Freie Universität Berlin

Stimuli-Sensitive Nanocarriers for Diagnostic and Therapy

Dissertation

zur Erlangung des akademischen Grades des

Doktors der Naturwissenschaften (Dr. rer. nat.)

eingereicht im Fachbereich Biologie, Chemie, Pharmazie

der Freien Universität Berlin

vorgelegt von

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Mai 2017

Diese Arbeit wurde unter der Anleitung von Prof. Dr. Rainer Haag im Zeitraum von Dezember 2012 bis Mai 2017 am Institut für Chemie und Biochemie der Freien Universität Berlin angefertigt.

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Disputation am 13.11.2017

Für Lena

Acknowledgements

First of all, I would like to thank Prof. Dr. Rainer Haag for the possibility to do my PhD thesis in his research group and for the scientific support during the last years under his supervision.

Prof. Dr. Nan Ma is gratefully acknowledged for co-refereeing this thesis.

Thanks to all the members of the AG Haag for the great support, collaborations, scientific discussions and the nice social environment at the group trips and elsewhere.

Furthermore, I would like to thank Dr. Dirk Steinhilber for the scientific support and the great time during my entire PhD inside and outside the laboratory.

Special Thanks to my former and present lab colleagues, Era Kapourani, Anja Stöshel, Fatemeh Zabihi, Dr. Xuejiao Zhang, Dr. Rahul Tyagi, Michael Unbehauen, Dr. Wei Chen, Felix Reisbeck, Wanjun Liang, Dr. Florian Paulus, Dr. Dirk Steinhilber and Manoj Kumar Muthyala for the great time in the 33.04 lab. Thanks to Dr. Pradip Dey and Dr. Dr. Sumati Bhatia for the nice discussions about nanogels and their crosslinking. Furthermore, I would like to thank Jutta Hass, Dr. Pamela Winchester, Eike Ziegler and Dr. Wiebke Fischer for the great support concerning all administrative issues.

Moreover, I would like to thank the AG Calderon for the great time in lab 33.05 and in the “Coffee” Office for the fruitful chemical and non-chemical related discussions. Thanks to Prof. Dr. Marcelo Calderón, Dr. Mazdak Asadian Birjand, Dr. Julián Bergueiro Álvarez, Michael Giulbudagian, Dr. Ana Sousa Hervés, Dr. Maria Molina Soler, Dr. Harald Rune Tschiche, Laura Voßen, Loryn Fechner, Katrin Michel, Dr. Stefanie Wedepohl, Emanuel Glitscher, Enrico Miceli, Gregor Nagel, Ernesto Osorio and the many visiting researchers.

Special thanks to all my cooperation partners. Especially to Falko Neumann and Prof. Dr. Nan Ma for the support regarding all biological issues. Thanks to Dr. Fitsum Feleke Sahle and Prof. Dr. Jürgen Lademann, the experts in dermatology at Charité Berlin. Thanks to Dr. Virginia Wycisk and PD Dr. Kai Licha for their knowledge and input regarding all dye labeling processes and to Fatemeh Zabihi, Katja Obst, Dr. Guy Yealland and Prof. Dr. Sarah Hedtrich (Institute for Pharmacy, FU Berlin). Thanks to Svenja Ehrmann and Jose Luis Cuellar-Camacho for the Nanogel

Characterization by Cyro-TEM and AFM. Furthermore, I am thankful to Yinan Zhong, Zhiyuan Zhong for their work at Soochow University (China). Special thanks to Daniel Stöbener and Dr. Florian Paulus for the nice time in the “Polyglycerin Manufaktur” and in Polymer Science. Here I would also like to thank my other Polymer Science Collogues: Dr. Benjamin Ziem, Karolina Walker, Gregor Nagel, Christoph Schlaich, Dr. Emanuel Fleige, Dr. Tobias Becherer, Dr. Jonathan Vonnemann, Dr. Qiang Wei and all the P.S. students for the nice time. Moreover, I would like to thank the people from the NMR department, for the countless measurements.

Thanks to the group of Prof. Dr. Jin Chang for the scientific support, the collaborations and the great time during my research stay as visiting scientist at the Tianjin University, School of Materials Science and Engineering, China.

Thanks to Schaggy, Alma, Rodrigo, Robby, Philipp, Harry, Anja, Olli and Andy, who have accompanied me through my studies.

Thanks to the “Dahlem Kickers” with Coach Dr. Mazdak Asadian Birjand and his assistant Dr. Olaf Wagner plus all current and past members for the nice soccer games. Thanks to the “Brachen” group for the nice volleyball matches at Landsberger Allee and at Nordbahnhof.

I would like to thank all my friends who supported me throughout my whole PhD.

A very special thanks to my family, especially my parents and grandparents for the overall support during my whole education.

Very special thanks to my wife Lena for the outstanding time. I am grateful for our common taken path and excited for the future way as a family.

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1 Introduction

1.1 Nanomedicine

The early concept of nanomedicine emerged by the lecture “There’s plenty of room at the bottom” of Robert Feynman in 1959. His long-term vison of nanorobots which can be helpful for medical use was further developed by writings of Drexler and Freitas in the 1980s to 2000s. First publications, which used the term nanomedicine appeared from that time. In general, it describes the use of nanosized tools in the field of medicine for drug delivery, disease diagnostics, and biological sensing. The nanoscale is the defined area from 100 nm down to the size of single atoms. In that scale, the properties of materials can differ from those of larger materials, such as the overcome of biological barriers and cellular membranes. However, many carriers above the size limit of 100 nm fulfil this important requirement. Due to this fact, carriers in the size range up to 1000 nm were often designated as “nano”.[1-3]

Nowadays, the idea of nanorobots has been developed into many different directions and many nanomaterials got into our everyday life. Cosmetics, sunscreens, strain resistant coatings on surfaces and textiles, as well as nano-materials in tires and electronics are only a few examples.[4] Also, the field of nanomedicine has emerged since the last couple of years. Especially nano-structured carriers based on organic and/or inorganic materials were designed to transport therapeutic or diagnostics cargos into diseased tissue. Liposomes, polymeric micelles, polymeric nanoparticles, polyplexes, dendrimers, and nanogels are the architectural products of assembled lipids, amphiphiles, linear or dendritic polymers. Among these also carbon nanotubes, nanocrystals, quantum dots, inorganic nanoparticles, and DNA-like scaffolds as well as mesoporous materials like silica or alumina were used. This toolbox of synthetic or natural building blocks can be used to build up carriers in the nanometer scale range and conjugate, complex, or encapsulate the desired cargo and release it at the place of interest. These systems are also called Drug Delivery Systems (DDS).[5-8]

A general scheme of stimuli responsive DDS for the transport and release of drugs is illustrated in Figure 1. Here, the main objective is to solubilize and protect therapeutic drugs with materials, that are easy to functionalize and have a higher uptake to the target tissue or cells than to the healthy one. These carriers should furtherly provide a colloidal stability for a prolonged plasma half-life and a low rate for aggregation. The related drugs can be encapsulated or complexed into the nanocarrier (A) or chemically conjugated to the

transport-system (B). The release of the cargo will be realized by natural or external triggers in the targeted environment. Helmut Ringsdorf developed the concept of stimuli-responsive nanocarriers for a DDS based on synthetic polymers (B) in 1970. Besides a responsive linker group for the controlled drug release, sugar groups or antibodies can be bound to the macromolecular backbone which can target receptors or antigens at the desired site and increase the carrier uptake. Solubilizing moieties improve the bioavailability of the polymer-drug conjugate. Based on that initial idea many polymer therapeutics were developed for the use as nanomedicine.[9, 10]

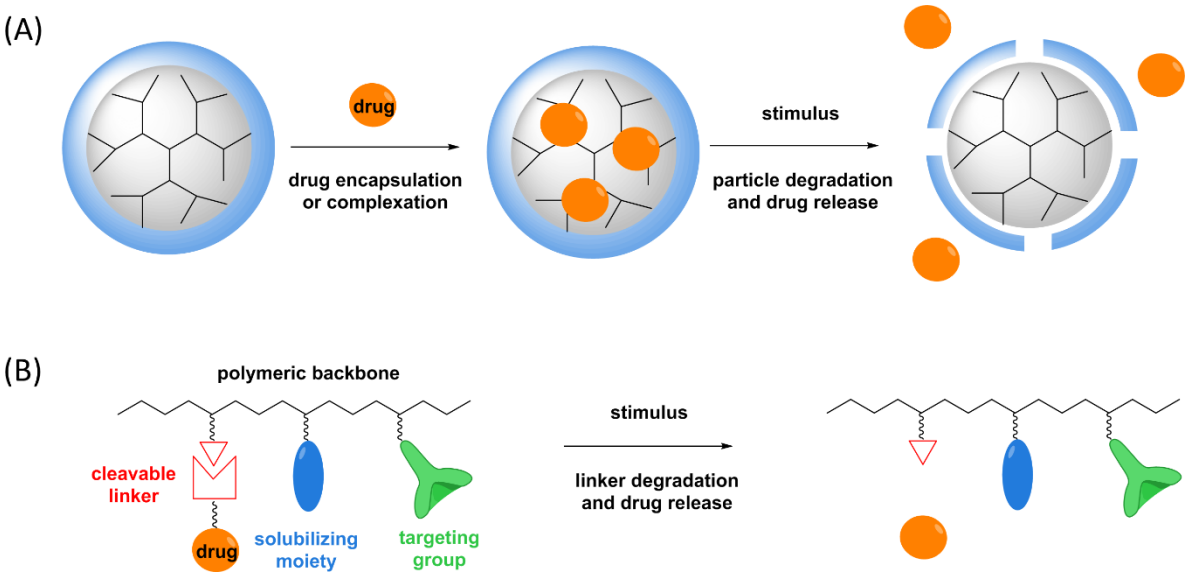

Figure 1. Mechanism for drug encapsulation, complexation by DDS (A), or conjugation to polymeric DDS (B), adapted from literature Fleige et al.[7] and Haag et al.[10]

The field of polymer-therapeutics includes polymer–drug and polymer–protein conjugates, polymeric micelles, and non-viral polymeric vectors for gene delivery (will be discussed in more detail in chapter 1.3).[11]

The advantages of such carrier systems to the conventional delivery of single drug molecules or genetic materials and examples of stimuli-responsive nanocarriers will be explained in more detail in the following chapters.

1.2 Administration Routes and Biological Barriers

In order to achieve a therapeutic effect, the DDS has to reach the target site. It needs to overcome extracellular as well as intracellular barriers of the human body. The type of barriers depends on the administration of the DDS. The main administration routes for the application of pharmaceuticals are: parenteral (intravascular, intramuscular, or subcutaneous), enteral (oral, sublingual, or rectal), and topical (dermal, inhalational, or through mucous membranes of the body).[12] The successful drug administration depends on the biochemical properties of the drug and the target. Since the majority of the pharmaceuticals to be transported are non-natural, hydrophobic, and of low molecular weight, they have many limitations regarding their transport ability *in vivo*.[13, 14]

For the intravascular application (injection), the drug reaches the blood stream first. Small drugs and therapeutic biomolecules like genetic material (DNA or siRNA) as well as proteins have a short half-life in the blood stream. Particles in the size range below 6-10 nm are subject to the fast-renal clearance of the kidneys (see Figure 2). Furthermore, the therapeutic biomolecules cannot pass the cell walls without any support and are recognized by the immune defense or degrade by enzymes. The circulatory half-life of naked siRNA in the bloodstream is less than 5 minutes.[15-17] Besides that, the overall distribution of small molecule drugs to healthy and diseased tissue can be problematic, due to the fact that they are, in case of anticancer drugs, highly cytotoxic. Without any selectivity only a few percent of the drugs can reach the target site. High injection-doses and -rates result in increased side effects for the patient.[10] The same applies for the areas of biosensing and molecular imaging.[18-20]

To overcome these limitations and reduce the drug dosage and increase the selectivity, DDS faded into the spotlight. One of the objective in the application of DDS is the extension of the blood circulation and establish a “therapeutic window” with a continuous and targeted drug release for the patient. Inert poly(ethylene glycol) (PEG) and drugs or therapeutic biomolecules conjugated to PEG (PEGylation) are the most prominent polymers for this purpose. PEGylation of therapeutic agents results in a reduction of immunogenicity, an enhanced stability against nucleases (for the transport of genetic material) or enzymatic degradation and avoid a rapid renal clearance, due to the increased molecular weight and steric hindrance. Furthermore, the non-toxic, non-immunogenic and

non-antigenic polymer is approved by the food and drug administration (FDA). It's high solubility in water and many organic solvents simplifies the conjugation procedure.[21-24]

The DDS for therapeutic biomolecules like genetic material are often positively charged to complex and bind the negative charged cargo. The drawbacks of the positive charged carrier surfaces are the resulting interactions with blood proteins like albumin or other negative charged proteins.[25] These interactions cause an aggregation and a fast excretion of the DDS.[26] The young research field of the so called protein corona formation is increasing in the last couple of years and gives insides in the biological interactions of DDS in the blood stream.[27] With these observations novel DDS with "stealth" properties can be achieved. The gold standard, still is to PEGylate the DDS and by that decrease these kind of interactions, shield the charged carrier systems, and prolong the blood circulation time. [28-30] Another elegant way to acquire stealth effects was realized by the use of the molecular imprinting technique, to control the attachment of the components which forms the protein corona on DDS. Kataoka and co-workers developed nanocarriers which rapidly bind albumin exclusively in a controlled manner, when applied intravenously and use the generated albumin corona for stealth properties.[31]

In the next step, the DDS must be taken up by a (specific) cell. Hereby the enhanced permeability and retention effect (EPR effect) can cause in the passive enrichment of the DDS into the target tissue. Matsumura, Maeda,[32] and Jain[33] observed the size-mediated tumor accumulation mechanism of nanoparticles in the 1980s. The nanoparticles can passively be enriched in the tumor tissue because their endothelial cell layer in the capillaries is fenestrated and porous. In contrast to the tumor tissue, only small molecules can pass through the endothelial cells of healthy tissue. Besides the EPR effect, a reduced release of nanoparticles (bigger than 50 kDa) and a simultaneous increased release of small molecules (smaller than 40 kDa) could be detected in carcinoma. These effect can be explained by the lack of lymphatic drainage and a disturbed lymphatic system.[34]

To date, the EPR effect is the most important concept for DDS mediated anticancer therapy. However, the effect is very heterogeneous in humans and differs from those of tumor-bearing murine models. In this case, the field of nanomedicine should investigate more into clinical important tumor models, and tumors which present a very strong EPR effect (kaposi sarcoma, and head and neck tumors).[35, 36] However, also poorly permeable tumors like pancreatic tumors were penetrated by 30 nm DDS and achieved an antitumor effect, whereas bigger DDS did not penetrate these type of tumor tissue.[37]

Besides the passive targeting via the EPR effect, also endogenous stimuli can be used to enhance the uptake of drugs or therapeutic biomolecules and overcome the limitations of the EPR effect. Solid tumors for example have a high rate of glycolysis and by that an extracellular pH value below 7.0. Furthermore, tumors present hypoxic areas and have an increased amount of reactive oxygen species (ROS). Enzymes like glycosidase, lipase or phospholipase are also overexpressed in tumors. These distinctions can be used to develop stimuli responsive DDS for an enhanced efficiency and a triggered release of the cargo (will be discussed in more detail in chapter 1.4).[35]

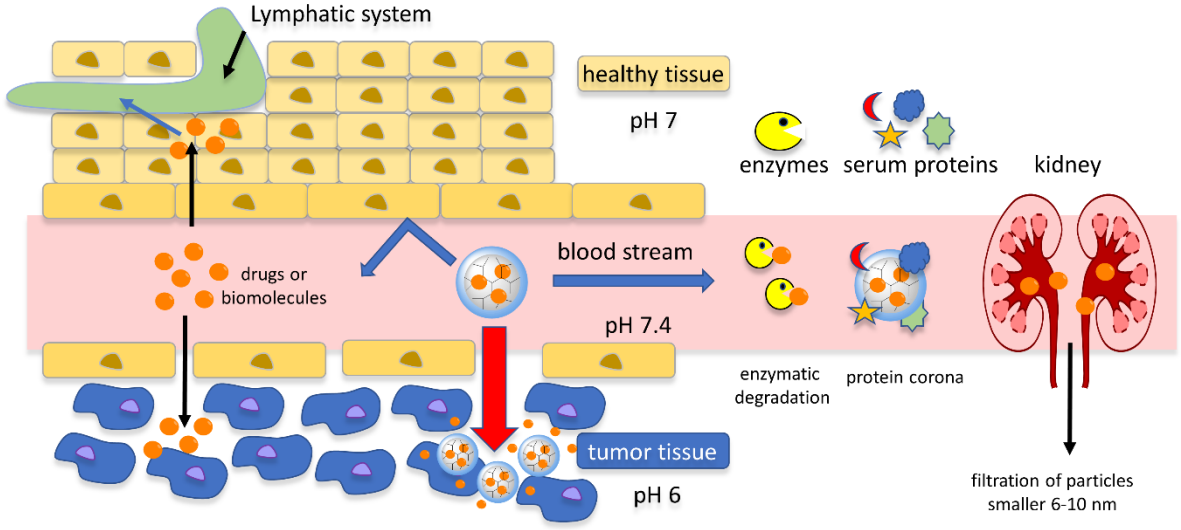

Figure 2. External barriers. Adapted from literature Haag et al.[10] and Kim et al. [36]

Besides the passive enrichment in the target tissue, there is also an active targeting for the DDS. Since synthetic DDS do not have any cell-specific targeting function for the accumulation and release, they must be introduced via specific ligands, which can be conjugated to the carrier. Here, the length of the spacer between the DDS and the ligand, as well as the number of targeting units play together with many other factors an important role.[38, 39]

After overcoming the extracellular hurdles, the DDS has to pass through the cell membrane into the cell interior. Here, the DDS has to escape from lysosomal degradation and enter the cytosol from the acidic endosomal vesicles (clathrin-mediated endocytosis). These vesicles reach pH values of 4.5 to 6 and are loaded with several degradation enzymes. Depending on the type of cargo, the DDS has to reach different destinations to achieve a therapeutic effect (see Figure 3).[40]

The chemotherapeutic agent's doxorubicin (DOX) and cisplatin are the most widely used anticancer drugs. After the release of these drugs from the DDS, they have to migrate

through the cytoplasm into the cell nucleus. Here the drug prevents or disturbs the cell division by induced DNA damage (DNA binding or crosslinking) or causes cell death due to the production of toxic free radicals.[41-43] On the other hand photosensitizers also can be used as nontoxic drugs which were activated by light. The cell death through necrosis or apoptosis is caused by a photochemical reaction cascades which produces a toxic reactive oxygen species. This process is called photodynamic therapy (PDT).[44, 45]

Besides anticancer drugs, also therapeutic biomolecules e.g. DNA or siRNA can be transported by DDS. The introduction of genes into cells of the human body to treat congenital or acquired diseases is called gene therapy. The development of this field of research started with the encoding of the DNA structure by Watson and Crick in 1953.[46] The next step stone was achieved by Fire and Mello, which discovered the mechanism for the targeted disruption of genes, the RNA interference (RNAi).[47] Tuschl and co-workers synthesized the first small interfering RNA (siRNA) which triggered a specific RNAi in mammalian cells.[48] The introduction of DDS loaded with genetic material into cell and their intracellular processing is called transfection. The decoding of the human genome in 2003 and the assignment of genes which are responsible for various diseases opened new possibilities for the gene therapy.[49]

Genetic material can be transported via viral and non-viral vectors. For the non-viral vectors, mainly cationic lipids or polymers were used. Their multiple positive charged groups can complex the negative charged phosphate backbone of the genetic material and transport thus in form of lipoplexes or polyplexes. For the complexation of genetic material with polycations helps the effect of multivalency. Multi- or polyvalent interactions are the simultaneous binding of multiple ligands (polycations) to a molecule or surface with multiple receptors (phosphate anions). These multivalent interactions are in general much stronger than those of monovalent correlations, but also reversible in biological systems.[50, 51] Furthermore, the size, charge, hydrophobicity, and the buffer capacity of the lipo- or polyplex play an important role for the successful transportation of the genetic material. Lipo- or polyplexes possess an increased transfection due to their proton sponge effect.[52]. This effect helps the DDS to get released from the endosomal vesicles and reach the cytosol (endosomal escape). Here, polycations induce an enormous number of protons when the pH level is dropped inside the vesicles. Due to their protonation, chloride ions and water molecules flow into the endosomal vesicles. These cause an increase of the osmotic pressure and a final burst of the vesicles. Thereby the lipo- or polyplex is getting released into the cytoplasm.[53] Besides the proton sponge effect, the protonated complex can also

interact directly with the endosomal membrane and destabilize it to get from the vesicle into the cytoplasm.[54]

After the “endosomal escape”, the genetic material has to reach the target destination. For the DNA, it is essential to migrate from the DDS to the cell nucleus. Inside the nucleus, the transcription into mRNA occurs which triggers the protein expression in the cytoplasm. The infiltrated DNA should alter the expression of existing genes, produce cytotoxic proteins, or generate prodrug activating enzymes by the enrichment of natural proteins.[55-57]

When siRNA (double-stranded RNA molecules with 21-23 base pairs) is used as cargo, it is sufficient to transport these into the cytoplasm. Here, the siRNA and protein units (produced by the target cell) form a RNA-induced silencing complex (RISC). The passenger strand of the siRNA is degraded and removed, whereas the guide strand binds to the complementary messenger RNA (mRNA) and cause its degradation by RISC. The important fact here is that the sequence of the siRNA can be designed to solve gene over-expressions and dysregulations, which are involved in human diseases like cancer, viral infections, or hereditary diseases.[58-60]

Besides the pH shift inside the cells, the differences of the glutathione and ATP level in the cytoplasm can also be used to design responsive DDS and realize a controlled release inside the target area (will be discussed in more detail in chapter 1.4).

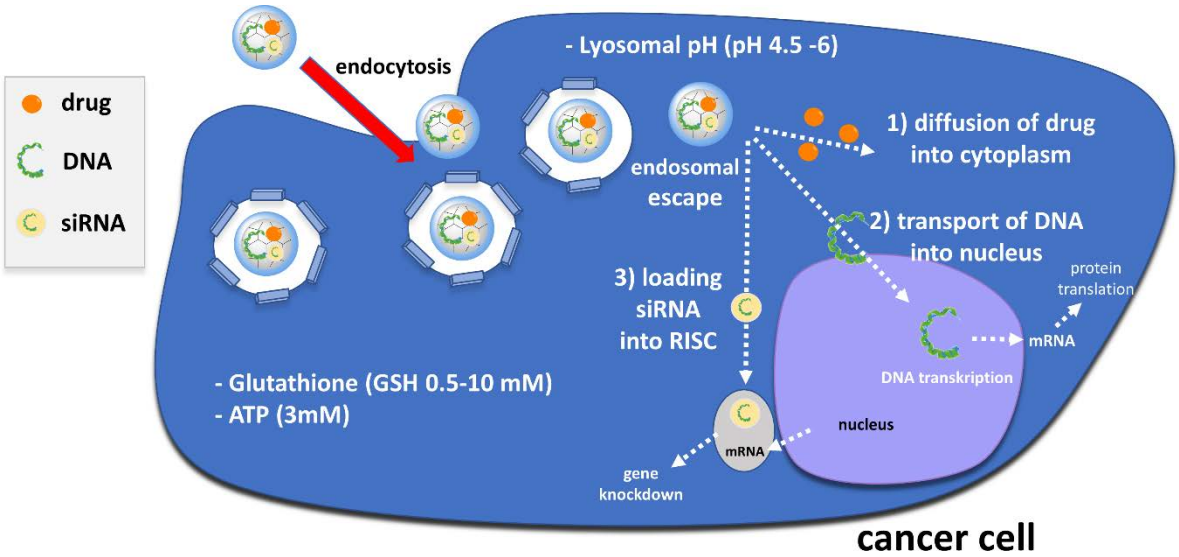

Figure 3. Internal Barriers. Adapted from literature Haag et al. [10] and Kim et al. [36]

An alternative to the intravascular application is the noninvasive topical administration of DDS, either dermal, inhalative or through mucous membranes of the human body. The topical application of drugs or therapeutic biomolecules for a targeted treatment of skin

diseases is called dermal and transdermal drug delivery. The skin is the outermost layer of the human body and its biggest organ. The principle task of the skin is the provision of a barrier, to protect the body from excessive water loss and prevent the entry of toxic agents, viruses, bacteria, dust, allergens, microbes, or other environmental threats into the organism. Most of them do not penetrate the human skin, as far as the barrier is not disrupted. The composition of the skin is shown in Figure 4. The uppermost skin layer is the stratum corneum (SC, 10-20 µm thickness). It is the most important layer for the barrier function and consists of terminally differentiated corneocytes embedded in a complex lipid matrix. The order of the SC is also known as "brick-and-mortal" pattern and prevent the absorption of particles bigger than 500 Da. The epidermis (50-100 µm thickness) consists of stratified keratinocytes which are also embedded in an extracellular lipid matrix. The dermis (1-2 mm) consists of various cell types and is a largely fibrous layer which provides the vasculature of the skin and its mechanical support. Furthermore, the hair follicle and sweat glands are anchored in this area.[61-63]

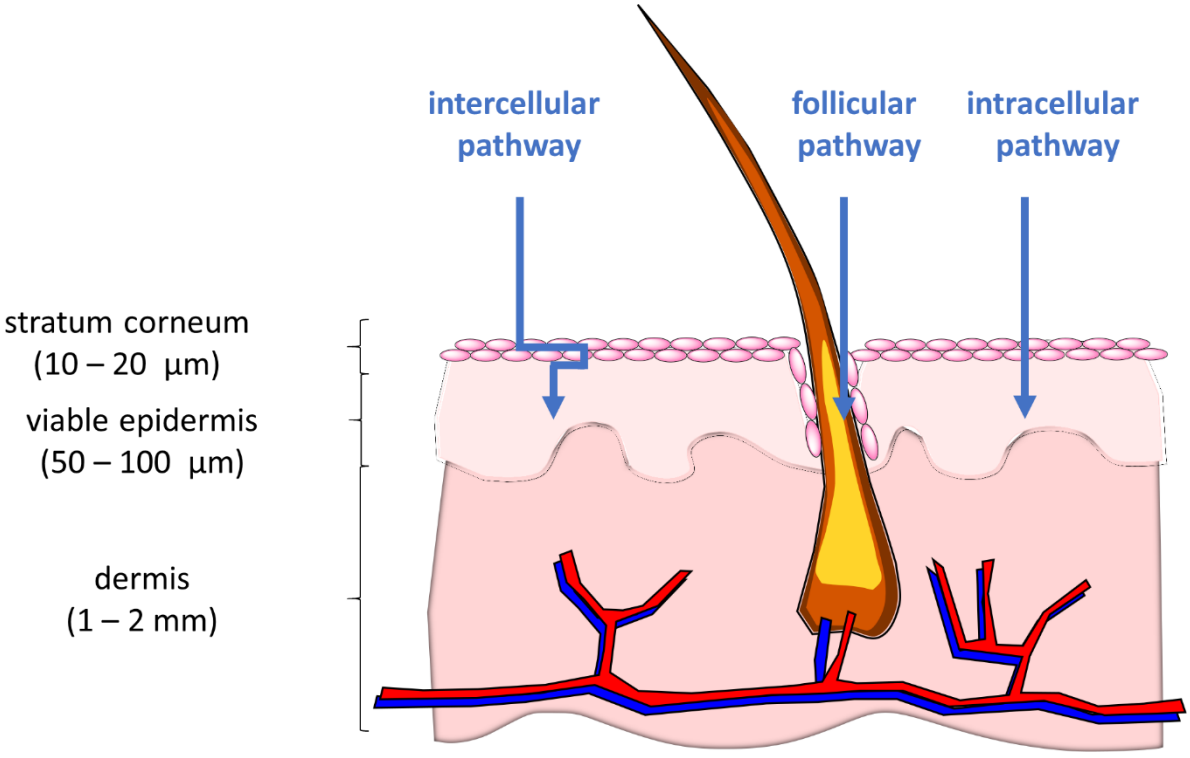

Figure 4. Skin barriers. Adapted from literature Prausnitz et al. [63] and Bolzinger et al. [65]

The three main penetration routes for drugs and DDS through the stratum corneum are the intercellular, the intracellular and the follicular pathway. Drugs or smaller DDS are suitable for the inter/intra- cellular route. It is known that hydrophobic drugs penetrate through the intercellular lipids of the SC (intercellular pathway), whereas hydrophilic drugs prefer to pass the SC through the corneocytes (intracellular pathway). Bigger DDS, above 100 nm

are mostly considered for the follicular pathway. However, inflammatory skin disease like atopic dermatitis, acne, and psoriasis can make the SC more permeable, also for DDS.[64-66]

Still larger DDS provides and great potential to overcome the SC barrier via the follicular route. Lademann and co-workers described a size-dependent uptake of particles in the size range of 100-1000 nm. Here, medium sized nanoparticles (500-700 nm) penetrated the hair follicle up to 1 mm due to the surface structure of the hair and the hair follicle.[67] The movement of the hair may serve as a pumping mechanism where the nanoparticles with an equal size to the keratin cells thickness on the hair surface were pumped into deeper layers.[68, 69] However, even if the particles of this size penetrate into the hair follicle, they will not pass the follicular barrier. Even though, these DDS could act as a drug reservoir and release their therapeutic cargo due to internal or external triggers. The cargo then could penetrate through the barrier and be internalized. The possible triggers and their consequences for the design of effective DDS will be discussed in more detail in chapter 1.4.

The treatment of inflammatory skin diseases is still challenging and requires tailor-made DDS for an effective transport and release of therapeutic pharmaceuticals. However, several strategies for the application of DDS in the field of dermal and transdermal drug delivery were already developed. Many different carrier systems were used to transport mainly hydrophobic anti-inflammatory drugs but also therapeutic genetic material.[70] Besides inflammatory skin diseases also skin cancer can be treated with photodynamic therapy. The local and noninvasive dermal delivery of photosensitizers into tumor tissues by DDS promises a great potential in this research field.[71, 72]

The entire chapter illustrates the complexity of the requirements for an effective drug delivery system to overcome the different barriers and release the therapeutic cargo (drugs or biomolecules) on the target site. Nevertheless, there are many examples for effective DDS in research, clinical studies, or already approved systems for the market. The following chapters will describe the development and design of polymer-based DDS in the nanometer range more in detail.

1.3 Polymer based DDS and the Scaffold Structures

Various Types of nanomedicine compounds were developed to realize the transport and release of pharmaceuticals including drugs or therapeutic molecules. The focus of this chapter describes the diverse polymer-based DDS like linear and dendritic polymers, micelles and nanogels as well as nonviral-vectors for the complexation of genetic material (polymers and polyplexes) and their preparation. Figure 5 illustrates the diversity of the carrier platforms for nanomedical use.[73, 74]

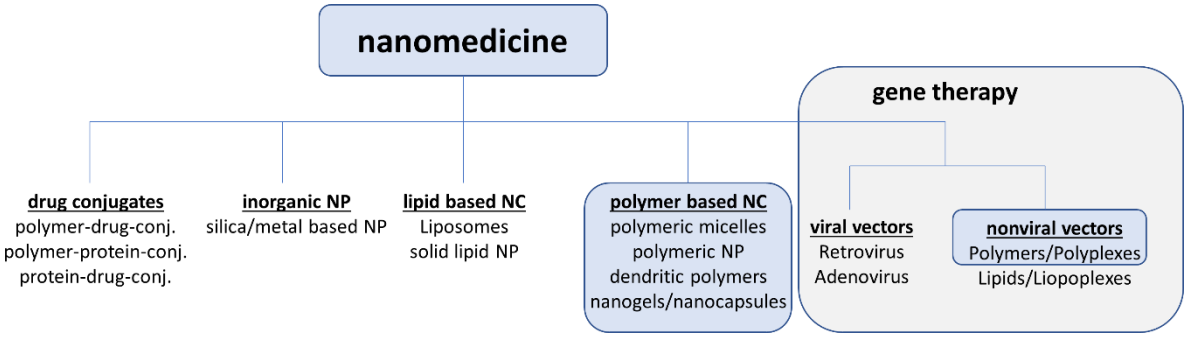

Figure 5. Schematic illustration of nanomedicine platforms. Conj.: Conjugates; NP: nanoparticles; NC: nanocarrier.

First of all, a toolbox of building blocks for the development of polymer-based carriers is needed. For this purpose, natural and synthetic polymers (linear and dendritic) can be used. Natural polymers such as chitosan, dextran, heparin or hyaluronic acid have already been used for DDS applications but will not be explained in more detail. The advantage of synthetic polymers over natural ones are the simple modification or functionalization, the fine-tuning of the mechanical properties and particle sizes, and the control over the degradation rates. However, factors like biocompatibility and “stealth properties” have to be required attributes for the synthetic carrier systems. [30, 75]

As already described in chapter 1.2, PEG is the most prominent synthetic polymer for the delivery of pharmaceuticals. The uncharged hydrophilic polymer is synthesized by an anionic ring opening polymerization of ethylene oxide. The resulting biocompatible linear polymer can be easily functionalized by its terminal hydroxy groups. An immense variety of functional groups e.g. -aldehydes, -amines, -alkynes, -azides, -thiols or -malemides were established.[76] However, PEG has only two or one (in case of mPEG) functional groups, which restricts the number of coupling reagents and hamper its applications.

Furthermore, studies reveal that PEG can induce immunogenic and antigenic body reactions and is non-biodegradable.[30, 77, 78] Therefore, new biocompatible polymers have been developed to overcome these drawbacks.

Synthetic poly(amino acid)s were investigated as alternatives to PEG. The most common hydrophobic polymers are poly(glutamic acid) (PGlu), poly(lysine) (PLys) and poly(aspartic acid) (PAsp). They can degrade *in vivo* into their corresponding amino acids or to oligomers with 4 to 9 repeating units by using different enzymes. These fragments can be metabolized by physiological pathways afterwards. The biodegradability of the poly(amino acid)s is a superior advantage when compared to PEG.[79-81] Poly(amino acid)-modified surfaces of nanoparticles showed a prolonged blood circulation similar to PEG. The combination of the hydrophobic poly(amino acid)s with hydrophilic PEG can form diblock polymers which combine the beneficial properties of both polymers. The resulting copolymers can be used as drug delivery system in form of polymeric micelles (will be discussed in more detail in a later paragraph of this chapter).

Furthermore, biodegradable aliphatic polyesters like poly(lactide) (PLA) and the related copolymer poly(lactide-co-glycolide) (PLGA), and poly(e-caprolactone) (PCL) have been studied extensively in the field of nanomedicine (mainly in form of copolymer with PEG and coatings) and are applied in medical products like surgical suture materials or biodegradable stents, but will not be explained in more detail.[82-84]

Another class of synthetic hydrophilic polymers are poly(vinyl)alcohols (PVA)s, which has been approved by the FDA. The polymer is commercial available and synthesized by radical polymerization of vinyl acetate, followed by partial hydrolysis of the acetate groups. The water-soluble and biocompatible polymer can be easily functionalized, due to the multiple hydroxy groups in the polymer backbone. These properties combined with its low toxicity make the polymer interesting for biomedical applications.[85, 86] However, incomplete hydrolysis of the acetate groups in the PVA backbone results in an inhomogeneous interior and can cause solubility problems. The low drug affinity and the uncontrollable release behavior may hamper its applications.

Similar to PEG and PVA, polyglycerol (PG) belongs to the group of biocompatible, non-cytotoxic and water-soluble polymers.[87] Dendritic polyglycerol (dPG) is traditionally prepared by an anionic ring-opening polymerization of glycidol (monomer). The deprotonated initiator 1,1,1-trimethylpropane (TMP) enables in combination with a slow monomer addition and a rapid cation exchange equilibrium a good control over the molecular weight, polydispersity index (PDI) as well as the multibranched structure.[88, 89]

Dendritic polymers were introduced in the early 1980s and named after the greek word ‘dendron’ which means tree. The dendritic polyglycerol, up to its name, possesses a tree-like architectural structure with multifunctional hydroxy groups inside the backbone and predominantly at the surface (see Figure 6).[90, 91] Due to that fact the dPG surface can be functionalized with a variety of active moieties and remains water soluble.[92] Further biocompatibility properties of dPG are high protein resistance[93] and good *in vivo* compatibility with high plasma half-life.[94] Various synthetic manufacturing processes of polyglycerol have been established in the last couple of years, which have an influence of the polymeric scaffold, its architecture and size. Brooks and coworkers developed a emulsion type polymerization of glycidol to extend the maximum of the molecular weight of the dPG up to 1 MDa.[95] Haag and co-workers developed a method for a cationic polymerization of glycidol by citric acid at ambient and solvent free conditions. This green chemistry approach resulted in degradable polyglycerol units with a molecular weight of 1-2 kDa.[96] Furthermore, linear polyglycerol can be synthesized for example by using acetal-protected glycidol derivate. The “protected glycidol” monomer ethoxyethyl glycidyl ether (EEGE) was polymerized and the protective groups were removed by acidic hydrolysis. The resulting linear polyglycerol and its conjugates, similar to the dendritic one are useful for many biomedical applications.[93, 97]

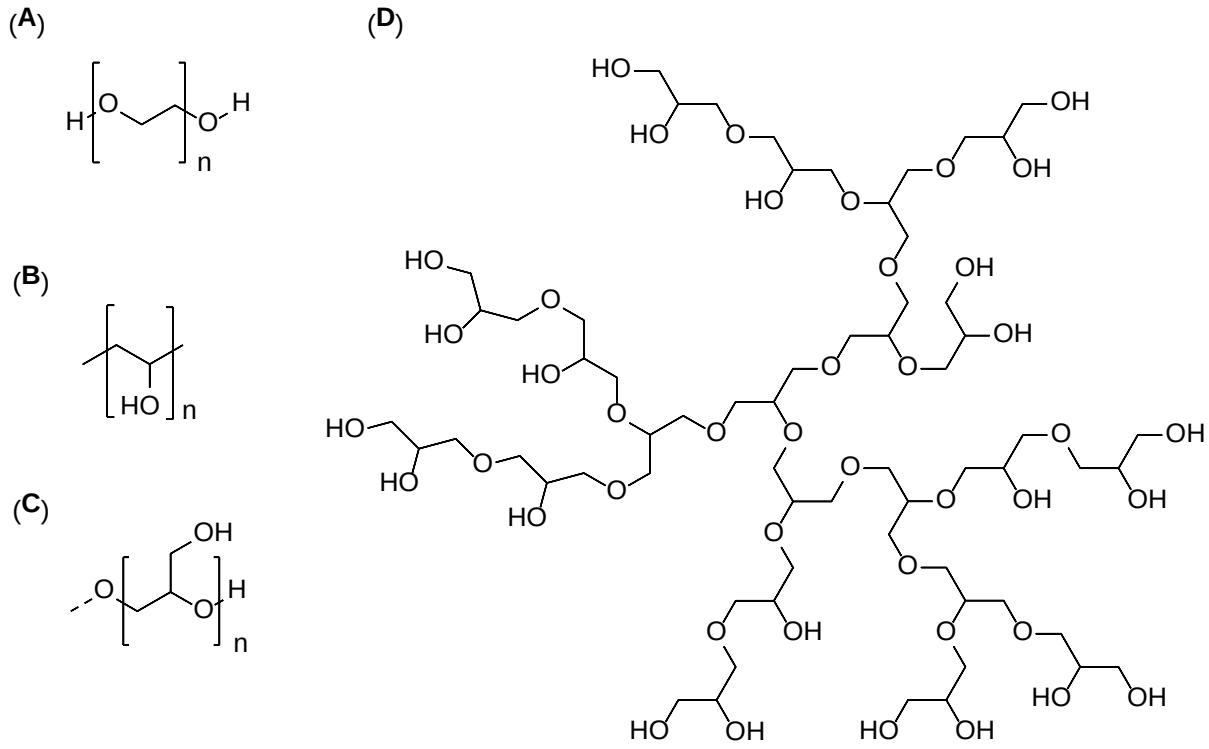

Figure 6. Chemical Structure of polyethylene glycol (PEG) (A), poly(vinyl alcohol) (PVA) (B), linear polyglycerol (lPG), and dendritic polyglycerol (dPG) (D).

Besides the building blocks of synthetic polymers mentioned above, cationic charged polymers were developed to build architectures for the delivery of genetic material. However, all of them try to mimic the most successful class of natural transporter for gene delivery: viruses. For the viral gene therapy, manipulated viruses and their ability to infect cells are used to transport and release genetic material with a high gene transfer efficiency. Here, the natural viral genome is replaced by therapeutic genes.[98] Although the viruses have been weakened in their pathogenic effects and their reproducibility, they still have an immunogenic and toxic potential. Furthermore, viruses have a limited targeting functionality for specific cell types. The low capacity for genetic material and their limited production quantity hamper their *in vivo* applications.[99] The most frequent used virus types are adeno- and retroviruses.[100]

Non-viral vectors have a lower transfection efficiency compared to viral vectors. However, their simplified production and handling offers lower risk to humans compared to viral carrier systems.[101] Non-viral vectors are represented by a variety of cationic lipids or polymers, which complex the anionic charged phosphate backbone of the genetic material and transport thus in form of lipoplexes or polyplexes.[102]

Mayhew and Juliano discovered in 1973 that the polyplex formation of the biodegradable poly(amino acid) poly-*L*-lysine (PLys) and RNA leads to an enhanced accumulation of genetic material in cancer cells.[103] The primary amines of the PLys (see Figure 7A) are positively charged at physiological pH values and can complex the charged nucleic acids. However, PLys shows very low gene transfer efficiency, most probably due to the low buffer capacity and the deficiency of secondary or tertiary amino groups. The latter ones have pka values between physiological and lysosomal pH, which can improve an endosomal escape due to the proton sponge effect and increase the gene transfer efficiency. Nevertheless, PLys- transferrin-conjugates were one of the first non-viral vectors for targeted gene delivery.[104]

Moreover polyamidoamine (PAMAM) dendrimers, which are globular, cascade-like polymers which consist of an alkyl-diamine core, tertiary amine branching points and primary amines at the surface, can be used for gene delivery applications (see Figure 7B). The size and surface charged of the PAMAM can be altered by the number of the dendron generation.[105] Haensler and Szoka discovered the potential of the "starburst dendrimer" for gene delivery and identified a connection between dendrimer generation and gene transfer efficiency. Thus, dendrimers of generation 5 showed a significant higher transfection efficiency than dendrimers of generation 3. Here, a coherence between the diameter and shape (generation 3: star shaped, generation 5: globular structure) of the dendrimer and gene transfer efficiency was discovered.[106] On the other hand, PAMAM dendrimers offer a large number of secondary and tertiary amino groups, which support the proton sponge effect and promote endosomal escape, thus increasing gene transfer efficiency compared to PLys.

Polyethylenimine (PEI) (see Figure 7C) and its potential for gene delivery was discovered in 1995. PEI is one of the most effective non-viral gene vector. Since every third atom in the polymer is a proton acceptor, it is an ideal proton sponge. With a large number of primary (terminal), secondary (linear) and tertiary (dendritic) amines, PEI has excellent gene transport efficiency besides a high density of amino groups.[107] PEI is commercially available in different molecular weights and in branched and linear constitution.

The dendritic polyglycerol described above can also be modified by a three-step synthesis (mesylation, azidation, and staudinger reduction) to a dPG-amine.[108] Through functionalization a gene delivery carrier with the positive properties of the dPG can be produced. Various differentiations in polymer size,[109] functionalization grade[110] and architectures[111, 112] were tested in several *in vitro* and *in vivo* experiments.[113-116] In

addition to the functionalization to the dPG amine, other groups relevant for gene therapy such as spermine derivatives,[117] oligo-amines,[118-120] or amino acids[121, 122] were coupled to the multifunctional hydroxy groups of the dPG and results in effective carriers for gene delivery.

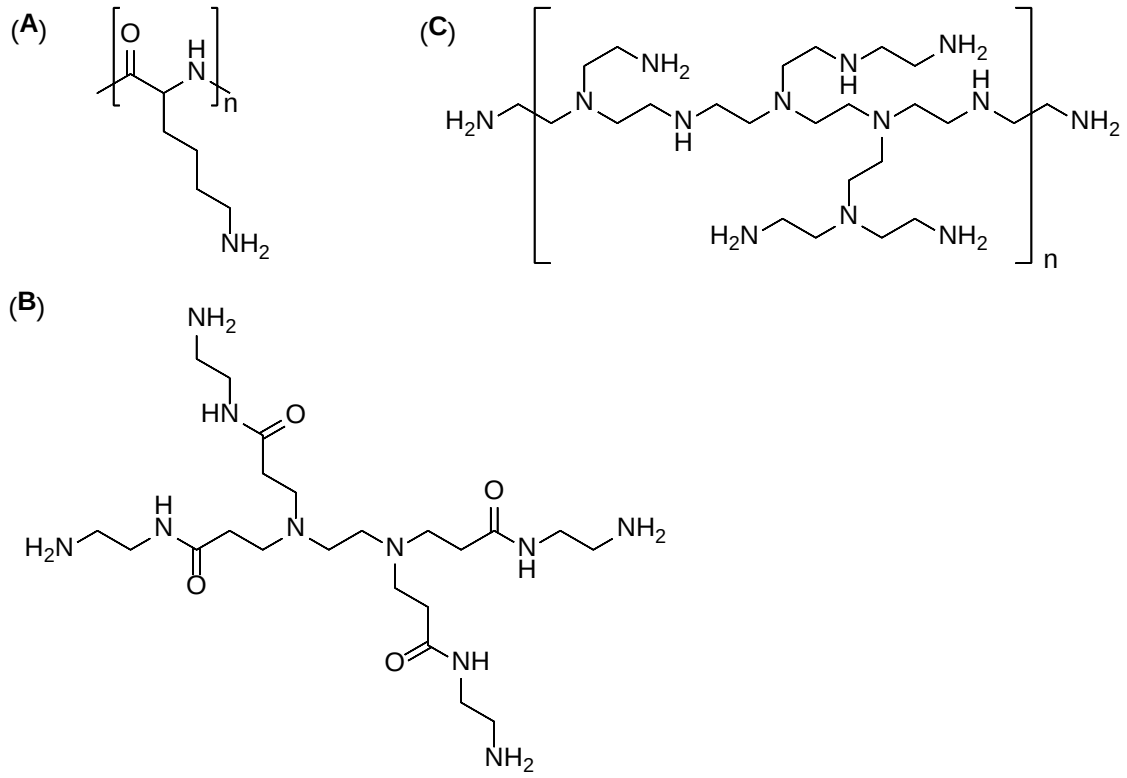

Figure 7. Chemical Structure of polylysine (PLys) (A), polyamidoamine (PAMAM) dendrimer generation 0 (B) and branched polyethylenimine (PEI) (C).

A variety of polymeric building blocks for the complexation and transport of drugs and genetic materials were described in the previous paragraphs. However, especially for gene therapy, highly positive charged cationic polymers are needed for an efficient delivery of genetic material (polyplexes). The drawbacks of these polymers are the associated cytotoxicity of the multiple amine groups which not only bind the genetic material, but also disturb the cellular compartments. To improve these limitations and further enhance the shielding and transport efficiency and increase the control over the release of the relevant therapeutic cargo, several types of nanosized DDS were developed. Besides linear and dendritic polymers, which can covalently bind to a drug (polymer-drug-conjugate) or complex genetic material (polyplex), more specialized carrier types like core shell nanoparticles, polymeric micelles and nanogels has been established (see Figure 8). The mentioned carrier types needed advanced production processes to fulfill the requirements for an efficient nanosized DDS. Especially for the delivery of therapeutic materials

(proteins or genetic material) mild reaction conditions and bioorthogonal chemistry is needed to bind or encapsulate and transport an intact cargo. Furthermore, the installation of stimuli-responsive moieties can increase the efficiency of a controlled cargo release by external and internal triggers. In the following chapter these complex carrier types and their production processes will be explained more in detail.

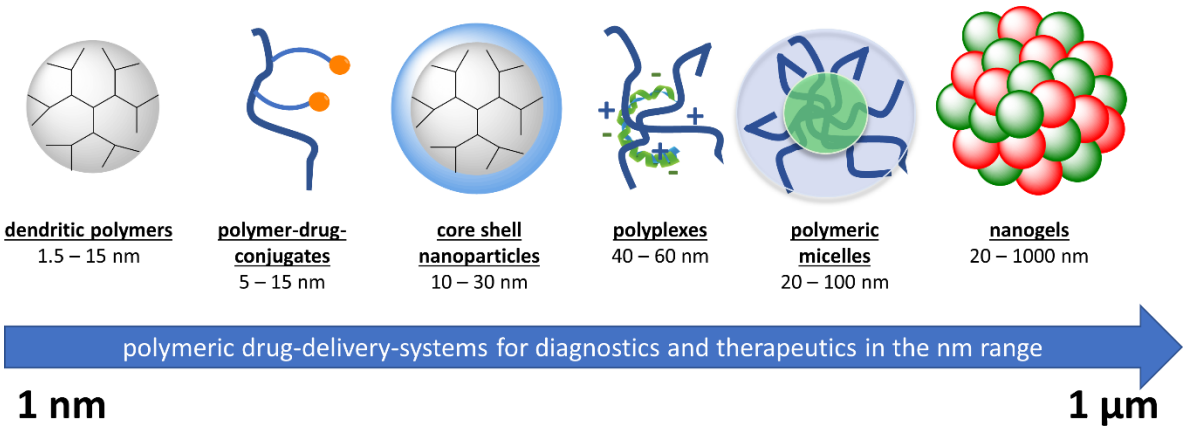

Figure 8. Types of polymeric DDS adapted from literature Khandare et al.[91]

A possibility to achieve functional DDS in the nanometer range is the synthesis of polymeric core-shell or core-multishell (CMS) particles (see Figure 8). Here, dendritic polymers can be modified with at least one covalent shell which results in stable core/shell architecture to encapsulate therapeutic drugs or dyes in a non-covalent manner. Among PAMAM dendrimers, branched PEI, dendritic polyglycerol and other dendritic scaffolds were successfully used to build such structures.[123, 124] The size of these DDS can be defined in the range of 10-30 nm. Furthermore, the encapsulation of the therapeutic drugs is driven by noncovalent interactions e.g. ionic, H-bonding, or van der Waals interactions, and not covalently (in case of polymer-drug-conjugates). By that, various drugs can be encapsulated and improve the applicability of these carriers.[125] For dendritic polymers with multifunctional groups in the backbone and at the surface it is possible to design carriers with hydrophilic or hydrophobic compartments inside the core or at the shell. The mainly toxic and hydrophobic therapeutic drugs are efficiently shielded by the encapsulation process.[126, 127] Stimuli responsive carrier systems enable a controlled release of the encapsulated cargo and will be described in chapter 1.4.

Core-multishell nanoparticles (CMS) are a further development of this carrier class. These CMS particles mimic natural liposomes, which are represented in cellular membranes and vesicles which are responsible for regulations and transportation in living

organisms. They consist of amphiphilic molecules (for example, phospholipids) which are formed by self-association into bilayers. Synthetic CMS nanocarriers have been synthesized by attaching mPEG-b-PLA block copolymers to a dendritic polyester (Boltorn)[128] or mPEG-b-PGlu to a branched PEI.[129] Furthermore, aliphatic building blocks of dicarboxylic acids were monofunctionalized with PEG. These "double shell" was covalently bound to a branched PEI and later to a biocompatible dendritic polyglycerol core (see Figure 9).

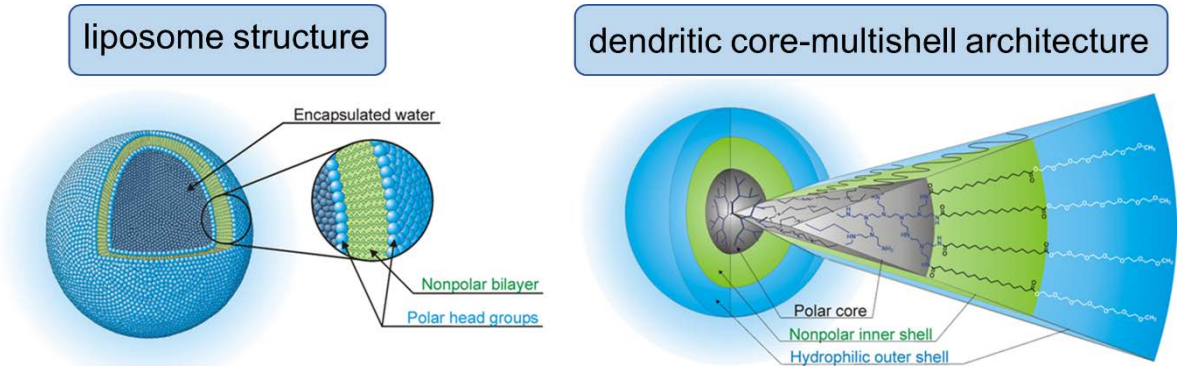

Figure 9. CMS architectures based on PEI cores, mimics natural liposome vesicles with aliphatic non-polar inner shell, and a hydrophilic core and outer shell. Figure adapted with permission of Quadir et al.[131] Copyright 2008 Elsevier.

With this architecture, it is possible to transport hydrophilic and hydrophobic drugs and dyes in aqueous media and organic solvents. Based on this universal transport behavior, they were described as "chemical chameleons".[130, 131] The CMS nanocarriers were modified regarding their building blocks (stimuli responsive and degradable cores[132, 133] and shells[134, 135]) and tested successfully *in vitro*[136-138] an *in vivo*[139, 140] regarding their skin penetration and transport properties of therapeutic drugs for skin diseases.

An alternative approach is the development of micellar architectures. They can be constructed by the supramolecular assembly of amphiphilic polymers which consists of at least one hydrophobic and one hydrophilic building block. First applications of polymeric micelles as DDS in the nanoscale came up in the late 1980s.[141] Numerous block copolymers have been synthesized to form self-assembled micelles and transport therapeutic drugs, contrast or imaging agents, proteins or genetic material.[142] The process of self-assembly occurs if the block copolymer concentration in the media reaches the critical micelle concentration (CMC), which is also called critical aggregation concentration (CAC) if polymers are used. By that, hydrophobic drugs can be encapsulated inside the hydrophobic core domain of the micelle, whereas charged genetic material, proteins or

peptides (of hydrophilic nature) can be entrapped inside the micellar compartments which present contrary charged blocks. These types are also called polyion micelles (PIC micelles).[143] Driving forces for the formation of polymeric micelles can either be hydrophobic or ionic interactions of the inner block. The biodegradable polyester PLA, PCL and PLGA, as well as the biodegradable poly(amino acid)s: PAsp, PLys, PGlu mentioned before are mainly used for the hydrophobic part. For the hydrophilic part, e.g., poly(ethylene oxide) (PEO), poly(N-isopropyl acrylamide) (PNIPAm) and the most common polymer PEG are used.[144-147] Recently, also degradable polymeric micelles with a dPG shell were successfully used for anticancer drug delivery.[148, 149]

Similar to the CMS nanocarriers, polymeric micelles allow the encapsulation of various therapeutic drugs and imaging agents but also hydrophilic biomolecules like proteins and genetic material. Their tunable parameters by the usage of various building blocks and their size in the nanometer range improves their delivery efficiency. However, most of the micelles were prepared and analyzed in aqueous solutions or buffers. *In vivo* applications in human blood serum can cause unwanted micellar destabilization and degradation.[150] The crosslinking of the micellar core or shell can help to avoid these limitations and further improve the stability of the micellar architecture. Here, a good average of the crosslinking density must be found to realize stability for the transport, but also lability for the cargo release. Stimuli-responsive cross linkers (will be described in chapter 1.4) can realize a functional micellar architecture for an effective delivery.[151]

Nevertheless, the amphiphilic copolymers which are required for the production of these micellar architecture are similar to surfactants and might interact or denature the encapsulated biomolecules. Furthermore, the control of the size, shape and elasticity for both, core-multishell nanoparticles and polymeric micelles is limited regarding their production procedures.

To overcome these limitations and improve the control of the nanomaterial properties, polymeric nanogels were developed by using various strategies. In general, “nanogels” define aqueous dispersions of hydrogel particles which consist of crosslinked polymer chains and form 3D networks in the nanoscale. Nanogels can encapsulate biological active molecules through hydrogen bonds or hydrophobic interactions. Furthermore, charged nanogels can incorporate and transport oppositely charged therapeutic biomolecules like proteins or genetic materials.[152, 153] For the nanogel preparation, reactive monomers or macromonomers are loaded into nanoreactors which consists of stabilized droplets in media

or cavities in tailor-made templates. Inside these reactors the monomers were crosslinked to the desired nanogels which present the same size and shape of the template.

The most commonly used method to prepare nanogels is mini- and micro-emulsion. In both cases nanoreactors consists of small, narrowly distributed and stable emulsion droplets. In case of mini-emulsion (see Figure 10), nanoreactors were obtained when two immiscible phases are administered to high share (e.g. by ultrasonication). The produced droplets are stabilized by amphiphilic surfactants and co-stabilizers. The surfactants are distributed in the continuous phase (phase 2), whereas the co-stabilizer, the reactive monomers and the therapeutic cargo are solubilized in the droplet phase (phase 1). The co-stabilizer builds up an osmotic pressure inside the droplets which counteracts to the Laplace pressure to avoid diffusion from small to big droplets and stabilize the nanoreactors. Finally, the monomers inside the droplet can react and crosslink to the desired nanogels. Different polymerization methods e.g. radical, anionic, cationic as well as (ring-opening) polyaddition and polycondensation reactions can be used for the mini-emulsion process and results in hydrophobic (e.g. Polystyrene, PCL, or PLA based) or hydrophilic (e.g. PEO, or dPG based) nanogels.[154-157] However, the required high energy input by ultrasonication can hamper the application for the encapsulation of sensitive biological compounds like proteins or genetic material.

For the micro-emulsion technique, the nanogel size can be fine-tuned by the concentration of the surfactant, cross linkable monomer and the stirring speed during the formation of the emulsion.[158, 159] Nevertheless, the high amount of the amphiphilic surfactants and related purification problems of nanogels restricts the biomedical applications.

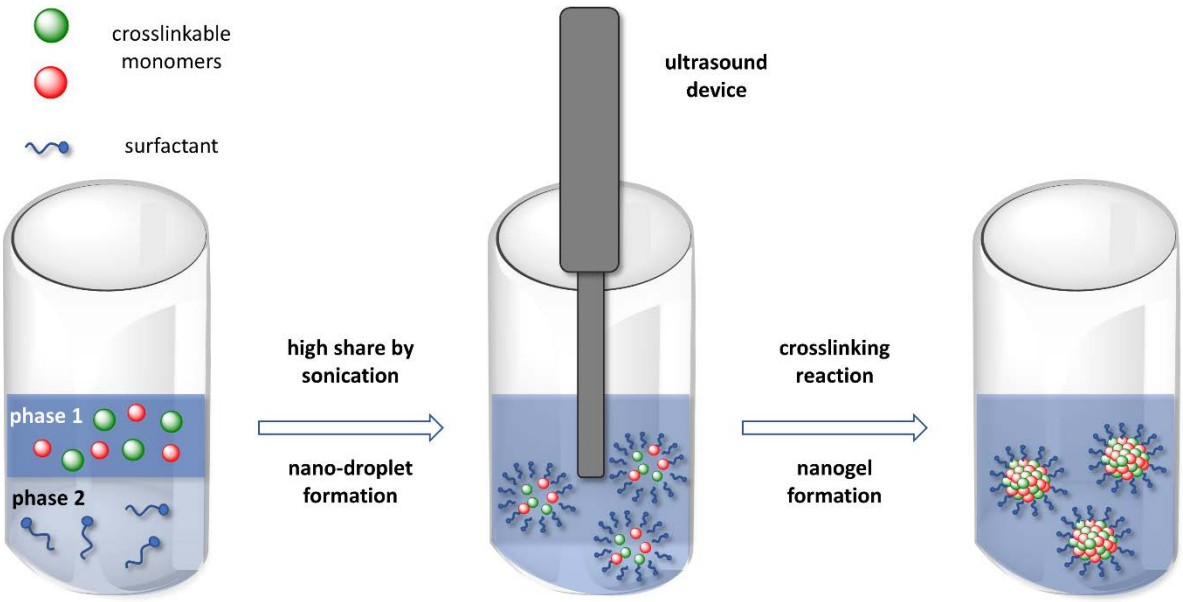

Figure 10. Nanogel preparation by mini-emulsion technique. Figure adapted from Landfester et al.[154]

Besides mini- and micro-emulsion, Whitesides and De Simone developed and improved a technique called soft lithography or PRINT® process which produces nanogels in tailor-made nanoreactors. By this moulding technique, monodispersed organic nanoparticles of nearly any structure (size, shape and composition) and function (cargo and surface) can be produced. In general, a silicon master-template with cavities of a certain size and shape is replicated to elastomeric replica molds using a photo-crosslinkable fluoropolymer. This mould is then filled with the desired liquid macromonomer (e.g. PLA or PEG-derivates) and crosslinked by an external trigger. The resulting nanogels can finally be removed from the mould and collected by a harvesting film.[160, 161] However, clean room conditions for the different moulds as well as chemical reactions which required external stimuli (e.g. UV-light triggered reactions) complicate biomedical applications.

To circumvent these limitations, the nanoprecipitation technique can be used to prepare nanogels under mild and surfactant free reaction conditions.

This technique was firstly applied, to produce hydrophobic nanoparticles consisting of polystyrene (PS), poly(methyl methacrylate) (PMMA) or PLGA. Here, the hydrophobic polymer is diluted into a polar organic solvent and precipitated in a large amount of non-solvent (mainly water). Both, solvent and non-solvent need to be miscible. The mixed solutions become a non-solvent for the hydrophobic polymers. By this process the macromonomers starts to form small aggregates (nucleation) which grow to bigger agglomerates until colloidal stability is achieved.[162] At adequate conditions, nanoparticles with a narrow polydispersity can be generated by this process.

The spontaneous emulsification process which forms an emulsion of small droplets is named by the Greek beverage: “Ouzo effect”. By mixing Ouzo with water, it becomes spontaneous milky due to the anethol (water-insoluble oil extracted from anis and ingredient of the Ouzo) which forms stable droplets which scatter the visible light.[163]

This nanoprecipitation method has been further developed to make it accessible for hydrophilic polymers and to use the desired nanogels for the transport and delivery of therapeutic biomolecules. The inverse nanoprecipitation method (see Figure 11) was firstly applied to produce dPG based nanogels by a copper-catalyzed azide–alkyne Huisgen cycloaddition (CuAAC). Here, hydrophilic and cross linkable dPG-conjugates were diluted in water (solvent) and precipitated into acetone (non-solvent). Due to the dilution process of the water into the acetone phase, small droplets were formed which act as nanoreactor for the active dPG-conjugates. Due to the mild and surfactant free preparation conditions, therapeutic proteins could be encapsulated into the nanogel matrix within this process and lead to defined nanogels of 100-800 nm in size.[164]

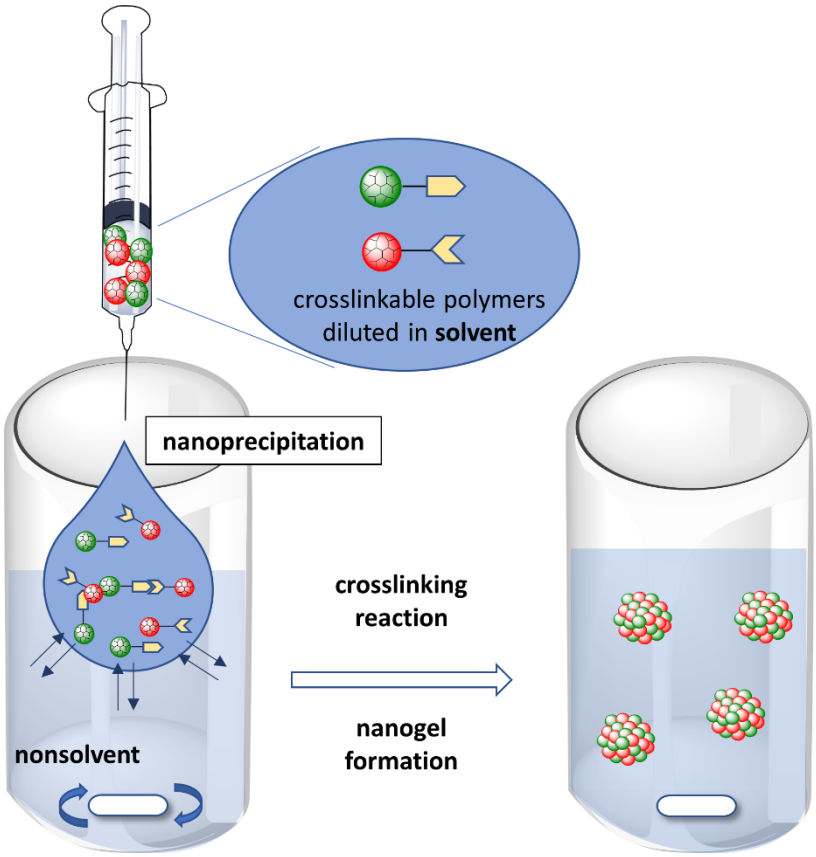

Figure 11. Nanogel preparation by the inverse nanoprecipitation technique. Figure adapted from Steinhilber et al.[164]

The described inverse nanoprecipitation process was further used for the preparation of nanogels with various polymeric building blocks and different crosslinking strategies.[165, 166] The mild and surfactant free process was used to successfully encapsulate and transport therapeutic proteins, genetic material, anti-inflammatory and anticancer drugs *in vitro* and *in vivo* (see Table 1). Ideal crosslinking reactions for the gelation of the nanogels should be free of any catalyst, bioorthogonal, show fast reaction kinetics, and react at ambient reaction conditions. The term bioorthogonal was defined by Bertozzi in 2003 and describes chemical reactions which do not react or interact with other groups of the biological cargo and environment. Besides the bioorthogonal CuAAC[167], the copper free strain-promoted azide-alkyne cycloaddition (SPAAC) was developed to ensure bioorthogonal reactions without the cytotoxic copper catalyst.[168] This reaction type was used to prepare responsive nanogels based on dendritic and thermo-responsive linear polyglycerol-conjugates to encapsulate the anti-inflammatory drugs dexamethasone and tacrolimus, or fluorescent model dyes for the dermal therapy.[169] Additionally, other non bioorthogonal crosslinking methods were used to encapsulate therapeutic biomolecules or anticancer drugs inside stimuli-responsive nanogels which will be explained more in detail in chapter 1.4.

Table 1. Selected covalent crosslinking reactions for the nanogel formation by inverse nanoprecipitation. Adapted from literature.[165]

Reaction	Reacting Groups	Polymeric Carrier	Encapsulated Cargo
CuAAC	azide and alkyne	dPG, PVA	therapeutic proteins,[164] DOX[170, 171]
SPAAC	azide and cyclooctyne	dPG, lPG	Dexamethasone, Tacrolimus, Dye[169, 172, 173]
Boronic–diol complexation	boronic acid and diols	dPG	therapeutic proteins,[174] MTX[175]
Thiol–disulfide exchange reaction	thiol and disulfide	dPG	DOX,[176] microRNA[120]
Photo crosslinking	acrylate	PVA	PTX[177]

Abbreviations: CuAAC, copper-catalyzed azide–alkyne Huisgen cycloaddition; SPAAC, Strain-promoted azide-alkyne cycloaddition; dPG, dendritic polyglycerol; PVA, poly(vinyl alcohol); lPG, linear polyglycerol; DOX, doxorubicin; MTX, Methotrexate; PTX, paclitaxel

Some of the carrier systems mentioned above were already approved in the market or being in clinical research. In 1990, Adagen® as first polymer-protein conjugate was approved by the FDA. The PEGylated protein increased the circulation lifetime and reduced the immunogenicity compared to protein alone. Besides polymer-therapeutics, also liposomal formulations and protein-drug conjugates of anticancer drugs were approved by the FDA since that time. Furthermore, polymeric micelles and non-viral polymeric vectors play an

important role in the admission of nanomedicine for drug and gene therapy in these days. Genexol® is a biodegradable micellar formulation of the anticancer drug paclitaxel and a poly(ethylene glycol)-poly(*D,L*-lactide) copolymer and approved since 2001. The CALAA-01 nanoparticle is a formulation of a cyclodextrin polymer and an adamantane–PEG conjugate which was developed by M. E. Davis. It was the first in human Phase I clinical trial for gene-based cancer therapy. However, trials have been terminated. The technology of micellar nanoparticles made of PEG-b-poly(amino acid) copolymers by K. Kataoka and T. Okano is further continued by the company NanoCarrier Co., Ltd. They are interested in approving these micellar platforms for anticancer drug and gene delivery application (Table 2).[36, 178, 179]

Table 2. Selected nanomedicine for cancer therapy or immune deficiency based on enzyme, drug or gene delivery. Listed carriers are approved or in clinical trials. Adapted from literature [36, 178, 179]

Nanocarrier Type	Name	Drug	Status	Ref.
Liposomes	Doxil®	DOX	approved 1995	[178]
	Myocet®	DOX	approved 2000	[178]
	Visudyne®	verteporfin	approved 2000	[178]
Polymer-Drug-Conj.	Opaxio®	PTX	approved 2012	[178]
	Zinostatin stimalamer®	neocarzinostatin	approved 1994	[178]
Polymer-Protein-Conj.	Adagen®	ADA	approved 1990	[178]
Protein-Drug-Conj.	Abraxane®	PTX	approved 2005	[178]
Polymeric Micelles	Genexol®	PTX	approved 2001	[178]
	NC-6004 Nanoplatin™	cis platin	phase 3	[179]
	NK-105	PTX	phase 2/3*	[179]
	NC-6300	epirubicin	phase 1	[179]
	PIC micelles	RNAi based	preclinical	[179]
Lipid NP	DCR-MYC	RNAi based	phase 1b/2	[36]
Polymer NP	CALAA-01	RNAi based	phase 1	[36]
Polymer implant	siG12D LODER	RNAi based	phase 2	[36]

Abbreviations: DOX, doxorubicine; PTX, paclitaxel; Conj., conjuagtes; ADA, adenosine-deaminase; NP, nanoparticle; Ref., reference; phase 2/3*, phase 2 for stomach cancer, phase 3 for breast cancer

To further improve the efficiency of polymeric nanocarriers types, “smart” DDS were developed to enable a controlled and triggered release of the therapeutic cargo by internal or external stimuli. In the following chapter, selected carriers will be described which make use of the natural or disease caused environment.

1.4 Natural Triggers and Examples for Stimuli-Responsive Carriers for Drug and Gene Delivery

The different administration routes of DDS which are described in chapter 1.2 and the associated barriers that must be overcome enables a variety of modifications inside the architecture of the nanocarriers which implement a control over the release of the therapeutic cargo. To release the cargo in a controlled way, the DDS can be modified with different groups that are sensitive to natural (internal) and external triggers (see Figure 12).

The uptake of DDS into the cell could occurs via endocytosis which results in a pH drop, starting from pH 7.2-7.4 in the extracellular matrix, to pH 5-6.5 inside the endosome and to pH 4.5 inside the lysosomal compartments. This pH drop provides one possibility to develop responsive DDS. Moreover, phosphatase, protease, and esterase are enzymes which occur in the intracellular matrix and can be used to degrade tailor-made cleavage points inside the polymeric architecture of the DDS.[180] Furthermore, higher concentrations of the tripeptide glutathione (GSH) and adenosine triphosphate (ATP) inside the cytosol provides a possibility for the development of sensitive carrier systems.

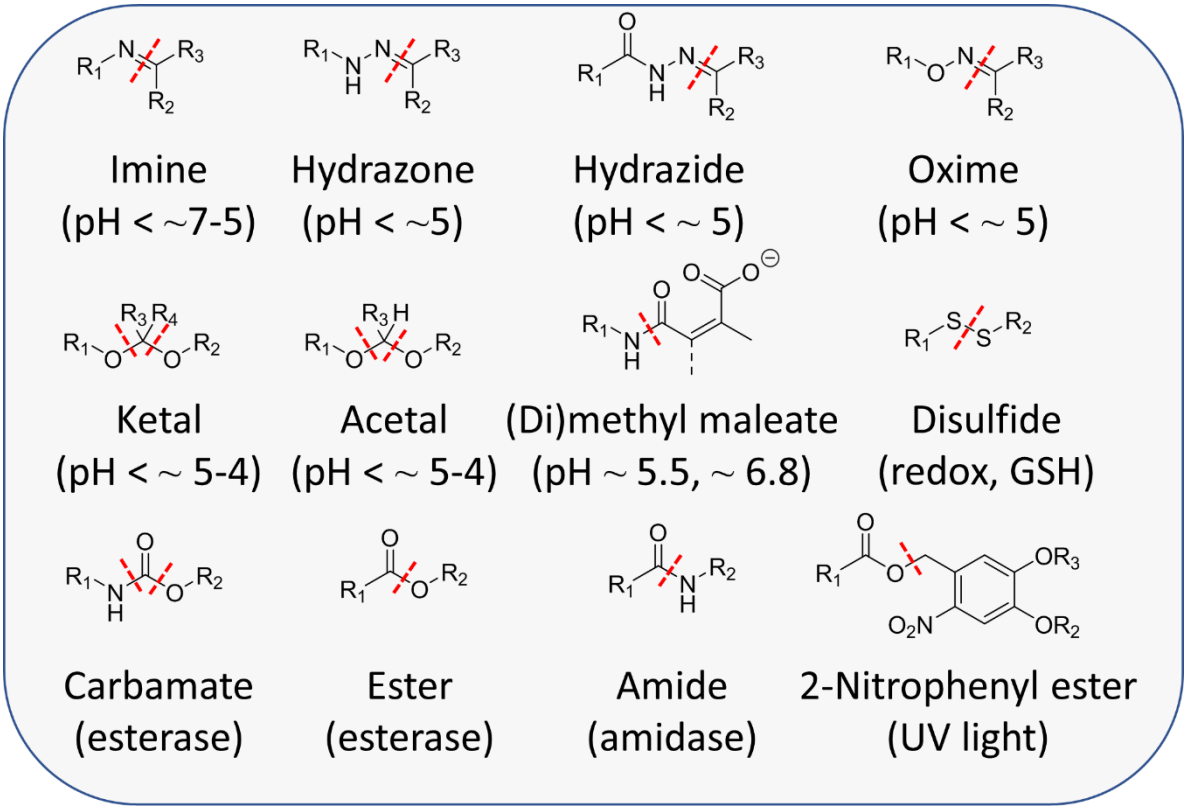

Figure 12. Cleavable linkers used for stimuli-responsive DDS. The dashed red line shows the bond that is broken upon activation by the corresponding stimulus which is given in parentheses. Figure adapted from Fleige et al.[7]

PH sensitive linkers to conjugate drugs or to incorporate them into the polymeric core (core-cleavable) or shell (shell cleavable) can be used to prepare pH responsive nanocarriers. Furthermore, pH sensitive crosslinkers or cleavage points can be used to degrade more complex architectures like polymeric micelles or nanogels in a controlled way and release the encapsulated therapeutic cargo at the target site. Functional groups, such as acetal, ketal, imine, hydrazone and hydrazide can be cleaved by a natural pH change.[7]

Haag and coworkers developed a polymer-drug-conjugated based on dendritic polyglycerol. Here, the anticancer drug doxorubicin was conjugated to the dPG via a pH sensitive hydrazone linker. The release of the anticancer drug was observed at pH values of 4 and 5, whereas almost no doxorubicin was released at pH values of 6 and 7. *In vivo* studies showed a complete tumor remission of 30 days without any side effects regarding weight loss of the tumor bearing mice.[181] Harada and co-workers used a similar approach and coupled a doxorubicin conjugate via a hydrazone linker to the PAMAM scaffold. They observed a seven-times higher cytotoxicity of the pH sensitive carrier compared to a stable amide linked polymer-drug-conjugate. These and other studies emphasize the importance of pH-sensitive linkage groups in polymer-drug conjugates for an effective transport and release.[182]

An acid degradable hydrazone linker was also used to conjugate anticancer drugs to the hydrophobic block of amphiphilic polymers. Kataoka and co-workers used this to form intelligent polymeric micelles. Block copolymers of PEG and poly(amino acid)s or PEG and PLA were used to transport and release anticancer drugs which were conjugated to sub-100 nm micelles. For all systems, an enhanced cytotoxicity against tumor-cells was measured.[183-185] Besides that, the advanced micellar architecture provides further possibilities for a pH responsive cargo release.

Lee et al. conjugated poly(histidine) (PHis) to polymeric micelles, consisting of PEG and PLA. By protonation of the PHis unit at pH values between 6.5 and 7 (extracellular tumor pH), the targeting ligand is exposed from the inner hydrophobic compartment to the surface. After successful cell uptake, the stimuli responsive micelle is further destabilized which leads to disassembling and releasing of the encapsulated doxorubicin.[186, 187]

Moreover, Kataoka and co-workers developed polyion complex (PIC) micelles with charge conversion properties by incorporation of an anionic citraconic amide side chain of the PEG-PAsp based micelle. The pH sensitive side chain selectively degrades at pH 5.5

(endosomal pH) which shifts the overall charge to a cationic compartment. The abrupt charge-conversion makes the PIC micelles promptly release the encapsulated cationic protein in response to the endosomal pH.[188]

A similar approach for charge-conversional nanogels was established by Wang and co-workers. The so called “positive (or negative) chameleon” exhibit a negative surface charge at physiological conditions which offers a decreased protein absorption. Slightly acidic conditions of pH 6.5 (extracellular tumor pH) result in a charge conversion to a positively charged surface which results in an enhanced cell uptake and increased cytotoxicity of the doxorubicin loaded nanogel.[189]

Another approach is the incorporation of pH sensitive cross-linkers which can degrade the complex architecture of polymeric nanogels or micelles and release the encapsulated cargo at the target site. Micellar architectures were crosslinked with pH sensitive ketal,[190] imine[191] or boronate ester.[192] These groups were either incorporated in the core, shell or in the core/shell interface of the micelle and reduce the premature cargo release under physiological conditions. However, after reaching the acidic environment around the tumor tissue or inside the target cell, the sensitive cross-linkers were cleaved and facilitate the cargo release. These pH sensitive cross-linked polymeric micelles are promising for enhanced delivery efficiency of many hydrophobic anticancer drugs. Furthermore, disulfides containing crosslinkers were used in an analogous approach to use the higher intracellular glutathione concentration for a disulfide exchange reaction and destabilize the micellar compartment in a similar manner to the pH sensitive micelles.[151]

In addition, stimuli responsive nanogels with acetal,[164, 177, 193, 194] ketal,[195] ester,[196] phosphate ester,[197] or disulfide[171, 176, 198, 199] containing crosslinkers were used to release their encapsulated cargo when the nanogel network is degraded by pH or reductive environment. Recent studies of B. Liu et al. analyzed the substituent effect for a library of pH sensitive acetals and ketals and their correlation to the polymeric nanogels and their encapsulation stability. The structural linker variations allow a high impact in the degradation kinetics and can result in differences of more than 6 orders of magnitude.[200]

However, especially highly cytotoxic cationic polymers which transport genetic materials need degradable alternatives which can reduce the toxicity and improve their application for *in vivo* experiments. Furthermore, targeted degradation of cationic DDS in the endosomal compartment can generate small molecules which increase osmotic pressure in vesicles and promote endosomal escape. Proton acceptors further introduce a carrier

swelling due to protonation and increase the release into the cytosol which improves the transfection efficiency.[201]

First approaches to solve this problem were dealing with the development of cleavable carriers which mimic the polymeric gold standard PEI. The branched PEI with a molecular weight of 25 kDa presents a high transfection efficacy but also an immense cytotoxicity. Wagner and co-workers synthesized pH cleavable cationic polymers based on oligoethyleneimine units (OEI) with a molecular weight of 800 Da which were connected via an acetal unit. The resulting polymer is stable in the extracellular matrix and provide a good binding ability for genetic material but split into small and biodegradable oligomers at the endosomal compartments. *In vivo* studies showed a good transfection efficiency and a higher biocompatibility of the degradable systems compared to the nondegradable controls.[202] Similar constructs of low molecular weight PEI connected via an degradable linker e.g. ortho ester, disulfides, ketals and carbamates were successfully tested.[203, 204]

Besides that, a polyspermine, was synthesized by a polycondensation, bearing a pH sensitive imine bond in the polymer backbone. After degradation in acidic environment only natural occurring spermine and bisformalehyde imidazole were left. The cationic polymer showed a good transfection efficiency and a lower cytotoxicity compared to PEI.[205]

Spermine and other oligoamines were also coupled via pH sensitive carbamate linkages to dPG,[117] by various acetal linkers to a polysaccharide dextran,[206] or applied in pH degradable dendrons and successfully tested *in vitro*.[207]

Furthermore, cationic nanogel structures with enzymatic,[208] redox,[209] or pH[210] sensitive cross-linkers were successfully tested for siRNA mediated gene silencing *in vitro* and *in vivo*. However, studies reveal that only sub-100 nm nanogels were efficient for RNAi based gene silencing, whereas bigger nanogels were accumulated in the lysosomal compartments and failed the transfection *in vitro*.[211]

Similar approaches regarding pH or redox degradable cross-linkers, degradable PEG shells or charge conversion was also applied for cationic micelles for gene delivery application but won't be described here in more detail.[36]

In case of topical application of nanocarriers, temperature differences of the skin can be used as a natural trigger. Recently, temperature responsive polymers were used to develop sensitive nanogels which benefit from the temperature gradient of 32°C at the skin surface to 37 °C in deeper layers of skin, to release therapeutic proteins or anti-inflammatory drugs in a controlled manner.[169, 212]

Furthermore, it is known that the surface pH of human skin is slightly acidic with a surface pH of 5.4 – 5.9 and that inflamed skin shows characteristics like a disrupted barrier function and an increased surface pH of ~ 0.5-units.[213] However, knowledge about a pH gradient in deeper areas of the skin or inside the hair follicles which could be used as natural stimulus for controlled delivery of therapeutic drugs and biomolecules is very limited so far. Nevertheless, the field of dermal, follicular and transdermal drug delivery has an enormous potential for tailor-made nanomedicine.

2 Scientific goal

An overview of the approaches for the developed nanocarriers and their application in this thesis is illustrated in Figure 13. The main objective of the thesis was the preparation of pH-cleavable core shell type carriers for the complexation and release of genetic material such as DNA and siRNA (**A**). The synthesis of degradable polymers should realize a release of the complexed genetic material at the target site by the loss of multivalent binding groups and reduce the toxic potential of the highly positive charged carrier at the same time. One possibility to further improve the transport quality is to encapsulate the genetic material into pH-sensitive nanogels.

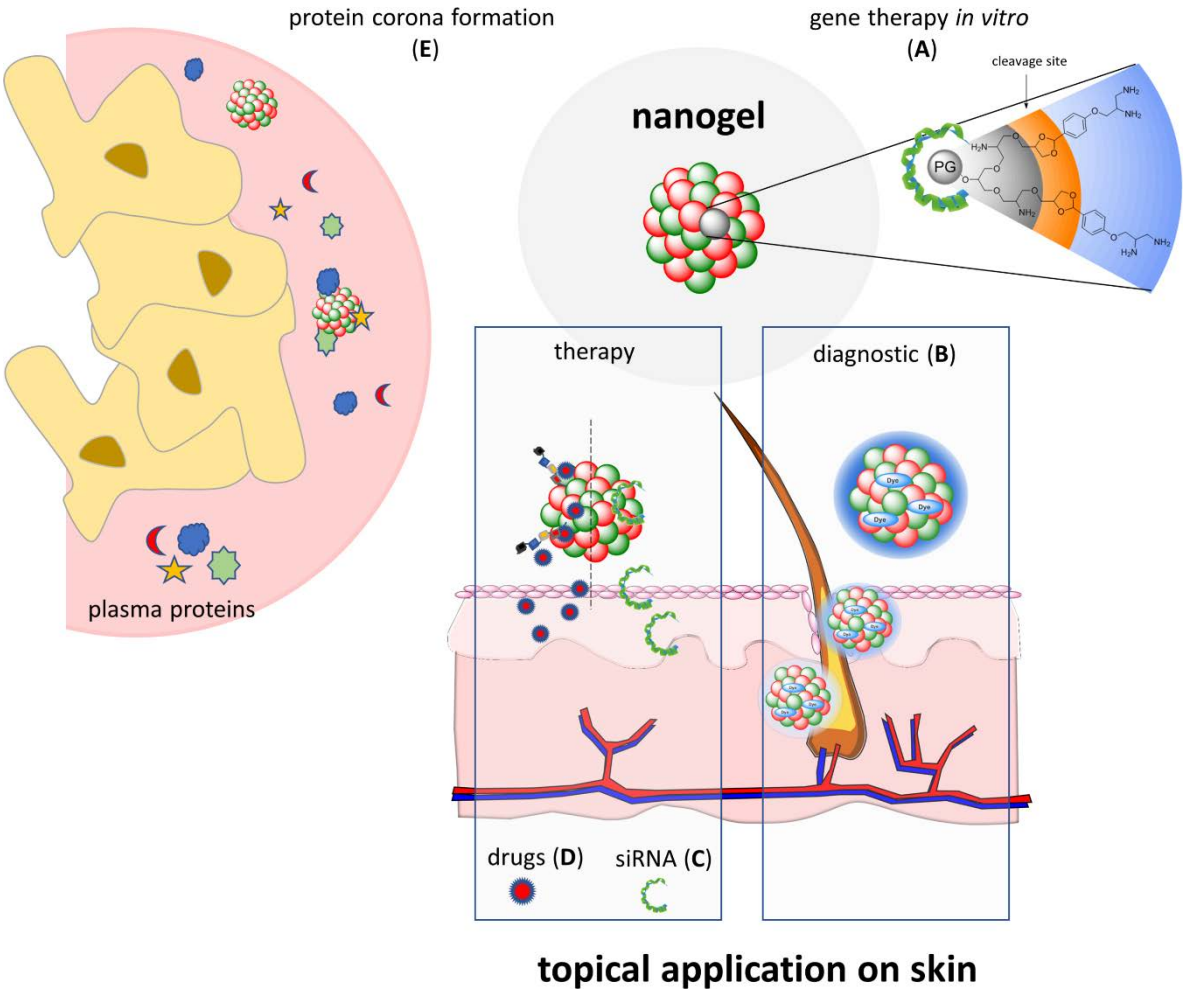

Figure 13. Schematic representation of the application for the nanogel in the field of nanomedicine.

With the successful establishment of the Collaborative Research Center 1112 “Nanocarriers: Architecture, Transport, and Topical Application of Drugs for Therapeutic

Use” the focus of the thesis shifted toward the development of nanocarriers and nanodiagnostics for the skin environment.

Besides the development of thermo-responsive nanocarriers, pH-responsive transporters in the nano range fade in the spotlight, to use the properties and natural stimuli of the skin for a controlled release of drugs and therapeutic biomolecules. However, for the synthesis of responsive nanotransporters a fundamental knowledge of the operational environment is needed. But so far not much is known about pH change inside the skin and hair follicles which is a promising uptake route for nanoparticles. To fill this knowledge gap, tailor-made pH-nanosensors should be developed to provide a clear answer to this question (**B**).

The know-how of therapeutic (**A**) and diagnostic (**B**) concepts was used to develop nanogels which transport and release genetic material or therapeutic drugs. Here, the main challenge was to develop a mild and biorthogonal strategy to encapsulate the sensitive genetic material into the three-dimensional nanogel network and make use of pH sensitive functionalities for a controlled release at the target site (**C**).

Moreover, it was important to establish methods to encapsulate and transport the hydrophobic drugs, which are mainly used for the therapy of skin diseases. Since the used nanogels were highly hydrophilic, a first attempt was made to bind and transport hydrophobic model drugs via functional nanogel-peptide conjugates (**D**).

Furthermore, the protein corona formation of different charged nanogels should be analyzed and compared with other nanocarriers to get insides into the biological interactions of those particles (**E**).

3 Publications and Manuscripts

3.1 Synthesis of pH-Cleavable dPG-Amines for Gene Delivery Application

Mathias Dimde, Dirk Steinhilber, Falko Neumann, Yan Li, Florian Paulus, Nan Ma, Rainer Haag*, *Macromolecular Bioscience*, **2016**, *17*, 1600190.

https://doi.org/10.1002/mabi.201600190

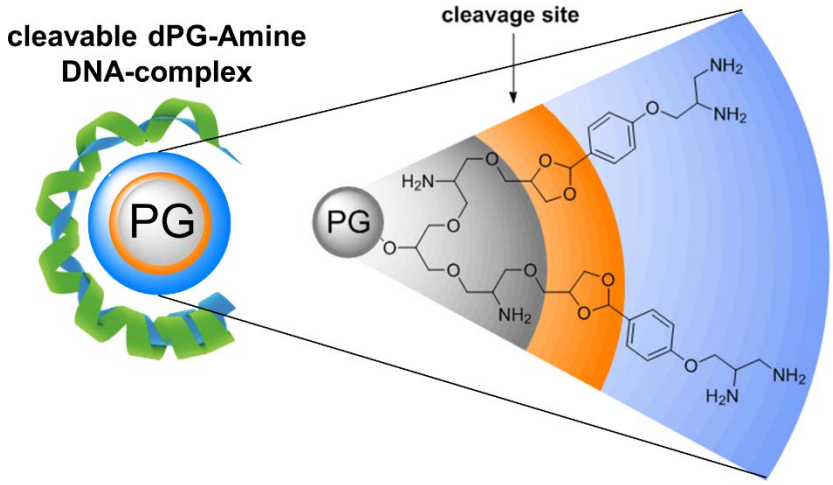

Figure 14. Adapted with permission of Dimde et al.[214] Copyright 2017 Wiley-VCH

The contribution of the author was the synthesis of the pH sensitive dPG-amines reported in this publication. Furthermore, the author characterized the gene carriers and their degradation behaviour. The manuscript was written by the author.

3.2 Synthesis and Validation of Functional Nanogels as pH-Sensors in the Hair Follicle

Mathias Dimde, Fitsum F. Sahle, Virginia Wycisk, Dirk Steinhilber, Luis C. Camacho, Kai Licha, Jürgen Lademann, Rainer Haag*, *Macromolecular Bioscience*, **2017**, *17*, 1600505.

https://doi.org/10.1002/mabi.201600505

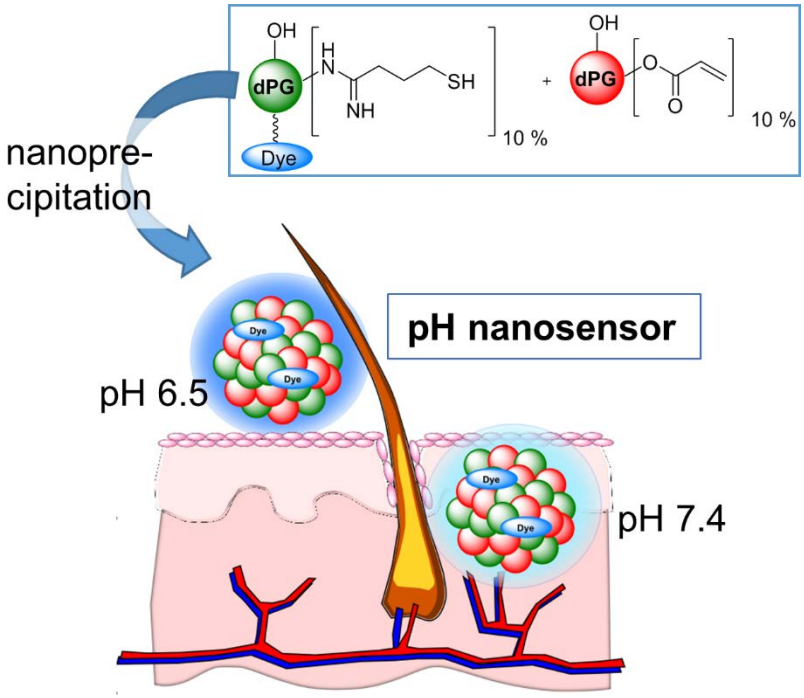

Figure 15. Adapted with permission from Dimde et al.[215] Copyright 2017 Wiley-VCH

In this publication, the author contributed with parts of the idea, as well as the synthesis, characterization and validation of the pH nanosensors. The author analyzed the data and wrote the manuscript.

3.3 Defined pH-sensitive Nanogels as Gene Delivery Platform for siRNA mediated in vitro gene silencing

Mathias Dimde and Falko Neumann, Felix Reisbeck, Svenja Ehrmann, Jose Luis Cuellar-Camacho, Dirk Steinhilber, Nan Ma,* Rainer Haag*, *Biomaterial Science*, **2017**, *5*, 2328-2336.

https://doi.org/10.1039/C7BM00729A

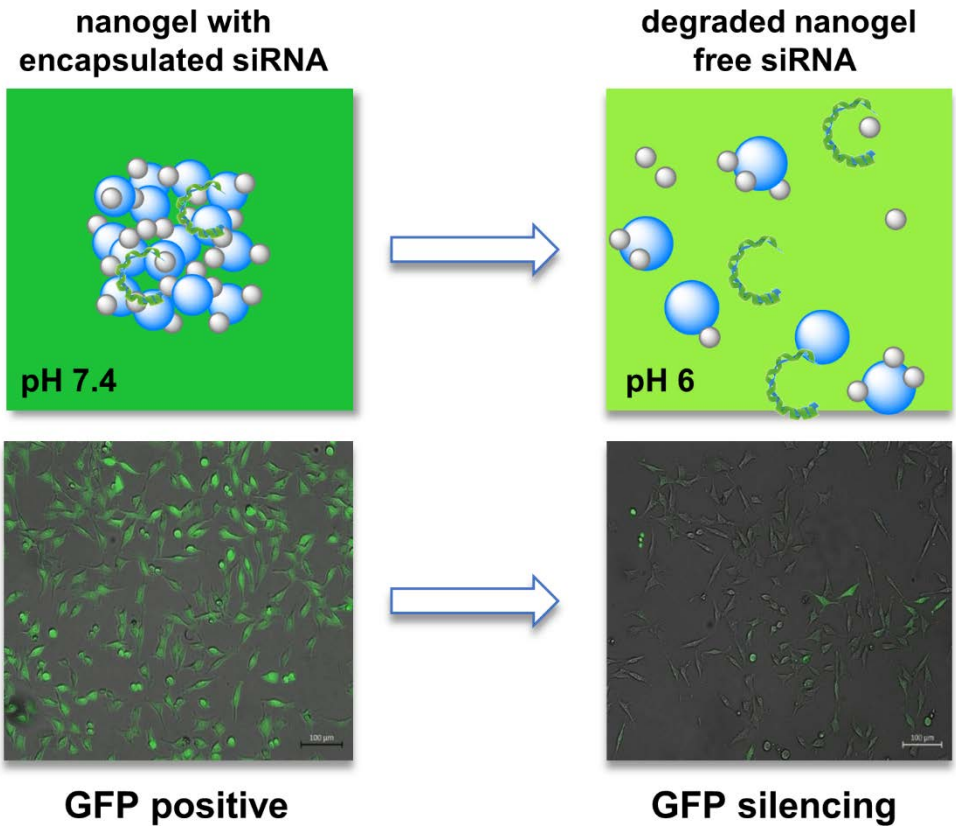

Figure 16. Adapted with permission from Dimde et al.[216] Copyright 2017 Royal Society of Chemistry.

In this publication, the author contributed with parts of the idea, the synthesis of the pH-sensitive nanogels, their characterization and the preparation of the manuscript.

3.4 Intradermal drug delivery by nanogel-peptide conjugates; specific and efficient transport of temoporfin

Fatemeh Zabihi, Sebastian Wieczorek, **Mathias Dimde**, Sarah Hedtrich, Hans G. Börner, Rainer Haag*, *Journal of Controlled Release*, **2016**, *242*, 35-41.

https://doi.org/10.1016/j.jconrel.2016.07.033

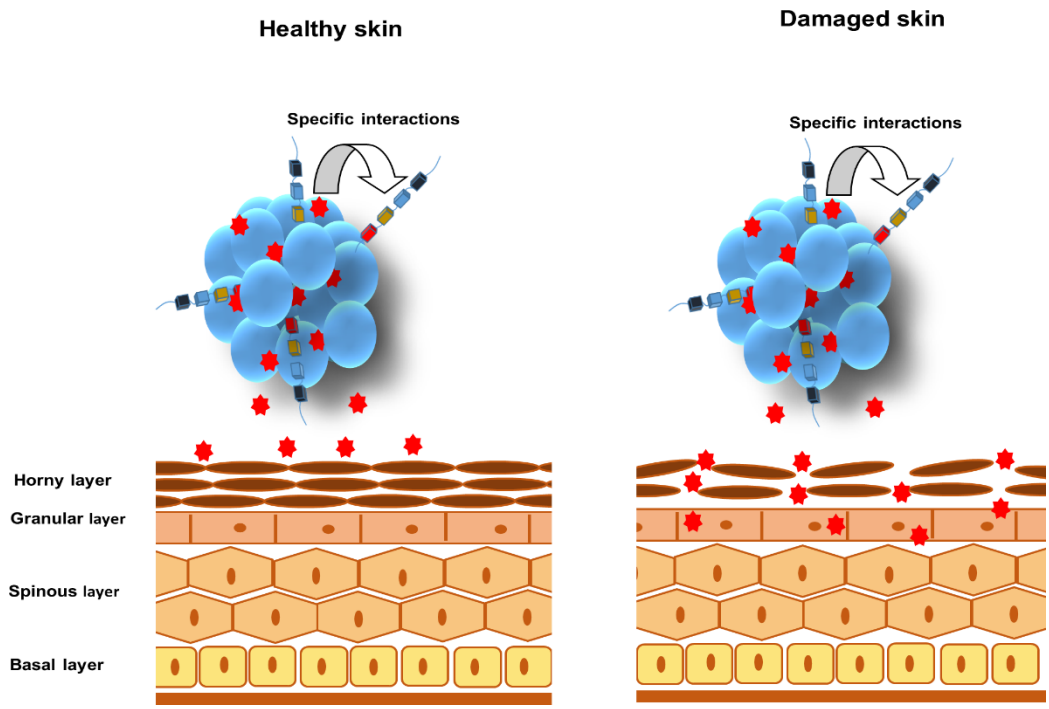

Figure 17. Adapted with permission from Zabihi et al.[217] Copyright 2016 Elsevier

In this publication, the author contributed with parts of the idea and the development of the standardized preparation protocol of the nanogel-peptide conjugates.

3.5 Protein corona formation on colloidal polymeric nanoparticles and polymeric nanogels: impact on cellular uptake, toxicity, immunogenicity and drug release properties

Katja Obst and Guy Yealland, Benjamin Balzus, Enrico Miceli, **Mathias Dimde**, Christoph Weise, Murat Eravci, Roland Bodmeier, Rainer Haag, Marcelo Calderón, Nada Charbaji, Sarah Hedtrich*, *Biomacromolecules*, **2017**, *18*, 1762−1771.

https://doi.org/10.1021/acs.biomac.7b00158

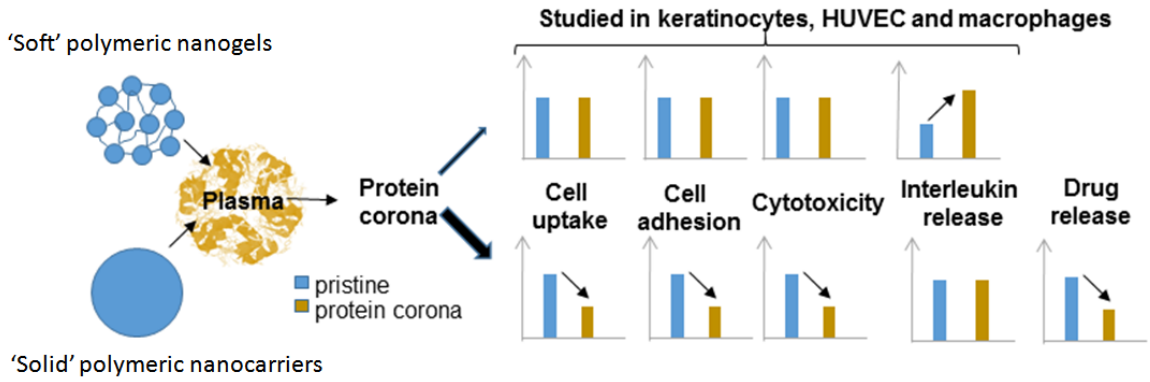

Figure 18. Adapted with permission from Obst et al.[218] Copyright 2017 American Chemical Society

In this publication, the author contributed with the synthesis and characterization of the "soft" polymeric nanogels based on dendritic polyglycerol with different surface charges.

4 Conclusion and Outlook

In this thesis, the overall goal was to develop novel stimuli-responsive nanocarriers which are able to overcome biological barriers and furtherly transport and release various therapeutic cargos to the target site. Furthermore, the aim was to develop a nanomedicine platform that shows the ability to protect the sensitive cargo and use natural stimuli for a controlled release. Besides therapy, the tailor-made nanocarriers are targeted to be adaptable for diagnostic approaches.

In the first project of this thesis, a pH degradable dPG-amine nanocarrier for the delivery of genetic material was developed. Due to the introduction of stimuli-responsive acetal groups the nanocarrier could be cleaved at acidic pH values which occur in the endosomal compartments and remained stable at neutral pH which appearrs in the extracellular matrix. Modified benzacetal moieties realized a fine-tune in the cleavage kinetics. The complexation and release of DNA out of nanocarriers was studied by various methods. The pH triggered cleavage of the outer amine shell results in the degradation of the multivalent amine architecture. By that, the ability to bind genetic material is reduced and a controlled release of DNA can occur. *In vitro* transfection demonstrated that pH cleavable dPG-amines could transfect HeLa cells with GFP-DNA and resulted in cell-compatible cleavage products with a significantly reduced toxicity compared to the non-degradable gold standard PEI. The synthesis of these degradable core-shell polymers realizes a new carrier platform for the complexation and controlled release of genetic material at the target site by the loss of multivalent interactions.

Additionally, a novel approach for the synthesis of dendritic polyglycerol based nanogels was developed. The Thiol–Michael nanoprecipitation method which operates under mild conditions and did not require any catalyst or surfactant was used to develop tailor-made nanogels in the size range of 100-1000 nm. The biocompatible nanogels were used for diagnostic as well as therapy for topical skin application.

Dye-labeled nanogels were used for the first time as pH-nanosensors to determine the pH of the hair follicle (HF) at different depth in an *ex vivo* porcine ear model. The non-toxic nanogels showed a high potential for the penetration via the follicular uptake route. An automated analysis of confocal laser scanning microscopy (CLSM) images helped to accurately determine the pH inside the HF. The pH gradient ranged from 6.5 on the skin surface to 7.4 in deeper areas of the HF with a sharp pH increase over the first 300 μm. This

finding provides a clear direction for the development of pH responsive DDS for follicular drug delivery.

Furthermore, functional nanogel-peptide conjugates were developed which could complex and release hydrophobic drugs into the skin. The conjugation of the tailor-made peptide chains to the polyglycerol based nanogel increased the loading capacity and binding specificity of the targeted therapeutic model drug significantly. Skin penetration tests showed efficient dermal delivery and release of a photosensitizer which can be used for photo-dynamic therapy against cancer.

Moreover, cationic nanogels based on dendritic polyglycerol and low molecular weight PEI were used for the delivery of siRNA. The genetic material could be encapsulated by the above-mentioned Thiol-Michael nanoprecipitation. The mild and bioorthogonal reaction enables the *in-situ* binding of GFP-siRNA into the nanogel matrix. Acetal moieties which were incorporated inside the nanogel realize a pH dependent degradation of the sub-100 nm carrier. *In vitro* transfection demonstrated that the pH cleavable nanogels could successfully silence GFP processing HeLa cells and were significantly less toxic compared to non-cleavable PEI. The cationic nanogel-platform will be investigated further for the delivery of anti-inflammatory siRNA for topical skin application.

Additionally, formation and impact of the protein corona on the dPG-based nanogels with a varied surface charge was analyzed. Here, nanogels with a higher surface charge showed an increased binding of the blood protein human serum albumin (HSA) which resulted in an increased hydrodynamic radius and reduced surface charge.

In the future, the nanogel manufacturing process could be upscaled to produce nanocarriers with a standard operation protocol in a one batch procedure to ensure further development to a nanomedicine platform. Recently, nanoparticles were prepared by nanoprecipitation process in a microfluidic device.[219] In general, this technique is used for the production of microparticles[220] which are useful for advanced drug delivery (e.g. the encapsulation of biological cargos like living cells[221]) and may be adapted to produce stimuli responsive nanogels in a continuous process in high quantity.

Furthermore, the nanogel-platform could be used to encapsulate various therapeutic proteins or therapeutic hydrophobic drugs. Due to the flexible nanogel size range the application area is widely spread. Smaller nanogels in the sub-100 nm area could be used for the intracellular delivery of encapsulated therapeutic cargo. In contrast, bigger nanogels could be applied to transport their freight into deeper areas of the hair follicle for

therapeutic dermal applications. Incorporated stimuli-responsive groups would realize a controlled release and the degradation to biocompatible residues.

5 Kurzzusammenfassung

Das übergeordnete Ziel dieser Arbeit war es neuartige Stimulus empfängliche Nanotransporter zu entwickeln, die biologische Barrieren überwinden und zusätzlich therapeutische Fracht transportieren und freisetzen können. Weiterhin sollte eine nano-medizinische Plattform hergestellt werden, der es möglich ist die sensible Fracht zu schützen und durch natürliche Stimuli kontrolliert am Zielort freizugeben. Neben therapeutischen Aspekten sollen die maßgeschneiderten Nanotransporter auch für diagnostische Ansätze genutzt werden.

Im ersten Projekt dieser Thesis wurde ein pH spaltbarer Nanotransporter entwickelt, der genetisches Material befördern kann. Auf Basis der Einführung von Stimulus empfänglichen Acetalgruppen kann der Nanotransporter in saurem pH Milieu gespalten werden. Diese treten in den endosomalen Kompartimenten auf und bleiben bei neutralem pH-Wert, wie er in einer extrazellularen Matrix auftritt, stabil. Modifizierte Benzacetal-gruppen ermöglichen dabei einen Feinschliff der Spaltkinetik. Die Komplexierung und die Freisetzung der DNA aus Nanotransportern wurde mit verschiedenen Ansätzen analysiert. Die pH-getriggerte Spaltung der äußeren Aminschale resultiert in einem Zerfall der multivalenten Amin-Architektur. Aufgrund dessen ist die Fähigkeit der Bindung von genetischen Material reduziert. Dies ermöglicht eine kotrollierte Freisetzung der DNA. Mit Hilfe von i*n vitro* Transfektions-Tests konnte gezeigt werden, dass pH-spaltbare dPG-Amine HeLa Zellen mit GFP-DNA transfizieren können. Die Spaltprodukte des Nanotransporters weisen im Vergleich zum abbauresistenten Goldstandard PEI eine signifikant reduzierte Toxizität auf. Die Synthese dieser spaltbaren Kern-Schale-Polymere ermöglicht eine neue Transportplattform für die Komplexierung sowie kontrollierter Freisetzung von genetischem Material, die durch den Verlust multivalenter Interaktionen genau am Zielort greifen kann.

Zusätzlich wurde ein neuer Ansatz für die Synthese von Nanogelen entwickelt, die auf dendritischen Polyglycerin basieren. Die Methode der Thiol-Michael Nanofällung, die unter milden Reaktionsbedingungen durchgeführt wurde, benötigt weder einen Katalysator noch Tenside, um maßgefertigte Nanogele im Größenbereich von 100-1000 nm herzustellen. Die biokompatiblen Nanogele wurden sowohl für die Diagnostik als auch für topische Hautanwendungen benutzt.

Zum ersten Mal wurden farbstoffmarkierte Nanogele als pH-Nanonsensoren genutzt, um den pH-Wert von Haarfollikeln (HF) in verschiedenen Tiefen in einem *ex vivo*

Schweineohrmodell zu bestimmen. Die nicht-toxischen Nanogele wiesen ein hohes Potenzial für das Eindringungsvermögen über den follikulären Aufnahmeweg auf. Mit Hilfe einer automatisierten Analyse durch konfokale Laser-Scanning-Mikroskopie konnte der pH-Wert innerhalb des HFs genau bestimmt werden. Der pH Gradient erstreckte sich von Werten von 6,5 auf der Hautoberfläche bis hin zu Werten von 7,4 in tieferen Arealen des HFs mit einem starken pH-Anstieg über die ersten 300 µm. Diese Erkenntnis gibt eine klare Richtung für die Entwicklung von pH-responsiven DDS für den follikulären Wirkstofftransport.

Weiterhin wurden funktionelle Nanogel-Peptid Konjugate entwickelt, die hydrophobe Wirkstoffe komplexieren und in der Haut freigeben können. Die Konjugation der maßgeschneiderten Peptidketten an Polyglycerin basierten Nanogelen konnte die Ladungskapazität sowie die Bindungsspezifität im anvisierten therapeutischen Modell-wirkstoff signifikant erhöhen. Tests zur Hautpenetration zeigten einen effizienten dermalen Wirkstofftransport sowie die Freigabe eines Photo-Sensibilisators der in der photo-dynamischen Krebstherapie eingesetzt werden kann.

Darüber hinaus wurden kationische Nanogele, basierend auf dendritischen Polyglycerin und niedermolekularem PEI, für den Transport von siRNA verwendet. Das genetische Material wurde dabei durch die oben bereits erwähnte Thiol-Michael Reaktion eingekapselt. Die milde, bioorthogonale Reaktion befähigt die *in situ* Bindung von GFP-siRNA in die Nanogelmatrix. Mittels Acetalgruppen, die in das Nanogel eingebettet wurden, konnte ein pH-abhängiger Abbau der sub-100 nm Träger realisiert werden. Mittels *in vitro* Transfektion wurde gezeigt, dass pH-spaltbare Nanogele erfolgreich die GFP Bildung von HeLa Zellen unterdrücken können. Zusätzlich zeigten sich diese als weniger toxisch verglichen mit nichtspaltbarem PEI. Die kationische Nanogelplattform wird weiter für den Transport anti-entzündlicher siRNA für topische Hautanwendungen untersucht werden.

Zusätzlich wurde die Bildung sowie der Einfluss der Proteinkorona auf dPG-basierte Nanogele mit unterschiedlichen Oberflächenladungen untersucht. Dabei zeigte sich, dass Nanogele mit einer höheren Oberflächenladung eine erhöhte Bindung des Blutproteins Humanalbumin aufweisen. Dies resultierte in einem erhöhten hydrodynamischen Radius sowie einer reduzierten Oberflächenladung des Nanogels.

In Zukunft wäre ein Upscale des Herstellungsprozesses von Nanogelen denkbar, um Nanotransporter mittels eines Standardprotokolls in einer ein-Batch Prozedur herzustellen, um die Entwicklung zu einer nanomedizinischen Plattform zu ermöglichen.

Erst kürzlich wurden Nanopartikel im Nanofällungsprozess mittels Mikrofluidik hergestellt.[219] Generell wird diese Technik für die Produktion von Mikropartikeln benutzt.[220] Diese sind besonders für die Anwendung des fortgeschrittenen Wirkstofftransports (z.B. die Einkapselung von biologischer Fracht wie lebenden Zellen[221]) nützlich. Außerdem besteht die Möglichkeit dieses Verfahren zu adaptieren, um Stimuli-sensitive Nanogele in einem kontinuierlichen Prozess in hohen Mengen herzustellen.

Weiterhin ist es möglich die Nanogelplattform zu nutzen, um verschiedene therapeutische Proteine oder hydrophobe Wirkstoffe einzukapseln. Aufgrund des weiten Größenbereichs der Nanogele ist das Anwendungsspektrum sehr breit. Kleinere Nanogele im sub-100 nm Bereich könnten für den intrazellulären Transport eines eingekapselten therapeutischen Wirkstoffes genutzt werden. Im Gegensatz dazu könnten größere Nanogele dazu genutzt werden, um ihre Fracht in tiefere Areale des Haarfollikels für therapeutische dermale Anwendungen zu transportieren. In das Nanogel eingebaute, Stimuli-sensitive Gruppen würden eine kontrollierte Freigabe und die Zersetzung zu biokompatiblen Abbauprodukten realisieren.

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8 Appendix

8.1 List of abbreviations

ADA	adenosine-deaminase
ATP	adenosine triphosphate
CAC	critical aggregation concentration
CLSM	confocal laser scanning microscopy
CML	critical micelle concentration
CMS	core-multishell
CuAAC	copper-catalyzed azide–alkyne Huisgen cycloaddition
DDS	drug delivery systems
DNA	deoxyribonucleic acid
DOX	doxorubicin
dPG	dendritic polyglycerol
EEGE	ethoxyethyl glycidyl ether
EPR effect	enhanced permeability and retention effect
FDA	food and drug administration
GSH	glutathione
HF	hair follicle
HSA	human serum albumin
lPG	linear polyglycerol
mPEG	poly(ethylene glycol) monomethyl ether
mRNA	messenger RNA
NP	nanoparticle
OEI	oligo-ethyleneimine
PAMAM	polyamidoamine
PAsp	poly(aspartic acid)
PCL	poly(e-caprolactone)
PDI	polydispersity index
PDT	photodynamic therapy
PEG	poly(ethylene glycol)
PEGylation	poly(ethylene glycol) conjugation
PEI	polyethylenimine
PEO	poly(ethylene oxide)
PG	polyglycerol
PGlu	poly(glutamic acid)
PHis	poly(histidine)
PIC	polyion complex

PLA	poly(lactide)
PLGA	poly(lactide-co-glycolide)
PLys	poly(lysine)
PMMA	poly(methyl methacrylate)
PNIPAm	poly(N-isopropyl acrylamide)
PS	polystyrene
PTX	paclitaxel
PVA	poly(vinyl)alcohol
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RNAi	RNA interference
ROS	reactive oxygen species
SC	stratum corneum
siRNA	small interfering RNA
SPAAC	strain-promoted azide-alkyne cycloaddition
TMP	1,1,1-trimethylpropane

Amino Acids denote the *L*-configuration.

8.2 List of publications

1) Sumati Bhatia, **Mathias Dimde**, Rainer Haag; *Multivalent glycoconjugates as vaccines and potential drug candidates*, Medicinal Chemical Communications **2012**, *5*, 862-878.
2) **Mathias Dimde**, Dirk Steinhilber, Falko Neumann, Yan Li, Florian Paulus, Nan Ma, Rainer Haag; *Synthesis of pH-Cleavable dPG-Amines for Gene Delivery Application*, Macromolecular Bioscience **2017**, *17*, 1600190.
3) Fatemeh Zabihi, Sebastian Wieczorek, **Mathias Dimde**, Sarah Hedtrich, Hans G. Börner, Rainer Haag; *Intradermal drug delivery by nanogel-peptide conjugates; specific and efficient transport of temoporfin*, Journal of Controlled Release **2016**, *242*, 35-41.
4) Yinan Zhong, **Mathias Dimde**, Daniel Stöbener, Fenghua Meng, Chao Deng, Zhiyuan Zhong, and Rainer Haag; *Micelles with Sheddable Dendritic Polyglycerol Sulfate Shells Show Extraordinary Tumor Targetability and Chemotherapy in Vivo*, ACS Applied Materials & Interfaces **2016**, *8(41)*, 27530–27538.
5) **Mathias Dimde**, Fitsum F. Sahle, Virginia Wycisk, Dirk Steinhilber, Luis C. Camacho, Kai Licha, Jürgen Lademann, Rainer Haag; *Synthesis and Validation of Functional Nanogels as pH-Sensors in the Hair Follicle,* Macromolecular Bioscience **2017**, *17*, 1600505.
6) **Mathias Dimde** and Falko Neumann, Felix Reisbeck, Svenja Ehrmann, Jose Luis Cuellar-Camacho, Dirk Steinhilber, Nan Ma, Rainer Haag; *Defined pH-sensitive Nanogels as Gene Delivery Platform for siRNA mediated in vitro gene silencing*, Biomaterial Science **2017**, *5, 2328-2336*.
7) Katja Obst and Guy Yealland, Benjamin Balzus, Enrico Miceli, **Mathias Dimde**, Christoph Weise, Murat Eravci, Roland Bodmeier, Rainer Haag, Marcelo Calderón, Nada Charbaji, Sarah Hedtrich; *Protein corona formation on colloidal polymeric nanoparticles and polymeric nanogels: impact on cellular uptake, toxicity, immunogenicity and drug release properties*, Biomacromolecules **2017**, *18*, 1762−1771.

8.3 List of Conference Contributions

Poster Presentations

1) **Mathias Dimde**, Dirk Steinhilber, Nan Ma, Rainer Haag

   Synthesis of pH-cleavable Polyglycerol Amines for the Complexation and Release of Genetic Material

   12th International Conference on Polymers for Advanced Technologies, Berlin, Germany, **2013**.

2) Daniel Stöbener, **Mathias Dimde**, Benjamin Ziem, Florian Paulus, Ronny Schulze, Dirk Steinhilber, Ingo Steinke, Pia Welker, Stefanie Wedepohl, Kai Licha, Jens Dernedde, Rainer Haag

   Biological Properties of Polyglycerol Sulfates – Influence of the Polymer Architecture

   Makromolekulares Kolloquium Freiburg, Freiburg i. Br., Germany, **2014**.

3) **Mathias Dimde**, Fitsum F. Sahle, Virginia Wycisk, Dirk Steinhilber, Kai Licha, Jürgen Lademann, Rainer Haag

   Investigation of dye labelled dPG-Nanogels as pH sensors in hair follicles

   SFB1112 International Conference on Dermal Drug Delivery by Nanocarriers, Berlin, Germany, **2016**.

   Poster Award

4) **Mathias Dimde**, Fitsum F. Sahle, F. Neumann, Virginia Wycisk, Luis C. Camacho, Dirk Steinhilber, Jürgen Lademann, Nan Ma, Rainer Haag

   Synthesis of dPG-Nanogels for pH sensing and Transport of Biomolecules into the Skin

   6th EuCheMS Chemistry Congress, Sevilla, Spain, **2016**.

Oral Presentations

1) **Mathias Dimde**, Rainer Haag

   pH-sensitive dendritic polymers for gene delivery applications

   FUB-TAU Joint Research Workshop, Berlin, Germany, **2013**.

2) **Mathias Dimde**, Rainer Haag

   Synthesis of pH responsive Nanogels

   TAU-FUB Joint Research Workshop, Tel Aviv, Israel, **2015**.

8.4 Curriculum vitae

Der Lebenslauf ist aus Gründen des Datenschutzes nicht enthalten.