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From Polymers to Functional Biomaterials

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This special issue of Macromolecular Bioscience highlights emerging applications of polymers for the synthesis of functional biomaterials. Polymers provide many unique features and in particular their structural flexibility enables tailoring material properties for the desired application. The individual structural optimization of a given polymer requires feedback systems, namely in vitro or in vivo studies, to fully explore and customize their properties. Consequently, most articles in this issue describe the optimization of polymers for biomedical applications particularly focusing on their in vitro and finally also their in vivo evaluation in relevant disease models. The great challenge is to tailor polymer properties to a specific biomedical, often therapeutic, need and to advance from fundamental materials research into clinical translation. Here, avoiding simplification and overgeneralization in the development of biomaterials will be a crucial step.[1]

In this special issue, variations of the dimensions, morphologies and functionalities will be demonstrated. Sizes ranging from the low nanometer regimes of polymers up to micrometer vesicles or hydrogels are presented. Functionalization includes the attachment of various drug molecules, proteins via residue-specific bioconjugation, such as antibodies for cellular targeting, organelle-specific reagents to control intracellular trafficking, which emerged as strategies to direct polymers to the desired location within the cell to improve therapeutic efficacy.

Due to the structural versatility of polymers, their applications can be broad. In this special issue, biomedical applications range from drug and gene delivery systems, immune therapies and wound healing of colloidal systems or hydrogels to local release formulations. This wide range of applications underlines the enormous variety of material properties polymers could provide and opens up a rich field for polymer research.

The first part of the special issues is devoted to the use of customized polymers for drug delivery applications.[2–4] In the first article, Harm-Anton Klok and co-workers review approaches of monitoring and directing the intracellular distribution of polymer-based nanomedicines, thus emphasizing the potential of organelle specific drug delivery.[5]

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The first research article in this section is contributed by Ulrich Lächelt and describes the development of sequence-defined oligoamide drug conjugates of pretubulysin and methotrexate for folate receptor-targeted cancer therapy. The authors could nicely demonstrate the development of a polymeric material with defined sequence and its in vitro and in vivo validation for the therapy of folate receptor-positive tumors in mice.[6]

Next, Martina Stenzel and co-workers report the application of fructose-coated micelles to overcome the poor delivery efficiency of the ruthenium complex dichlororuthenium (II) *p*-cymene (1,3,5-triaza-7-phosphaadamantane) in tumor spheroid models and invasion assays. The reported results demonstrate the potential of polymeric micelles to enhance bioavailability of poorly soluble drugs and underline the role of carbohydrates for enhancing cellular uptake.[7]

The approach reported by Zhiyuan Zhong and co-workers enhances the stability of self-assembled nanostructures, e.g. micelles or polymersomes, by additional cross-linking by bioreversible disulfide bond formation.[8,9] Disulfide bond formation was also used to attach a mertansine prodrug, while cRGD ligands enhance cellular uptake into B16F10 melanoma cells in vitro and in vivo. The presented poly(ethylene glycol)-*b*-poly(trimethylene carbonate)-*co*-(dithiolane trimethylene carbonate) polymers provide a flexible platform for the development of disulfide cross-linked drug delivery systems.[10]

In the following article, Marcelo Calderon and Rosa M. Reguera demonstrate that the conjugation of a Doxorubicin prodrug to PEGylated dendritic polyglycerols provides new perspectives in the therapy of Leishmaniasis, a parasite-induced disease with often fatal outcome. Since the amastigotes live in parasitophorous vacuoles inside macrophages, drug delivery systems are likely to improve therapeutic outcome.[11]

Additionally, Hans Börner and co-workers describe the development and optimization of block copolymer-based drug formulations. In their case, a tailor made peptide binder is combined with PEG to efficiently solubilize the anti-Alzheimer drug B4A1, which enhances bioavailability of the poorly soluble drug and thus enables inhibition of Tau-protein aggregation in vitro.[12]

The chapter on polymer-based drug delivery systems is completed by an article by Rainer Haag and co-workers. The authors present a study in which a non-toxic pH-responsive dendritic polyglycerol nanogel (dPG-NG) is developed. The article nicely demonstrates that the dPG-NGs penetrate the skin via the follicular pathway and are able to monitor the topical or intradermal pH in an ex vivo porcine ear model. The presented nanogel platform seems to provide a suitable basis for the development of intrafollicular drug delivery.[13]

The second part of this special issue comprises of articles reporting the synthesis of polymer-based gene delivery systems. The delivery of nucleic acids for gene editing in vivo provides enormous potential for a multitude of diseases, e.g.

cancer (immune) therapy,[14–16] or liver fibrosis,[17] but is more demanding in terms of delivery. The carrier needs to protect the nucleic acids from degradation in the blood stream, to transport it to the target cell, to bypass the lysosomal degradation (endosomal escape), and finally to release the functional siRNA or mRNA inside the cytosol. In case of pDNA, nuclear import needs to be accomplished additionally.[18] These complex requirements generate the need for sophisticated delivery systems, to which functional polymers will at least contribute.

The first article in the section is a feature article by Rudolf Zentel and co-workers, which highlights the use of cationic nanohydrogels for the therapeutic delivery of short pharmaceutically active oligonucleotides, e.g. small interfering RNA (siRNA) or cytidine-phosphate-guanosine (CpG). The authors discuss synthetic concepts and their use in the modulation of the immune system.[17]

In the following research article, María Vicent and Ernst Wagner describe the development of biodegradable gene delivery systems combining sequence defined tetraethylene pentamines among a polyglutamic acid backbone. Although, the developed transfection agents may be limited to local application, they displayed high transfection efficiencies in N2a neuroblastoma and 4T1 breast cancer cell lines.[19]

The third article was contributed by Stefan De Smedt and coworkers and describes the use of polymer-based siRNA formulations in high-pressure nebulization for RNAi after peritoneal administration. While this process did not alter transfection efficiencies, the presence of ascites fluid reduces substantially the efficiency of cationic cyclodextrine based siRNA formulations. In contrast, the effect is much less pronounced for lipofectamine-based siRNA polyplexes, which underlines on the one hand the importance of the cationic polymer and on the other the potential of high-pressure nebulization for an application in the peritoneal cavity.[20]

The chapter on polymer based gene delivery systems ends with an article by Pol Besenius and co-workers on supramolecular polymers for siRNA carrier systems. The authors designed histidine enriched dendritic peptide amphiphiles, which self-assembled into nanorods. Most importantly the alternating histidine and phenylalanine peptide trimers allow the assembly/disassembly at physiologically relevant pH and are able to complex siRNA, which is released after disintegration of the supramolecular structure.[21]

The third section of the special issue is devoted to polymer-based immune therapies. Immune therapies have seen an increased amount of attention during the last decades. Nanoparticle and thus polymer based immune therapies seem particularly interesting since the immune system evolved to care about nano-sized objects. In addition, potent immune responses often require co-delivery or co-presentation of several molecules, which can be accomplished by the use of polymers.[15,22,23]

In this respect polymers and self-assembled structures thereof provide a suitable platform to modulate the immune system in a way that immune responses can be specifically induced to fight pathogens and to treat bacterial or viral infections or even cancer. In this respect, Matthias Bartneck provides a comprehensive overview on "Immunomodulatory Nanomedicine". His article includes an overview on major immune cell types and their role in different diseases, selected therapeutic interventions as well as a critical perspective on novel developments in polymer-based immune therapies.[24]

In the following article Matthias Barz and co-workers report the development of polymersomes based on Polypept(o)ides[25,26] (PeptoSomes) for antigen-specific vaccination. In the presented approach, antigen (ovalbumin) and adjuvant (CpG) are co-encapsulated by dual centrifugation and co-delivered into dendritic cells (DC), which induces a DC mediated T-cell response in co-culture. The presented approach is in particular interesting since it enables the fast and efficient loading of antigens and adjuvants as well as combinations thereof into polymersomes, which can be specifically targeted to DCs.[27]

The fourth part of this special issue is devoted to the development of polymer-based colloidal nanoparticles and their in vitro or in vivo application. Although polymer-based colloids have so far failed to reach advanced clinical phases for systemic drug delivery and their pure size (>60 nm) may hinder applicability for drug delivery to cells of solid tumors,[28,29] they have been part of approved long active release systems for local administration[30,31] and bear enormous potential in immune therapies[32] or drug release by focused ultrasound.[33]

In the first article of this section Sebastian Perrier and co-workers report on the synthesis of polyacrylamide-stabilized polystyrene nanoparticles by surfactant-free reversible addition-fragmentation chain transfer (RAFT) emulsion polymerization. Fluorescent labeling enabled the investigation of in vitro and in vivo biodistribution. Interestingly, although the particles are rather small (11 nm and 22 nm), predominant accumulation in liver and lung after systemic administration is reported, which may indicate protein corona formation in the blood stream.[34]

The next article by Ulrich S. Schubert and co-workers describes the synthesis of amino-functionalized methyl methacrylate-based statistical copolymer modified with retinoic acid (RA) for targeting hepatic stellate cells in vitro and in vivo. The polymers are used to form cationic nanoparticles by utilizing the nanoprecipitation method, which are taken up by cells efficiently and accumulate as expected in liver after systemic application. While imaging of the dye labeled RA containing particles is possible, hepatobiliary clearance from the organism is not observed.[35]

In the following article, Katharina Landfester and Frederik Wurm employ polymeric surfactants and surfmers, polyglycidols containing allylic groups for covalent attachment during mini-emulsion polymerization or hydroxyl groups for urea formation during inverse miniemulsion technique using diisocyanates. With these chemical tools colloidal nanoparticles can be synthesized to which the polymeric surfactant is covalently attached. Although this approach should lead to stably covered colloidal nanoparticles, the authors report detectable formation of serum aggregates in citrated human plasma using dynamic light scattering. Nevertheless, these novel surfactants may be a promising alternative to conventional surfactants, because they are known to be biocompatible, provide functional groups for further modifications and lead to a protein corona comparable to PEGylated particles.[36]

In the last article of this section Twan Lammers and co-workers describe the optimization of the shell composition of poly(butyl cyanoacrylate) (PBCA) based microbubbles. The

authors investigated the molecular weight and polydispersity of polymers formed during the polycondensation reaction used for microbubble shell formation. Interestingly, all polymers of the PBCA shell of microbubbles display molecular weights below 40 kD, which may allow renal or hepatobiliary excretion.[37]

The fifth part of the special issue is devoted to the synthesis and application of hydrogels. This section nicely demonstrates that biomaterials are much more than polymers for drug or gene delivery or diagnostic systems. Polymers can be designed to be the building blocks for scaffolds in tissue engineering[38,39] or enhance wound healing by local drug release, pharmacological or antibacterial activity or antibacterial activity.[4,40]

In the first article of the section Tim Deming and co-workers, report the use of a hydrogel forming synthetic, cationic, and hydrophobic poly(amino acid) block copolymer as antimicrobial agent. In time-kill assays, solutions of a poly(L-lysine·hydrochloride)$_{100}$-*b*-poly(L-leucine)$_{40}$ copolymers show multi-log reductions in colony forming units of Gram-positive and Gram-negative bacteria, as well as yeast, including multidrug-resistant strains at concentrations as low as 10–100 mg L^{-1}. Moreover these polypeptide hydrogels provide an effective barrier to microbial contamination of wounds, as displayed by multi-log decreases of tissue-associated bacteria with deliberate inoculation of porcine skin explants, porcine open wounds, and rodent closed wounds with foreign body. Therefore, this outstanding work perfectly demonstrates the process of development of a polymeric antimicrobial biomaterial from its synthesis to in vitro and in vivo evaluation.[41]

In the second article, Holger Frey and co-workers introduce a novel PEG-based acid-labile macromonomer with methacrylate units that enable 3D cross-linking by photoinitiator-mediated free-radical polymerization. The synthesized PEG-ketal-diols and PEG-ketal-DMA polymers are stable in their lyophilized form, while they get rapidly degraded under acidic conditions (hydrolysis half-life times from 82.4 to 5.6 min). Hydrogels containing 0, 5, or 10 wt% of PEG-ketal-DMA and 100, 95, or 90 wt% of PEG-DMA, respectively, show visible disintegration at pH 5 when containing PEG-ketal-DMA, whereas no visible degradation is observed at all at neutral pH or for PEG hydrogels without PEG-ketal-DMA. Since these hydrogels are based on biocompatible components, they may be suitable for local release of bioactive compounds.[42]

The sixth and last section of the special issue on functional polymer-based biomaterials is devoted to protein-polymer conjugates.[43–46] Protein–polymer conjugates can combine the properties of biologic and synthetic materials, which can be individually adjusted to achieve therapeutic effects, thus leading to novel materials with unique properties. Protein biorecognition has already been used to replace deficient or deliver absent natural proteins and up-regulate or inhibit metabolic pathways, since enzymes can catalyze chemical reactions in vivo as well as in vitro. Often these biomolecules, however, possess limited stability or get rapidly excreted upon systemic administration, which creates a need for further modifications to overcome these limitations. Synthetic polymers can be the key to achieve enhanced stability and increase size without altering function or even provide additional functionality to enhance the therapeutic potential of proteins. This fusion of biological properties with chemical stability or reactivity provides protein-polymer conjugates their unique position as therapeutic entities and it is not very surprising that protein-polymer conjugates are widely used to improve the pharmacokinetic properties of therapeutic proteins.

Frederik Wurm and co-workers report the synthesis of poly(phosphate)-protein conjugates, in which biodegradable polyphosphates are covalently linked to the model protein bovine serum albumine (BSA). Therefore, polyphosphate polymers with molecular weights between 2000 and 33 200 g mol^{-1} have been synthesized by organo-catalyzed anionic ring-opening polymerization and ω-functionalized with a succinimidyl carbonate group, which enables the straight forward coupling to BSA. Notably, the synthesized protein-polymer conjugates are the first ones based on polyphosphates, which degraded upon exposure to human phosphodiesterases.[47]

The selected examples in this special issue demonstrate that there is not one material or one material class that provides all desired features. It is the delicate interplay between chemists, biologists and medical doctors that allows advancing the design of the polymeric material for translational applications. In this respect, many materials have been used as versatile tools to very successfully enhance our understanding of e.g. in vitro and in vivo transport pathways. Nevertheless, the ultimate goal of these interdisciplinary efforts should be the design of materials that could advance as safe and efficient treatments into clinical development and finally become approved diagnostic or therapeutic agents.

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