocompatibility of the coating was also demonstrated. The factors are distributed in the coating in the form of a fine suspension and released above all by diffusions and erosion processes. The

thickness of the layer, the amount of factors applied and the release kinetics can be adjusted simply by variation of the polymer/solvent/factor ratio. This is especially important since it permits responding flexibly to requirements. For infection prophylaxis, the initial release of antibiotics is required, but it is known from more recent studies that growth factors are important at different stages of the healing process. Time-controlled release of different factors could therefore be optimal for the successful stimulation of healing.

The figure shows a titanium nail (a) for intramedullary stabilisation of long bones. The polymer coating is shown in blue. The raster electron microscopic pictures show a cross-section of a titanium wire with the coating (b), a detail enlargement (c) and the coating with integrated gentamicin (arrows, d); scale bar: 20µm.

Scientific method and results

Our scientific approach to improving bone healing by the biologisation of implants is based on three steps:

1. Identification of the clinical problem (delayed healing, defect healing, bone infections)
2. The search for therapy options and their optimisation by improvement of the application format
3. Multistage evaluation of new developments, from in vitro to preclinical testing in suitable models.

The combination of basic research and clinical studies can yield new insights which ultimately lead to a human therapy.

The promising results of the experimental investigations prompted the decision to transfer this technology to the human application of gentamicin-coated intramedullary nails in especially critical situations. The first coated tibia nails (UTN Protect, Synthes®) were implanted in patients with complex open lower leg fractures in 2005. The indication for implantation of nails coated with antibiotics is given especially by severe open fractures since they entail an up to 30 higher risk of osteomyelitis compared to patients with closed fractures. A tibia nail (ETN Protect, Synthes®) with optimised design and a gentamicin coating has been CE-certified since 2011.

PolyAn GmbH

Molecular Design of Surfaces

The influence of surface characteristics on a wide range of chemical, physical and biological processes has been and is gaining increasing recognition in analytics. The end user applications in this dynamically developing field extend from molecular (human) diagnostics including pharmaceuticals research, through veterinary diagnostics as well as analytical issues in plant breeding and cultivation, to environmental analytics.

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PolyAn is a nanotechnology company engaged in molecular surface engineering (MSE). It develops and manufactures functional materials mainly for molecular diagnostics und research in the life science industry.

Today, some 1400 different molecular (human) diagnostic tests are available on the market worldwide. Multiplex tests, of which some 135 are already on the market or at the development stage, form the most dynamic segment in this market. The simultaneous measurement of several biomarkers permits both an increase in sensitivity/specificity and a reduction in costs. The surface of the materials used (microparticles, slides, 96 well plates, etc.) is thereby of quality-defining importance.

The solutions offered, however, often still come up against the following problems: With decreasing sample volumes, unspecific interactions occur frequently. At the same time, a low biomarker concentration in the samples often leads to insufficient sensitivity. In addition, there is often a need for combinations of customised functional matrices with mechanical or chemically robust materials suited for low-cost processing, for example by injection moulding.

Consumable material requirements

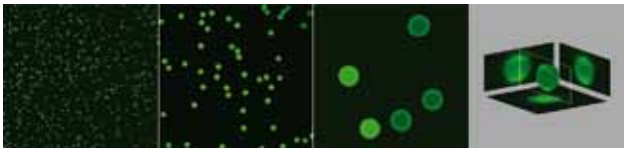
The preconditions for use of the materials in molecular diagnostics are low batch-to-batch variation of the surface characteristics and excellent homogeneity of the surface itself. The consumables should permit good and above all simple and robust handling and, in the case of optical readout procedures, be characterised by excellent optical properties such as low autofluorescence and high optical transmission. The use of the proprietary molecular surface engineering (MSE) technology of PolyAn makes it possible to produce consumables customised for applications in the field of multiplex diagnostics, which are characterised by:

a) Covalent anchoring of a specific nanoporous 3D functional matrix on polymer materials which are also suited for injection moulding;
b) Variation in the density of functional groups;
c) Variation of the surface morphology to reduce unspecific interactions; and
d) Adjustable surface hydrophilicity / hydrophobicity.

Substrate platforms for multiplex tests

In the field of multiplex analytics, a wide range of technological solutions based on the most diverse substrates and readout systems is available. This means that there is no uniform format on which readout and handling systems could build, but a number of substrate platforms have been established on which most multiplex tests run:

Bead arrays: Multiplex bead arrays are mainly read out using systems based on flow cytometry. Alternately, imaging processes like the system AKLIDES® are available which are usually more robust to operate.



Confocal Laser Scanning Microscopy (CLSM) images of fluorescence encoded PMMA beads. The image on the right nicely illustrates the homogeneous distribution of fluorophor in the bead (Source: BAM Bundesanstalt für Materialforschung und -prüfung).

For these applications PolyAn has introduced functionalised polymer beads on the market which are used for multiplex bead assays, to optimise cell assays and other applications. The integration of several fluorophores and the precise production in size classes between 2 – 25 µm with a standard size deviation of less than 2 – 5 per cent

permits production of a large number of well distinguishable bead populations for multiplex applications.

The 3D functional matrix on the surface of the particles ensures minimal aggregation and a low limit-of-detection. In selected immunological applications, the PolyAn beads consequently achieve a 10 times lower limit of detection than competitor products and a dynamic range of up to 400.

Planar arrays are spotted, for example on glass slides for medium or high-density arrays. These slides are fitted with reactive layers to couple biomarkers. In some products very thin slides (app. 120 µm) are also used, which are printed in large scale and then broken into smaller pieces to equip individual test kits.

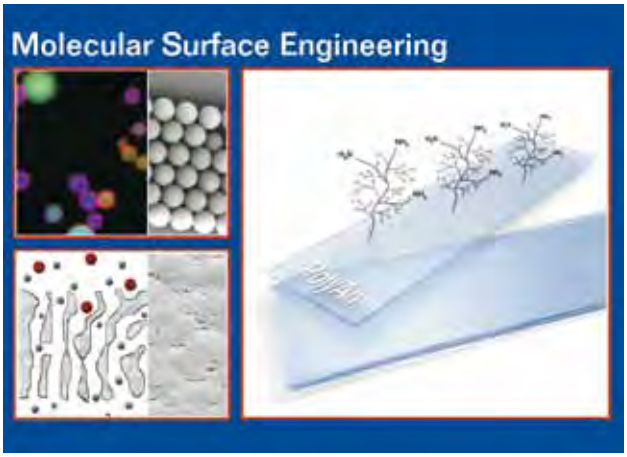
Plates (96, 384, 1536 well and other formats) are the most widely used format for classic ELISA tests. By using the different cavities of the plate for different biomarkers, this format can be rendered "multiplex-capable". In addition, a range of providers now also print arrays of low to medium density in 96 well plates.

Slides made of highly transparent polymer materials (HTA™ platform from Greiner) have also become established in diagnostic applications. They, too, can be fitted with reactive layers for coupling and have the additional advantage of being largely break-resistant and can be produced by injection moulding.

The PolyAn microarray slides are characterised by excellent homogeneity within the slides and from batch to batch as well as, in particular, by a high signal-noise ratio. In addition to plastic and glass slides, the product portfolio of PolyAn in the field of microarray slides also includes 96 well microplate formats. For fluorescence-based detection systems, PolyAn has also developed specific calibration solutions.

Molecular surface engineering in medical technology and other applications

In addition to slides and beads, PolyAn offers consumable and substrate materials for OEM applications which are tailored to specified customer requirements. They include highly robust wicking materials for sensor applications, sample vessels equipped with antifouling layers for cell analytics and reactive substrates for solid phase synthesis of complex molecules.



PolyAn's Moleculare Surface Engineering is used to enhance the performance of microparticles, microarray slides, 96-well plate formats and membranes (Source: PolyAn).

Freie Universität Berlin · Institute for Chemistry and Biochemistry

Multifunctional Polyglycerols as Innovative Biomaterials

The Dahlem Research Campus with its many local and neighbouring university and extramural research facilities, federal institutions and companies in the life science sector provides an outstanding research environment – above all for the field of biomaterials. One successful example is the cooperation of the research group headed by Prof. Dr. Rainer Haag at Freie Universität Berlin with mivenion GmbH on the research campus Dahlem, which has successfully characterized a new macromolecular diagnostic agent for inflammation imaging of rheumatoid arthritis.

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mivenion GmbH develops personalised solutions for medical diagnostics and therapy. Inflammatory diseases like rheumatoid arthritis form a focus area of these activities. A rheumatism scanner now available for clinical practice (Xiralite® X4) which was developed in cooperation with the Physikalisch-Technische Bundesanstalt (PTB) permits early diagnosis of active inflammation sites in the hands as well as monitoring treatment progress. Imaging is implemented by highly sensitive fluorescence markers.

Our research group at the Institute for Chemistry and Biochemistry at FU Berlin has profiled a macromolecular substance which promises to open up a new approach for the treatment and diagnosis of severe inflammatory diseases. We recently described the diagnostic use of this macromolecular agent in the journal Bioconjugate Chemistry. The therapeutic potential of this synthetic macromolecule which targets multiple proteins and has anti-inflammatory characteristics was published in the Proceedings of the National Academy of Sciences 2010 in the context of a successful cooperation between the university and industry. The foundations for the profiling were laid in the collaborative research centre 765 of the German Science foundation "Multivalency as a Chemical Organisation and Action Principle".

Polyglycerol sulfates target inflammations

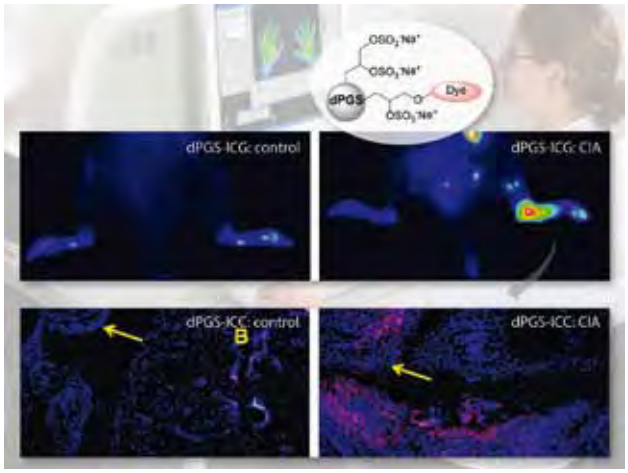
The new substance is a macromolecular conjugate of polyglycerol sulfate (dPGS) with a dye which fluoresces in the near infrared spectral range and can therefore be tracked in the organism using optical imaging methods. dPGS has a compact, highly branched chemical structure with a high number of functional groups on the periphery. These freely accessible groups are available for functionalization by coupling with targeting structures or dyes as optical diagnostic markers, for example. By detecting different target structures in inflamed sites – for example by interaction with L- and P-selectins on cell surfaces – it could be shown that the polyglycerol sulfate conjugate (dPGS) accumulates specifically in inflamed joints of an animal model for rheumatoid arthritis. It therefore represents an alternative to the available biologicals for specific imaging of inflammations. By coupling other therapeutically active substances, the targeted transport of these drugs can lead to an increase of effectiveness and a reduction of side-effects. Functionalized dendritic polyglycerols have been a focus of research in our research group for many years due to their excellent biocompatibility, protein-repellent properties and very good water solubility. As innovative biomaterials they promise to provide a wide range of applications in future.

Research Campus Dahlem – An ideal network for biomaterials research

The modern Research Campus Dahlem located in the southwest of Berlin has an impressive history as a top-ranked location for research and study. A number of institutes of the former Berlin University and federal agencys already located here at the start of the 20th century. In particular the opening of institutes of the Kaiser-Wilhelm-Gesellschaft founded in 1911, which was the precursor of today's Max Planck Society, swiftly made Dahlem into one of the world's leading natural science research locations where scientists like Albert Einstein, Werner Heisenberg, Max Planck, Otto Hahn and Lise Meitner conducted their research.

Today the sciece institutes at Freie Universität Berlin with their strong research profiles and the region's extramural research facilities pro-

vide a fertile environment for young start-ups and innovative small and mid-sized companies in the fields of biotechnology and biomaterials. The Institute for Chemistry and Biochemistry of the Freie Universität Berlin offers some of these companies – e.g. RiNA GmbH, Biolitec Berlin GmbH, nanopartica GmbH and mivenion GmbH – direct contact with scientists and the potential to establish intensive research cooperations. Mologen AG is a young biotech company located in the immediate neighborhood. In the field of biomaterials and drug delivery systems, research groups at FU Berlin from chemistry, biochemistry and pharmaceutical science are cooperating closely across disciplines – partly in joint labs – with researchers at Charité - Universitätsmedizin Berlin (Campus Benjamin Franklin), the research institutes of the Helmholtz Association (Helmholtz-Zentrum Geesthacht – Centre for Biomaterials Development in Teltow, HZG; Helmholtz -Zentrum Berlin für Materialien und Energie, HZB; Max-Delbrück-Centrum für Molekulare Medizin, MDC), the institutes of the Leibniz Association as well as the Max Planck and Fraunhofer Societies. The Federal Institute for Risk Assessment (BfR) and the Federal Institute for Materials Research and Testing (BAM) are located in the direct vicinity and integrated closely into the research network.



Biocompatible, functionalized polyglycerols for fluorescence imaging of inflamed tissue. Research cooperation with mivenion GmbH (background image: Fluorescence camera system Xiralite® X4, mivenion GmbH, Berlin). Source: K. Licha, M. Weinhart, R. Haag et al. Bioconjugate Chem. 2012, in press.

All these collaborative activites form a central part of the focus area "Nanoscale Functional Materials" which has been in operation at FU Berlin since 2008. We cooperate closely with the Helmholtz-Zentrum Geesthacht, Centre for Biomaterials Development in Teltow, for example in the project Poly4Bio "Polymers for Medicine" of the German Federal Ministry of Education and Research (BMBF) and the Helmholtz Virtual Institute for "Multifunctional Biomaterials in Medicine". Through the portfolio theme for technology and medicine "Multimodal Imaging" for which the Helmholtz-Zentrum Geesthacht serves as the coordinating centre, our working group is also networked closely with the Helmholtz Centres throughout Germany. The opening of the new Biomedizintechnikum II at the Centre for Biomaterials Development in Teltow (HZG) in December 2011 and the cooperation with the Berlin-Brandenburg Centre for Regenerative Therapies (BCRT) at the Charité give additional impetus to biomaterials research in the region.

Charité – Universitätsmedizin Berlin · Department of Radiology

Paccocath: The Balloon Catheter Coated with Paclitaxel for Treatment of Vascular Stenosis

Paclitaxel coating of balloon catheters (Paccocath®) is a novel approach to minimally invasive treatment of coronary and peripheral arterial stenosis. Short local delivery of paclitaxel during vessel dilation leads to a marked decrease in restenosis in the vessel segment treated. The Paccocath technology was invented at the Department of Radiology at the Charité in Berlin and then successfully developed to market maturity in cooperation with InnoRa GmbH and Saarland University. It is now being marketed by two companies, B. Braun and Medrad Inc., the latter a subsidiary of Bayer AG. Since the product was launched, sales have risen quickly and continuously. The Paccocath technology is the Charité's largest source of licence fee income from transfer projects to date.

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Minimally invasive procedures such as balloon dilation or stent implantation play a very important role in the treatment of arterial stenosis. Drug-coated stents were introduced in 2002; local drug delivery by these stents prevents reocclusion of the vessel (restenosis), a major side-effect of bare metal stents. The success of drug-coated stents indicated that the combination of medical devices and drugs might enable interventional treatment of previously untreatable or hard to treat diseases or prevent side-effects of local treatment.

The company InnoRa GmbH, founded in 2001, made precisely this combination of established and approved drugs with equally tested and certified medical devices the focus of its research and development activities. The development of Paccocath proceeded on the one hand from the observation that poorly water-soluble cytostatics (e.g., paclitaxel) dissolve far better in X-ray contrast agents and on the other hand from the fact that a large amount of contrast agent is used for imaging during an interventional procedure. The very first test in a restenosis animal model already showed that a high concentration of paclitaxel dissolved in the contrast agent prevents restenosis, indicating that short-term contact (around 60 seconds) of paclitaxel with the vessel wall is sufficient to bring about this effect. These findings were surprising because it was generally assumed that paclitaxel delivery for at least several days (as in the drug-coated stents) is required for restenosis prophylaxis. These findings were the basis for the use of balloon catheters as an effective drug delivery technology.

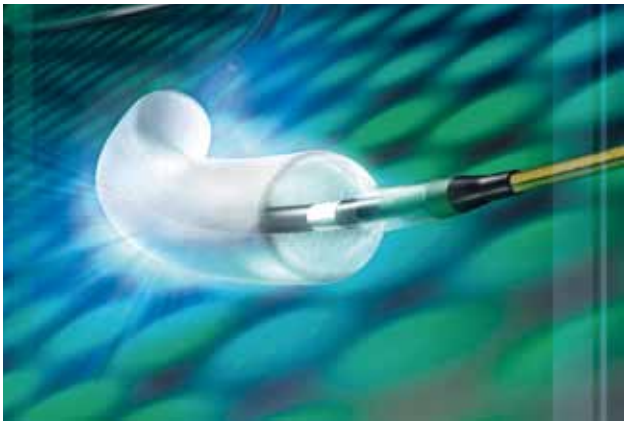

Figure 1: The SeQuent® Please paclitaxel-releasing balloon catheter from B. Braun

The Paccocath technology

The Paccocath balloon catheter coating contains a dose ensuring that sufficient paclitaxel is released into the vessel wall during the usually brief contact of the balloon with the wall during angioplasty (30-60 seconds) for long-term prevention of restenosis by inhibition of neointimal hyperproliferation. The overall strain on the organism from paclitaxel is so low that no systemic side-effects are to be expected from the applied dose, and no such effects have been observed in clinical studies. The first controlled clinical trial in patients with coronary in-stent restenosis demonstrated convincing effectiveness of Paccocath: after six months, the need for repeat intervention was reduced from 38 to 6 per cent.

The Paccocath balloon catheter is also effective in vascular areas where bare stents or drug-coated stents are not effective or not used such as leg artery stenosis. In a controlled clinical trial of patients suffering from leg artery stenosis, the need for a repeat treatment following vascular dilation with the Paccocath balloon catheter was reduced significantly from 52 to 15 per cent for up to two years following treatment.

Paradigm shift with the Paccocath technology

The convincing results of the preclinical and subsequent clinical studies refuted the then prevailing opinion that only continuous long-term drug release could reduce restenosis. It is already evident now that the Paccocath balloon catheter will be the preferred treatment for in-stent restenosis of the coronary vessels and for leg artery stenosis. The Paccocath technology should also become standard for smaller vessels or vessel branches.

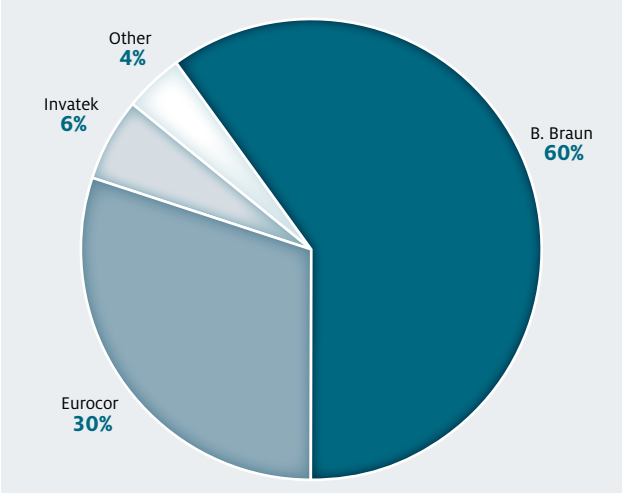

Figure 2: Drug eluting balloons market, global, key company shares, 2009 (Source: Global drug eluting balloons (DEB) pipeline analysis, opportunity assessment and market forecasts to 2016, GlobalData)

However, this paradigm shift may result in far greater changes in the interventional treatment of arterial stenosis. Presently, bare stents or, in the case of restenosis prevention, drug-coated stents are used to keep the vessel open, but the stent remains in the vessel permanently and may cause thrombotic complications later on. Since no foreign material remains in the vessel when balloon dilation alone is used, we are investigating new technologies which may permit short-term stabilization of a stenotic vessel after dilation in combination with drug-coated balloons.

Use and market potential

The invention was filed for two patents worldwide by the Charité in 2001 and 2002. In 2003, the company B. Braun, whose Vascular Systems Division is located in Berlin, acquired a global (excluding North America) nonexclusive licence for use of the Paccocath balloon catheter in coronary vessels. This swift move enabled B. Braun to gain early certification and market launch of its product (the paclitaxel-coated SeQuent® Please balloon catheter, Figure 1) in Europe and Asia.

In late 2005, the Schering company (now part of Bayer AG) acquired the two Charité patents. The licence agreement entered into with B. Braun by the Charité remained unaffected. Schering aimed to develop its own balloon catheter primarily for the treatment of peripheral vascular lesions. This development has since been taken over by the Bayer subsidiary Medrad (USA). In 2011, Medrad obtained European approval for its product and started marketing it under the name Cotavance®. To enable an early market launch of the catheters for leg arteries, InnoRa had already tested a modified coating for balloon catheters from the Invatec company (now Medtronic). These products were launched in 2009. Figure 2 presents an overview of the 2009 market shares of drug-coated balloon catheters. It should be noted that the Eurocor catheter (with a different coating technology) was withdrawn from the market due to ineffectiveness and replaced with a version more similar to Paccocath.

The Paccocath technology is currently still at the beginning of broader application, but its market potential was recently estimated by Cambridge Consultants to be approx. € 500 million annually in the United States alone (Market Strategy Report, Cambridge Consultants, 2009).

celares GmbH

Improvement of the Pharmacological Properties of Biopharmaceuticals by PEGylation

Biopharmaceuticals development has made tremendous progress in recent years. Many biological substances like peptides, proteins or nucleic acids are currently being developed or already approved for human therapy. However, their short half-life and their immunogenicity in patients have proved a barrier to broader application. celares GmbH solves these problems by targeted modification using polyethylene glycol, a procedure otherwise known as PEGylation.

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PEGylation – A special form of drug delivery

Although PEGylation is generally used in the same breath as the term "drug delivery", this is strictly speaking not what it is. Typical drug delivery formats do not change the active ingredient, but only serve to transport it across boundaries in the body – e.g. the skin, the blood-brain barrier, the lung alveoli and the mucous membranes of the mouth, nose or intestines. Other delivery forms release an active ingredient in a particular way, e.g. as a "slow release" formulation. What celares GmbH has done, on the other hand, is to couple polyethylene glycol chemically and hence usually permanently to the active ingredient. This method creates a new PEG substance whose physicochemical and significantly improved pharmacological properties differ markedly from those of the unmodified substance. The method is therefore also called an „enhancing technology". Adagen®, Macugen®, Neulasta®, Oncaspar®, Pegasys®, PEG-Intron® and Somavert® are prominent examples of approved drugs based on PEGylated active ingredients which have been used successfully for many years and have improved many therapies decisively.

Targeted modification

Polyethylene glycol can principally be bound to almost every position of a biological agent. In particular proteins and peptides have many natural bonding locations in the form of amino acids. The aim thereby is to implement targeted and reproducible PEGylation in selected locations by choosing the appropriate reaction chemistry. celares GmbH has developed a range of strategies for doing so and applied them successfully in customer projects. Modifications of the N-terminal amino acid, of cystein residues or artificial amino acids have proven especially efficient and non-aggressive. They occur only once or rarely in the peptide or protein and can therefore be modified selectively with PEG. Over the years, celares GmbH has established systematic screening procedures which permit the determination of a suitable PEGylation for every pharmaceutical agent. Three PEG agents developed by celares GmbH are now at the clinical trial stage and others are due to reach it shortly.

PEG reagents with defined structures

One field of specialisation at celares GmbH are PEG reagents with defined structures and masses called CelaSYS-PEG. PEG reagents are normally not homogenous substances, but mixtures of PEG chains of different lengths. This feature is also called "dispersity" (from the Latin "dispergere" for distribute). celares GmbH can produce branched PEG reagents by convergent syntheses which do not display any dispersity and therefore provide uniform product quality. This is of interest especially in areas where defined structures and dimensions are critical.

One example are the Affilin® tetrameres. They are presently being developed by Scil Proteins GmbH in Halle as pharmaceutical agents. Affilines® are scaffold proteins which can identify and block specific target structures in patients in high specificity in a manner similar to antibodies. If the target structures are receptors or cell proteins which occur in exposed position on the cell surface in clusters, the action of the Affilines® can be enhanced by multimerisation. It is thereby essential to set the spaces between the individual Affilin® molecules precisely to fit those in the cluster of the target structure. This has been achieved by use of a 4-arm PEG reagent produced by celares GmbH. In addition to enhanced effectiveness, this results in extending the agent's dwell period in the body. Since the Affilin® monomers are smaller proteins of only 14,000 Dalton they are normally secreted rapidly through the kidneys. By formation of a tetramere, the total size of the agent is increased to more than 56,000 Dalton, so that natural secretion through the kidneys is slowed significantly.

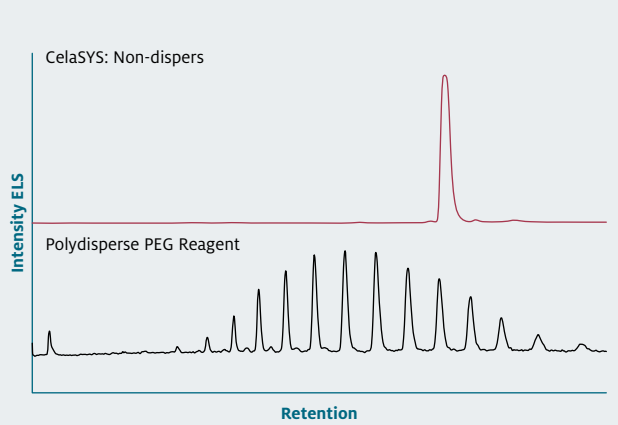

Figure 1: HPLC analysis of CelaSYS-PEG and polydisperse PEG. CelaSYS is monodispers resulting in one single signal. Polydispers PEG is inhomogeneous and shows several signals.

Customised polymers for a new application

The internal projects at celares GmbH concern the use of polymers for other applications. Two extensive R&D projects are currently in progress at celares GmbH.

A cooperation project with Beuth University of Applied Sciences in Berlin funded by the German Federal Ministry of Economic and Technology is investigating the development of PEGylated liposomes with improved carrier characteristics. The PEGylation of liposomes originally served essentially to prolong the half-life in patients or improve agent stability. Today, however, there are many new applications in the field of targeting or diagnostics which pose especially high demands on PEGylated liposomes. Here, celares GmbH wants to open up new possibilities by the use of non-dispersed and branched PEG.

The second project is a major alliance initiative with partners from science and industry and concerns the development of "Biocompatible siRNA nano transporters for targeted mRNA knock-down in vivo". The transport systems for ribonucleic acids (RNA) and specifically siRNA are of tremendous importance for the development of new efficient therapeutic agents and new diagnostic tools. The potential of siRNA in medical applications has been known for some time, but their use usually fails due to the lack of delivery systems that are not either too toxic or inefficient. The aim of this alliance project is therefore to develop a new, highly efficient and less toxic polymer-based nano-transport system for RNA.

Figure 2: GMP production of activated PEG at celares GmbH

PEG production in pharmaceutical quality

The production of activated PEG reagents for the modification of pharmaceutical substances requires both extensive experience and special production systems. In 2010 celares GmbH invested more than one million Euros in its facility in Berlin-Buch for the establishment of cleanrooms and the installation of machinery and equipment. The centrepiece is a modern 60L reactor for chemical synthesis of the PEG reagents on a multi-kilogram scale. The entire process complies with the latest GMP guidelines, and this ensures that the products can be used in the pharmaceutical sector. The establishment of this production facility means that celares GmbH now belongs to only a handful of companies worldwide who can manufacture PEG in the quality required for pharmaceutical applications.

ALRISE Biosystems GmbH

Controlled Drug Delivery as a Focus Area in the Pharmaceuticals Industry

Drug delivery generally aims to achieve systemic circulation of a pharmaceutical substance to generate a specified pharmacological effect. The active ingredient and its drug delivery system must meet specific requirements depending on the different delivery routes. Over the past decades, drug delivery has therefore become an increasingly important focus area in drug development up to the approval stage. The ImSus® technology platform facilitates controlled release of the most diverse active pharmaceutical ingredients by encapsulation in polymer particles.

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Different routes to the target

Drugs are delivered mainly by the peroral, transdermal and parenteral administration routes. Peroral administration is the simplest and most common one. However, both the first pass effect and the potentially short half-life of the pharmacological agent present considerable barriers for a range of potent drugs. In addition, the physicochemical properties of the active ingredient – e. g. its hydrophobicity, degree of ionisation or molecular weight – play an important role in absorption following oral administration. Peptides and proteins, for example, are generally not available orally due to their instability in the acidic milieu of the stomach.

Transdermal delivery permits systemic circulation of the active substance under circumvention of the first pass effect, but the use of this route is largely restricted by the molecular weight and physicochemical properties of the pharmaceutical ingredient. Examples of transdermal delivery products are hormone patches (contraceptives), pain patches (fentanyl and buprenorphine) and Rivastigmine TTS (morbus Alzheimer).

Parenteral administration also avoids the first pass effect. The main routes are intravenous, intramuscular and subcutaneous injections. In particular in intramuscular and subcutaneous delivery, the injection volume involves restrictive formulation requirements. In intravenous administration of particulate drug delivery systems, on the other hand, the size of the injected particles must be observed because microparticles with a diameter large r than 5 µm could block the capillaries.

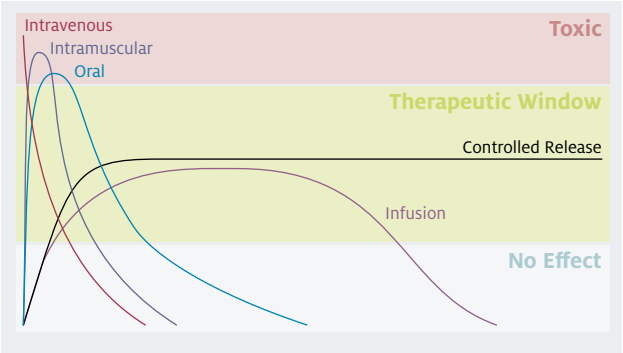

Figure: Schematic presentation of the plasma concentration of a pharmaceutical ingredient with a short half-life as a function of time under use of different application routes

Controlled active ingredient release

In the field of parenteral application, specifically using the intramuscular and subcutaneous route, intelligent drug delivery systems have been a focus of pharmaceutical research for many years. In addition to targeted drug delivery, i.e. application of an active ingredient to the specific target cell, controlled drug delivery has been of particular interest. Controlled drug delivery formulations can contain a pharmaceutical ingredient in a dose for up to several months. This is achieved, for example by encapsulation of the ingredient in a biodegradable and compatible matrix from which the ingredient is released following parenteral application by diffusion from the matrix followed by a release due to its erosion. The established matrix materials are, in particular, the copolymers of lactic acid and glycolic acid (PLGA) which have been used in medicine for several decades and are approved by the US approval authority FDA for parenteral application (e.g. Lupron Depot®). Other important application methods include liposomal delivery systems. Encapsulation of the active ingredient in a polymer matrix or liposomes additionally protects it against e.g. enzymatic depletion, whereby sensitive pharmaceutical ingredients can be made available only by controlled drug delivery. In addition, a microparticle formulation of the active ingredient can be administered in a dose sufficient for several days or even months by

a single injection, since the encapsulated pharmaceutical ingredient release from the depot occurs controlled within the window of the therapeutic index. This reduces administration frequency and hence leads to improved compliance and greater therapeutic success.

These benefits are especially important in the case of diseases of the central nervous system. For example, effective schizophrenia therapy with the antipsychotic drug risperidone (Risperdal Consta®) became possible only by means of a controlled drug delivery formulation based on PLGA microparticles. In addition to Risperdal Consta®, two other antipsychotic drugs, olanzapine and paliperidone are marketed for controlled drug delivery (Zypadhera®, Invega Sustenna®). Here, controlled release of the drugs is not achieved by release of the encapsulated active ingredient, but by the dissolution kinetics of the prodrug olanzapine pamoate or paliperidone palmitate, respectively.

Potentials for cancer therapy

In addition, controlled drug delivery systems offer great potential for chronic diseases and cancer therapy. Here, optimal use of the respective pharmacodynamics and compliance are of prime interest.

PLGA-based controlled drug delivery systems include the formulations of the GnRH analogues leuprorelin, triptorelin and goserelin, which are used in palliative therapy of prostate cancer. The products Lupron Depot®, Decapeptyl® and Zoladex® are marketed as 1- and 3-month depots. For leuprorelin and triptorelin, 6-month depots are also approved. The pharmacological effect of GnRH analogues is based on reduction of the testosterone in plasma below castration level to suppress hormone-dependent growth of the prostate carcinoma. Controlled drug delivery formulations of GnRH analogues are also approved for the indications breast cancer and endometriosis. Other peptides and proteins can also be made available systemically by parenterally applied controlled drug delivery systems. This is an indispensable precondition for the potential commercial success of these pharmaceutical substances.

Fewer side-effects

In cytostatic drug formulations, the undesired range of side-effects can be reduced by a controlled drug delivery system. The active pharmaceutical ingredient paclitaxel is a good example. The marketed formulation Taxol® leads to allergic and even anaphylactic reactions when infused due to the surfactant Cremophor® used as an excipient. These side-effects are avoided by the development of a controlled drug delivery formulation for paclitaxel in albumin nanoparticles (Abraxane®).

Looking ahead

Controlled drug delivery systems will continue to grow in importance as a technology that increases therapeutic effectiveness, reduces the range of side-effects and makes hydrophobic pharmaceutical ingredients available for therapeutic application. Modern drug delivery systems will also permit making drugs with difficult physicochemical characteristics available for new therapies. This is the aim of the ImSus® technology which provides a state-of-the-art platform for the development of controlled drug delivery formulations of pharmaceutical substances. The fast and economical microparticle formation process and the excellent reproducibility of drug encapsulation and release performance are superior to established technologies. In addition to the formulation of new chemical/biological entities, the ImSus® technology is also suited perfectly for lifecycle management and the generic pharmaceuticals industry.

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BioTOP is a joint initiative of the state of Berlin and the state of Brandenburg under the umbrella of the TSB Innovationsagentur Berlin GmbH and takes part in the Cluster Management Health Capital. BioTOP is funded by the federal state of Berlin, the federal state of Brandenburg and the Investitionsbank Berlin, co-funded by the European Union (European Fund for Regional Development). Investing in your Future