FRIEDRICH-SCHILLER-
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JENA

Collaborative Research Center 1278
PolyTarget

Conference of the CRC PolyTarget 2020

Cell Specific Strategies Against Inflammation -
Transferring Basic Research into Application

Scientific Programme

07 - 08 September 2020 | Jena (Germany)

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GENERAL INFORMATION

Date
07 - 08 September 2020

Venue
Friedrich Schiller University Jena
Jena Center for Soft Matter (JCSM)
Lecture hall
Philosophenweg 7
07743 Jena
Germany

Scientific board
Prof. Dr. Michael Bauer
Prof. Dr. Dagmar Fischer
apl. Prof. Dr. Michael Gottschaldt
PD. Dr. Stephanie Höppener
Prof. Dr. Ulrich S. Schubert
Prof. Dr. Oliver Werz

Organizers

PolyTarget
SFB 1278

JCSM
Jena Center for Soft Matter

Supported by

DFG Deutsche Forschungsgemeinschaft
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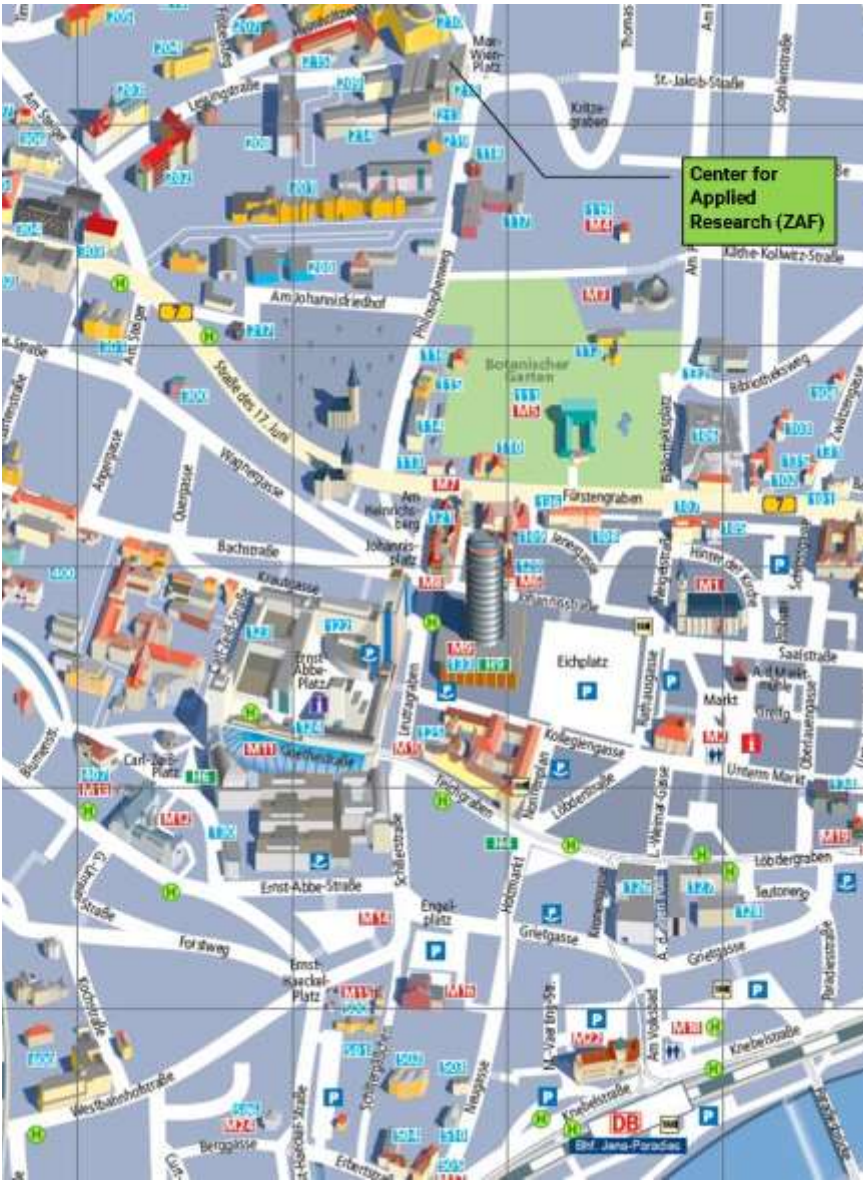
MAP OF THE CONFERENCE VENUE



SCIENTIFIC PROGRAMME

Monday, 07 September 2020

09:00 am — REGISTRATION

10:00 am — CONFERENCE OPENING

Welcome notes

Prof. Dr. Ulrich S. Schubert
Friedrich Schiller University Jena, Jena (DE)

Prof. Dr. Dagmar Fischer
Friedrich Schiller University Jena, Jena (DE)

Prof. Dr. med. Michael Bauer
University Hospital Jena, Jena (DE)

SESSION 1

Session Chair: Prof. Dr. Ulrich S. Schubert

10:30 am — L01: INVITED LECTURE

Chemical evolution of sequence-defined peptide-like carriers for nucleic acid delivery

Prof. Dr. Ernst Wagner
Ludwig Maximilians University Munich, Munich (DE)

11:15 am — L02: CONTRIBUTED LECTURE

How architecture, sequence and composition of polymers influence the transport of genetic material

Dr. Anja Träger
Friedrich Schiller University Jena, Jena (DE)

11:45 am — SHORT BREAK (AIRING)

11:50 am — L03: CONTRIBUTED LECTURE

Polymeric nanoparticles with neglectable protein corona

Prof. Dr. Rudolph Zentel
Johannes Gutenberg University Mainz , Mainz (DE)

SCIENTIFIC PROGRAMME

12.20 pm — LUNCH BREAK

SESSION 2
Session Chair: Prof. Dr. Michael Bauer

01:30 pm — L04: INVITED LECTURE
The microbiome as a therapeutic target in inflammatory diseases
Prof. Dr. Christoph Kaleta
Kiel University , Kiel (DE)

02:15 pm — SHORT BREAK (AIRING)

02.20 pm — L05: CONTRIBUTED LECTURE
Impact of polymer micelle design on interaction with biological systems
Dr. Johannes C. Brendel
Friedrich Schiller University Jena, Jena (DE)

02.50 pm — L06: INVITED LECTURE
Academic drug discovery: Ficts, facts and phantasy
Prof. Dr. Stefan Laufer
University of Tübingen, Tübingen (DE)

03:35 pm — COFFEE BREAK AND POSTER SESSION

SESSION 3
Session Chair: Prof. Dr. Dagmar Fischer

04.40 pm — L07: INVITED LECTURE
Keeping the promise of nanomedicines?
Prof. Dr. Gerrit Borchard
University of Geneva, Geneva (CH)

SCIENTIFIC PROGRAMME

05:25 pm	L08: SHORT PRESENTATION Drug delivery systems to target *Aspergillus fumigatus* conidia inside macrophages *Katherine González Rojas* *Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena (DE)*
05.40 pm	L09: SHORT PRESENTATION Circumventing the drawbacks of conventional nanoparticle preparation - innovative non-toxic and sustainable formulation methods *Christian Grune* *Friedrich Schiller University Jena, Jena (DE)*
05.55 pm	SHORT BREAK (AIRING)
06:00 pm	L10: INVITED LECTURE "Inert" pharmaceutical polymers...not exactly "inert"… *Prof. Dr. Dr. Lorenz Meinel* *University of Würzburg, Würzburg (DE)*
06.45 pm	ORGANISATIONAL REMARKS *Prof. Dr. Ulrich S. Schubert* *Friedrich Schiller University Jena, Jena (DE)*
07.00 pm	END OF SCIENTIFIC PROGRAMME

SCIENTIFIC PROGRAMME

Tuesday, 08 September 2020

SESSION 4
Session Chair: Prof. Dr. Oliver Werz

08:45 am — L11: INVITED LECTURE
(Programmed) cell death and immunity
Prof. Dr. Hamid Kashkar
University of Cologne, Cologne (DE)

09.30 am — L12: CONTRIBUTED LECTURE
siRNA loaded nanoparticles based on vitamin A functionalizeed polymethacrylates to target hepatic stellate cells for anti-inflammatory applications in vivo
Dr. Stephanie Schubert
Friedrich Schiller University Jena, Jena (DE)

10:00 am — SHORT BREAK (AIRING)

10:05 am — L13: SHORT PRESENTATION
The dual mPGES-1/FLAP inhibitor TG-201 encapsulated into polymeric nanoparticles overcomes plasma protein binding and displays high effectiveness in whole blood
Christian Kretzer
Friedrich Schiller University Jena, Jena (DE)

10:20 am — L14: SHORT PRESENTATION
Formulation of liver-specific PLGA-DY-635 nanoparticles loaded with the protein kinase C inhibitor Bisindolylmaleimide I
Blerina Shkodra
Friedrich Schiller University Jena, Jena (DE)

10:35 am — COFFEE BREAK AND POSTER SESSION

SCIENTIFIC PROGRAMME

SESSION 5
Session Chair: Prof. Dr. Ulrich S. Schubert

11:00 am — L15: INVITED LECTURE
Parenteral controlled drug release – concepts and surprises
Prof. Dr. Karsten Mäder
Martin Luther University Halle-Wittenberg, Halle a. d. Saale (DE)

11:45 am — L16: CONTRIBUTED LECTURE
New applications of analytical ultracentrifugation in the diversity of a collaborative research center: From small polymers to nanoparticles
Dr. Ivo Nischang
Friedrich Schiller University Jena, Jena (DE)

12:15 pm — L17: INVITED LECTURE
Anaphylatoxins as rheostats of immune cell function
Prof. Dr. Jörg Köhl
University of Lübeck, Lübeck (DE)

01:00 pm — FAREWELL
Prof. Dr. Ulrich S. Schubert
Friedrich Schiller University Jena, Jena (DE)

01:10 pm — LUNCH BREAK

02:00 pm — CRC POLYTARGET PI MEETING
PI's only

04.30 pm — END OF SCIENTIFIC PROGRAMME

SOCIAL PROGRAMME

Monday, 07 September 2020

07:30 pm

CONFERENCE DINNER

Botanical Garden Jena
Fürstengraben 26
07743 Jena

Enjoy a great evening in the relaxed atmosphere of the "green lung of Jena"!

Abstracts of Oral Contributions

ABSTRACTS OF ORAL CONTRIBUTIONS

L01: Chemical evolution of sequence-defined peptide-like carriers for nucleic acid delivery

Ernst Wagner[a]
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It took 50 years from first transfections to the approval of gene therapies as medical drugs. Nine gene therapy products, 2 siRNAs, and 8 oligonucleotides reached the market. Controlled, targeted intracellular delivery remains the key bottleneck for the success of such therapeutic macromolecules. Different chemical evolution approaches are pursued for further refinements of synthetic nanocarriers.[1, 2] Our strategy focuses on a bioinspired sequence-defined process including (i) artificial amino acids active in specific delivery steps, (ii) precise assembly into sequences by solid phase-assisted synthesis, (iii) screening for a defined delivery task and selection of top candidates, followed by random/educated variation for a next selection round. The optimal sequence and delivery motifs of nanocarriers depends on the type of cargo[1,2] and application, as will be outlined for pDNA,[3] siRNA,[4-7] mRNA, PMOs[8], or Cas9/sgRNA.[9] Comparing related nanoformulations, surprising differences in tumour-targeted activity *in vitro* and *in vivo* were observed.

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ABSTRACTS OF ORAL CONTRIBUTIONS

L02: How architecture, sequence and composition of polymers influence the transport of genetic material

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The controlled transport of genetic material or proteins in eukaryotic cells is essential for the development of new therapeutics based on different genetic materials. Due to their flexibility and easier large-scale production, polymeric systems are of great interest. Cationic polymers are able to complex the negatively charged genome to promote cell uptake. When taken up by endosomal processes, the complexes of polymer and genome must reach their site of action, the cytoplasm or the cell nuclei. Therefore, the endosomal membrane, a major intracellular hurdle, must be crossed. Here we present polymers in which different strategies have been used for successful gene delivery through efficient endosomal release mechanisms. First, polymeric micelles of block copolymers whose amines are pH-dependently charged were investigated. In addition, successful gene expression with pH-independent guanidinium functionalities was also observed. Finally, the integration of hydrophobic units can further improve membrane interaction. Calcein release studies, lipid interaction studies, confocal laser scanning microscopy and flow cytometry were used to further investigate these interactions between material and cells. It will be shown that the integration of membrane-active monomers as well as the modified architecture can significantly increase transfection efficiency. The more efficient polymers have even been able to express genes in immune or blood cells that are considered to be difficult or almost impossible to express. In addition, the integration of stealth monomers or a protective polymer layer has reduced the toxicity of the systems. The results presented here will show different design strategies.

ABSTRACTS OF ORAL CONTRIBUTIONS

L03: Polymeric nanoparticles with neglectable protein corona

Irina Alberg[a], Rudolf Zentel[a]
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The current understanding of nanoparticle-protein interactions indicates that they rapidly adsorb proteins upon introduction into a living organism. The formed protein corona determines thereafter identity and fate of nanoparticles in the body. The present study evaluates protein affinity of 3 core-crosslinked polymeric nanoparticles with long circulation times, differing in the hydrophilic polymer material forming the particle surface, namely poly(*N*-2-hydroxypropylmethacrylamide) (pHPMA), polysarcosine (pSar) and poly-(ethylene glycol) (PEG). This includes the nanotherapeutic CPC634, which is currently in clinical phase II evaluation. To investigate possible protein corona formation, the nanoparticles were incubated in human blood plasma and separated by asymmetrical flow field-flow fractionation (AF4). Notably, light scattering showed no detectable differences in particle size or polydispersity upon incubation with plasma for all nanoparticles, while in gel electrophoresis minor amounts of proteins could be detected in the particle fraction. Label-free quantitative proteomics was additionally applied to analyze and quantify the composition of the proteins. It proved that some proteins were enriched, but their concentration was significantly less than one protein per particle. Thus, most of the nanoparticles are not associated with any protein. Therefore, this work underlines that polymeric nanoparticles can be synthesized, for which a protein corona formation does not take place.

References

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ABSTRACTS OF ORAL CONTRIBUTIONS

L04: The microbiome as a therapeutic target in inflammatory diseases

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It is increasingly becoming apparent that the microbiome is a key player in human health and disease. In my talk, I will cover the role of the microbiota as a therapeutic target in inflammatory diseases focusing on aging. In a first part, I will talk about the type 2 diabetes medication metformin for which we could show that the microbiota produces a compound that mediates the actual effect of metformin on the host.[1] In a second part, I will talk about about essential changes in the interaction between the microbiota and its host in the context of aging and how we can systematically identify such interactions using metabolic modelling. With aging we observe that there is a loss of interaction between host as well as microbiota that potentially contributes to aging-associated diseases and which can be reversed through fecal microbiome transplants. Finally, in an outlook, I will cover next-generation microbiome-centered therapies that rather than using unspecific whole-microbiome transplants directly modulate disease-associated changes in metabolic interactions between host and microbiota.

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ABSTRACTS OF ORAL CONTRIBUTIONS

L05: Impact of polymer micelle design on interaction with biological systems

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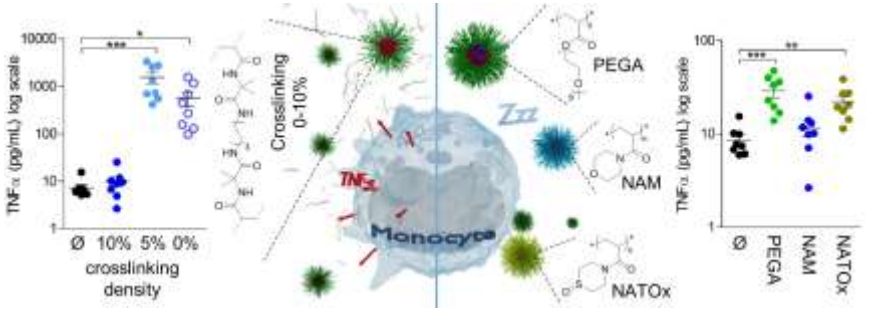
Self-assembly processes based on supramolecular interactions are key elements in nature to form well-defined structures from small building blocks. Materials science has heavily borrowed these processes to engineer a wealth of new materials with unique properties. Considering polymer chemistry, the self-assembly of amphiphilic block copolymers into micelles or vesicles has undoubtedly attracted most attention and found widespread application[1]. Particularly in drug delivery, these polymer assemblies promise unprecedented solutions for the selective and localized application of active pharmaceuticals circumventing often detrimental side effects.

Despite continuous efforts, the influence of several aspects in the design of polymer nanomaterials remains unclear or controversial. In many cases limited control on the overall structure of the applied materials remains a bottleneck in the design of detailed systematic studies. Our focus, therefore, is the design of well-defined assemblies which can be controlled in terms of shape, dimensions, function, and stability.[2] The latter, for example, is related to the often dynamic or soft character of such assemblies, which may cause undesired interactions or behaviour in contact with biological media. In particular, the crosslinking of polymer micelles was found to be crucial to minimize inflammatory response of immune cells, which is key for efficient drug delivery vectors.[3] On the contrary, differences in the chemistry of shell were found to have only a minor influence, if neutral hydrophilic polymers are considered (see Figure).

Overall, our aim is to gain better control over all design aspects in polymer self-assembly, which gives us an opportunity to systematically evaluate the impact of structure variations (e.g. shape, size, surface chemistry) on biological interactions and responses.

ABSTRACTS OF ORAL CONTRIBUTIONS



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ABSTRACTS OF ORAL CONTRIBUTIONS

L06: Academic drug discovery: Ficts, facts and phantasy

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More than 60% of all drug candidates entering clinical development are coming no longer from in-house research of big pharma but from small companies, often spin-offs from universities or directly from academia. Big pharma is facing problems of reduced R&D productivity and mature products coming close to the patent cliff.

Cost pressure forces pharmaceutical industry more and more to cut internal early drug discovery, on the other hand, partnering with academic institutions became more and more important. In the USA, this had lead to >100 academic drug discovery units.

General aspects of this development, chances for academia but risks and incompatibilities as well will be presented and controversially discussed in the talk. Special aspect is given to current developments in Germany.

Our research in RNAi- and Crispr/Cas9 based functional genomics especially focuses on the identification of new cancer genes and therapeutic targets in therapy-resistant solid tumors. For such studies, clinically relevant mouse tumor models, which closely resemble the human disease, are available. Specifically, we are combining so called mosaic mouse models with stable RNAi technology to dissect tumor suppressor networks in gastrointestinal tumors and to identify and validate new therapeutic target genes. Together with a limited number of other laboratories worldwide, we have the expertise to conduct RNAi screens for new cancer genes directly in orthotopic and immunocompetent cancer mouse models *in vivo*.

To best translate data from our unique RNAi platform into new cancer therapies, we recently systematically connected our RNAi expertise with the research areas virtual screening/modelling and medicinal chemistry to build an academic drug discovery unit, designated TuCAD2 (Tübingen Centre for Academic Drug Discovery). Our unit was recently approved as a member of the worldwide acting Academic Drug Discovery Consortium. TuCAD2 represents an interfaculty and interdisciplinary endeavor and was founded by the Dept. of Pharmaceutical/Medicinal Chemistry and the Dept. of Internal Medicine VIII.

In our talk we will discuss the pivotal role of academic drug discovery infrastructures for rapidly translating validated therapeutic target structures into clinical applications and will give examples of a novel and promising drug for the treatment of liver cancer or liver regeneration which entered the phase of clinical testing only 13 month after completion of pivotal preclinical proof of concept.

ABSTRACTS OF ORAL CONTRIBUTIONS

L07: Keeping the promise of nanomedicines?

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The application of nanotechnology to the development of advanced therapeutics has brought about a number of so-called "nanomedicines". Examples of such drugs already introduced into the market include glatiramoids (Copaxone®) and its generics, liposomal formulations (Doxil®), and nanoparticles such as iron-carbohydrates (Venofer®) and albumin-paclitaxel (Abraxane®). Though showing high variability in terms of size, shape, materials used, etc., they share complex structures, which cannot be characterized in their entirety. Being mostly of synthetic origin and their preparation often including a self-assembly step, such drugs are referred to as non-biological complex drugs (NBCDs). Their size and attributes at the molecular scale confer these systems certain properties that impact their interaction with their biological environment, and thus influence PK/PD and safety profiles.

The challenge, though, persists in the correlation of physico-chemical parameters ("critical quality attributes", CQA) of nanomedicines to their clinical activity and toxicity. As an example, efforts have been undertaken to draw conclusions from the built-up and composition of the so-called biomolecular corona covering the particle surface upon introduction into a biological environment. FDA has recently issued a guidance draft on products containing nanomaterials, and even though this draft guidance is suggesting a list of properties of nanomedicines, among them corona formation, to be tested, it lacks true guidance on individual (groups of) nanomedicines. It presumes levels of knowledge of product behaviour that are typically not available, neither for NDA nor for generic, ANDA products, and leaves room for interpretation.

Especially the comparability of efficacy and safety between originator and follow-on nanomedicine products is of concern, as several pre-clinical and retrospective and prospective clinical studies have revealed. Although studies showed that these products do have a different efficacy and safety profile than the originator product, they are deemed to be interchangeable in clinical practice.

Regulatory authorities know several pathways for the registration of a drug. According to the US Food, Drug and Cosmetics Act, drugs may be approved as originators (505(b)(1), NDA), as generics (505(j), ANDA) or as "products closely related to innovators" (505(j)). Next to originator and generic pathways, the latter is also described by the European Medicine Agency as "hybrid" pathway and can be applied for medicines whose approval partially relies on data from a

reference (originator) medicine and partly on new clinical data. To date, most NBCDs have been approved by the generic pathway, and follow-on products, so called nanosimilars or “complex generics” are entering the market.

In addition, the scale-up of nanomedicine preparation from the lab to commercial scale requires a controlled manufacturing procedure assured through in-process controls and their specifications, as the term “the process is the product” does certainly also apply for these non-biologic complex drugs. As recently suggested through the European project “GoNanoBioMat”, the application of a safe-by-design concept to nanomedicine development us posed to increase human and environmental safety of such therapeutics.

This presentation will discuss several issues related to the development of nanomedicines that are encountered beyond the lab scale but may be already considered at an early stage.

ABSTRACTS OF ORAL CONTRIBUTIONS

L08: Drug delivery systems to target *Aspergillus fumigatus* conidia inside macrophages

Katherine González Rojas[a, b], Gauri Gangapurwala[c], Julien Alex[c], Thomas Orasch[a], Antje Vollrath[c], Christine Weber[c, d], Justyna Czaplewska[c], Stephanie Höppener[c, d], Thorsten Heinekamp[a], Carlos Guerrero-Sánchez[c, d], Ulrich S. Schubert[c, d], Axel A. Brakhage[a, b]

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Aspergillus fumigatus is the most important airborne fungal pathogen of the immunocompromised patient. The fungus causes life-threatening pulmonary aspergillosis, which has a mortality rate of 60 to 90%. Several virulence factors have been identified, which allow the fungal spores (conidia) to survive inside macrophages. Therefore, to deliver antifungals to intracellular persistent conidia, we generated polymer-based particles and polymer-drug conjugates.

The particles were made up of poly(lactic-co-glycolic)acid (PLGA) and were designed to have a size of approximately 800 nm to be phagocytosed by macrophages. The particles were first tested without antifungal payload with RAW 264.7 macrophages. They were non-toxic at concentrations of 100 µg m^{L-1} and their highest uptake monitored by imaging flow cytometry was between 3 and 4 h, reaching 65% of macrophages containing particles. The co-localization of conidia in a phagolysosome (PL) with particles was quantified and analyzed by microscopy. We observed particles in 1% of PL containing conidia, when using a particle concentration of 10 µg mL^{-1}. Transmission electron microscopy allowed detecting the co-localization of the particles at the conidium-containing PL.

The polymer drug conjugates were based on hydrophilic poly(poly(ethylene glycol) methyl ether methacrylate) PmPEGMA and hydrophobic poly(methyl methacrylate) (PMMA) with covalently attached caspofungin. These polymer conjugates were tested for their antifungal activity and cytotoxicity. Both polymer conjugates were active against *A. fumigatus* despite the increase in the minimal effective concentration (MEC) ranging from 2.5 to 8 mgL^{-1} compared to pristine caspofungin (MEC: 0.03 mgL^{-1}). They were not toxic for RAW 264.7 macrophages and HeLa cells.

The results obtained with these two strategies will found the basis to develop an improved intracellular drug delivery.

References

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[2]S. Amin, A. Thywissen, T. Heinekamp, H. P. Saluz, A. A. Brakhage, Int. *J. Med. Microbiol.* **2014**, *304*, 626-636.
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ABSTRACTS OF ORAL CONTRIBUTIONS

L09: Circumventing the drawbacks of conventional nanoparticle preparation - innovative non-toxic and sustainable formulation methods

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Biodegradable nanoparticles based on poly(D,L-lactide-co-glycolide) (PLGA) are a widely used approach for drug delivery. They are formulated by standard preparation methods like the emulsion-diffusion-evaporation method which suffer from the use of organic solvents potentially toxic to the human health and environment. Strong efforts must be made to purify the final product from solvent residuals, to protect the operator and to recycle the solvent which is often associated with high costs in industry. This study aims to develop alternative formulation techniques for drug-loaded PLGA nanoparticles using different non-toxic and sustainable solvents[1,2] to circumvent the drawbacks of conventional preparation methods. The obtained nanoparticles showed comparable particle sizes, polydispersity and zeta potentials to the conventional method while process time and steps were effectively reduced. Depending on the type of solvent, a modified crystallinity of the nanoparticles through plasticizing effects could be observed allowing a modified drug release. The amount of residual solvents was negligible giving the particles an excellent biocompatibility after local and systemic administration in a shell-less hen's egg test. As a proof of concept, anti-inflammatory natural product-derived compound (i.e., indirubin derivative 6BIG-OE) and a synthetic drug (atorvastatin) were encapsulated to demonstrate the general eligibility of the alternative methods. The anti-inflammatory effects of the nanoparticles in human monocytes showed a comparable efficiency between standard and alternative formulation methods. In conclusion, the conventional emulsion-diffusion-evaporation method was successfully replaced by fast, simple, non-toxic and cost-efficient techniques avoiding all the downsides of organic solvents.

References

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ABSTRACTS OF ORAL CONTRIBUTIONS

L10: "Inert" pharmaceutical polymers ... not exactly "inert" ...

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Pharmaceutical polymers are used in most formulations e.g. to protect the active pharmaceutical ingredient (API) during manufacturing and storage. These pharmaceutical polymers are often referred to as inert pharmaceutical ingredients contradicting their essential functional roles including interaction with the API, impacting its dissolution rate, mediating supersaturation phenomena, etc. This contribution highlights the impact of selected pharmaceutical excipients on API solubilization within the gastrointenstinal tract (GIT), and API permeation through the mucus layer overlaying the GIT epithelium. The interpretation of the molecular interplay between bile salts, lecithin, APIs, and selected pharmaceutical polymers as well as the interaction of these partners with mucin and the dynamics of these interactions within mucin layers was detailed by NMR, analytical ultracentrifugation, flux studies, dynamic light scattering and isothermal titration calorimetry with all information emulating into molecular dynamics simulations.

ABSTRACTS OF ORAL CONTRIBUTIONS

L11: Cell death and immunity

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In an average human adult billions of cells die each day to counter mitosis. Cellular death also occurs under pathological conditions such as infection or in response to cytotoxic therapeutics. It is increasingly evident that dying cells actively coordinate the fade of the affected tissue by emitting a plethora of different inflammatory mediators. Based on the nature of cellular death process these provoke beneficial or detrimental immune actions and ultimately culminate in tissue repair/regeneration or collateral and irreversible tissue damage. Not surprisingly, the molecular link between cellular death and inflammatory signalling is currently considered as a fundamental process governing tissue functionality and represents the central objective of our research group.

Caspases are cysteine proteases that have been initially and extensively studied during the course of apoptosis. Accumulating evidence indicated that these proteases also coordinate and decisively control necrotic cell death and tissue response. Our recent and ongoing studies focus on the role of caspases during infection. The results obtained indicated that caspase activity is a versatile cellular action that can switch between different modes of cell death, in particular, in response to a pathogen, thus ensuring cell death as a pivotal cellular innate immune response. These data point at beneficial and detrimental outcomes of pharmaceutic inhibition of caspase activity as a therapy in infectious and inflammatory disorders.

ABSTRACTS OF ORAL CONTRIBUTIONS

L12: siRNA loaded nanoparticles based on vitamin A functionalizeed polymethacrylates to target hepatic stellate cells for anti-inflammatory applications *in vivo*

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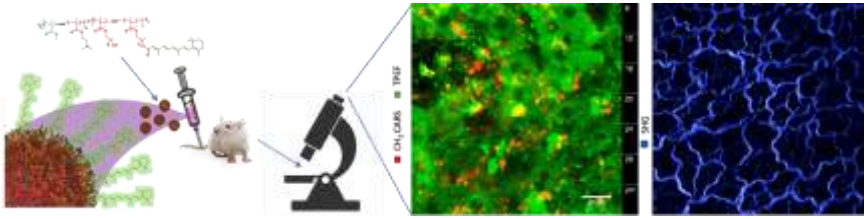
The specific role of hepatic stellate cells (HepSC) in vitamin A uptake and storage offers the ability to purposefully use this function as a targeting moiety at least in early stages of their transformation. Therefore, a library of vitamin A functionalized polymethacrylates that contain cationic and also stealth moieties was synthesized. The polymers were formulated into siRNA bearing nanoparticles that are fully characterized regarding their size, loading capacity, protection ability, surface functionality, cytotoxicity, and interaction with the retinol binding protein. The nanoparticles were investigated in dynamic *in vivo* studies in mice by nonlinear multimodal microscopy in order to evaluate their ability to target HepSC for future anti-inflammatory applications.



ABSTRACTS OF ORAL CONTRIBUTIONS

L13: The dual mPGES-1/FLAP inhibitor TG-201 encapsulated into polymeric nanoparticles overcomes plasma protein binding and displays high effectiveness in whole blood

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Prostanglandin (PG)E2 and leukotrienes (LT) belong to the superfamily of lipid mediators (LMs), generated by the cyclooxygenase and 5-lipoxygenase pathways in white blood cells, which promote inflammatory reactions[1]. TG-201 is a dual inhibitor of microsomal prostaglandin E2 synthase-1 (mPGES-1) and 5-lipoxygenase-activating protein (FLAP). Despite certain advantages to common antiinflammatory drugs[2], TG-201 is an acidic and highly lipophilic drug with a strong tendency to bind to plasma protein, thus, limiting its effectiveness in human whole blood (HWB) or in vivo. To overcome these hurdles, we encapsulated TG-201 into biodegradable polymeric nanoparticles (NPs). We used poly (D,L-lactide-co-glycolide) (PLGA) and acetalated dextran (Ace-dex) to formulate monodisperse NPs via nanoprecipitation. Physicochemical properties of the NPs were characterized with dynamic light scattering. Drug loading and drug release were determined by UV-VIS spectroscopy and analytical ultracentrifugation, respectively. Furthermore, we investigated the whole biological profile of free TG-201 and TG201-loaded NPs in different leukocytes and observed that the encapsulation into NPs lowered the detrimental effects of TG-201 on cell viability and increase the effectivness of the compound. In the human whole blood (HWB) assay, we observed that Ace-dex NPs enhanced the efficiency of

TG-201 by 10-fold. Finally, when HWB was incubated with LPS to prime leukocytes, mimicking low-grade inflammation prior to treatment, we found that Acedex NPs inhibit LTB4 formation with high effectiveness at low concentrations, displaying significant improvement versus the free drug. Conclusively, our data reveal the feasibility of a novel pharmacological approach using dual FLAP/mPGES-1 inhibitors encapsulated into polymeric NPs with high efficiency in HWB.

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ABSTRACTS OF ORAL CONTRIBUTIONS

L14: Formulation of liver-specific PLGA-DY-635 nanoparticles loaded with the protein kinase C inhibitor Bisindolylmaleimide I

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Bisindolylmaleimide I (BIM-I) known as a protein kinase C inhibitor is a promising drug candidate in the regulation of inflammation-related diseases, especially in the case of liver disease, but also as an adjunct therapy in the treatment of cancer. However, its low solubility and its direct interaction with the human ether-à-go-go-related gene (hERG) ion channels may prevent its development into an efficacious drug. Hence, to improve the drug solubility and its cell-specific delivery, BIM-I was formulated into nanoparticles prepared from poly(lactic-co-glycolic acid) (PLGA) coupled to the dye DY-635 as a targeting moiety. DY-635 provides two functions: (i) as a fluorescent dye it serves as a diagnostic tool; and (ii) with specificity towards OATP transporters—abundantly expressed in hepatocytes and cancer cells—it improves the delivery of the drug to the liver and cancer cells.

PLGA-DY-635 nanoparticles loaded with BIM-I were prepared via nanoprecipitation and characterized with respect to their size, dispersity, and morphology using dynamic light scattering, analytical ultracentrifugation and cryogenic transmission electron microscopy. The diameter of the particle was between 20 to 70 nm and a difference in size between the drug-loaded and unloaded particles was observed with all techniques. The cytotoxicity of PLGA-DY-635[BIM-I] particles was investigated in human and murine cell lines including a hepatocyte and fibroblast cell line. The liver-specific delivery of the particles was investigated *in vivo* utilizing intravital microscopy.

In vitro studies demonstrated that (i) the NPs efficiently prevented the phorbol 12-myristate 13-acetate-mediated PKC activation; (ii) the encapsulation of the

inhibitor into polymeric NPs decreased its' cytotoxicity; and (iii) the NPs were highly taken up in the hepatocytes. Finally, based on the *in vivo* evaluation, the NPs showed a considerable improvement of the survival probability in mice, compared to the free drug. Conclusively, our NP formulation strategy can be used as an example to produce multi-functional delivery systems that improve the bioavailability of poorly soluble drugs and provide an enhanced cell-specific uptake.

ABSTRACTS OF ORAL CONTRIBUTIONS

L15: Parenteral controlled drug release – concepts and surprises

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The presentation will discuss concepts of controlled drug delivery for parenteral applications. Control includes the location (biodistribution) and the kinetics of the drug release. The impact of the size, the polymer properties and drug characteristics on the release rate and the biofate of the polymeric carrier will be discussed.

Drug release mechanisms might change simply due to the different size of polymeric carriers. Diffusion controlled release is often achieved easily in microparticles. However, drug release from nanoparticles is in many cases difficult to control for non-covalently incorporated drugs due to the short diffusion lengths. Therefore, covalent linkage is often necessary.

Different examples for polymeric drug conjugates, including different stimulus sensitive drug delivery systems and their in vivo performance will be shown. Finally, the unintended accumulation of different nano-carriers in the ovaries and adrenals will be shown and discussed.[1,2]

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ABSTRACTS OF ORAL CONTRIBUTIONS

L16: New applications of analytical ultracentrifugation in the diversity of a collaborative research center: From small polymers to nanoparticles

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One of the historically most important techniques to study macromolecules and colloidal structures in solution is that of analytical ultracentrifugation (AUC). Via its modern, though seldom implementation in the 21st century, it can be considered a sophisticated technique in solution analytical chemistry. A particular promising application area is that of polymers and nanoparticles (NP) for the nanomedicines since it allows the *in situ* study of a large range of sizes of structures in solution via adjustable centrifugal forces and multi-detection concepts. In context of the CRC PolyTarget, we develop and use this technology for small functional polymeric structures, their colloidal assemblies as well as complex nanoparticulate and multifunctional nanomedical entities intended for potential translation toward inflammation and inflammation-related diseases.

In this contribution, we introduce the very basic measurement principles of AUC. This includes, among others, sedimentation velocity, sedimentation equilibrium, and synthetic boundary experiments. The power of AUC emanates from its possible core role in combination with other separation / fractionation techniques coupled to multi-detection, such as concentration sensitive, light scattering, and viscometric detection. Most importantly, AUC can answer questions outside the realm of, or in addition to, typically employed techniques. We underpin the newly discovered power of AUC for pharmapolymers,[1] functional polysaccharides,[2] nanoparticle formulations based on degradable polymers,[3] and also self-assemblies of varying structural properties. The application for the study of multi-component systems is particularly envisaged as well as opportunities to study the interaction of biologically relevant fluids with multifunctional NP entities.

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ABSTRACTS OF ORAL CONTRIBUTIONS

L17: The multiple roles of complement in health and disease

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The complement system senses invading pathogens as well as environmental or self-derived antigens by pattern recognition molecules of the canonical classical and lectin pathways. This function is critical to our sustained health and survival. It results in a cascade of proteolytic events leading to the cleavage of C3 into the anaphylatoxin (AT) C3a and the opsonin C3b by pathway-specific canonical C3 convertases. Consecutively, such C3 convertases build the framework for C5 convertases that cleave C5 into the AT C5a and C5b. In addition to classical and lectin pathway activation, the thioester in C3 can be directly activated by any nucleophilic attack leading to the activation of the so-called alternative pathway, driving strong cleavage of C3 and C5. Eventually, C5b forms the nucleus for non-proteolytic terminal pathway activation leading to the formation of the soluble (s)C5b-9 complex in the circulation of the poring-forming membrane attack complex (MAC) on cell surfaces which can drive cell lysis.

The Köhl lab is interested in the biology of the complement system. A particular focus is on the multiple functions of ATs C3a and C5a in the networks of innate and adaptive immune responses. The ATs play important roles as mediators of inflammation. Further, they regulate and control multiple innate and adaptive immune responses through binding and activation of their cognate G protein-coupled receptors, i.e. C3a receptor (C3aR), C5a receptor 1 (C5aR1) and C5a receptor 2 (C5aR2), although the latter lacks important sequence motifs for G protein-coupling. The lab has generated several floxed reporter mice to track and cell-specifically delete C3aR, C5aR1 and C5aR2. Based on their pleiotropic functions, the ATs contribute not only to tissue homeostasis but drive, perpetuate and resolve immune responses in many inflammatory diseases including infections, malignancies, autoimmune as well as allergic diseases.

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P02	The biopolymer bacterial banocellulose as a platform for nail applications *Tom Bellmann* *Friedrich Schiller University Jena, Jena (DE)*
P03	Study of nanomedicines in biologically-relevant fluids of varying complexity *Gizem Cinar* *Friedrich Schiller University Jena, Jena (DE)*
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P05	Smart delivery systems for anti-inflammatory 6-bromo-indirubin-3'-glycerol-oximether (6BIGOE) that modulates cytokines and lipid mediators by inhibition of TLR4/GSK3β-signaling in human monocytes *Anna Czapka* *Friedrich Schiller University Jena, Jena (DE)*
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P08	Defining the degree of crystallinity for PLA-based polymer blends and nanoparticles in solid state and suspension using Raman spectroscopy *Frederike Gladigau* *Friedrich Schiller University Jena/Leibniz Institute of Photonic Technology Jena, Jena (DE)*
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P10	Guanylated cellulose as a highly biocompatible non-viral vector for pDNA delivery *Juliana Hülsmann* *Friedrich Schiller University Jena, Jena (DE)*
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Abstracts of Poster Presentations

ABSTRACTS OF POSTER PRESENTATIONS

P01: Drug delivery systems to target Aspergillus fumigatus conidia inside macrophages

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We describe the synthesis of hydrophilic poly(poly(ethylene glycol) methyl ether methacrylate) PmPEGMA and hydrophobic poly(methyl methacrylate) (PMMA) caspofungin conjugates by a post polymerization modification of copolymers containing 10 mol% pentafluorophenyl methacrylate (PFPMA), which were obtained via reversible addition fragmentation chain transfer copolymerization. The coupling of the clinically used antifungal caspofungin was confirmed and quantified in detail by combination of ^{1}H-, ^{19}F- and diffusion ordered nuclear magnetic resonance spectroscopy, UV-Vis spectroscopy and size exclusion chromatography. The trifunctional amine-containing antifungal was attached via several amide bonds to the hydrophobic PMMA but sterical hindrance induced by the mPEGMA side chains prohibited intramolecular double functionalization. Both polymer-drug conjugates revealed activity against important human-pathogenic fungi, *i.e.*, two strains of *Aspergillus fumigatus* and one strain of *Candida albicans* (2.5 mg L^{-1} < MEC < 8 mg L^{-1}, MIC50 = 4 mg L^{-1}) whereas RAW 264.7 macrophages as well as HeLa cells remained unaffected at these concentrations.[1]

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ABSTRACTS OF POSTER PRESENTATIONS

P02: The biopolymer bacterial banocellulose as a platform for nail applications

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Common nail diseases, e.g. onychomycosis or nail psoriasis, can have a negative impact on patients quality of life due to impairment in daily activities and stigmatization. Local therapy formulations often lack efficacy because of low permeability of the nail plate, which can be increased by effective hydration of the keratinous network.[1]

Bacterial nanocellulose (BNC) is a biotechnologically synthesized three-dimensional network of nano-sized fibers that is capable to bind high amounts of water and water-miscible substances, that has already been utilized for various medical applications.[2]

In the present study BNC was loaded with glycerol as a hydration enhancing factor and urea as a keratolytic agent. The so obtained patches were designed as a moisturizing medical device, that could be used for the pre-treatment of diseased nails to enhance the efficacy of other therapeutic measures, like laser therapy. Additionally, the Boswellia extract Boswellin® Super was further added to BNC patches to develop a locally applicable anti-inflammatory drug delivery system. The patches were physicochemically characterized regarding transparency, fluid binding characteristics and mechanical strength. Furthermore, efficacy regarding wetting capacity and release properties was determined by utilizing keratin films as a nail plate model.

The formulated patches demonstrated no impairment of the mechanical stability and the fluid binding characteristics of the material, while the transparency could be increased. The wetting capacity of the patches was increased due to the incorporation of glycerol and sufficient amounts of anti-inflammatory boswellic acids were released from the drug delivery system.

In conclusion, we demonstrated that BNC patches displayed a therapeutic platform for nail applications with a simplified handling and capability to hydrate the nail plate effectively.

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P03: Study of nanomedicines in biologically-relevant fluids of varying complexity

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The large volume and diversified nanomedicine market, undergoing a rapid growth, particularly demands a quantitative assessment of their physicochemical properties. While synthetic tailoring and formulation strategies of such systems are steadily increasing, the development of suitable analysis techniques before application in *in vitro* and *in vivo* settings, particularly humans, is urgently required.

We demonstrate an in situ assessment of multifunctional biodegradable nanoparticle (NP) entities as core components of nanoscale drug delivery systems (NDDS)s by making use of analytical ultracentrifugation (AUC) and multi-detection at variable speed and time profiles. We present our experimental investigations of NDDSs comprising NPs based on biodegradable poly(lactide-co-glycolide) (PLGA), containing a physically-encapsulated anti-inflammatory drug, and being stabilized in solution by the surfactant poly(vinyl alcohol) (PVA). The NPs, prepared by nanoprecipitation, as well carry a cell-targeting, organ-specific dye, covalently bound to their constituting PLGA pristine polymer.

Under globally dilution-free conditions and an overall conserved mass balance, the AUC with multi-detection could unveil a multitude of solution components and decode, *inter alia*, the degradation dynamics in long-term storage experiments.[1] The fade and solution state of such NDDS components, were made accessible by the uniquely adaptable measurement settings. This includes degradation of NPs through a concerted bulk and surface erosion mechanism, possible aggregation phenomena, the associated quantitative study of the release of the anti-inflammatory drug from the NPs, and the fade of the cell-targeting dye unit. It was possible to address the concerted changes of these components and formulation substances of the NPs, last but not least also the role of the utilized surfactant present in the formulations.[1]

Next to repeatable and quantitative studies of formulations in simple aqueous media, experiments were also conducted in buffer solutions, cell culture media (CM) without and with added human serum (HS) as well as by direct dilution in HS. Alongside, we show assessment of changes and solution integrity concerted, and *on par*, with each other in the same experiment. The investigation of the integrity and degradation of NDDSs in biological fluids near to application conditions of nanomedicines, is believed being the up-and-

coming tool for the study of future nanomedicines, since existent standard methods are certainly not sufficient to address this challenge.

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ABSTRACTS OF POSTER PRESENTATIONS

P04: Automated image analysis of cargo delivery by nano- and polymer-particles

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In modern medicine, the proper targeting and dosage of disease-combating compounds is of primary importance. With organ-specific delivery of the active agents by utilizing nanoparticles and polyplexes (NP, PP), the pathogenic conditions can be treated with minimal side effects whilst using the proper dosage. It is also crucial to precisely characterize the progression of the disease, in order to be able to measure the severity of the conditions and the efficacy of the treatments provided by the NP and PP-based drug delivery. Automated image analysis algorithms provide the necessary tools to track the drug delivery and to quantify the pathogenic conditions. Here, we developed a series of systems biology approaches that are able to characterize the spatio-temporal behaviour of NPs and PPs both during their organ-targeted migration processes, as well as around the time point of the cargo release. We apply morphokinetic characterization tools in order to identify the local anatomy of the delivery area even without organ-specific labelling. As an example, we apply tools that are able to measure the severity of liver fibrosis and cirrhosis based on structural signals provided by label-free microscopy methods. Based on a combination of deconvolution and 3D or 4D (3D plus time) surface and point segmentation, we are also able to track the movements of NPs and PPs in live cells either based on fluorescence signals from the cargo, or via label-free image analysis methods.[1, 2] We also developed a toolkit to analyse Multi-Spectral Optoacoustic Tomography experiments using an automated clustering algorithm that provides objective measures about the pathogenic conditions of the liver or the kidneys.

In summary, our approaches represent the necessary symbiosis of medical, biological and mathematical approaches that is necessary to solve the complex problems of personalized, targeted disease treatment.

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ABSTRACTS OF POSTER PRESENTATIONS

P05: Smart delivery systems for anti-inflammatory 6-bromo-indirubin-3'-glycerol-oximether (6BIGOE) that modulates cytokines and lipid mediators by inhibition of TLR4/GSK3β-signaling in human monocytes

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The natural product indirubin is a bis-indole alkaloid contained as active ingredient in the traditional Chinese remedy Danggui Longhui Wan.[1] Indirubin derivatives are known to exhibit anti-inflammatory properties by modulating cytokine release.[2] In a structure-activity-relationship analysis, 6-bromo-indirubin-3'-glycerol-oxime-ether (6BIGOE) was identified as the most potent derivative able to inhibit pro-inflammatory cytokine and prostaglandin E_2 release in human monocytes while increasing anti-inflammatory IL-10 levels. However, the mode of action of 6BIGOE is unknown, and matters of instability and targeted delivery need to be addressed. Here we show that effects of 6BIGOE are mediated via inhibition of TLR4/GSK3β-signaling pathway. We found that 6BIGOE acts as a GSK3β inhibitor in the cell leading to enrichment of β-catenin but does not influence levels of GSK3β phosphorylation. Using an ultra-performance liquid chromatography tandem mass spectrometry approach, we found that 6BIGOE decreases pro-inflammatory cyclooxygenase (COX)-derived product formation via inhibiting COX-2 expression. To improve bioavailability and to overcome unfavorable physicochemical properties, 6BIGOE was encapsulated into nanoparticles (NP) consisting of modified poly(lactic-co-glycolic) acid for efficient delivery. Different encapsulation methods and polymers were evaluated for efficacy in human monocytes and whole blood assays. First results indicate that these NP are suitable as delivery systems for targeting monocytes and macrophages. We conclude that 6BIGOE is a potent modulator of inflammation-related cytokines and lipid mediators, which benefits from encapsulation into NP for targeted delivery, therefore highlighting it as promising candidate for treatment of inflammatory diseases.

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ABSTRACTS OF POSTER PRESENTATIONS

P06: Tuning the core-corona ratio of polymeric micelles for the oligonucleotide delivery to liver parenchymal cells

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The reticuloendothelial system (RES) of the liver clears nanocarriers regardless their size and surface properties, eventually creating a protective barrier for liver parenchymal cells.[1] Consequently, cell-type specific targeting towards hepatocytes remains challenging for gene delivery systems. Structural properties have strong influence on tissue penetration, stealth and clearance.[2] Despite the carrier size and surface charges, the interaction between the nanocarriers with their cargo and the cargo's properties have been proven to alter the in vivo pharmacokinetic and biodistribution profile.[3] Here we demonstrate the in vivo biodistribution of siRNA micelles with different core-corona ratios, allows tuning for passive cell type specificity in the liver, overcoming the reticuloendothelial barrier. EN15 and EN76 micelles were prepared by complexation of siRNA with PEG-b-PAGE. EN15 with 15 PAGE repeating unit results in a PEG:PAGE ratio of 0.6:1, while EN 76 with 76 repeating unit results in ratio of 0.1:1. The hydrodynamic diameter differed between EN15 (50 nm) and EN76 (70 nm) Both EN15 and EN76 show significant cell uptake and efficient gene silencing (knock down from 1 to 3 log2 -fold change). Both micelles were injected intravenously to FVB/N mice. In vivo biodistribution of both micelles were analysed by IVM on mouse liver for 45 min. Results depict that even without targeting moiety, EN15 with high PEG ratio (0.6:1) accumulate passively in hepatocytes while EN76 with lower PEG ratio (0.1:1) are recognized and cleared by Kupffer cells, the local tissue macrophages in the liver (Fig 1). Core-corona ratios of micelles determine their recognition and the biodistribution by RES cells. EN15 micelles with high PEG density have a favourable core-corona balance and is a promising candidate to passively deliver oligonucleotides into hepatocytes.

ABSTRACTS OF POSTER PRESENTATIONS

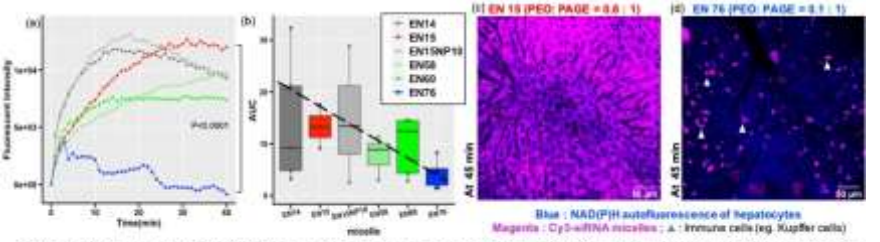

Figure 1 (a) Representative curves of Cy5-siRNA micelles fluorescent intensities in hepatocytes quantified over 45 min unbiased by automated algorithm. Two-sided t-test, α=0.05, n=6 (b) Area under the curve (AUC) was calculated from 1 to 30 min to quantify hepacellular uptake; Intravital Microscopy images of (c) EN15 and (d)EN76 taken at 45 min.

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ABSTRACTS OF POSTER PRESENTATIONS

P07: ROS-sensitive polymeric micelles for selective degradation in primary human monocytes from patients with chronic inflammatory diseases

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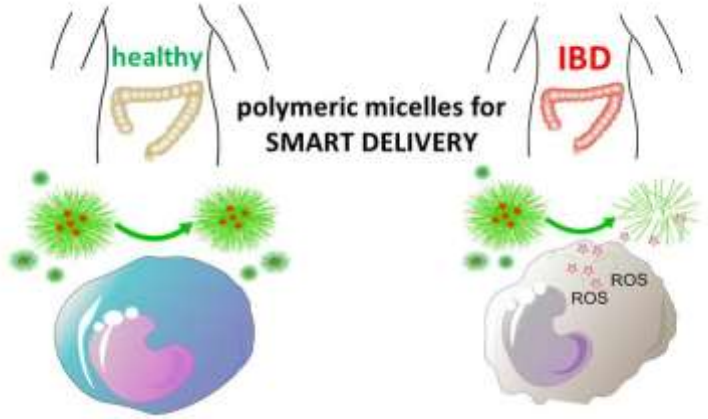
Chronic inflammatory disorders, as inflammatory bowel disease (IBD), are characterized by increased levels of reactive oxygen species (ROS) in inflamed areas of the gastrointestinal tract and in mononuclear cells of the patients. These increased levels of ROS open up new opportunities for targeted drug delivery to inflamed intestinal areas only.[1] To avoid detrimental effects on non-inflamed parts of the intestine, such a specific delivery of anti-inflammatory therapeutics to inflammation is paramount. Recently, we have demonstrated that oxidation-responsive polymeric nanostructures of various shapes can be efficiently prepared via one-pot polymerization-induced self-assembly (PISA).[2] The micelles selectively degrade in the presence of H_2O_2, due to the oxidation of the core-forming thioether functionality. Moreover, the degradation time can be tuned by the variation of the molar mass of the core-forming block. Based on these results, we hypothesized that such degradation process can be triggered in a similar way by the incubation with stimulated monocytes isolated from 15 patients with IBD. Our first results reveal that PMA-induced ROS release from monocytes in patients with active IBD resulted in a substantial degradation of the polymeric micelles ($p=0.0123$). Furthermore, micelles incubated with unstimulated monocytes from patients with active IBD significantly degraded even without PMA stimulation in contrast to monocytes isolated from 8 healthy donors ($p=0.0003$). These findings demonstrate that the presented materials may be suitable for selective release in presence of activated immune cells as they are found in inflamed tissue.



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P08: Defining the degree of crystallinity for PLA-based polymer blends and nanoparticles in solid state and suspension using Raman spectroscopy?

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Polymer crystallinity is an important property of nanomaterials, affecting *e.g.* release kinetics.[1] Renowned methods to determine crystallinity are Wide Angle X-ray Scattering (WAXS) and Differential Scanning Calorimetry (DSC). Both are usually applied to solid material. In this contribution, Raman spectroscopy is used to define the crystallinity of polymeric material in solid state and in suspension.

The nanomaterials were synthesized within the CRC Polytarget[1]and are based on biodegradable and biocompatible poly(lactic acid) (PLA).[1, 2] Both stereo-isomers of PLA, the L- (PLLA) and D-form (PDLA), were used in the study as well as stereocomplexes (SCs) formed from both isomers. Certain amounts of amorphous 3-ethylglycolide (EtGly) were incorporated into the PLA copolymers to alter the degrees of crystallinity while keeping a constant hydrophobicty. The different blends were used to synthesize PLLA/PDLA SCs[1] from which nanoparticles (NPs) with various EtGly contents were created.

Raman spectroscopy was used to characterize the crystallinity of the solid raw material, the different SCs and the NPs in a non-destructive and label-free manner. Using an in-house fabricated heating chamber, the glass transition and melting point could be determined from the Raman data of the polymers. As known from literature[2], the characteristic changes in the C=O stretching band (around 1760 cm-1) proved to be a good indicator for crystallinity. 2D correlation spectroscopy specified these changes appeared within 3 vibrational bands, whose fit parameters were used to define the degree of crystallinity from the spectra.

With this method NPs can also be analysed in suspension using dielectrophoretic capturing[3] to keep them in the laser focus.

Financial support from the DFG via SFB 1278 PolyTarget (A06, Z01) is highly acknowledged.

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ABSTRACTS OF POSTER PRESENTATIONS

P09: The influence of hydrophobicity on gene delivery and nuclear factor-kappa B suppression of poly(methacrylamide)s

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Endothelial barriers represent a significant hurdle for the transport of therapeutic compounds into tissue cells, especially for macromolecules like plasmid DNA (pDNA) or small interfering RNA (siRNA).[1] Looking for alternatives to poly(ethylene imine), terpolymers containing N-(2-hydroxy-propyl) methacrylamide (HPMA) and N-(3-guanidinopropyl)methacrylamide (GPMA) were used in this study.[2] Different hydrophobic moieties inspired by the amino acids tryptophan, phenylalanine and tyrosine were added to the polymers to investigate the influence of hydrophobicity on the transfection efficacy. Polyplexes were obtained after incubation with pGL3 pDNA or mouse RelA SMARTpool siRNA and physicochemically characterized according to binding efficacy, release properties and protection from enzymatic degradation. Particle sizes ranging from 180 to 200 nm and endosomal release, which was studied by endosomal leakage assay using calcein as a fluorescent dye, gave strong evidence for sufficient transfection efficacy. Moreover, toxicological characterization was performed on L-929 mouse fibroblasts as well as sheep blood.

To investigate the anti-inflammatory behavior of the polyplexes carrying siRNA on mouse brain endothelial cells (bEnd.3), quantification of NF-kB mRNA expression was conducted by real time quantitative PCR (RT-qPCR) showing significant knockdown of transgene expression.

In conclusion, the results show strong evidence for highly efficient pDNA as well as siRNA delivery *in vitro* in combination with high cell viability and low hemotoxicity.

Acknowledgment: The authors would like to thank the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - project number 316213987 - SFB 1278 (projects A02, B03 and Z01).

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ABSTRACTS OF POSTER PRESENTATIONS

P10: Guanylated cellulose as a highly biocompatible non-viral vector for pDNA delivery

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Natural polymers like cellulose or dextran lately play an increasing role as non-viral gene delivery agents due to their fast synthesis, low cytotoxicity and high biocompatibility compared to synthetic polycationic carriers like poly(ethylene imine) (PEI).[1] To condensate negatively charged genetic material under physiological conditions, polysaccharides can be modified with cationic charge bearing moieties inspired by the amino acid arginine. In this work 6-deoxy-6-(2-aminoethyl) amino cellulose was modified with a guanylation agent and resulting in *6*-deoxy-*6*-(2-guanidiniumethyl)-amino-cellulose. Guanylated cellulose successfully complexed plasmid DNA (pDNA) at nitrogen to phosphate ratios (N/P) ranging between 2 and 40 qualitatively as well as quantitatively. Moreover, protection from enzymatic degradation was measured, showing intact complexes after treatment with DNAse I. The particle size of complexes was determined by nanoparticle tracking analysis (NTA) resulting in particle sizes from 80 to 200 nm. Complexes also showed highly effective transfection potential assessed on CHO-K1 cells for complexes containing pGL3 pDNA and guanlyated cellulose with low cytotoxicity. Cell viability measurements were performed on L-929 mouse fibroblasts showing high biocompatibility. To give proof of sufficient endosomal release of complexes, calcein dye release assay was performed using fluorescence microscope analysis.

In conclusion this guanylated cellulose is a promising non-viral vector, which shows a high binding affinity together with high transfection efficiency and low cytotoxicity.

Acknowledgment: The authors would like to thank the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - project number 316213987 - SFB 1278 (projects A02, B03 and Z01).

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ABSTRACTS OF POSTER PRESENTATIONS

P11: Novel light-responsive polymers based on 1-hydroxypyrene: Synthesis and self-assembly studies

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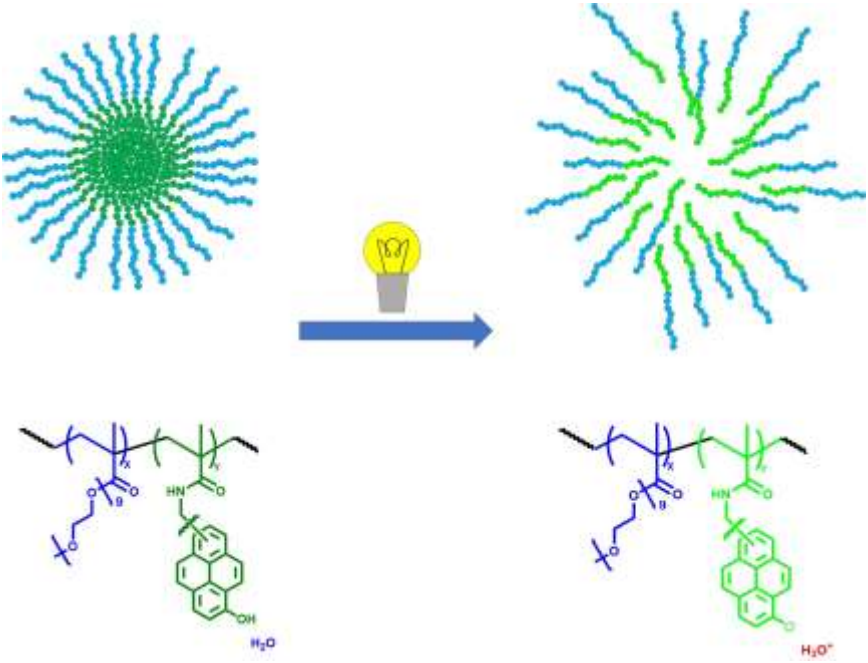
The utilization of the polymers as drug delivery systems often requires their stimuli-responsive association and dissociation. Although the temperature- or pH-responsive self-assembly is more studied, light-responsive polymers are also of considerable interest for biomedical applications. One of the examples of reversible photoswitches are photoacids, where a significant increase of acidity is observed upon excitation by light of appropriate wavelength leading to so-called excited-state proton transfer (ESPT). The most prominent class of photoacids known so far are hydroxyarenes (phenols, naphthols, pyrenols, etc.).

Recently, several polymeric photoacids containing 1-naphthol were carefully studied in our group.[1,2,3] Here, we report on the synthesis and investigation of macromolecular photoacids based on pyrenols. In contrast to naphthols, where the ESPT requires UV excitation, pyrenol-based photoacids can be activated by visible light, which is more appropriate for living tissue.

All polymers were synthesized by reversible addition-fragmentation chain transfer polymerization (RAFT). The solution behavior of all copolymers was studied by a series of physico-chemical methods such as dynamic light scattering, scanning and transition electron microscopy.

This research was supported by the DFG within the collaborative research center SFB 1278 POLYTARGET, project A03.



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P12: Aspects of cryo-transmission electron microscopy analysis of soft matter structures

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The analysis of soft matter structures has to be carried out with caution as structural parameters are easily disturbed. In that aspect cryo-Transmission Electron Microscopy (TEM) provides ideal possibilities to gain more structural information of soft matter samples, such as, micelles or vesicles. Key issue is here the image-based characterization of such systems in their nearly native state by freezing the samples in a thin aqueous film. High cooling rates of the water layer ensure the formation of vitrified ice where no crystallization of the water occurs. This strategy visualizes the distribution as well as the shape of the soft matter objects by minimizing drying aggregates and clustering. In this sense, cryo-TEM investigations provide an unprecedented tool to access the shape, size and distribution of soft matter materials. In our research this characterization tool is extensively used to visualize the structure of designed co-polymer systems which are able to self-assemble in aqueous solutions. The resulting shape of the formed self-assembled structures depends critically on a number of parameters which can be fine-tuned during their synthesis. This complex, and partially sensitive interplay of the relationship between molecular architecture and the self-assembly behaviour are investigated and carefully compared with complementary techniques.

Next to the design criteria for desired supramolecular architectures, cryo-TEM investigations can contribute moreover to the investigation of responsive nanoparticle systems. In this sense, the degradation of vesicular structures by changes of the pH value or the design of nanoparticles which can be used as probes for the development of reactive oxygen species are discussed. Such aspects are of importance for studying, e.g. drug release or to monitor inflammatory processes. We will introduce illustrative examples how cryo-TEM investigations can be utilized for an in-depth characterization of such systems.

P13: Evaluation of a library of PEO42-b-PAGE40 polymers with a variety of different amine groups for their use in mammalian cell-transfecting polyplexes

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The application of gene therapy is an emerging topic of the treatment of inflammation and inflammatory diseases. One promising strategy for the delivery of negatively charged nucleic acids such as plasmid deoxyribonucleic acid (pDNA) involves (reversible) polyplex formation with cationically charged polymeric materials.

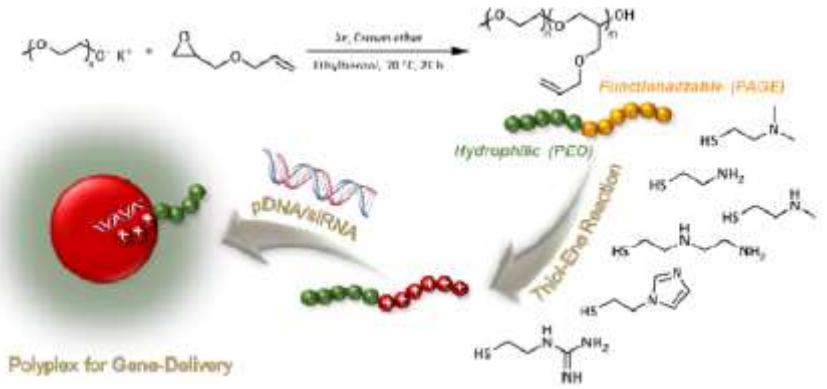
Herein, we present a poly(ethylene oxide)-block-poly(allyl glycidyl ether) (PEO-b-PAGE) system, which was synthesized by anionic ring-opening polymerization and modified by subsequent thiol-ene click reaction to bind pDNA for gene-delivery as depicted in Fig. 1. Thiols with different nitrogen-containing chargeable moieties like amines, ethylenediamine, guanidine, or an imidazole moiety were prepared.

These functionalized block copolymers were studied regarding their functionality in biological systems, including analyses of pDNA-interaction, biocompatibility, cellular uptake, and transfection efficiency achieved by the pDNA loaded polyplexes. The block copolymers were compared to each other as well as the considered “gold standard” in gene therapy, linear poly (ethyleneimine) (l-PEI).

Most polymers exhibited promising physicochemical properties. Binding and release of pDNA was comparable to that of l-PEI, except for the polymer carrying the imidazole moiety. In comparison to l-PEI, lower toxicities were observed. Whereas uptake into cells usually was efficient, the transfection efficiencies varied greatly. The polyplexes carrying a primary amine, a diamine, an ethylenediamine or a guanidinium moiety exhibited promising characteristics and in part outperformed l-PEI.

Our study, to our knowledge is the first comparison of a library of PEO-b-PAGEs with different nitrogen-carrying moieties. This improves the understanding of the biological interactions with PEO-b-PAGEs based on different functionalized moietie.

ABSTRACTS OF POSTER PRESENTATIONS



ABSTRACTS OF POSTER PRESENTATIONS

P14: DNA binding with core-crosslinked temperature- and pH-responsive micelles

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Among various possible assemblies that polymers can show in aqueous solution, micelles present a versatile tool to accomplish the delivery of genes into target cells through endocytosis. Micelles mainly consist of amphiphilic block copolymers including a positively charged segment to enable the complexation of genetic material.[1] Here, responsive block copolymers that are sensitive to different stimuli offer an advantage due to the possibility to switch between distinct properties in order to overcome the various barriers faced in gene delivery. The most frequently reported examples for double stimuli-responsive block copolymers feature a temperature- and pH-responsive segment.[2]

Therefore, we synthesized a diblock copolymer with N,N-diethyl acrylamide (DEAm) as the temperature-responsive first block, representing the core-forming segment of the block copolymer. The homopolymer of DEAm shows an LCST (lower critical solution temperature) behavior at approximately 33 °C,[3] which is close to body temperature. The pH-responsive second block consists of aminoethyl acrylamide (AEAm), which carries positively charged primary amino groups in the physiologically relevant pH-range. Additionally, a monomer based on thiol groups, 2-(pyridin-2-yldisulfanyl)ethyl acrylate (PDSAc), was incorporated into the core-forming segment of the diblock copolymer. It served as a reactive site for dithiol-based crosslinking, providing the necessary stability for the micelles to maintain their structure even at temperatures below the cloud point.

The self-assembly of the amphiphilic diblock copolymer into micellar structures above the cloud point of the solution was proven by fluorescence spectroscopy. The behavior of the micelles before and after crosslinking was studied by dynamic light scattering and analytical ultracentrifugation at varying concentrations and temperatures. Finally, the DNA binding ability of the double stimuli-responsive micelles was examined with regard to an application as a gene delivery vector. In conclusion, it was demonstrated that defined temperature- and pH-responsive micelles could be formed above the cloud point of the solution and that those can be stabilized below that temperature by core-crosslinking via thiol-bonds.

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ABSTRACTS OF POSTER PRESENTATIONS

P15: Biocompatible nanocarriers with HDAC inhibitor activity from modifyable polysaccharide valproates

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Polysaccharides (PS) are valuable polymers for medical applications as they are non-toxic, biocompatible and biodegradable. Their structure and, hence, properties can be widely modified. Valproic acid (VPA) is a histone deacetylase inhibitor (HDACi) that is known for its anti-inflammatory as well as for its anti-cancer activity.[1] VPA can be bound to PS via an ester linkage with DS values above 2.0. These PS valproates can then be shaped to nanoparticles (NPs).[2]

The PS valproates can be modified with functional groups, e.g. sulfuric half esters[3], amino groups, dyes etc. Further, the introduction of different linker systems can be used to improve the release of VPA or VPA prodrugs. In addition, functional groups or dyes can enable specific cell or organ targeting. The encapsulation or coupling of additional drugs offers the possibility to design multi-drug systems. Thus, these multifunctional polysaccharide derivatives can be used as versatile and adaptable nanocarrier systems.

The introduction of sulfuric acid half ester moieties efficiently improved the intracellular VPA release. The NPs are taken up rapidly, are non-toxic *in vitro* and *in vivo*, and are able to induce histone H3 hyperacetylation. This enables the application of these NPs in a variety of treatment approaches, especially in the context of inflammatory diseases and defined cancer types.

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P16: Nebulized polyester nanoparticles to overcome bacterial biofilms and resistance in pulmonary infections

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Pseudomonas aeruginosa and Burkholderia cepacia complex cause difficult to treat pulmonary infections related to high mortality especially with diseases like cystic fibrosis. Due to the formation of biofilms containing the microbial cells surrounded with secreted polymer, the bacteria achieve higher resistance against antibiotics.[1] Therefore, inhalable antibiotic treatment has to overcome the obstacles arising from mucus and biofilm barriers as well as various biofilm -related resistance mechanisms of the biofilm-embedded bacteria.[2]

In this study, poly(lactic-co-glycolic acid) (PLGA) and poly(ethylene glycol) (PEG)-grafted PLGA nanoparticles (NP) were investigated as carriers of tobramycin (Tb) for lung application as well as mucus and biofilm penetration. In biofilm experiments, Tb-loaded PEG-PLGA NP demonstrated improved bacterial killing of biofilm-embedded and mucus-covered bacteria in comparison to the free drug. Blends of NP and Tb displayed no antimicrobial activity indicating that only Tb-loaded NP can reach the biofilm-embedded bacteria. Additionally, an ex ovo shell-less hen's egg test on the chick area vasculosa (HET-CAV) was used to investigate the biocompatibility of the nebulized NP on a complex biological surrounding using vibrating-mesh technology confirming in vitro cytotoxicity data. Stability and efficacy of released Tb after nebulization were evaluated on a modified setup of the HET-CAV utilizing Pseudomonas aeruginosa (Pa) infected eggs.

In conclusion, we demonstrated that polyester NP displayed excellent properties as biocompatible drug delivery systems for antibiotics with improved deposition and efficacy in deeper biofilm layers and therefore, providing a therapeutic benefit in biofilm-associated pulmonary infections. Moreover, nebulization offers a highly suitable approach to deliver NPs efficiently to the deep lungs.

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ABSTRACTS OF POSTER PRESENTATIONS

P17: Core A06 - Controlling the degradation behavior of polymeric nanoparticles by structurally tailored thermal properties

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Polyesters represent biodegradable and biocompatible polymers making them useful for medical applications.[1] In particular, applications aiming at release of actives from polyester-based nanoparticles are in focus of our research. The degradation and release behavior is affected by the crystallinity of the polyesters[2], however, the exact role of this feature is difficult to quantify as additional factors such as the hydrophilicity play a major role as well. Our approach is therefore directed to obtain tailor-made polyesters that vary in their crystallinity but maintain the same hydrophilic / hydrophobic balance (HHB). In this contribution, we show the synthesis and the characterization of homo and co-polyesters featuring the same HHB as PCL or PLA by the synthesis of tailor-made polyesters and by blending.[3] The polyesters were obtained via ring opening polymerization (ROP) of lactones or substituted glycolides yielding well-defined homo- and co-polymeric specimens. In addition, blending led to stereocomplexation of PLA based polyesters. The resulting materials were characterized by means of size exclusion chromatography, nuclear magnetic resonance spectroscopy, matrix assisted laser desorption ionization mass spectrometry, differential scanning calorimetry, thermogravimetric analysis, wide angle X-ray scattering (WAXS) and atomic force microscopy (AFM) to assess the structural, thermal and mechanical properties of the bulk samples. Stable aqueous nanoparticle suspensions of varying size could be prepared by changing the concentration during the nanoprecipitation. Nanoparticles from all the polymers were investigated in detail by AFM, dynamic light scattering and scanning electron microscopy. As proof of concept, fluorescence spectroscopy of pyrene loaded nanoparticles for the PLA and PCL series proved same hydrophobicity, validating our approach. All techniques consistently hinted towards an altered internal structure of the nanoparticles with constant HHB.

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P18: Characterizing nanoparticle - cell interaction by using model membrane systems and advanced fluorescence microscopy

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Within the Polytarget-project C05, we aim to investigate nanoparticle–cell membrane interactions by using live-cell as well as model membrane systems, thereby optimizing imaging techniques. We here present three of our recent projects. i) A Guanidin-group is used by several polytarget-groups to enhance the cellular uptake of particles. With a simple model system, consisting of large -unilamellar vesicles, we try to understand the underlying mechanism of the membrane interaction. ii) We set up a robust system for spectral unmixing. This enables us to distinguish several dyes within the same sample based on their different spectral properties, even when they have a spectral overlap. We apply this technique while imaging nanoparticles together with polarity-sensitive membrane dyes, such as NR12A, whose emission spectrum shifts upon changes of the environmental lipid order. Since the availability of such dyes is limited, spectral unmixing helps us to better understand membrane interactions. iii) We further optimized the microscope setup. Previously, an inexpensive, yet powerful, homogenous illumination solution was investigated. We now try to make this applicable even to ultrafast microscope techniques, such as iSCAT microscopy.

P19: Organic solvent-free nanoparticle formulation of statistical cationic-hydrophobic terpolymers for gene delivery

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Cationic polymers are promising candidates as gene delivery vectors due to their ability to bind and protect genetic material through electrostatic interactions. A polymeric carrier should be stable in the presence of serum proteins and assist in the endosomal release to deliver its cargo. Introducing additional hydrophobic moieties improves the efficiency in vitro by enhancing the interaction with cellular membranes.[1] However, introduction of a substantial hydrophobic character can lead to reduced water solubility and imposes a challenge in encapsulation of genetic material. Further, commonly applied formulation methods for water insoluble polymers, usually involve harsh conditions such as sonification and organic solvents, impairing intactness of the genetic material and limiting the loading efficiency of the nanoparticles.[2, 3]

The aim of this study was to develop a mild, organic-solvent-free formulation method to enable the encapsulation of large amounts of genetic material into stable nanoparticles by cationic, hydrophobic polymers. We therefore, synthesized well-defined P(DMAEMA-*co*-BMA-*co*-MMA) (PBMD) *via* RAFT polymerization with compositions inspired by the commercially available polymer Eudragit E. The statistical terpolymers are pH-responsive and soluble in acidic aqueous solutions. By applying the organic-solvent-free formulation approach for the encapsulation of pDNA, stable nano-sized particles were prepared with high pDNA concentrations. The particles were characterized by dynamic light scattering (DLS), analytical ultracentrifugation (AUC), and *cryogenic* transmission electron microscopy (*cyro*-TEM). Furthermore, the pDNA binding/release capability of the polymers and complex stability in the physiologically relevant range from pH 5 (endosomal) to pH 7.4 (blood) were investigated. Subsequently, the pDNA particles were tested *in vitro* for their transfection efficiency (expression of EGFP-encoded plasmid) in comparison to the gold standard lPEI. High transfection efficiency was achieved at relatively low pDNA concentrations (5 to 10 times less than that required for lPEI) at short incubation times (< 4 h). The formulation approach was further succesfully applied for complexation and delivery of various types of genetic material (e.g. siRNA, mRNA).

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ABSTRACTS OF POSTER PRESENTATIONS

P20: Celastrol - a natural compound as a treatment for inflammatory skin diseases

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Celastrol, a pentacyclic triterpenoid, is a pharmacologically active natural compound present in Thunder God Vine (TGV). Although TGV is known as a poisonous plant, its root contains severval pharmaceutical active compounds, e.g. triterpenoids or alkaloids. It has been used in traditional Chinese medicine as a treatment for inflammatory and autoimmune diseases, e.g. rheumatoid arthritis. Molecular targets of this natural compound have already been investigated. Celastrol directly inhibits the IKK-α and -β kinases which play an important role in the NFκB-pathway.[1] Unfortunately, its use is highly limited by the poor water-solubility, high lipophilicity and thus its poor bioavailability.

In this study celastrol-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NP) were prepared with different preparation methods, comparing the already established and frequently used solvent ethyl acetate with novel non-toxic and sustainable solvents polyethylene glycol 400 (PEG 400) and Cyrene™.[2,3] Size, polydispersity and zeta potential of the NPs were characterized to compare the different preparation methods. Additionally, biocompatibility and toxicology of free celastrol was characterized by an *exo ovo* shell-less hen's egg test on the chick area vasculosa (HET-CAV).

Celastrol-NP prepared by these different preparation methods were comparable in size and polydispersity. Zeta potential, respectively, was high enough to stabilize the NP in suspension. Free Celastrol showed no toxic effects with concentrations between 1 µM and 100 µM. Only with concentrations above 500 µM toxic effects were observed.

In conclusion, we were able to encapsulate celastrol by different encapsulation techniques and showed the biocompatibility of the compound and its nanoformulation within therapeutically relevant concentrations.

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Publisher: Jena Center for Soft Matter (JCSM)
Photos: DominoPlus Jena / Erdinc Sezgi (University of Oxford), Prof. Dr. Christian Eggeling (FSU Jena) / Ranjat M from Pixabay / Anne Günther/FSU
Layout: Communications Office FSU Jena

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